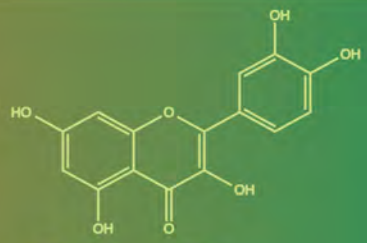
NUTRACEUTICALS Basic Research/Clinical Applications



ANTIOXIDANT NUTRACEUTICALS

PREVENTIVE AND HEALTHCARE APPLICATIONS

Edited by
Chuanhai Cao • Sarvadaman Pathak
Kiran Patil

CRC Press
Taylor & Francis Group

Antioxidant Nutraceuticals

Nutraceuticals: Basic Research and Clinical Applications

Series Editor: Yashwant Pathak, PhD

Herbal Bioactives and Food Fortification: Extraction and Formulation,
D. Suresh Kumar

Nanotechnology in Nutraceuticals: Production to Consumption,
edited by Shampa Sen and Yashwant Pathak

Handbook of Metallonutraceuticals,
edited by Yashwant V. Pathak and Jayant N. Lokhande

Nutraceuticals and Health: Review of Human Evidence,
edited by Somdat Mahabir and Yashwant V. Pathak

Marine Nutraceuticals: Prospects and Perspectives,
Se-Kwon Kim

Nutrigenomics and Nutraceuticals: Clinical Relevance and Disease Prevention,
edited by Yashwant Pathak and Ali M. Ardekani

Food By-Product Based Functional Food Powders,
edited by Özlem Tokuşoğlu

Flavors for Nutraceuticals and Functional Foods,
M. Selvamuthukumaran and Yashwant Pathak

Antioxidant Nutraceuticals: Preventive and Healthcare Applications,
edited by Chuanhai Cao, Sarvadaman Pathak, and Kiran Patil

For more information about this series, please visit: https://www.crcpress.com/Nutraceuticals/book-series/CRCNUTBASRES

Antioxidant Nutraceuticals
Preventive and Healthcare Applications

Edited by
Chuanhai Cao
Sarvadaman Pathak
Kiran Patil

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Printed on acid-free paper

International Standard Book Number-13: 978-1-4987-3703-6 (Hardback)

We dedicate this book to all the ancient and modern scientific wisdom, the physicians, and scientists who pioneered all aspects of medicine, the pharmacists who prescribe and the patients who benefit. Advancement in science is not possible without students with inquisitive minds, who strive to become the best providers.

We express our gratitude to Adryan Perez and Richard Nguyen for their hard work to make this book a success. We sincerely thank all our contributing authors as without them there is no book, and our families, as without their support we would not be editors.

Sincerely
Sarvadaman Pathak,
Kiran Patil, and Chuanhai Cao

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Contents

Foreword

Promotion of longevity, vitality, strength, mental health and wellness in general, are all rooted in food.

Charaka Sambita 1.25.350

Charaka (2000 BCE), the father of Ayurveda, the science of life, has propounded the wisdom that food sustains life, augments health and wellness, and prevention of disease. Hippocrates (460 BCE), the father of modern medicine, had echoed the same insight into food in his oft-quoted teaching, *Let food be thy medicine and medicine be thy food*. This wisdom of yore has developed the concept of a new class of food supplements termed *nutraceuticals*, which bridged together foods and drugs.

To start with the understanding of nutraceuticals, they are categories of food, which not only provide necessary nutrition but also act to prevent diseases and promote strength and vitality. Nutraceuticals over the course of the past two decades have grown exponentially and have currently reached the US$200 billion mark. It is further expected to reach the approximately US$300 billion mark in the next 10 years. With this stupendous growth, the precise definition of nutraceuticals has become fuzzy and now includes multiple categories of food comprising a wide variety of classifications that includes food additives that provide medical or health benefits; products isolated or purified from food; medicines that are not associated with food; isolates that provide concentrated nutrients in the form of pills, tablets or other forms; commodities derived from foods in the form of medicines with demonstrated physiological benefits; and a range of products that have properties of health promotion, including the concept of enhanced performance. With commercial growth of this range of food supplements, regulatory processes have

also developed with a good deal of variations across countries and even within a country as applied to particular types of nutritional supplements. This has led to efforts to redefine nutraceuticals and classify them on pharmaceutical, physiological, and therapeutic grounds. Some of the classes are already well-recognized, such as antioxidants, probiotics, phytopharmaceuticals, energy foods, and so on. Defining these classes with scientific precision and using this research as guidelines for regulatory purposes will serve to address the present chaotic stage of the vast ocean of nutraceuticals that are emerging.

This book, *Antioxidant Nutraceuticals: Preventive and Healthcare Applications,* usefully bridges present gaps in the literature on this subject. The content by experienced authors portrays a wide view of the topic in a total of 19 chapters. The authors discuss diverse topics ranging from historical perspectives and traditional systems to antioxidant nutraceuticals in the prevention and treatment of acute diseases. The chapter on current status of market and future trends illustrates the background of the topic. This book addresses many clinical specialties like cancer, dementia, diabetes, preeclampsia, heart diseases, depression, inflammatory diseases, skin care, ocular diseases, and lung diseases. A chapter on pharmacology and pharmacokinetics provides an in-depth analysis of possible mechanisms of natural antioxidants. The topic also covers pharmaceutics approach through probiotic applications as health drinks for disease prevention.

An important chapter of this book, "Antioxidant Nutraceuticals: Historical Perspective and Applications in Various Traditional Systems Worldwide," is particularly noteworthy. Ayurveda, in particular, is known across the world for its fundamental contributions on concepts of health and personalized therapeutic approaches. The theory of Ayurveda and Yoga suggests that nutrition is not only for nourishment of the body but also to nourish mind and spirit. Charaka Samhita, Sushruta Samhita, and many other classics are resources for discovery of new nutraceuticals and health supplements. Ayurveda has described properties of fruits, spices, herbs, animal products, minerals, and medicated recipes for specific diseases. An effort to integrate such research with modern medical practices is the need of the hour.

The methodology for research investigating nutraceuticals has posed a challenge on the background of nutritional genomics that decodes variations of nutritional effects. We know that reactive oxygen species (ROS) play a major role in the pathogenesis of several diseases. There is lack of clarity in the role of ROS in origin and progression of many diseases. Another challenge is antioxidant paradox that several clinical trials have reported. Antioxidant supplements have demonstrated little or no preventative, or therapeutic effect in large doses. There is a need to revisit traditional knowledge and practices and support them with current

advances of biology and medicine. Ongoing and prospective research worldwide on this important subject can only enhance the future editions of this book.

Dr. Patil, Dr. Pathak, and Dr. Cao have to be commended in coordinating this effort to produce this publication, which addresses a felt need of defining application of antioxidants in wide-ranging clinical conditions.

This book makes useful reading not only for stimulating research on this important subject for scientists but also provides useful guidance for practitioners of medicine and medical students.

Dr. Gururaj Mutalik
Former Director of the World Health Organization
at the United Nations

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Series Preface

In biology, both in plant and animals, abiotic components or abiotic factors are nonliving chemical and physical parts of the environment that affect living organisms and the functioning of ecosystems. Abiotic factors and the phenomena associated with them underpin all biological systems. *Abiotic stress* is defined as the negative impact of nonliving factors on the living organisms in a specific environment. Various abiotic stress processes lead to the overproduction of reactive oxygen species abbreviated as ROS. These are highly reactive species causing damage to proteins, lipids, carbohydrates, and other biological systems leading to low—severe toxicity. These also damage the tissues and cells resulting in a large number of diseases and disorders.

Many natural cellular processes in our bodies lead to formation of free radicals that are also part of the ROS. If these highly reactive substances aren't neutralized, they can cause damage in our bodies, which can lead to inflammation. A consistently high state of inflammation is considered to be a precursor to many common conditions in older adults, such as cardiovascular diseases, neurodegenerative diseases, and various types of cancer.

Our bodies do have a mechanism to create antioxidants to balance this damage due to free radicals or ROS. Antioxidants bind to free radicals and suppress their damage. However, since we are exposed to additional free radicals from pollution, cigarette smoke, pesticides, radiation, and some processed foods, we need to also take, in addition, antioxidants to neutralize the free radicals.

In recent years, increasing attention has been given to understand the abiotic stress processes and how the nature is dealing with such processes. This led to looking at various natural components, which

have exhibited protective effects that have been ascribed often as antioxidant effects. Traditionally, reactive oxygen intermediates (ROIs) were considered to be toxic by-products of aerobic metabolism, which were disposed of using antioxidants. However, in recent years, it has become apparent that plants actively produce ROIs as signaling molecules to control processes such as programmed cell death, abiotic stress responses, pathogen defense, and systemic signaling.

Recent technological advances in understanding these processes including microarray studies and the development of mutants with altered ROI-scavenging mechanisms provide new insights into how the steady-state level of ROIs is controlled in cells. These raise several intriguing questions about the relationships between ROI signaling, ROI stress, and the production and scavenging of ROIs in the different cellular compartments; several researchers are dedicating their time and efforts in this area and many of them are focusing significantly on natural antioxidants.

New insights from genetic analyses of ROS detoxifying and signaling mutants are shedding light on the complexity and roles that ROS plays in plants. Considering recent ROS-induced genome-wide expression analyses, the possible functions and mechanisms for ROS sensing and signaling in plants are comparable to those in animals.

A variety of antioxidant compounds derived from natural products (Nutraceuticals) have demonstrated neuroprotective activity in either *in vitro* or *in vivo* models of neuronal cell death or neurodegeneration, respectively.

The World Health Organization reported that cardiovascular disease (CVD) is the leading cause of death globally, resulting in 17.5 million deaths in 2012. Antioxidants may benefit many health factors that lead to CVD, including blood pressure, cholesterol, and circulation.

Arthritis and diabetes, two significant concerns for the aging population, involve inflammatory processes that generate extra free radicals. The antioxidant nutraceuticals have been shown to improve joint health by reducing inflammation caused by free radicals and may also help to reduce blood sugar among diabetics.

The antioxidant nutraceuticals market is growing significantly. The 2014 data reported 14.8 million for vitamin A, 331 million for vitamin C, green tea supplements over 60 million, and so on, and it is growing day-by-day.

The scope of the CRC series on *Nutraceuticals: Basic Research/Clinical Applications* aims at bringing out a range of books edited by distinguished scientists and researchers who have significant experience in

scientific pursuit and critical analysis. This series will address various aspects of the nutraceutical products, including the historical perspective, traditional knowledge base, analytical evaluations, green food to processing, and applications. This series will be very useful to not only the researchers and academicians but also as valuable reference books for personnel in the nutraceuticals and food industries.

The purpose of the inclusion of this particular book titled *Antioxidant Nutraceuticals: Preventive and Healthcare Applications* in the series is to cover the recent trends in this area, which is significantly enhanced with the advent of understanding about the ROS and related preventive processes as mentioned earlier. This series has successfully included several titles in the area of nutraceuticals and functional foods, including *Handbook of Metallonutraceuticals*, *Marine Nutraceuticals, Nutraceuticals and Human Health: Review of Human Evidence, Herbal Bioactives for Food Fortification*, *Handbook of Nutraceuticals Volume I: Ingredients, Formulations, and Applications, Handbook of Nutraceuticals Volume II, Scale-Up, Processing, and Automation*, and *Nanotechnology and Nutraceuticals: Production to Consumption*.

The forthcoming titles in the series include *Seaweed Bioactives*: *Health Benefits and Potential Applications, Nutrigenomics and Nutraceuticals: Recent Developments and Market Trends,* and *Food By-Product Based Functional Food Powders*.

Antioxidant Nutraceuticals: Preventive and Healthcare Applications is edited by three scientists: Dr. Chuanhai Cao, PhD, Dr. Sarvadaman Pathak, MD, and Dr. Kiran Patil, MD. Some of the top-ranking scientists in this field have contributed to this book and I am sure this book will be very useful to the academicians and industry people equally.

This series has proven to be a very good resource to the academicians, industrial scientists, and students in the area of nutraceuticals basic research and clinical applications. We request scientists and academicians working in this field to contribute to this series.

Yashwant Pathak
College of Pharmacy, University of South Florida Health

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Preface

The term antioxidant refers to a substance that inhibits oxidation, especially to counteract the deterioration of stored food products. The term nutraceutical refers to a food-based substance with potential health benefits. Nutraceuticals cover a broad spectrum of substances, including antioxidants. Antioxidant nutraceuticals have been used widely in ancient medical systems for preventive and curative ailments. In today's modern medical system, nutraceuticals are considered beneficial but have unsubstantiated claims with regard to specific ailments, thus further research needs to be conducted in this area. It is also important to determine if nutraceuticals are necessary in western populations with adequate nourishment from food.

This book further explores the antioxidant properties and benefits of nutraceuticals and expert authors and scientists have contributed chapters based on their research. This book covers historical aspect and development, current and future market trends, prostate cancer, general cancer prevention, Alzheimer's disease and dementia, Parkinson's disease, general well-being, obstetric applications, and ophthalmic applications, among others. It also covers research, both epidemiological and scientific, based on each organ system, as well as novel delivery approaches for enhanced absorption for antioxidant nutraceuticals.

This book will be a good resource for students, clinicians, researchers, public health officials, and the general public, who are all interested in the area of nutraceuticals and their application in human health and disease.

Sarvadaman Pathak

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Editors

Chuanhai Cao is an experienced and independent neuroimmunologist, vaccinologist, and a dedicated nutraceutical researcher with a tenure-track assistant professor position in the Department of Pharmaceutical Sciences at the College of Pharmacy, University of South Florida (USF), Tampa, Florida. Dr. Cao has been working with the clinical core of the Florida Alzheimer's Research Center (FADRC, NIH funded center) for more than 8 years and has combined his contemporary immunology and vaccine skills with molecular biology to search for novel biomarkers and therapies for neurodegenerative diseases. He believes that most diseases occur due to changes in the immune system, and that targeting the immune system is a promising solution to diseases.

Sarvadaman Pathak went to the University of Houston for undergraduate studies with a concentration in biochemistry and premedicine. Following that, he pursued a doctor of medicine degree from Avalon University School of Medicine, Youngstown, Ohio, summa cum laude. He was educated partially in Belize and Mexico with all clinical experience in Chicago, Illinois. After graduating from medical school with honors, he focused on research and worked at the University of South Florida, Tampa, Florida. In 2013, he completed a 1-year clinical fellowship in traditional Chinese medicine, including Chinese herbalism, with a focus on eastern–western integrative medicine at the Dalian Medical University in Dalian, Liaoning province in Mainland China. In 2017, he completed a master's level program and graduated from the Harvard Medical School, Boston, Massachusetts, with a focus on clinical trials and drug development. Currently, he works as a clinical research

director in a hospital and outpatient setting. Sarvadaman has traveled to over 35 countries and has had a fascination with traditional medicines of the world since his childhood.

Kiran Patil was trained in both the United States and India and has a lot of interest in the Ayurvedic system of medicine, which has been practiced for more than 3000 years in India. He is currently working as a neurologist in Pittsburg, Pennsylvania, with more than 15 years of experience in research and medical practice.

Contributors

Oyinlola Adeyanju
University of South Florida
Tampa, Florida

Adeleke Badejo
Pacific University
Hillsboro, Oregon

Manish Bodas
Central Michigan University
Mount Pleasant, Michigan

Chuanhai Cao
University of South Florida
Tampa, Florida

Runu Chakraborty
Jadavpur University
Kolkata, India

Patrick Chan
Western University of Health Sciences
Pomona, California

Danielle Dantuma
University of South Florida
Tampa, Florida

Arpita Das
Babes-Bolyai University
Cluj-Napoca, Romania

Nandini Doshi
Emory University
Atlanta, Georgia

Anjali Hirani
University of South Florida
Tampa, Florida

Mark C. Houston
Vanderbilt University
Nashville, Tennessee

Ying Huang
Western University of Health Sciences
Pomona, California

Shannon Kelly
University of South Florida
Tampa, Florida

P. Latha
Sree Vidyanikethan College of Pharmacy
Tirupati, India

Uyen Le
Sullivan University
Louisville, Kentucky

Bell Loo
Sullivan University
Louisville, Kentucky

Ashim Malhotra
Pacific University
Hillsboro, Oregon

Thea Moore
University of South Florida
Tampa, Florida

Rohini Nimbalkar
University of South Florida
Tampa, Florida

Hiep Nguyen
Mercer University
Macon, Georgia

Richard Nguyen
University of South Florida
Tampa, Florida

Sarvadaman Pathak
University of South Florida
Tampa, Florida

and

Harvard University
Boston, Massachusetts

Yashwant Pathak
University of South Florida
Tampa, Florida

Kiran Patil
St. Clair Hospital
Pittsburgh, Pennsylvania

Garrett Pehote
Central Michigan University
Mount Pleasant, Michigan

Adryan Perez
University of South Florida
Tampa, Florida

Aishwarya Potdar
University of South Florida
Tampa, Florida

Charles Preuss
University of South Florida
Tampa, Florida

Kumar Rajendran
VIT University
Vellore, India

Avipsha Sarkar
VIT University
Vellore, India

Shampa Sen
VIT University
Vellore, India

Suyansh Sharma
University of South Florida
Tampa, Florida

Gregory L. Smith
CBC Biotechnologies
Tampa, Florida

Kyle Sutherland
University of South Florida
Tampa, Florida

Thomas Towers
University of South Florida
Tampa, Florida

Andrew Tran
University of South Florida
Tampa, Florida

Radouil Tzekov
University of South Florida
Tampa, Florida

Ailyn Valladares
University of South Florida
Tampa, Florida

Neeraj Vij
Central Michigan University
Mount Pleasant, Michigan

and

Johns Hopkins University
Baltimore, Maryland

Monika M. Wahi
Vasanta Health Science
Boston, Massachusetts

Jeffrey Wang
Western University of Health Sciences
Pomona, California

Zhijun Wang
Western University of Health Sciences
Pomona, California

Chen Xie
Western University of Health Sciences
Pomona, California

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

1

Antioxidant Nutraceuticals

Historical Perspective and Applications in Various Traditional Systems Worldwide

Chuanhai Cao and Kyle Sutherland

Contents

1.1 Introduction

1.1.1 Nutraceuticals definition

Different definitions for the term *nutraceutical* have been described over time. It is hard to gauge how big the nutraceutical market will be because the definition itself can be taken vaguely. For example, a nutraceutical could be described as something that has both nutritional and medical benefit. To this point, all foods could be considered nutraceuticals.

Nutraceuticals were first described by Dr. Stephen DeFelice and the Foundation for Innovation Medicine in 1989 in New York due to the heavily increasing number of research done in this area (Andlauer and Furst 2002; Kalra 2003; Pathak 2009). This research was believed to develop foods for the future, where the lines were obscure between food and drug. DeFelice described the term as, "a substance that is a food or part of food and provides medical and health benefit." At this time, nutraceuticals were used to describe nutrients that were isolated from foods, dietary supplements, genetically engineered foods, and specific diets designed to increase a specific nutrient intake. The described nutraceutical food itself may be medically active, such as garlic or soybeans, or components of the food may be active, such as omega-3 fatty acids found in salmon and other fish.

This later portion was described further by Zeisal in that this was a "diet supplement that delivers a concentrated form of a presumed bioactive agent from food" (Zeisel 1999). These bioactive agents are those normally present in the foods we eat, but are increased in concentration and delivered as an object that people would not consider being a food.

The definition was updated in 2007 when Dr. Lockwood slightly redefined nutraceuticals using previous definitions as something that "describes a medicinal or nutritional component that includes a food, plant, or naturally occurring material which may have been purified or concentrated, and that is used for the improvement of health by either preventing or treating disease" (Lockwood 2007).

So then we can describe nutraceuticals as following the concurrent two definitions: nutraceuticals (1) are nutrition derived from food or plants that are naturally occurring, and that they (2) are specifically used for the prevention or improvement of disease or well-being. It is the combination of nutrients and their use as pharmaceuticals.

The term that is commonly heard today is referring to *superfoods*, or those which are nutrient rich and are particularly beneficial toward health. Most of

these foods you find will be rich in flavonoids, antioxidants, and specific fatty acids that many say can prevent or treat chronic conditions, such as cancer or heart disease (Gebauer et al. 2006). Some of the most beneficially described nutrients are those that are labeled as *antioxidants* (Shibamoto et al. 2008; Caffrey 2015) (**Table 1.1**).

Table 1.1 Nutraceutical Categories/Examples

Category Title	Nutrient or Supplement	Foods Containing Nutrient
Isoprenoids (Terpenoids)	Carotenoids	Carrots, carrot juice, sweet potatoes, apricots
	Saponins	Soybeans, legumes, and garlic
	Tocopherols	Oils, nuts, seeds, whole grains
Phenolic compounds	Isoflavones	Berries, coffee, tea
	Lignin	Whole grains, wheat, legumes, nuts, seeds
Proteins/Amino acids	Amino acids	Meats, chia seeds, soybeans, spirulina, pumpkin, almonds, avocados
	Indoles	Cruciferous vegetables: collard and mustard greens, broccoli, kale, cabbage, turnips
	Choline	Egg yolk, beef, fish, pork, soybean oil, chicken
	Folate	Spinach, turnips, broccoli, bokchoy, parsley, romaine lettuce
Carbohydrate derivatives	Ascorbic acid (vitamin C)	Citrus fruit, strawberries, sweet peppers, broccoli
	Oligosaccharides	Leeks, onions, asparagus, Jerusalem artichoke
Fatty acid and lipids	N-3 PUFA	Oils (flaxseed, walnut, and canola), nuts, seeds (Vesper et al. 1999)
	MUFA	Olive oil, canola oil, avocados, olives, peanut oil, nuts (Vesper et al. 1999)
	Sphingolipids	Dairy products, eggs, soybeans (Ferreyra et al. 2012)
	Lecithin	Soy, egg yolk, vegetable oils
Minerals	Calcium	Dark green vegetables, dairy products, okra, almonds, fish
	Potassium	Beans, dark green vegetables, potatoes, squash, bananas, mushrooms
	Zinc	Oysters, beef, wheat germ, spinach, nuts, pumpkin, and squash seeds
Microbial	Probiotics	Yogurt, sauerkraut, sourdough bread, miso, kimchi
	Prebiotics	Garlic, onions, leeks, asparagus, bananas
Antioxidants		Chocolate, pomegranates, cranberries, blueberries, beans, artichoke, pigmented potatoes

Source: Crowe, K. M. and Francis, C., *J. Acad. Nutr. Diet.,* 113, 1096–1103, 2013.

1.1.2 Antioxidant nutraceuticals

Antioxidant is a broad term that is used to describe any substance (vitamin, mineral, or enzyme) that can protect the cells of the body from oxidative damage, usually due to free radicals (Hart and DeAngelo 2013).

Free radicals are produced for a variety of reasons within the body and can be produced through radiation, environmental stress, or by the normal process of mitochondrial and cells. Early on in life, the enzymes and processes of the body are better able to handle the production of free oxygen radicals, but as the body ages, so does the handling of these components. This develops the need for a higher intake of antioxidants so that a person's body is better equipped to handle the ever increasing number of free radicals produced in the slowly failing system. These chemicals produced are highly reactive and can interact with a number of components in the cells, such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA), cellular proteins, and the lipids of the membranes.

Aging itself and almost every disease state that can affect the human body is thought to be caused, in part, by oxidative damage and the buildup of free radical damage over one's lifetime. Therefore, all problems associated with aging should receive some benefit, if we could just learn how to utilize antioxidants efficiently to reduce oxidative stress (Bourassa and Tardif 2006).

1.1.3 Antioxidant nutraceuticals categories and examples

There is some classification of antioxidant molecules. Within the body there exist a number of enzymatic antioxidants, which have the ability to break down and remove free radicals. Most of these go through the process of converting the free radical into an intermediary, hydrogen peroxide, and then will convert this into water. Many of these enzymes utilize metal atoms, such as zinc, copper, and iron.

Other antioxidants that are not enzymatic in nature are molecules that have the ability to negate the action of free radicals. Most of this is due to the structure of the molecules, and many are large with phenolic groups to engage the effect of the free electrons in the radicals. Some of these important antioxidant molecules include vitamin E, vitamin C, and beta-carotene (**Table 1.2**).

1.1.4 Biochemical components of antioxidant nutraceuticals

The stresses of an oxidative environment have led to organisms developing these defense mechanisms (Zipperer 1902). As mentioned previously, there are the enzymatic antioxidants such as superoxide dismutase, glutathione peroxidase, and catalase. However, we are interested in the nonenzymatic nature of the nutraceutical antioxidants.

Chocolate and derivatives from the cocoa bean contain some of the highest concentrations of phenolic antioxidants in foods, and because of this, cocoa products have some of the highest antioxidant activity (**Figure 1.1**; **Table 1.3**).

Table 1.2 Antioxidant Examples

Type	Function	Current Research
Flavonoids	In plants, these molecules protect against UV-B radiation and pathogen infection (Muhamad et al. 2010)	Possible antiviral action in humans (Peterson et al. 2012), lower coronary heart disease, and stroke (Johnson 2002)
Beta-carotene, carotenoids, vitamin A	Precursor to vitamin A; ability to absorb UV and light energy	Slows cognitive decline, decreases risk of certain cancers and eye disease (Valko et al. 2015)
Vitamin E (tocopherol)	Fat-soluble antioxidant; natural antioxidant in the body protecting tissues from oxidative damage and upkeep of immune health	Some protection of arteries against damage, some protection to toxins, eye disorders, neurological disease, and diabetes
Vitamin C (ascorbate/ ascorbic acid)	Water-soluble antioxidant; aid in growth and repair of body tissues (collagen) and to heal wounds	Boosts immune system, strengthens blood vessels, increases levels of glutathione, and restores active vitamin E
CoQ10, glutathione	Master antioxidant molecules in the body, similar to vitamin E; prevent free radical damage in cytosol and mitochondria	Lower risk of cancers, cardiovascular disease, and inflammatory diseases (Cadenas 1997; McCarty and DiNicolantonio 2015)

Source: Crowe, K. M. and Francis, C., *J. Acad. Nutr. Diet.*, 113, 1096–1103, 2013.

Figure 1.1 *(a) Picture of cocoa pods ready for harvest on a cocoa tree; each pod can contain 30–50 cocoa beans to be used for further processing (From Katz, D. L. et al., Antioxid Redox Signal 15, 2779–2811, 2011.); (b) structure of procyanidin; and (c) structure of catechin. The structures in Figure 1.1a and c are biochemical, phenolic antioxidant molecules found in cocoa products. (From Kukongviriyapan, U. et al., Can. J. Physiol. Pharmacol. 90, 1345–1353, 2012.)*

Table 1.3 Foods Highest in Antioxidants

Food	Antioxidant Ability (Total Oxygen Radical Absorbance Capacity—Mean Value per 100 g)
Baking chocolate, unsweetened (1 square)	14479 μmol TE
Cranberry (1 cup)	10542 μmol TE
Blueberry (1 cup)	9621 μmol TE
Wine, table, red (5 oz.)	5693 μmol TE
Gala apple (1 apple)	5147 μmol TE

Source: Haytowitz, D. and Bhagwat, S., USDA database for the oxygen radical absorbance capacity (ORAC) of selected foods, release 2, Agriculture and Agricultural Research Service (Ed.), USDA National Nutrient Database for Standard Reference, Washington: DC, 2010.

These phenolic compounds of flavonoids, such as catechin and procyanidin, are composed of tricyclic structures that act as free radical scavengers during periods of oxidative stress. These molecules can also chelate iron and copper atoms, inhibit other enzymes, and can upregulate the body's antioxidant defenses. Both catechin and epicatechin have shown to increase nitric oxide production, impacting the vascular endothelium, and these polyphenols have also shown anti-inflammatory function (Kukongviriyapan et al. 2012).

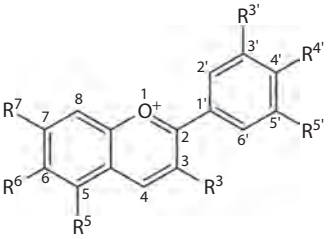
A couple of other well-known antioxidants are berries, such as blueberries, cranberries, and pomegranates (**Figure 1.2**; **Table 1.3**). These foods have been shown to be high in other flavonoids, such as quercetin and anthocyanins. Quercetin in the animal model has been shown to reduce oxidative stress brought on by the release of a toxic protein, lipopolysaccharide (LPS), by harmful bacteria (Richter et al. 1999). These nutraceuticals worked to alleviate lipid peroxidation and was able to restore the cell's antioxidant capacity. Quercetin also has shown activity in the cell related to antiproliferative and antineoplastic capabilities. It can induce apoptosis and arrest the cell cycle in the early G1 phase (Choi et al. 2001; Murphy et al. 2003). Anthocyanins also work as powerful antioxidants. These molecules are thought to inhibit some of the oxidative processes linked to the creation of tumors and cancers (Kaspar et al. 2011).

Other examples of antioxidant foods besides cocoa products and berries are those of green and root vegetables, such as artichokes and Russet potatoes. Both the artichoke's leaves and head are rich in phenolic compounds such as benzoic derivatives, flavonoids, and tannins.

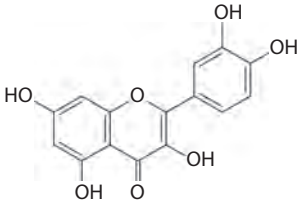
Also, potatoes are one of the highest consumed vegetables per capita in the American diet. Pigmented potatoes, such as sweet, and especially, purple potatoes (**Figure 1.3**), contain high numbers of phenolic acids, anthocyanins, and carotenoids. Knowing each of these facts, scientists have been researching for years the protective effects of the molecules within these vegetables (Zeisel 1999; Teow et al. 2007).

(a)

(b)



(c)

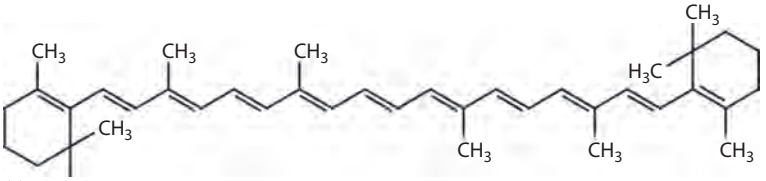


(d)

Figure 1.2 *(a) Cranberries off the vine, (b) split pomegranate with seeds, (c) general structure of anthocyanin, and (d) structure of quercetin. The structures in Figure 1.2c and d are biochemical phenolic antioxidant molecules found in many berry products, and there is a noticeable similarity to the tricyclic flavonoids found in cocoa in Figure 1.1.*

(a)

(b)



(c)

Figure 1.3 *(a) Typical coloring of a sweet potato, (b) coloring of purple potatoes, and (c) structure of beta carotene found in these root vegetables.*

In almost all foods, some nutrients can be found acting as antioxidants. Plants themselves produce these molecules to protect against their forms of oxidative damage, and this protection can be passed onto humans through ingestion for the most part. Phenols, flavonoids, and carotenoids are among the most common, and in effect with vitamins C and E, these molecules can help to clear the body of these oxygen radical waste products.

When radical waste products begin to build up, they can cause many kinds of disease as you will explore throughout the remaining chapters. However, these nutrients can act to halt and even regress the disease progression in some cases. This is the use of nutrients or functional foods as a means of treating diseases and disorders; the very definition of nutraceuticals.

1.1.5 Regulation of nutraceuticals

Within the past decade, the marketplace of nutraceuticals has grown so vastly that it is hard for the U.S. government to regulate all the products coming onto the market. Many of the more advocated products, such as fish oils, amino acids, ubiquinol, and glucosamine, can be found in an aisle in the local pharmacy or wellness store. Even if you go to a vitamin marketplace online, you can find a selection of all kinds of supplements, and most likely, they will have a section for antioxidants as well.

U.S. Congress, through the Dietary Supplement Health and Education Act (DSHEA) in 1994, made up the framework for the regulation of all dietary supplements. The FDA required that for the supplement to be on the market, it must be labeled as such. The manufacturer must also place a disclaimer stating that the supplement has not been evaluated by the FDA, and that it is "not intended to diagnose, treat, cure, or prevent any disease." Because of this, manufacturers have relatively free reign on what they put or how much or little they put into their products (Zeisel 1999). More recently, investigations have been carried out on some of the top national retailers, studying the concentration of the advertised supplement within each pill. The finding was that almost four out of five products did not contain any of the herbs on their label, and even prompted the NY State attorney general to get involved (O'Connor 2015).

Although maybe not to the level of strict regulation that the FDA puts on prescription drugs and food additives, people are wondering if tighter control on the supplement industry might do more good than harm. People tend to assume that these supplements are relatively safe, as they are not prescribed; however, for example, an excess of vitamin B6 found in many vitamin B complexes, exceeding a dose of 300 mg per day can cause a level of nerve damage in some cases with people experiencing numbness in their extremities (Schaumburg et al. 1983; Smith 1990). It is true that too much of one thing is not good for anyone and in the case of supplements, studies done to examine

the minimal levels of toxicity and side effects, approved by the FDA, would at least offer another degree of protection to people who take them at will.

Aside from regulation, further studies on nutraceuticals would only help to show that these molecules have benefit in the human body, and cement the position that nutraceuticals can be beneficial. These molecules and beneficial foods have been used for thousands of years by ancient cultures all over the world. You can find foods in the paintings, sculpture, and art of the previous societies, and some even worshiped the food by presenting it as one of the highest offerings in their religions. Some foods are sacred, only to be eaten on special occasion. In all of these examples, there is an understanding in these ancient societies that certain foods could have more benefit as others.

1.2 Nutraceuticals and historical perspectives

In all species, food equates to life. The use of nutraceuticals can be found throughout the history. The father of modern medicine, Hippocrates, stated, "Let food be thy medicine, and medicine be thy food." Humans have always cared and taken an interest in the integrity of what they were eating. Important minds such as Hippocrates were watching what people were eating and the effect these foods would have on the person. He stressed that "differences of disease depend on nutriment" (Jones 1923). This was the thinking in 377 B.C. In the societies before modern time, people came to realize this after years and years of studying what people eat and what it does to them. Here, we will examine the historical perspective of the various antioxidant foods and nutraceuticals used over the history of mankind.

1.2.1 Ancient India

Much of the culture of ancient India was put down in texts called the *Vedas*, which is Sanskrit for *knowledge*. These texts are some of the oldest in the Indian world and are some of the oldest scriptures of Hinduism. Mentioned multiple times within these texts are descriptions of food, and as they believed, food was thought to be the source of physical survival, stamina, and strength. Food was also seen to have medicinal and healing attributes. "Therefore, even now when one who is afflicted gets better he asks for food. Then they are hopeful for him thinking, 'He asks for food; he will live.'" This was described in the Vedas when Prajapati (the *lord of people*) was weary from fighting off death and evil, and he asked for food to be revitalized (Wujastyk 1995).

Within the ancient sanskrit works, there is also the description of various plants and vegetables, and the works go into detail on how to use different parts of the plants for medicinal purposes. A selection of the hundreds of medicinal plants used is mentioned in Sections 1.2.1.1 through 1.2.1.3 (Grover et al. 2002; Ravindran and Babu 2004).

1.2.1.1 Castor oil

Much of the ancient Indian medicinal text is written in the *Susruta Ayurveda*, which was compiled around 2000 BCE. It is the ancient writings of the traditional Hindu medicines native to the Indian region. Castor oil is mentioned within these writings for its use as a stimulant laxative to treat constipation. Another close Asian country, China, used the castor bean plant to restore the body's *chi*, and the thinking was that this product could be used by the body where it was needed most.

The high concentrations of vitamins in castor oil, especially vitamin E, make for its use as a potential antioxidant when treating gastrointestinal problems.

1.2.1.2 Ginger

Many of the ancient people and monks of the Indian region looked for roots to use as medicinal products. Roots are fairly stable and can be used and stored for a lifetime. These medicinal products were usually never taken as a solid or soft food themselves but were added to other nutrition as a medicinal additive. One of the earliest conceived uses of what we call today a *nutraceutical*.

The people viewed ginger as the healing gift from their creator, and this plant was found throughout the ancient Indian and Chinese systems (Mehlhorn et al. 2014). Today, India is the largest cultivator of the ginger plant, contributing to about 30%–40% of the world production. It was not typically used as a spice, as it is more commonly today, but was seen as a medicine. In the ancient Indian language, it was called *mahabhbeshaj, mahaoushadhi*, which means *the great cure, the great medicine*. In the ancient ayurvedic text, the use of the ginger root was recommended for elephantiasis, gout, and impairment of the body due to indigestion. In fact, when European explorers were scouting the region, they found that the native people had large cultivations of aloe, pepper, ginger, cinnamon, and myrobalan plum plants.

Ginger contains a large concentration of gingerol, an antioxidant compound chemically related to capsaicin in chili peppers and piperine in black pepper. These compounds give the relative *spiciness* to each of these plants and plant roots. In animal models, this compound has been shown to induce hypothermia and treat rheumatoid arthritis, as well as being cytotoxic to blood and lung cancer cell lines.

1.2.1.3 Pomegranate

The earliest cultivation of the pomegranate was in the Indus Valley, at the foothills of the Himalayan mountains. In Sanskrit, this fruit is called *dadima* and in Hindi and Urdu it is called *anar*.

In the Ayurveda texts, its mentioned use is to treat heart and blood conditions, to stimulate one's appetite, as an antiemetic, and as an antidiarrheal. The fruit is also a symbol of fertility and prosperity. When ripe, the fruit is

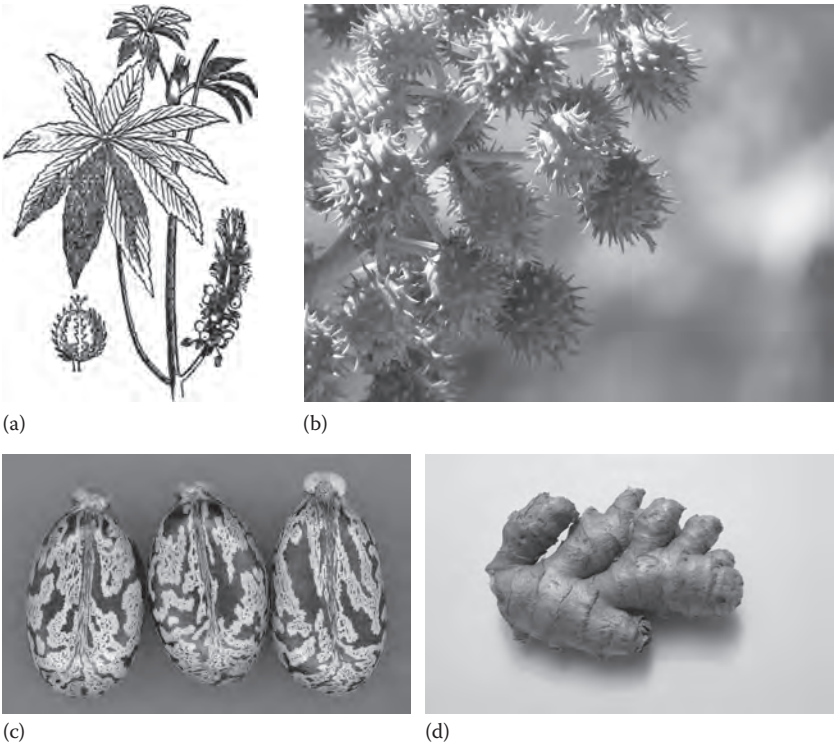


Figure 1.4 *(a) Picture of a typical castor plant, (b) castor seeds on the castor plant, (c) cultivated castor seeds ready for processing, and (d) typical picture of a ginger root.*

described as resembling a heart, and it may be that is why it was used for cardiovascular conditions, along with the juice and seeds resembling blood. The fruit, as described previously, has immense antioxidant potential and is one of the earliest examples of an antioxidant nutraceutical (**Figure 1.4**).

1.2.2 Ancient Chinese

Traditional Chinese medicine (TCM) goes back to 2000 BCE and encompasses many of the traditional practices such as acupuncture, tai chi, and moxibustion and herbal medicine. In the United States alone, more than an estimated 5 million people according to the National Health Interview Survey have used these types of treatment. One of the major herbal texts, Shennong's *Materia Medica*, was thought to be about by one of China's first herbalists, Shennong, who lived around 2800 BCE and allegedly had tried hundreds of Chinese plants, and later imparted his knowledge of medicinal and poisonous plants to other farmers.

Another Chinese text is *Huangdi Neijin* or *The Yellow Emperor's Inner Canon*. It was written in 500–200 BCE and in the format of a question and answer-type piece between the mythical emperor Huangdi and his ministers.

The first text of the two-part series, Suwen, gives the foundation of Chinese medicine and its diagnostic foundings.

Finally, one of the oldest clinical textbooks in the world comes from ancient China, at the end of the Han dynasty in 220 CE. The *Shanghan Lun* is known in English as the *Treatise on Cold Damage Disorders*, and many traditional Chinese medical students still learn from this today. The text combines the Yin and Yang of the body with five phases of possible drug therapy. It is one of the earliest Chinese medical texts describing symptoms as clinically useful patterns that herbalists and ancient medical personnel could target for treatment.

1.2.2.1 Astragalus (huangqi)

Traditionally, astralagus was used to balance the life force, or qi, within the human body. The plant is harvested after 4 years, and the flat, yellowish roots are used medicinally. This herb is used to enhance a person's overall health and is supposed to increase resistance to disease. It is also a noted antioxidant, antiviral, and antibiotic. Clinically, it is used on people who show general weakness, diarrhea, fatigue, lack of appetite, chronic illnesses, and to increase once vitality and levels of qi. Today, this is one of the largest health-related plants used in China and other countries of Asia (Biggs 1995).

This herb contains unique polysaccharides that are thought to stimulate the immune system (**Figure 1.5**). These compounds also show strong antioxidant and antitumor activity within an animal model (Ramesh et al. 2012).

Figure 1.5 *The plant products, mainly the roots of the astragalus plant, are used to generally make a tea marketed for immune health.*

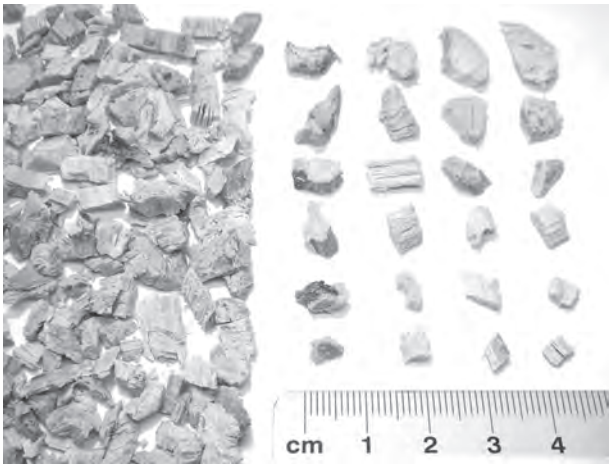
1.2.2.2 Licorice (gancao)

Another plant used for its roots, licorice, has been used through the ancient civilizations of China, India, Egyptians, and Romans. Its sweet tasting root was used for its rejuvenating properties, and in TCM, it was clinically used for sore throats and bouts of food poisoning. In China today, licorice is the second most prescribed herb (McCoy 2013).

Compounds of this plant root have been shown to be effective against forms of LDL oxidation when tested in the lab (Sipos et al. 2004). LDL oxidation is a key factor in the early formation of atherosclerotic lesions, and use of licorice may be used to curb or prevent such processes (**Figure 1.6**).

(a)

Figure 1.6 *(a) Picture of a typical* Glycyrrhiza uralensis *licorice plant.* *(Continued)*



(b)

Figure 1.6 (Continued) *(b) Dried licorice root that was often chewed for its sweetness and medicinal properties.*

1.2.2.3 Ginseng (renshen)

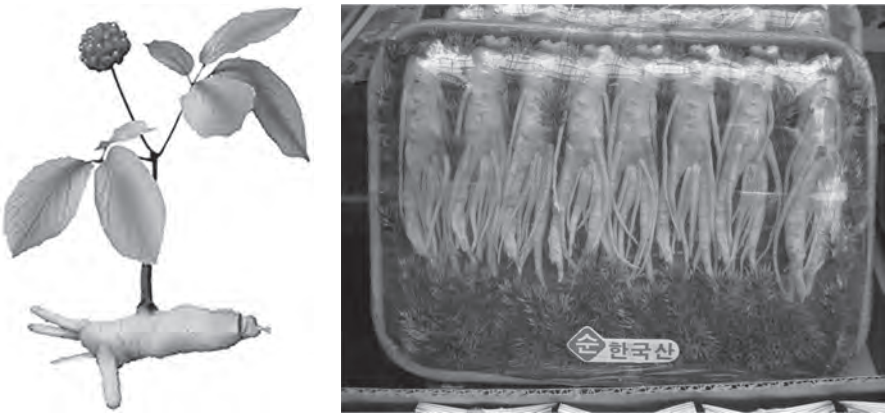
The term was given to the ginseng plant because its roots sometimes resembled the shape of a man. In Chinese, the term ginseng means *man root* or *essence*. Because of the peculiar shape, the plant and its roots were thought to be divine, and these plants were extremely valuable. The demand in China was so high at one point in 200 CE that it nearly exterminated the wild Chinese ginseng supply. The herb was thought to increase energy, enhance athletic performance, improve mental function, and enhance immunity.

In the animal model, when fed a steady diet of red ginseng water extract, these animals tended to show significantly less oxidative damage (**Figure 1.7**). The method of action is thought to be a reduction in the lipid peroxidation and restoration of antioxidant capacity, curbing the processes of oxidative stress (McGovern et al. 2009).

1.2.2.4 Wolfberry (goji)

Mentioned in Shennong's *Materia Medica*, wolfberry or goji berries have been used for millennia in ancient China. Over time, people began to think and watch that the people who would routinely drink a goji tonic would tend to live longer. The fruit and its plant were correlated to vitality and longevity in ancient China.

The, usually dried, red berries have high concentrations of vitamin C, beta-carotene, and other phenolic compounds, making this fruit an early used antioxidant that is still widely used today (**Figure 1.8**).



(a) (b)

Figure 1.7 *(a) Picture of a typical ginseng plant, from root up to the red flower and (b) packaged ginseng roots for sale, resembling shapes of little persons.*

Figure 1.8 *Picture of a typical ripened goji berry or wolfberry plant. The red berries are usually harvested and dried out.*

1.2.3 Ancient Egyptians

The medical advances in ancient Egypt were some of the best in the world. They were renowned for their knowledge and application of bandages, recognizing infection and inflammation, and their use as various medicinals and herbs. The *Ebers Papyrus* was the standard medical text in this civilization, written in about 1500 BCE (Rajasekaran et al. 2005). Although most of the Egyptian medical practice was based around the idea that magic and spirits caused illness, the study of illness and symptoms lead to a system of treatment. Different herbal treatments, consisting of plants and tree resins, were put into the wine (Miladi and Damak 2008).

1.2.3.1 Aloe vera

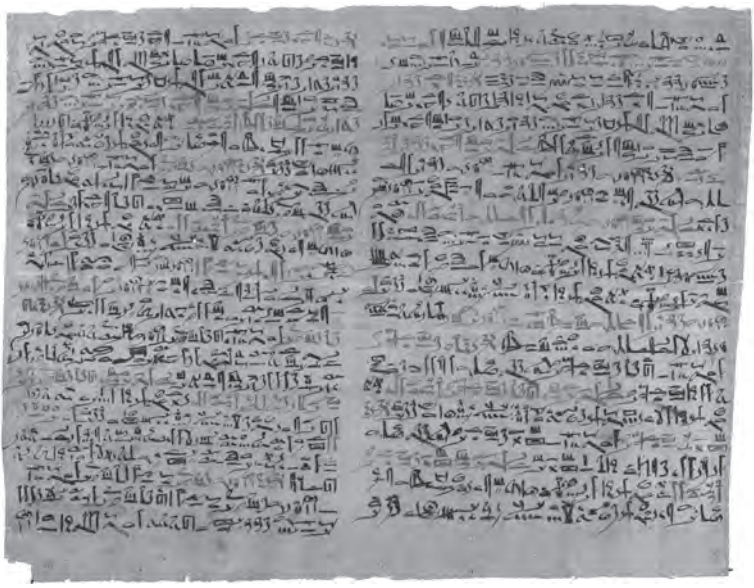
One of the plants from the *Ebers Papyrus* originated from the Middle East and Arab regions and was carried to the Asian continent around 600 BCE by the Arab traders. The Egyptians thought that this plant was a religious icon, and would often hang it in their doorway to protect themselves from evil. You can find the drawings of the aloe plant on the walls of their temples and tombs, mainly shown being used to treat different burns. In the Indian Ayurveda texts, it is mentioned to help manage pain and painful conditions.

The Egyptians used the plant to aid in the preparation of the papyrus scrolls and was used medicinally for early treatment of tuberculosis. The plant was recommended for wounds due to its analgesic and antibacterial properties and was used externally to treat rashes, burns, and sunburns.

The antioxidant potential of aloe vera has been studied extensively over recent years. The plant, when fed to diabetic rats at concentrations of 300 mg/kg, has shown to decrease levels of blood glucose, glycosylated hemoglobin, and increased hemoglobin numbers (Saada et al. 2003). Overall, the free radical potentials of the plant have been assayed by multiple research teams, and this may be why it is so effective to treat irradiation and burns (Chithra and Leelamma 1999) (**Figure 1.9**).

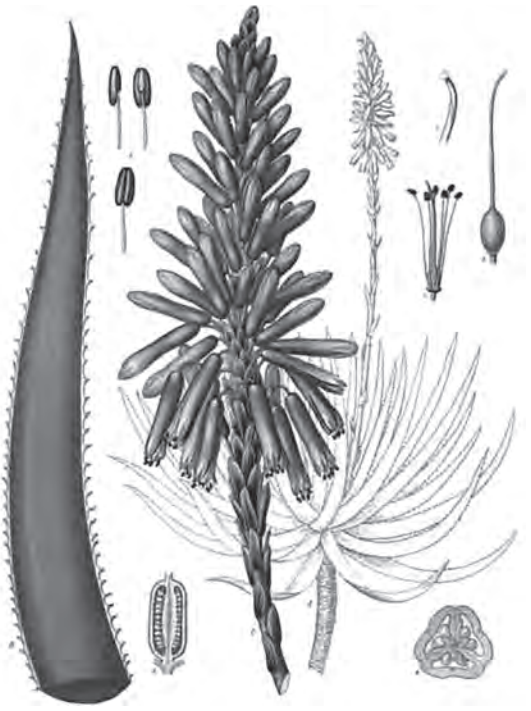
1.2.3.2 Coriander

Coriandrum sativum was thought to aid in digestive problems, and all parts of the plant were utilized. Mainly used as a tea, coriander helped for stomach pain and urinary problems, such as cystitis. Its fruits were found in the tomb of the King Tutankhamun and other ancient Egyptian graves. Externally, coriander was used to treat ulcers and other skin conditions. The oil derived from the plant was useful as an antibacterial.



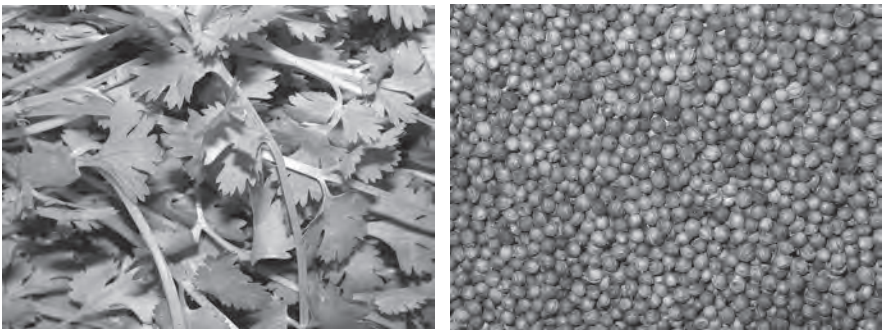
(a)

(b)



(c)

Figure 1.9 *(a) The Ebers Papyrus writing, (b) temple carvings of the ancient Egyptian medical devices and plants, and (c) drawing of a typical aloe vera plant.*



(a) (b)

Figure 1.10 *(a) Coriander leaves and (b) coriander seeds used for their oils.*

In an animal model, the coriander seeds were found to decrease the levels of lipid peroxidation, and overall the activity of antioxidant enzymes increased when compared to the control (Morrow 2011) (**Figure 1.10**).

1.2.3.3 Honey

In ancient Egypt, the bee was thought to grow from the tears of the sun god, Ra, and land on the sands of Egypt. The bee's by-product, honey, was used to treat many infections, and the *Ebers Papyrus* mentions the value of adding honey, wine, and milk to most food products. The substance was added to bandages and dressings because of its antimicrobial abilities. One specific patient found with ankylosing spondylitis or inflammatory arthritis of the spine and joints was found to have consumed honey in his last days as a form of medication (Youdim and Deans 2000) (**Figure 1.11**).

Figure 1.11 *Shown is the common hieroglyph for the bee in ancient Egypt.*

Today, honey is still known for its various antimicrobial properties, but the compound also has some different antioxidant substances. There are a number of different polyphenols, carotenoids, and flavonoids.

1.2.4 Ancient Sumerians

These ancient people lived in Mesopotamia, located at current day Syria, Iraq, and Turkey, between the Tigris and Euphrates rivers. Much of the source text from this period comes in the form of cuneiform tablets, and many of these have been destroyed or not well preserved. As the government during this time took the form of a theocratic culture, healers and priests took on the role of healing the people. Illness was seen as being sent by the gods and was carried by demons and other evil spirits. However, different forms of empirical therapies took aim at combating these spirits and helping the lessening of the patient's symptoms (Cumo 2013).

1.2.4.1 Acacia

It is also known as the thorn tree and produces gum arabic and a polyphenol known as tannins. These substances today are used in adhesives, pharmaceuticals, and various dyes and inks. These plants tend to grow in environments with little water, making them a hardy plant in ancient Egypt. The plant's resin is mentioned in the *Ebers Papyrus* as being used in various eye problems, as well as put into the wounds and applied to skin diseases. It was also used surgically to set broken bones (**Figure 1.12**).

The acacia tree and its resin have large antioxidant potentials. The tree bark was found to reduce the number of superoxide radicals and reduced intracellular hydrogen peroxide when coincubated with a human leukemia cell line (Golbidi et al. 2011).

1.2.4.2 Myrrh

This is another gum resin that is obtained from different thorny, flowering trees, all related to the species genus *Commiphora*. The substances were mainly used in the ancient times as a medicinal and embalming ingredient.

In modern medicine, myrrh can be used as an analgesic for a toothache and as an antiseptic in toothpaste and mouthwashes, among other things. It has various antioxidant properties, and compounds known as sesquiterpenes have been shown to inhibit tumor growth (Brower 2005).

1.2.4.3 Thyme

Thyme is a common herb used in many different food dishes today. This plant was majorly used for embalming by the ancient Egyptians. Medicinally, its oil can be extracted and is utilized as an antiseptic, and can be found today in

(a)

(b)

Figure 1.12 *(a) A common picture of an acacia tree in Africa and (b) a drawing of the birds of ancient Egypt in what is depicted as an acacia tree.*

Table 1.4 Antioxidants in Ancient Civilizations

Ancient Civilization	Ancient Text	Medicinal Products Used
India	*Ayurveda* texts, *Suśrutasamhitā*, *Carakasaṃhitā*	Castor oil, ginger, pepper, cinnamon, garlic, pomegranate
China	*Huangdi Neijing, The Emperor's Inner Canon*	Ginseng, astragalus, cinnamon, ginger, licorice, rhubarb, wolfberry
Egypt	*Ebers Papyrus*	Honey, garlic, hibiscus, onion, castor oil, coriander
Sumerian	Cuneiform tablets	Acacia, aloe vera, balsam apple, myrrh, onion, poppy, thyme

many types of mouthwashes. Oil of thyme was placed on bandages to help heal wounds, and the active nutraceutical, thymol, can be effective against different types of fungi. The plant could be boiled and drunk as a tea to treat bronchitis and cough.

Thyme oil contains different terpenoid molecules, such as myrcene, linalool, thymol, and carvacrol. Thymol is a known antioxidant and has been widely studied in different application systems. Significant declines were found in the aging rat brain with regards to the levels of superoxide dismutase and glutathione peroxidase when fed with thymol oil (Wolfe 2003) (**Table 1.4**).

1.3 Nutraceutical application in worldwide traditional systems

1.3.1 Asian medicine and nutraceuticals today

The modern market of nutraceuticals as it is known today took its start in 1980s in Japan, where the modern technologies began to infiltrate the ancient uses of folk medicine (Jones 1923). Although nutraceutical use has always been a primary market in the Asian–Pacific region, the use of these products today is ever more important. Over the past couple of decades, the eating habits of the region have changed to a more Western diet in some areas. Analysts are beginning to see patterns of irregular eating, increases in junk food consumption, and poor lifestyle habits starting to affect the lives of these people. With the increase in these pitfalls comes the increase in a number of diseases caused by stress and poor eating habits, and these can be accompanied by relations to nutritional deficiencies.

Products such as green tea and omega-3 fatty acids are taken for weight loss and cardiovascular health. With the buying habits of the people causing a rise in demand for dietary supplements and preventative nutraceuticals, more and more manufacturers are coming to these markets to provide the supply.

1.3.2 Nutraceuticals in western medicine

It was the father of western medicine, Hippocrates, who saw the value in food and food products. This proves to be true in the United States and Europe over the past couple of decades, as these are some of the largest markets for nutraceuticals. A 2001 Harris Interactive poll showed that 72% of Americans surveyed take supplements, and 53% of those surveyed believed that taking nutraceuticals offered some benefit that they could not get from pharmaceuticals or conventional medicine. Out of all the people questioned, 95% of the people were satisfied with the supplements they were taking (Brower 2005).

However, regulation of these products is becoming more and more of a debate. Many advocacy groups are pushing for tougher oversight of these products, especially after the case of ephedra. The FDA allowed ephedra to remain on the market, despite there being more than 100 cases of serious side effects such as heart attack, stroke, and death (Wolfe 2003). With the popularity of these products growing, nutraceuticals may come to play a key role in the treatment of various diseases.

References

Andlauer, W. and P. Furst. 2002. Nutraceuticals: A piece of history, present status and outlook. *Food Res Int* 35 (2):171–176. doi:10.1016/S0963-9969(01)00179-X.

Biggs, R. D. 1995. Medicine, surgery, and public health in ancient Mesopotamia. In: Sasson J. M. (Ed.). *Civilizations of the Ancient near East*. Peabody, MA: Hendrickson Publishers.

Bourassa, M. G. and J. C. Tardif. 2006. *Antioxidants and Cardiovascular Disease*. New York: Springer.

Brower, V. 2005. A nutraceutical a day may keep the doctor away. *EMBO Reports* 6 (8):708–711. doi:10.1038/sj.embor.7400498.

Cadenas, E. 1997. Basic mechanisms of antioxidant activity. *Biofactors* 6 (4):391–397.

Caffrey, C. 2015. *Superfoods*. Ipswich, MA: Salem Press.

Chithra, V. and S. Leelamma. 1999. Coriandrum sativum changes the levels of lipid peroxides and activity of antioxidant enzymes in experimental animals. *Indian J Biochem Biophys* 36 (1):59–61.

Choi, J. A., J. Y. Kim, J. Y. Lee, C. M. Kang, H. J. Kwon, Y. D. Yoo, T. W. Kim, Y. S. Lee, and S. J. Lee. 2001. Induction of cell cycle arrest and apoptosis in human breast cancer cells by quercetin. *Int J Oncol* 19 (4):837–844.

Crowe, K. M. and C. Francis. 2013. Position of the academy of nutrition and dietetics: Functional foods. *J Acad Nutr Diet* 113 (8):1096–1103. doi:10.1016/j.jand.2013.06.002.

Cumo, C. 2013. *Encyclopedia of Cultivated Plants: From Acacia to Zinnia*. Santa Barbara, CA: ABC-CLIO.

Ferreyra, M. L. F., S. P. Rius, and P. Casati. 2012. Flavonoids: Biosynthesis, biological functions, and biotechnological applications. *Front Plant Sci* 3:222. doi:10.3389/fpls.2012.00222.

Gebauer, S. K., T. L. Psota, W. S. Harris, and P. M. Kris-Etherton. 2006. n-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. *Am J Clin Nutr* 83 (6 Suppl):1526s–1535s.

Golbidi, S., S. A. Ebadi, and I. Laher. 2011. Antioxidants in the treatment of diabetes. *Curr Diabetes Rev* 7 (2):106–125.

Grover, J. K., S. Yadav, and V. Vats. 2002. Medicinal plants of India with anti-diabetic potential. *J Ethnopharmacol* 81 (1):81–100.
Hart, L. M. S. and L. DeAngelo. 2013. *Antioxidants*. Ipswich, MA: Salem Press.
Haytowitz, D. and S. Bhagwat. 2010. USDA database for the oxygen radical absorbance capacity (ORAC) of selected foods, release 2. Agriculture and Agricultural Research Service (Ed.), USDA National Nutrient Database for Standard Reference, Washington, DC.
Johnson, E. J. 2002. The role of carotenoids in human health. *Nutr Clin Care* 5 (2):56–65.
Jones, W. H. S. 1923. *Hippocrates, Volume I: Ancient Medicine*. Cambridge, MA: Harvard University Press.
Kalra, E. K. 2003. Nutraceutical-definition and introduction. *AAPS PharmSci* 5 (3):27–28. doi:10.1208/ps050325.
Kaspar, K. L., J. S. Park, C. R. Brown, B. D. Mathison, D. A. Navarre, and B. P. Chew. 2011. Pigmented potato consumption alters oxidative stress and inflammatory damage in men. *J Nutr* 141 (1):108–111. doi:10.3945/jn.110.128074.
Katz, D. L., K. Doughty, and A. Ali. 2011. Cocoa and chocolate in human health and disease. *Antioxid Redox Signal* 15 (10):2779–2811. doi:10.1089/ars.2010.3697.
Kukongviriyapan, U., K. Sompamit, P. Pannangpetch, V. Kukongviriyapan, and W. Donpunha. 2012. Preventive and therapeutic effects of quercetin on lipopolysaccharide-induced oxidative stress and vascular dysfunction in mice. *Can J Physiol Pharmacol* 90 (10):1345–1353. doi:10.1139/y2012-101.
Lockwood, B. 2007. *Nutraceuticals*, 2nd ed. London, UK: Pharmaceutical Press.
McCarty, M. F. and J. J. DiNicolantonio. 2015. An increased need for dietary cysteine in support of glutathione synthesis may underlie the increased risk for mortality associated with low protein intake in the elderly. *Age* (*Dordr*) 37 (5):96. doi:10.1007/s11357-015-9823-8.
McCoy, J. 2013. *Medicinal Plant Pathogens*. In J. Davis and W. S, Persons (Eds.) *Growing and Marketing Ginseng, Goldenseal & Other Woodland Medicinals*, (2nd ed.). Minneapolis, MN: Consortium Book Sales & Distributors.
McGovern, P. E., A. Mirzoian, and G. R. Hall. 2009. Ancient Egyptian herbal wines. *Proc Natl Acad Sci USA* 106 (18):7361–7366. doi:10.1073/pnas.0811578106.
Mehlhorn, H., Z. Wu, and B. Ye. 2014. *Treatment of Human Parasitosis in Traditional Chinese Medicine, Parasitology Research Monographs*. Berlin, Germany: Springer-Verlag.
Miladi, S. and M. Damak. 2008. In vitro antioxidant activities of Aloe vera leaf skin extracts. *J Soc Chim Tunisie* 10:101–109.
Morrow, J. A. 2011. *Encyclopedia of Islamic Herbal Medicine*. Jefferson, NC: McFarland.
Muhamad, M., L. Y. Kee, N. A. Rahman, and R. Yusof. 2010. Antiviral actions of flavanoid-derived compounds on dengue virus type-2. *Int J Biol Sci* 6 (3):294–302.
Murphy, B. T., S. L. MacKinnon, X. Yan, G. B. Hammond, A. J. Vaisberg, and C. C. Neto. 2003. Identification of triterpene hydroxycinnamates with *in vitro* antitumor activity from whole cranberry fruit (*Vaccinium macrocarpon*). *J Agric Food Chem* 51 (12): 3541–3545. doi:10.1021/jf034114g.
O'Connor, A. 2015. New York attorney general targets supplements at major retailers. *New York Times*, February 3, 2015.
Pathak, Y. V. 2009. *Handbook of Nutraceuticals Volume I: Ingredients, Formulations, and Applications*. Boca Raton, FL: CRC Press.
Peterson, J. J., J. T. Dwyer, P. F. Jacques, and M. L. McCullough. 2012. Associations between flavonoids and cardiovascular disease incidence or mortality in European and US populations. *Nutr Rev* 70 (9):491–508. doi:10.1111/j.1753-4887.2012.00508.x.
Rajasekaran, S., K. Sivagnanam, and S. Subramanian. 2005. Antioxidant effect of Aloe vera gel extract in streptozotocin-induced diabetes in rats. *Pharmacol Rep* 57 (1):90–96.
Ramesh, T., S. W. Kim, S. Y. Hwang, S. H. Sohn, S. K. Yoo, and S. K. Kim. 2012. Panax ginseng reduces oxidative stress and restores antioxidant capacity in aged rats. *Nutr Res* 32 (9):718–726. doi:10.1016/j.nutres.2012.08.005.
Ravindran, P. N. and K. N. Babu. 2004. *Ginger: The Genus Zingiber, Medicinal and Aromatic Plants—Industrial Profiles*. Boca Raton, FL: CRC Press.

Richter, M., R. Ebermann, and B. Marian. 1999. Quercetin-induced apoptosis in colorectal tumor cells: Possible role of EGF receptor signaling. *Nutr Cancer* 34 (1):88–99. doi:10.1207/s15327914nc340113.

Saada, H. N., Z. S. Ussama, and A. M. Mahdy. 2003. Effectiveness of Aloe vera on the antioxidant status of different tissues in irradiated rats. *Pharmazie* 58 (12):929–931.

Schaumburg, H., J. Kaplan, A. Windebank, N. Vick, S. Rasmus, D. Pleasure, and M. J. Brown. 1983. Sensory neuropathy from pyridoxine abuse. A new megavitamin syndrome. *N Engl J Med* 309 (8):445–448. doi:10.1056/nejm198308253090801.

Shibamoto, T., K. Kanazawa, F. Shahidi, and C. T. Ho. 2008. Functional food and health: An overview. ACS Symposium Series. https://global.oup.com/academic/product/functional-food-and-health-9780841269828?cc=us&lang=en&#.

Sipos, P., H. Gyory, K. Hagymasi, P. Ondrejka, and A. Blazovics. 2004. Special wound healing methods used in ancient Egypt and the mythological background. *World J Sug* 28:211–216.

Smith, B. K. 1990. Eaters, food and social hierarchy in ancient India: A dietary guide to a revolution of values. *J Am Acad Relig* 58 (2):177–205.

Teow, C. C., V. Truong, R. F. McFeeters, R. L. Thompson, K. V. Pecota, and C. G. Yencho. 2007. Antioxidant activities, phenolic and ß-carotene contents of sweet potato genotypes with varying flesh colours. *Food Chem* 103 (3):829–838.

Valko, M., K. Jomova, C. J. Rhodes, K. Kuca, and K. Musilek. 2015. Redox- and non-redox-metal-induced formation of free radicals and their role in human disease. *Arch Toxicol* 90 (1):1–37. doi:10.1007/s00204-015-1579-5.

Vesper, H., E. M. Schmelz, M. N. Nikolova-Karakashian, D. L. Dillehay, D. V. Lynch, and A. H. Merrill, Jr. 1999. Sphingolipids in food and the emerging importance of sphingolipids to nutrition. *J Nutr* 129 (7):1239–1250.

Wolfe, S. M. 2003. Medicine. Ephedra—scientific evidence versus money/politics. *Science* 300 (5618):437. doi:10.1126/science.1081910.

Wujastyk, D. 1995. Kenneth G. Zysk: Asceticism and healing in ancient India: Medicine in the Buddhist monastery. [viii], 200 pp. New York and Oxford: Oxford University Press, 1991. £30. *Bulletin of the School of Oriental and African Studies* 58 (3):573–573. doi:10.1017/S0041977x00013240.

Youdim, K. A. and S. G. Deans. 2000. Effect of thyme oil and thymol dietary supplementation on the antioxidant status and fatty acid composition of the ageing rat brain. *Br J Nutr* 83 (1):87–93.

Zeisel, S. H. 1999. Regulation of "nutraceuticals". *Science* 285 (5435):1853–1855.

Zipperer, P. 1902. *The Manufacture of Chocolate and Other Cacao Preparations*. M. Krayn.

2

Antioxidant Nutraceuticals

Present Market and Future Trends

Adryan Perez, Richard Nguyen, Ailyn Valladares,
and Sarvadaman Pathak

Contents

2.1 Introduction

In 1989, Stephen DeFelice coined the term *nutraceutical* by combining both *nutrition* and *pharmaceutical* to describe any substance that is considered food or part of a food that may provide health benefits, including the prevention or the treatment of the disease (Andlauer and Fürst 2002). Since then, the global nutraceuticals market has increased so significantly that it is predicted to reach a value of US$278.96 billion by 2021 as stated in a market report from Transparency Market Research (Nutraceuticals Market 2015e). The shift in outlook among the population from seeking a cure for diseases to seeking

methods of prevention has aided the growth of the nutraceuticals market. It is expected for pharmaceutical companies and nutraceutical companies to combine in order to revolutionize the way current treatments for prevalent diseases are approached.

Humans have used nutraceuticals for thousands of years as remedies for ailments. However, today very few unindustrialized countries rely solely on this traditional medicine for the healthcare of their populations. Regions such as the United States, Europe, and the Asia–Pacific have successfully incorporated modern medicine into their healthcare system and are now looking to combine the two forms to achieve the best outcome for patients.

2.2 Global markets

Nutraceuticals have a large global market that is expected to continuously increase as the healthcare system shifts to methods of prevention rather than waiting for the disease onset. The nutraceutical market consists of many categories of products ranging from supplements used to improve health, prevent chronic diseases, and postpone the aging process (Meštrović 2015). Although nutraceuticals have existed in some form throughout the world, market growth has varied among global regions.

2.2.1 United States

The use of dietary supplements in the United States (U.S.) has increased over the past 30 years and currently more than one half of adults report using one or more dietary supplements (Joshi 2015; Nicastro et al. 2015). This could be partly due to the population becoming increasingly concerned with living a healthy lifestyle in order to combat the rising cost of healthcare. According to the data in the 2007–2010 National Health and Nutrition Examination Survey, a national representative, cross-sectional, population-based survey, the actual reason for which most consumers reported to using dietary supplements was to *improve* (45%) or *maintain* (33%) overall health (Bailey et al. 2013). This trend of increased health promotion is likely to remain the same due to the increase in health education throughout the population. The nutraceutical industry is contributing to, and exploiting, this health trend by seeking to continually expand its prevalence in the U.S. market by promoting healthy living and disease prevention.

2.2.2 Europe

Contrary to the market in the United States and other parts of the world, Europe has much more stringent regulations regarding the control of nutraceuticals. These rigid regulations often force companies to make major reformulations of their products in order to distribute their products to countries such as part

of the European Union (EU) (Ottaway 2002). Given the many pieces of legislation applying to the process of having functional foods or nutraceuticals ready for the market, significant time and money must be invested (Coppens et al. 2006). These European legislations regarding nutraceuticals are increasing the safety of the supplements and are also adding to the increase in popularity of the supplements by establishing credibility. Regardless of the stiff nutraceutical regulations, Europe is still expected to grow at a compound annual growth rate (CAGR) of 7.2% from 2014 to 2019 (Europe Nutraceuticals Market 2015c).

The nutraceutical market in the EU is not evenly distributed among its countries being that Italy, Germany, the United Kingdom, and France make up two-thirds of the market (Heller 2008). Thus, these companies have the leverage to impact further nutraceutical development and research on the remaining one-third of the European nutraceutical market.

2.2.3 Asia–Pacific (India, Japan, and China)

The nutraceuticals market in the Asia–Pacific is estimated to grow at a CAGR of 7% from 2014 to 2019 (Asia Pacific Vitamins 2015a). Accompanying the global trend of the increasing nutraceutical market, the Asia–Pacific market is expected to continue to expand. This growth is due, in part, to the increased education about health and nutrition as well as the proliferating middle-class population capable of purchasing nutraceutical products (Asia Pacific Vitamins 2015a). Given the improving standard of living of the Asia–Pacific market, consumers are making the switch to healthier diets and are seeking affordable products such as nutraceuticals, which have the ability to prevent or ameliorate common health issues as well as promote longevity.

2.3 Growth of nutraceuticals

Nutraceuticals vary widely in their potential health benefits to their consumers. A reason the nutraceutical industry has seen such growth is the wide variety of products available that may be beneficial to vastly different lifestyles. From protein powders that aid in recovery for athletes, to probiotics for individuals with poor gastrointestinal health, there is an applicable nutraceutical product.

In conjunction to their wide ranging applicability, physicians, the media, and research data have played a critical role in the nutraceutical market by increasing public awareness about the benefits nutraceuticals may have on healthy living and preventative care. The nutraceutical industry has also increased its market growth by appealing to consumers concerned with cancer prevention, as well as brain and cardiovascular health.

Nutraceuticals hold promise for the treatment as well as the prevention of many diseases because of their many nutritional properties. The more educated Americans depend on preventive measures for healthcare maintenance.

If more research is conducted on nutraceuticals, it may further enhance understanding and increased usage for preventive health.

Physicians and scientists are under pressure to provide the best possible care for the population. As modern treatments are unable to provide cures for various ailments, these professionals are seeking the use of nutraceuticals to bridge a gap in healthcare to provide better preventative care for the population. This shift in mindset among professionals contributes to the growth of the nutraceutical industry as they are informing patients of their wide-ranging benefits.

The media is the primary source of information for most of the population in industrialized countries. The media has had both beneficial and detrimental effects on the nutraceutical industry, but it has recently demonstrated a positive impact in their role in medicine. This growth of nutraceutical use has required many food, beverage, and supplement entities to alter their slogans in order to adapt and contribute to the movement.

The food industry is on its way to becoming a research-oriented industry similar to the pharmaceutical industry (Pandey et al. 2010). Due to the increase in research, food companies are now modifying their products to be more nutritionally dense. In doing so, it ensures the consumers will meet all the nutritional needs in one meal. The promulgation of higher quality of sustenance per capita can potentially boost sales in such a competitive market. Adding nutraceuticals into the field can give a company a fairly large advantage due to its proclaimed health advantages. One division of foods that partook in the nutraceutical expansion is breakfast cereals. With enriched and fortified cereals, consumers can receive proper sustenance in their meals.

Applying science-based research into the nutraceutical and functional food industry will make companies and the industry as a whole more competitive by building the confidence of the consumers (Matthyssens et al. 2008). By commercializing the science of nutraceuticals, consumers will have the ability to make conscious decisions in health in regards to food. Furthermore, it would benefit the uninformed consumer by meeting their nutritional needs. One such example is the consumption of thiamine-enriched rice by persons in underdeveloped countries who no longer have to be concerned with a thiamine-deficient diet (Salcedo et al. 1950).

2.3.1 Cancer prevention

Noteworthy research conducted by Dr. Jed Fahey, a nutritional biochemist, and a faculty research associate at Johns Hopkins School of Medicine, Baltimore, Maryland specifically investigates the chemopreventive properties of cruciferous vegetables. He discusses diets rich in crucifers mentioning that they effectively aid in preventing cancer as well as other chronic diseases due to their *very high concentrations of glucosinolates* (Fahey et al. 2012). In a clinical

study conducted in Qidong, China, he and his team were able to analyze broccoli sprout-derived drinks and how sulforaphane, the chemoprotective agent in the broccoli sprouts, was metabolized by the body via urine samples of the local population (Egner et al. 2011). In addition to broccoli, Fahey studied the *Moringa oleifera* tree, which has been gaining rapid popularity in the nutraceutical world due to its *anti-cancer, hypotensive, hypoglycemic, and antibiotic properties*. Dr. Fahey comments that without clinical studies being conducted on actual individuals—rather than *in vitro*—it's "impossible to specify the dose necessary to produce a specific beneficial effect in humans" (Olson and Fahey 2011). This statement emphasizes the need for clinical studies in the nutraceuticals research world.

2.3.2 Brain health

Many neurodegenerative diseases still have no successful cure and scientists are looking to nutraceuticals for potential methods of prevention and treatment. As the population reaches higher ages due to better standards of living and healthcare, these late onset diseases are becoming increasingly prevalent and if nutraceuticals are demonstrated to be efficacious against these disorders, the nutraceutical market can expect continued growth.

Alzheimer's disease (AD) is a neurodegenerative disorder caused by oxidative stress leading to the aggregation of the endogenous protein amyloid beta. Research using antioxidant nutraceuticals for treatment of AD has shed light on their potential in disease treatment and prevention. Specifically, research on a transgenic mouse model for AD has demonstrated that treatment with caffeinated coffee has the ability to reduce both brain and plasma amyloid beta levels (Arendash and Cao 2010; Cao et al. 2009). A similar study included treatment of APP + PS1 double transgenic mouse model with the common nutraceutical and endogenous hormone, melatonin. The findings in the study provide support for the long-term melatonin therapy as a primary or complementary strategy diminishing the progression of AD (Olcese et al. 2009).

Similar to AD, Parkinson's disease (PD) is also a proteopathic disease. It is theorized that the etiology of PD is caused by the aggregation of the endogenous protein alpha-synuclein forming plaques (Lewy bodies) leading to the death of dopaminergic neurons in the substantia nigra (Hsu et al. 2000). It is suspected that the protein aggregation is caused by an imbalance in the immune system. It is known that the immune system degrades with increasing age and maintaining the immune system in a balanced form found at younger ages may be the solution for disease prevention. Nutraceuticals found to affect or strengthen immune function might play a critical role in the prevention of debilitating neurological diseases such as PD. Antioxidant nutraceuticals such as epigallocatechin 3-gallate (EGCG) (Tan et al. 2008), quercetin (Haleagrahara et al. 2013), and rosmarinic acid (Ren et al. 2009) have shown potential in the treatment of PD. Continued future investment in

research on the manner antioxidant nutraceuticals impact brain health will benefit the nutraceutical market and offer promising preventative measures for these diseases.

2.3.3 Cardiovascular health

A significant antioxidant nutraceutical market exists in the prevention of heart disease, which is responsible for nearly every one in every four deaths occurring in the United States (Heart Disease Facts 2015d). According to 2009–2012 data, 32.6% of U.S. adults more than 20 years of age have elevated blood pressure (Mozaffarian et al. 2015). Currently, coronary artery disease (CAD) is the most common type of heart disease in the United States and can cause heart attacks by affecting blood flow to the heart (Coronary Artery Disease 2015b). Various antioxidant components in nutraceuticals can reduce the risk for heart disease, including flavonoids and coffee.

Flavonoids are a group of polyphenols derived from plants that have antioxidant capabilities. They can be found in many fruits and vegetables as well as in many beverages such as cocoa, tea, and wine (Zuchi et al. 2010). It has been demonstrated that people with very low dietary intakes of flavonoids have increased risk of coronary heart disease (Knekt et al. 1996), and flavonoid consumption is inversely correlated with mortality from coronary artery disease (Hertog et al. 1995).

Specifically, the wine market has taken advantage of the wine's ability to improve heart health since the 1970s. Moderate wine consumers, in juxtaposition to those who abstain or to those who drink in excess, have been associated with a lower risk of death as a result of cardiovascular diseases (Klatsky 1999). If further research is conducted supporting the use of flavonoids in promoting cardiovascular health, more foods and supplements containing flavonoids can be expected to reach the market.

Coffee is one of the most commonly consumed beverages worldwide and is one of the top sources of caffeine and polyphenols in the American diet (Bhatti et al. 2013). The effect of coffee and cardiovascular disease has been a topic of much controversy, but recent further research has swayed toward the demonstration of the cardiovascular benefits of coffee. In particular, a recent study concluded that moderate coffee consumption was inversely associated with cardiovascular disease risk with the lowest risk at a consumption of three to five cups of coffee per day (Ding et al. 2014).

People currently use coffee on a daily basis in order to take advantage of its caffeine, which acts as a stimulant to maintain wakefulness and focus. Many companies have been very successful in marketing for coffee to the point where coffee consumption has become a trend among much of the population. If continued research is released in support of coffee consumption, it is expected for the market to continue to rise as more companies enter the market.

2.4 Nutraceuticals market

As mentioned previously, nutraceuticals are not regulated in the same manner as pharmaceuticals. A reason that they are not regulated the same is that nutraceuticals do not, and cannot, make claims to prevent much less cure for any disease. Because the nutraceutical industry experiences less regulation, it may be inferred that it is easier for them to be profitable and expand their use. However, there is a need for tighter nutraceutical regulation in order to undergo similar development of pharmaceuticals.

2.4.1 Nutraceutical regulation

Legislative regulation plays a significant influence in the nutraceutical market. Minimal regulation of the nutraceutical market is beneficial to nutraceutical companies by reducing costs, but can potentially be harmful to consumers due to a lack of product research. Contrarily, tighter regulations benefit consumer health but raise product costs compared to less researched nutraceuticals. In the pursuance of taking full advantage of the many potential benefits of nutraceuticals, it is necessary to enforce stricter regulations of products reaching the market.

Nutraceuticals have undoubtedly gained popularity in recent years in an effort to bridge a perceived gap in the healthcare system left by modern medicine. However, in order for nutraceuticals to continue their momentum and become an integral part of modern day health systems, it is necessary to put them under the same scrutiny as pharmaceuticals. By investing the research of these products, nutraceutical companies gain the ability to prove that their products may be a staple to maintaining health and wellness and not just a complement to a normal diet. Without the well-established validation of these products consumers cannot justify the added expenditure associated with *functional food* products compared to the conventional ones.

Ensuring the safety of nutraceutical products entering the market comes with the need for a development process parallel to that of pharmaceuticals. Currently, pharmaceutical development requires identification of mechanisms of action and active constituents, chemical standardization based on the active compounds, biological standardization based on the pharmacological activity, preclinical evaluation of toxicity and potential for drug–botanical interactions, metabolism of active compounds, and finally, clinical studies of safety and efficacy (van Breemen 2015). Unfortunately, manufacturers of dietary supplements have little incentive to invest in further product research because the natural raw materials for the dietary supplements generally cannot be patented (Umhau et al. 2012). Although, if these dietary supplements can be proven to be effective through research, consumers will benefit from cost savings as well as health benefits (Umhau et al. 2012). The lack of policing and the lower market barrier to the entry of nutraceuticals compared to

pharmaceuticals many times diminish their reputation among scientists and consumers. In order for nutraceuticals to continue to compete against or work in conjunction with the pharmaceutical industry they must increase the developmental regulations of supplements.

Other factors hinder nutraceutical advancement in the health system such as the National Institutes of Health (NIH) budget cuts leading to decreased funding for nutrition research (Davis and Ohlhorst 2014). These budget cuts may act as a driving force for nutraceutical companies to merge with pharmaceutical companies in order to remain a part of the health market.

2.4.2 Future of nutraceuticals

The future of nutraceuticals continues to be promising as increasing amounts of people seek out the benefits of natural products for preventative care. Natural products bode well with the population, as they are many times believed to be a better alternative to modern medicine even when they have not proved to be advantageous. This expanding progression toward natural products and healthy lifestyles is not going to diminish as the population becomes more knowledgeable and the proliferation of the nutraceutical market is expected to follow the suit.

Many common nutraceuticals are naturally occurring products that have the capacity to be produced at a much more affordable rate compared to pharmaceuticals. Pharmaceutical products typically entail high costs due to the amount of money and time required in research and development. The current cost-effectiveness development of nutraceuticals contributes to the overall growth and expansion of the nutraceutical industry. However, if nutraceutical development costs begin to mimic the price of pharmaceutical drug development, it is expected for the industries to merge. The affiliation of the two industries will act to mitigate costs and provide better products for consumers due to more demanding legislative regulations.

2.5 Merging nutraceuticals and pharmaceuticals

With the increase in research in nutraceuticals, they have recently become more credible, intercalating themselves as a potentially prominent source of medicine. However, the disparity between nutraceuticals and pharmaceuticals is that one is more natural and the other is synthetically based, respectively. Albeit, for years, it has been considered the alternative to the traditional medicine, it may be more efficacious to amalgamate the two entities. Given this, it is expected for the nutraceutical industry to combine with the pharmaceutical industry in order to become a more powerful component of the healthcare sector. The merging of the two industries is also advantageous to patients due to the cost of disease prevention being much more

affordable compared to the curing after the disease onset. The potential synergistic effects of the collaboration of the two industries would only add to the efficacy of healthcare by promoting disease prevention and decreasing drug interactions.

2.5.1 Disease prevention

The cooperation of the two industries is essential for the better health outcomes of consumers. Research regarding antioxidant nutraceuticals addresses the prevention and the remedying of the existing conditions by the exploitation of natural bioactive components through sustenance, whether be it foods, drinks, or supplements. Utilizing these studies can be advantageous when addressed to disease prevention.

Many current diseases (aging, endothelial degeneration, autoimmune disorders, etc.) are due to the accumulation of free radicals. Subscribing and implementing antioxidants as a prescribed daily regimen can act as a remedy to the said ailments. From brain health to heart health, nutraceuticals are a potential way to provide disease prevention.

2.5.2 Drug interactions

Currently, a lack of regulation within the nutraceutical industry is leading to a promotion of products of questionable effects with potentially dangerous drug interactions with established pharmaceuticals. These negative effects may be negated by increasing research funding to nutraceuticals to create a synergistic effect between the two industries. Without the investigation of drug interactions between nutraceutical supplements and pharmaceutical products, healthcare providers suggesting nutraceuticals may be causing harm to their patients. Nonetheless, many preventative nutraceutical supplements are safe to consume, but caution must be observed when introducing a new supplement to the current regimen.

2.6 Conclusion

Nutraceuticals hold great potential in becoming an integral part of the modern healthcare system. In order for the nutraceutical industry to accomplish a supportive reputation, products need to undergo more vigorous research and investigation before being introduced to the market. As further research is conducted on antioxidant nutraceutical efficacy, it is expected that the market size will continue to expand. Currently, many antioxidant nutraceuticals have demonstrated correlation with better health, but due to the many contributing factors more research needs to be conducted to establish causation.

References

Andlauer, W. and P. Fürst. 2002. Nutraceuticals: A piece of history, present status and outlook. *Food Res Int* 35 (2–3):171–176. doi:10.1016/S0963-9969(01)00179-X.

Arendash, G. W. and C. Cao. 2010. Caffeine and coffee as therapeutics against Alzheimer's disease. *J Alzheimers Dis* 20 (Suppl 1):S117–S126. doi:10.3233/JAD-2010-091249.

Asia Pacific Vitamins (Nutraceuticals) Market By Application and By Geography–Trends and Forecast up to 2019. 2015. Research and Markets Accessed October 20, 2015. https://www.nutraceuticalbusinessreview.com/news/article_page/AsiaPacific_vitamins_market_by_application_trends_and_forecast_up_to_2019/108325.

Bailey, R. L., J. J. Gahche, P. E. Miller, P. R. Thomas, and J. T. Dwyer. 2013. Why us adults use dietary supplements. *JAMA Intern Med* 173 (5):355–361.

Bhatti, S. K., J. H. O'Keefe, and C. J. Lavie. 2013. Coffee and tea: Perks for health and longevity? *Curr Opin Clin Nutr Metab Care* 16 (6):688–697.

Cao, C., J. R. Cirrito, X. Lin, L. Wang, D. K. Verges, A. Dickson, M. Mamcarz et al. 2009. Caffeine suppresses amyloid-beta levels in plasma and brain of Alzheimer's disease transgenic mice. *J Alzheimers Dis* 17 (3):681–697.

Coppens, P., M. F. da Silva, and S. Pettman. 2006. European regulations on nutraceuticals, dietary supplements and functional foods: A framework based on safety. *Toxicology* 221 (1):59–74. doi:10.1016/j.tox.2005.12.022.

Coronary Artery Disease (CAD). 2015. Centers for Disease Control and Prevention Accessed October 23, 2015. http://www.cdc.gov/heartdisease/coronary_ad.htm.

Davis, C. D. and S. Ohlhorst. 2014. The Future of Nutrition Research at the National Institutes of Health. *Adv Nutr* 5 (5):537–540. doi:10.3945/an.114.006403.

Ding, M., S. N. Bhupathiraju, A. Satija, R. M. van Dam, and F. B. Hu. 2014. Long-term coffee consumption and risk of cardiovascular disease: A systematic review and a dose-response meta-analysis of prospective cohort studies. *Circulation* 129 (6):643–659. doi:10.1161/CIRCULATIONAHA.113.005925.

Egner, P. A., J. G. Chen, J. B. Wang, Y. Wu, Y. Sun, J. H. Lu, J. Zhu, Y. H. Zhang, Y. S. Chen, and M. D. Friesen. 2011. Bioavailability of sulforaphane from two broccoli sprout beverages: Results of a short-term, cross-over clinical trial in Qidong, China. *Cancer Prev Res* 4 (3):384–395.

Europe Nutraceuticals Market by Applications; by Types; by Country–Analysis and Forecast (2014–2019). 2015. Research and Markets Accessed October 21, 2015. https://www.micromarketmonitor.com/pressreleases/europe-nutraceuticals.html.

Fahey, J. W., S. L. Wehage, W. D. Holtzclaw, T. W. Kensler, P. A. Egner, T. A. Shapiro, and P. Talalay. 2012. Protection of humans by plant glucosinolates: Efficiency of conversion of glucosinolates to isothiocyanates by the gastrointestinal microflora. *Cancer Prev Res* 5 (4):603–611.

Haleagrahara, N., C. J. Siew, and K. Ponnusamy. 2013. Effect of quercetin and desferrioxamine on 6-hydroxydopamine (6-OHDA) induced neurotoxicity in striatum of rats. *J Toxicol Sci* 38 (1):25–33. doi:10.2131/jts.38.25.

Heart Disease Facts. 2015. Centers for Disease Control and Prevention. Accessed October 23, 2015. http://www.cdc.gov/heartdisease/facts.htm.

Heller, L. 2008. The four markets dominating EU supplements. Accessed October 20, 2015. https://www.nutraingredients.com/Article/2008/05/06/The-four-markets-dominating-EU-supplements.

Hertog, M. L., D. Kromhout, C. Aravanis, H. Blackburn, R. Buzina, F. Fidanza, S. Giampaoli et al. 1995. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch Intern Med* 155 (4):381–386.

Hsu, L. J., Y. Sagara, A. Arroyo, E. Rockenstein, A. Sisk, M. Mallory, J. Wong, T. Takenouchi, M. Hashimoto, and E. Masliah. 2000. α-Synuclein promotes mitochondrial deficit and oxidative stress. *Am J Pathol* 157 (2):401–410.

Joshi, S. 2015. Vitamin supplementation in the elderly. *Clin Geriatr Med* 31 (3):355–366. doi:10.1016/j.cger.2015.04.004.
Klatsky, A. L. 1999. Moderate drinking and reduced risk of heart disease. *Alcohol Res Health* 23 (1):15–24.
Knekt, P., R. Jarvinen, A. Reunanen, and J. Maatela. 1996. Flavonoid intake and coronary mortality in Finland: A cohort study. *BMJ* 312 (7029):478–481.
Meštrović, T. 2015. What are Nutraceuticals? News Medical: Life Sciences & Medicine.
Mozaffarian, D., E. J. Benjamin, A. S. Go, D. K. Arnett, M. J. Blaha, M. Cushman, S. de Ferranti et al. 2015. Executive summary: Heart disease and stroke statistics—2015 update: A report from the American heart association. *Circulation* 131 (4):434–441.
Nicastro, H. L., R. L. Bailey, and K. W. Dodd. 2015. Using 2 assessment methods may better describe dietary supplement intakes in the United States. *J Nutr* 145 (7):1630–1634.
Nutraceuticals Market—Global Industry Analysis, Size, Share, Growth and Forecast 2015–2021. 2015. https://www.news-medical.net/health/What-are-Nutraceuticals.aspx.
Olcese, J. M., C. Cao, T. Mori, M. B. Mamcarz, A. Maxwell, M. J. Runfeldt, L. Wang et al. 2009. Protection against cognitive deficits and markers of neurodegeneration by long-term oral administration of melatonin in a transgenic model of Alzheimer disease. *J Pineal Res* 47 (1):82–96.
Olson, M. E. and J. W. Fahey. 2011. Moringa oleifera: Un árbol multiusos para las zonas tropicales secas. *Revista mexicana de biodiversidad* 82 (4):1071–1082.
Ottaway, P. 2002. Dietary Supplements, Nutraceuticals & Functional Foods In Europe. Accessed October 20, 2015.
Pandey, M., R. K. Verma, and S. A. Saraf. 2010. Nutraceuticals: New era of medicine and health. *Asian J Pharm Clin Res* 3 (1):11–15.
Matthyssens, P., K. Vandenbempt, and L. Berghman. 2008. Value innovation in the functional foods industry: Deviations from the industry recipe. *Brit Food J* 110 (1):144–155. doi:10.1108/00070700810844830.
Ren, P., H. Jiang, R. Li, J. Wang, N. Song, H. M. Xu, and J. X. Xie. 2009. Rosmarinic acid inhibits 6-OHDA-induced neurotoxicity by anti-oxidation in MES23.5 cells. *J Mol Neurosci* 39 (1–2):220–225. doi:10.1007/s12031-009-9182-y.
Salcedo Jr, J., M. D. Bamba, E. O. Carrasco, G. S. Chan, I. Concepcion, F. R. Jose, J. F. DeLeon, S. B. Oliveros et al. 1950. Artificial enrichment of white rice as a solution to endemic beriberi. Report of field trials in Bataan, Philippines. *J Nutr* 42 (4):501–523.
Tan, L. C., W. P. Koh, J. M. Yuan, R. Wang, W. L. Au, J. H. Tan, E. K. Tan, and M. C. Yu. 2008. Differential effects of black versus green tea on risk of Parkinson's disease in the Singapore Chinese Health Study. *Am J Epidemiol* 167 (5):553–560. doi:10.1093/aje/kwm338.
Umhau, J. C., K. Garg, and A. M. Woodward. 2012. Dietary supplements and their future in health care: Commentary on draft guidelines proposed by the food and drug administration. *Antioxid Redox Signal* 16 (5):461–462. doi:10.1089/ars.2011.4402.
van Breemen, R. B. 2015. Development of safe and effective botanical dietary supplements. *J Med Chem* 58 (21):8360–8372. doi:10.1021/acs.jmedchem.5b00417.
Zuchi, C., G. Ambrosio, T. F. Lüscher, and U. Landmesser. 2010. Nutraceuticals in cardiovascular prevention: Lessons from studies on endothelial function. *Cardiovasc Ther* 28 (4):187–201. doi:10.1111/j.1755-5922.2010.00165.x.

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

3

Antioxidant Nutraceuticals and Prostate Cancer

Zhijun Wang, Patrick Chan, Chen Xie, Ying Huang, and Jeffrey Wang

Contents

3.1 Introduction

3.1.1 Benign prostate hyperplasia

Benign prostate hyperplasia (BPH) is one of the leading causes of urinary dysfunction in men. It is a noncancerous, chronic, and progressive urological disease that primarily affects aging males. While less than 10% of men under the age of 40 years suffer from BPH, the frequency increases to 70% in 61–70-year-old men (Berry et al. 1984). Most males will develop histological features of BPH and enlargement of the prostate gland with advancing age.

The etiology for BPH is unclear. It is possibly caused by imbalanced sex hormones. Prostate growth is dependent on intact testes because patients who undergo castration prior to puberty do not develop BPH (Rand 1895). The hallmark characteristic of BPH is an abnormal overgrowth of the prostate. The development of BPH entails static and dynamic mechanisms. The static process involves the bladder outlet obstruction (BOO) due to the enlargement of the prostatic tissue, which constricts the prostatic urethra and the neck of the bladder. The dynamic process involves constant stimulation of the prostate by dihydrotestosterone (DHT), resulting in increased smooth muscle tone and hyperplasia. Adrenergic stimulation of α_1 receptors in the prostate further causes smooth muscle contraction and constricts the urethral lumen (Lepor et al. 1988, Kobayashi et al. 1993). DHT stimulates prostate growth by modulating stromal cell insulin-like growth factor (IGF) axis (Le et al. 2006). The proliferation of smooth muscle and epithelial cells results in subsequent enlargement of the prostate gland and causing lower urinary tract symptoms (LUTS) (Lee et al. 1997). LUTS include urinary urgency and frequency, nocturia, incomplete voiding of the bladder, and weak urinary stream. BPH can impact the quality of life but is not typically considered a life-threatening condition.

The diagnosis of BPH and prostate cancer is based on the evaluation of LUTS, digital rectal examination (DRE) and physical examination, urinalysis, and the presence of the prostate specific antigen (PSA), and urinary frequency and volume (Abrams et al. 2013).

3.1.2 Prostate cancer

Prostate cancer is the second-leading cause of cancer death among males in the United States (Siegel et al. 2013). Constant PSA screening and surveillance may prevent long-term prostate cancer metastases and reduce mortality (Schroder et al. 2009 and 2012, Vickers et al. 2013, Amin et al. 2014). In the United States, there are 220,800 new cases of prostate cancer and 27,540 deaths in 2015 (Howlader 2015). The 5-year survival rate from 2005 to 2011 was 98.9%. There is a 14% lifetime risk of developing prostate cancer for men. The median age of diagnosis is 66 years. Since 1992, there is a downward trend of new cases and deaths from prostate cancer. The overall trend for prostate cancer

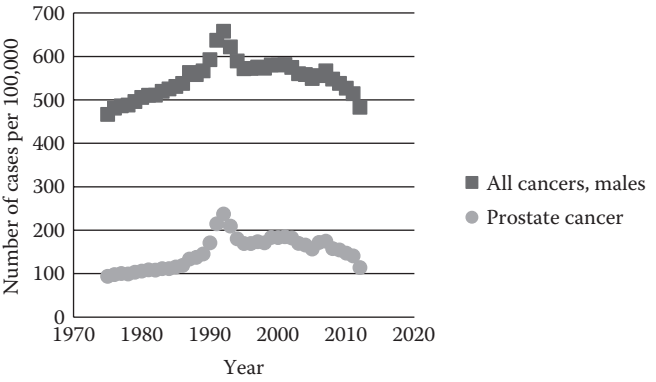


Figure 3.1 *Trends of prostate cancer and all cancers in men in the United States from 1975 to 2012. (From Howlader et al. 2015.)*

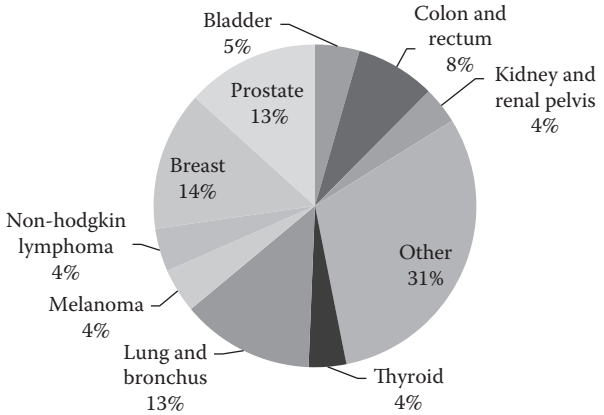


Figure 3.2 *Estimated percentage of new cases by cancer in the United States in 2015. (From National Cancer Institute 2015.)*

incident mirrors to all cancers in men (**Figures 3.1** and **3.2**, [Howlader 2015]) and about 26% of all cancers in men in 2015 is due to the malignancy of the prostate.

Several factors are associated with an increased risk of prostate cancer development. These include heredity, ethnical group, age, inflammation, obesity, and daily habit (smoking, dietary, exercise, and so on) (Aune et al. 2015). There is a genetic component associated with prostate cancer. Men with first-degree relatives afflicted with prostate cancer have a two-to-four fold higher risk than the general population (Goldgar et al. 1994, Johns and Houlston 2003). The homeobox *HOXB13* gene has been postulated to predispose patients

to prostate cancer (Ewing et al. 2012). The pathology of prostate cancer is complex and may involve a number of molecular mechanisms. The androgen receptor (AR) is a nuclear receptor that, when stimulated by testosterone or DHT, translocates to the nucleus to stimulate gene transcription (Suzuki et al. 2003). AR's function is to stimulate normal prostate growth during development (Yeh et al. 2002). However, AR intracellular signaling has also been implicated and is crucial during the pathogenesis of prostate cancer (Ryan and Tindall 2011). This is the rationale for the development of drugs that reduce the concentration of androgens that stimulate AR signaling.

The decision to treat is based on the patient's overall life expectancy, assessment of risks, tumor staging, PSA, and Gleason score (Thompson et al. 2007). Watchful waiting for low-grade cancer (6 or lower Gleason score) has an 18%–30% 15-year mortality rate (Albertsen et al. 1998). This rate jumps to over 60% in patients with Gleason scores above 6. There are currently no FDA-approved drugs for the prevention of prostate cancer. Chemoprevention of prostate cancer is based on empirical treatment with androgen deprivation and/or antioxidant therapy to minimized DNA damage (Walsh 2010).

3.1.3 Pharmacotherapy for benign prostate hyperplasia and prostate cancer

The treatment of BPH is reliant on the evaluation of LUTS. If LUTS is bothersome, pharmacotherapy along with modifying fluid and food intake is the standard of treatment (McVary et al. 2011). Drugs available for the treatment of BPH are listed in **Table 3.1**.

Initial management of prostate cancer is active surveillance (Parker 2004). For localized prostate cancer, treatment options include radical prostatectomy, hormone therapy/androgen deprivation therapy (ADT), and radiation (Thompson et al. 2007). ADT includes AR inhibitors, luteinizing hormone-releasing hormone (LHRH) agonist, and antagonists (**Table 3.2**). However, after several years of ADT, patients may develop androgen-independent progression of prostate cancer (Feldman and Feldman 2001). Prostate cancer may eventually progress to become androgen resistant or castration resistant. In advanced or metastatic prostate cancer, chemotherapy agents are used (**Table 3.2**).

Table 3.1 Drugs Used in the Treatment of Benign Prostate Hyperplasia

Drug Category	Examples
α_1-Adrenergic receptor blockers	Alfuzosin, doxazosin, silodosin, tamsulosin, terazosin
5-α-Reductase inhibitors	Dutasteride, finasteride
Anticholinergic agents	Tolterodine

Table 3.2 Drugs Used in the Treatment of Prostate Cancer

Chemotherapy	Hormone Therapy	Vaccine	Others
Cabazitaxel	*Androgen receptor inhibitors*	Sipuleucel-T	Abiraterone acetate with prednisone
Carboplatin	Bicalutamide		Ketoconazole
Docetaxel	Enzalutamide		
Doxorubicin	Flutamide		
Estramustine	Nilutamide		
Etoposide	*LHRH/GnRH agonists*		
Mitoaxantrone	Goserelin		
Paclitaxel	Histrelin		
Vinblastine	Leuprolide		
Vinorelbine	Triptorelin		
	LHRH/GnRH antagonists		
	Degarelix		

3.1.3.1 α_1-Adrenergic receptor blockers

Prostatic smooth muscle contraction is stimulated by the activation of α_1-adrenergic receptors (Lepor et al. 1988, Kobayashi et al. 1993). The predominance of α_1-adrenergic receptors localized on the prostate smooth muscle is the rationale for the use of α_1-adrenergic receptor blockers (**Table 3.1**) (Walden et al. 1999). α_1-Adrenergic receptor blockers (α-blockers) have been the mainstay of BPH therapy for decades (Caine et al. 1976). Antagonism of the α_1-adrenergic receptors reduces smooth muscle tone and improves urinary flow rate and LUTS. A meta-analysis indicates similar efficacy across the class of α_1-adrenergic receptor blockers (Djavan and Marberger 1999, Boyle et al. 2001).

A Cochrane review of eight clinical trials using 5-α-reductase inhibitors in the prevention of prostate cancer was performed (Wilt et al. 2010). The results showed a risk reduction in being diagnosed with prostate cancer. However, the effect of 5-α-reductase inhibitors on all-cause mortality was inconclusive.

3.1.3.2 5-α-Reductase inhibitors

The conversion of testosterone to DHT is mediated by the enzyme 5-α-reductase. DHT is a potent stimulant of prostatic growth. Finasteride and dutasteride inhibit 5-α-reductase and therefore reduce DHT levels (Bramson et al. 1997, Bartsch et al. 2002, Gormley et al. 2002). Both drugs are indicated for the treatment of BPH (Gormley et al. 2002, Roehrborn et al. 2002). However, sexual dysfunction is associated with the use of 5-α-reductase inhibitors (Gacci et al. 2014). The use of 5-α-reductase inhibitors in the prevention of prostate cancer is controversial. In a randomized, placebo-controlled trial

(Prostate Cancer Prevention Trial), the appearance of prostate cancer was prevented or delayed in patients receiving finasteride (Thompson et al. 2003 and 2013). Unfortunately, the development of high-grade prostate cancer was more prevalent in the finasteride treatment group.

3.1.3.3 Hormone therapy/androgen deprivation therapy

ADT has been employed in the treatment of BPH and prostate cancer since the 1970s (Caine et al. 1975). ADT reduces the amount of testosterone production.

AR inhibitors are nonsteroidal anti-androgens (**Table 3.2**). AR antagonists competitively inhibit the binding of testosterone and DHT on nuclear AR in prostatic tissue. This results in reduced gene expression; therefore, decreasing prostatic growth (Feldman and Feldman 2001).

LHRH, also termed gonadotropin-releasing hormone (GnRH), overstimulates LHRH receptors in the pituitary gland, causing desensitization and downregulation of the LHRH receptors. Subsequently, the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) are suppressed and decreasing testosterone release from the gonads (Tolis et al. 1982, Engel and Schally 2007). LHRH antagonists block LHRH receptors in the pituitary gland, decreasing the release of FSH and LH (Schally et al. 2001).

Clinical guidelines do not recommend ADT as a standard therapy for localized prostate cancer, but rather as an adjuvant therapy following radical prostatectomy or radiation (Thompson et al. 2007).

3.2 Antioxidant nutraceuticals and mechanisms of action

3.2.1 Reactive oxygen species and free radicals

Reduction and oxidation (redox) are two typical chemical reactions in many essential biological processes, such as cellular respiration, immune response, metabolic functions, and energy generation (Fox et al. 2005, Bindoli and Rigobello 2013, Manda et al. 2015). Redox reactions involve the transfer of electrons between elements: oxidation is the loss of electrons or an increase in oxidation state, whereas reduction is the gain of electrons or a decrease in oxidation state (Bindoli and Rigobello 2013).

Reactive oxygen species (ROS) and free radicals are produced as natural by-products of the normal metabolism during the redox reactions in biological systems (Gupta et al. 2012b, Phaniendra et al. 2015, Salisbury and Bronas 2015). A free radical is an atom or a molecule that has one or more unpaired electrons, whereas ROS is a chemically reactive molecule containing oxygen. Oxygen radicals, or radical ROS, are the most common free radicals in the biological system. ROS can also be non-radical, such as hydrogen peroxide, organic hydroperoxide, and ozone.

ROS and free radicals can be a double-edged sword in cellular processes depending on their cellular levels. In biological systems, they are critical for normal body functions and play important roles in various cell-signaling pathways at their normal physiological concentrations (Oberley 2002). However, at higher levels, ROS and free radicals can be toxic and may damage cells by causing oxidative stress. The main harmful effects of ROS and free radicals are DNA damage, oxidations of polyunsaturated fatty acids in lipids, oxidation of amino acids in proteins, and deactivation of specific enzymes by oxidation of their cofactors (Byersdorfer 2014, Giancaspero et al. 2014, Kermanizadeh et al. 2015). The damaged cells can release more free radicals and create additional oxidative stress to surrounding cells. Interestingly, it has been found that a modest level of ROS/free radicals can promote tumor cell growth (Gupta et al. 2012b, Gupta-Elera et al. 2012), whereas at excessive levels they can inhibit tumor growth or induce cell apoptosis/necrosis (Renschler 2004).

3.2.2 Oxidative stress and prostate cancer

Free radicals and ROS are essential in the stimulation of signaling pathways in response to changing intra- and extracellular environmental conditions. Elevated free radical and ROS levels caused by an increased ROS generation or loss of antioxidant defense may result in cellular oxidative stress. Oxidative stress is associated with several pathological conditions, including inflammation, infection, and carcinogenesis (Khandrika et al. 2009). In particular, chronic elevated levels of ROS are known to induce somatic mutations and neoplastic transformations, which may stimulate normal cells into cancerous cells. Superoxide, hydrogen peroxide, and hydroxyl radicals are the most studied and common ROS/free radicals involved in cancer development, including prostate cancer (Gupta et al. 2012b, Gupta-Elera et al. 2012).

As free radicals and ROS can cause cell senescence and their levels increase with age, the risk of developing prostate cancer increases dramatically for men over 50, and about 60% cases are diagnostic in men older than 65 (Stangelberger et al. 2008, American Cancer Society 2015). It has been found that oxidative stress is required for the aggressive phenotype of prostate cancer (Sauer et al. 2001). Thus, antioxidants may protect the human body against prostate cancer. The general mechanisms of developing prostate cancer by oxidative stress include DNA damage, aging, involvement of androgen activity, and activating nuclear receptors (androgen receptor), although the exact cellular mechanism is still poorly understood.

3.2.2.1 DNA damage

DNA impairment is one of the major damages caused by ROS and free radicals, which can directly interact with DNA molecules and cause damage to cell function. These include modification of bases, DNA strand break, DNA–DNA cross-link, and DNA–protein cross-link (Maynard et al. 2009,

Scott et al. 2014). Cells possess cellular repair systems to protect themselves from oxidative lesions. However, if cells fail to effectively repair damaged DNA due to deficient DNA repair capability, the DNA damage can remarkably affect its structure and function, leading to cell mutation. Physiologically, the body has the ability to remove mutated cells by inducing cell apoptosis or necrosis. However, if mutated cells elude the body's defensive system, they can become cancerous and may further develop into cancer. The exposure of prostate cells to abnormal level of free radicals/ROS has been found to play an important role in the initiation of prostate cancer (Jena 2012).

3.2.2.2 Aging

As mentioned earlier, prostate cancer is a highly age-related disease. Increasing evidence has shown that a high level of free radicals and ROS is associated with the aging process (Minelli et al. 2009). Thus, cell senescence induced by ROS/free radicals may contribute to the initiation of prostate cancer. The mitochondrion is the main organelle in eukaryotic cells for energy conversion by producing ATP via cellular respiration, which releases ROS. Excessive ROS may cause the mutation of mitochondrial DNA (mtDNA), and the accumulation of such somatic mutations may cause deficiencies in the electron transport chain and further increase in the production of ROS (Slimen et al. 2014, Pinto and Moraes 2015). The accumulation of mtDNA somatic mutations appears to be an indicator of human age-related diseases, including prostate cancer (Khrapko and Turnbull 2014).

3.2.2.3 Androgens

Androgens play an important role in the progression of prostate cancer and the imbalance of androgen levels may cause carcinogenesis (Attard et al. 2015). Androgen signaling is one of the major pathways of ROS generation in prostate epithelial cells. ROS produced by the androgen signal pathway may contribute to the development of prostate cancer (Chignalia et al. 2015).

Androgens can activate the activator protein 1 (AP-1) transcription factor JunD and form androgen–JunD complex. The latter can induce spermidine/spermine N1-acetyl transferase (SSAT), which initiates a major polyamine oxidation pathway and generates excessive hydrogen peroxide in polyamine-rich prostatic epithelial cells. The inhibition of androgen–JunD complex can downregulate the transcription of SSAT, resulting in a decrease of cellular ROS (Mehraein-Ghomi et al. 2014). The increase in ROS by the androgen pathway was also related to the elevation of p66Shc protein levels. p66Shc is an oxidase, which can increase ROS levels by oxidizing Cyt C in the mitochondria or through SOS-mediated Rac1 activation at the cell membrane. Excessive ROS can inactivate prostatic acid phosphatase, leading to the activation of ErbB-2. This subsequently promotes the proliferation of prostate cells. The addition of antioxidants can suppress cell proliferation (Veeramani et al. 2012).

Fatty acids are an important source of energy in cells. Its oxidation in the mitochondria can generate ATP with ROS as the by-product. Androgens have been found to increase the uptake of fatty acids in prostate cancer cells, and to induce the transcription of carnitine palmitoyltransferase, which is the rate-limiting enzyme in the oxidation of mitochondrial fatty acids (Lin et al. 2010).

3.2.2.4 Nuclear receptor

AR and estrogen receptor β (ERβ) are the two main steroid hormone receptors expressed in prostate epithelial cells. AR is a ligand-dependent nuclear receptor, which can be activated by androgenic hormones such as testosterone, DHT, and androstenedione, whereas ERβ is one of two main types of estrogen receptor.

Hyperactivation of AR is associated with prostatic inflammation and carcinogenesis. Upon activation, AR translocates to the nucleus and binds specific androgen response element in the promoter area of its downstream genes. One of the most well-known genes is the PSA gene. It has been found that hydrogen peroxide production by flavin-dependent monoamine oxidase KDM1A is necessary for the activation of PSA transcription, although the specific mechanism is unclear. Oxidation inhibition was found to reduce AR-dependent PSA expression and such reduction was reversed by the addition of hydrogen peroxide (Shiota et al. 2011).

In contrast to AR, ERβ may have beneficial effects of suppressing cancer development. Studies have shown that ERβ is sensitive to ROS and its activity can be inhibited by ROS (Grubisha and DeFranco 2013). When subjected to ROS such as hydrogen peroxide, its second zinc finger motif can be oxidized and is unable to form a homodimer, and thus cannot activate the transcription of E-cadherin. The suppression of E-cadherin transcription has been reported to be related to prostate carcinoma progression (Rubin et al. 2001).

3.2.2.5 Mechanisms of antioxidation

Controlling and maintaining ROS and free radicals within the normal concentration range is very important for cellular homeostasis. To maintain such balance, cells possess an antioxidant system to scavenge ROS and free radicals. Antioxidants play important roles in the cellular defense system against oxidative stress. Antioxidants are molecules, which can safely interact with free radicals/ROS and terminate the chain reaction before vital molecules are damaged by removing free radical intermediates or inhibiting other oxidation reactions. The interaction can be via a simple chemical reaction for nonenzymatic antioxidants or by stimulating antioxidant enzymes for enzymatic oxidants (Jiao and Wang 2000, Mancuso et al. 2007, Liu et al. 2014) (**Figure 3.3**).

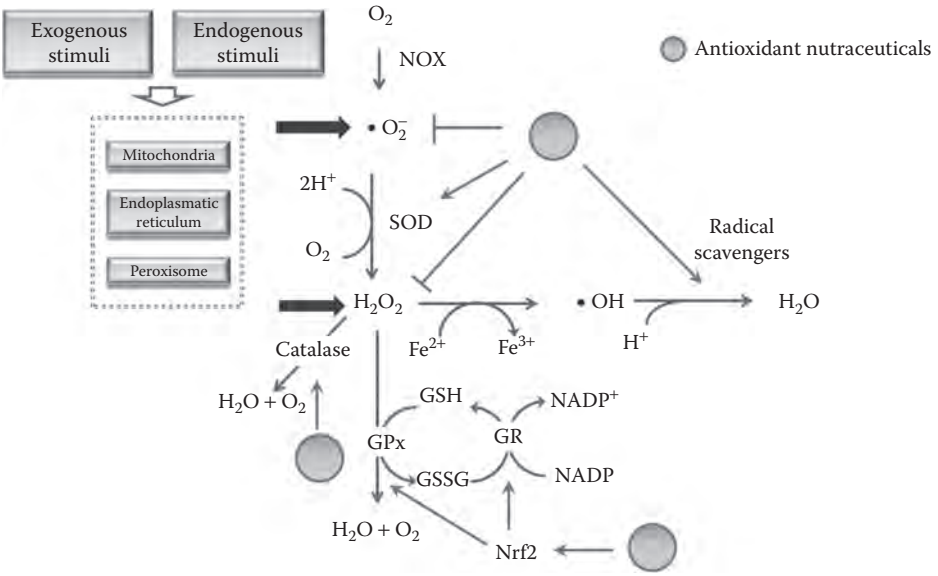


Figure 3.3 *The generation of ROS/free radicals and the mechanisms of antioxidant nutraceuticals. ROS/free radicals are usually produced in mitochondria, endoplasmatic reticulum, and peroxisome by both exogenous and endogenous stimuli. Nutraceuticals can eliminate ROS/free radicals by stimulating the enzymatic system or scavenging radicals.*

3.2.2.6 Nonenzymatic antioxidants

Vitamin C and E are the major antioxidants in the biological system. Vitamin C is a water-soluble antioxidant that can neutralize free radicals and ROS, whereas vitamin E is the major lipid-soluble antioxidant, which protects cellular membranes from oxidative damage by trapping peroxyl radicals (Odin 1997).

Glutathione (glutamyl–cysteinyl–glycine tripeptide) is another important intracellular antioxidant. The free –SH group in cysteine is highly reactive and can remove radicals effectively by oxidizing itself. The oxidized glutathione can be reduced via a redox cycle involving NAPDH (Shen et al. 2010, Yusuf et al. 2010, Singh et al. 2015).

3.2.2.7 Enzymatic antioxidants

Three main enzymes involved in the enzymatic antioxidant system include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Weydert and Cullen 2010). SODs catalyze the conversion of two superoxide molecules into hydrogen peroxide and oxygen. SODs are metal-containing enzymes that depend on bound manganese, copper, or zinc for their antioxidant activity. In human, the manganese-containing enzyme is the

most abundant in the mitochondria, whereas the zinc or copper forms are predominant in the cytoplasm. SODs are highly inducible enzymes, and thus are important targets for many antioxidant nutraceuticals (D'Alessandro and Zolla 2011). Catalase is located in peroxisomes in eukaryotic cells. It degrades hydrogen peroxide to water and oxygen. Hydrogen peroxide can be generated by SODs and other signal pathways. GPx is a group of enzymes, the most abundant of which contain selenium. These enzymes can also degrade hydrogen peroxide. GPx can also reduce organic peroxides to alcohols, providing another antioxidant mechanism for eliminating toxic oxidants. In addition to these enzymes, glutathione S-transferase, ceruloplasmin, hemoxygenase, and possibly several other enzymes may participate in the enzymatic control of oxygen radicals and their products.

A number of nutraceuticals have been identified to induce the expression of antioxidant enzymes. Bilirubin, uric acid, flavonoids, carotenoids, rainbow trout (*Oncorhynchus mykiss*) extract supplemented with sage, mint, and thyme oils can stimulate the activity of SOD, G6PD, and GPx (Sönmez et al. 2015). Soy isoflavones can protect cells from free radical-induced DNA damage in both normal and cancerous prostate tissues by upregulating antioxidant enzymes such as glutathione reductase, glutathione S-transferase, and SODs.

Although the specific mechanisms for the induction of enzymatic activity have not been identified, the role of the activation of nuclear factor-erythroid-2-related factor 2 (Nrf2, also known as NFE2L2), a transcription factor, has been well studied. Nrf2 is an important cell-defensive gene regulating cellular redox homeostasis. Under normal conditions, Nrf2 is located in the cytoplasm and is degraded rapidly by Kelch-like ECH-associated protein 1 (Keap1) and Cullin 3 by ubiquitination (Cheung and Kong 2010, Ma and He 2012, Lee et al. 2013). In response to oxidative stress, Nrf2 relocates to the nucleus where it binds to the promoter region and induces the transcription of antioxidative genes such as SOD, GPx, glutathione S-transferase, NAD(P)H:quinone oxidoreductase-1, and phase-II drug-metabolizing enzymes (Lee and Surh 2005, Hu et al. 2010).

Other than regulating antioxidant enzymes, several factors can regulate ROS activity in normal cells. NF-κB transcription factors (nuclear factor kappa-light-chain-enhancer of activated B cells) are also redox-related factors, which were found to play a role in promoting prostate cancer (Acharya et al. 2010). NFκB transcription factors are protein complexes composed of homodimers or heterodimers of NF-κB and Rel proteins. NF-κB regulates the expression of multiple genes involved in cell proliferation, apoptosis, angiogenesis, and metastasis. The NF-κB dimers are sequestered in the cytoplasm by a family of IκB inhibitors. In response to stimulation, such as proinflammatory cytokines (e.g. tumor necrosis factor, interleukin-1, and bacterial lipopolysaccharide) and free radicals/ROS, IκBs are rapidly phosphorylated and then undergo ubiquitination and proteolysis by the 26S proteasome, resulting in activation of NF-κB. The activated NF-κB translocates to the nucleus and promotes

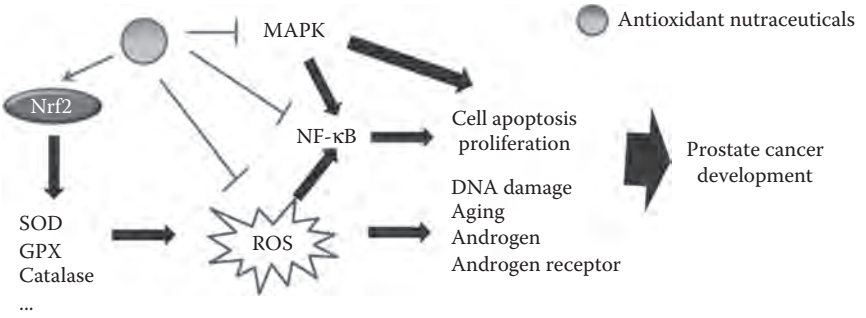


Figure 3.4 *The potential mechanisms of antioxidant nutraceuticals in the prevention of prostate cancer: elimination of ROS or inhibition of MAPK and NFκB.*

transcription of its target genes. It has been reported that intracellular ROS can regulate NF-κB activity, although the exact molecular mechanism remains to be elucidated. The inhibition of NF-κB may block ROS damage.

Mitogen-activated protein kinases (MAPKs) are involved in cellular response to various stimulations such as heat shock, proinflammatory cytokines, and oxidative stress. Three subgroups of MAPKs exist in human, i.e., extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs), and p38 MAPKs. Growth factors and mitogens can prevent cell apoptosis under normal condition via the Ras/Raf/MEK/ERK signaling cascade. ROS can regulate MAPKs to stimulate cell growth or cell death, depending on the signal intensity and duration (Eguchi et al. 2003).

In summary, the mechanisms of antioxidants involved in the prevention of prostate cancer remain to be fully elucidated. Nrf2 is the main cell-signal pathway regulating antioxidant systems, but other cellular mechanisms such as NF-κB and MAPK pathways are also involved (**Figure 3.4**).

3.3 Examples of antioxidant nutraceuticals for prostate health

Herbs have a long history of being used for the prevention and treatment of human diseases. Many of them have been recently developed into a class of dietary supplements termed nutraceuticals. Antioxidant activity is one of the most outstanding properties of nutraceuticals. Because of the complex nature of their composition and property of each of the compound contained within, nutraceuticals usually display a spectrum of activities, collectively contributing to their beneficial effects (Wang et al. 2013a). Preclinical and clinical studies of antioxidant nutriceuticals in the prevention of prostate cancer are summarized in **Table 3.3** and **Table 3.4**, respectively.

Table 3.3 Preclinical Studies of Antioxidant Nutraceuticals in the Prevention of Prostate Cancer

Agent	*In Vitro/In Vivo* System	Effects and Mechanism	Reference
Saw palmetto	LNCaP	Induces growth arrest and apoptosis; inactivates STAT3 and AR signaling	Yang et al. 2007
Saw palmetto	PC, PC-3, LNCaP	Inhibits proliferation; induces apoptosis; downregulates inflammation-related genes IL-6, CCL-5, CCL-2, COX-2, and iNOS)	Silvestri et al. 2013
Saw palmetto	PC-3, LNCaP	Complete mitochondrial depolarization; release of cytochrome c; caspase 9 activation	Baron et al. 2009
Saw palmetto	LNCaP, DU145	Induces apoptosis	Hostanska et al. 2007
Saw palmetto	PC-3	Inhibits proliferation; stimulates apoptosis; reduces Akt phosphorylation; reduction in the mitochondrial potential; decreases in PIP2 level	Petrangeli et al. 2009
Saw palmetto	RWPE-1, PWR-1E, PC-3, DU145, LNCaP, HaCaT	Radiosensitizes normal prostate cells	Hasan et al. 2010
Lycopene	Xenograft rat model	Inhibits tumor cell growth	Campbell et al. 2004
Lycopene	LNCaP	Inhibits cell growth; inhibits AR gene element activity and expression	Zhang et al. 2010
Polyphenon E	TRAMP mice	Prevents early and metastatic progression of prostate cancer	Kim et al. 2014
Nano-EGCG	22Rv1 tumor xenograft model	Inhibits tumor growth and secreted PSA level	Khan et al. 2014
SFN from broccoli	PC-3, DU145, LNCaP	Induces apoptosis; activates caspases, ERK1/2, and AKt; increases p53 and bax	Fimognari et al. 2008a, b
SFN from broccoli	PC-3, LNCaP	Inhibits HDAC activity	Ho et al. 2011
SFN from broccoli	LNCaP, 22Rv1, C4-2, PC-3	Downregulates CXCR4 expression	Sakao et al. 2015
PEITC from broccoli	PC-3	Reduces migration; inactivates Akt; suppresses VEGF and EGF expression and G-CSF secretion	Wu et al. 2009

(*Continued*)

Table 3.3 (*Continued*) Preclinical Studies of Antioxidant Nutraceuticals in the Prevention of Prostate Cancer

Agent	*In Vitro/In Vivo* System	Effects and Mechanism	Reference
Resveratrol	PC-3, DU145, LNCaP	Inhibits cell growth; induces cell cycle arrest and apoptosis; lowers both intracellular and secreted PSA	Hsieh and Wu 1999
Resveratrol	*In vivo* transgenic rat for adenocarcinoma of prostate model	Suppresses prostate cell growth	Seeni et al. 2008
Muscadine grape skin extract/resveratrol	PrEC, RWPE-1, WPE1-NA22, WPE1-NB14, WPE1-NB26	Inhibits cell growth; promotes apoptosis without affecting normal epithelial cells; reduces Akt activity; alters level of DJ-1; resveratrol arrests cell growth at G1-S phase but does not induce apoptosis	Hudson et al. 2007
Muscadine grape skin extract	ARCaP, LNCaP	Reverts EMT and ROS-mediated tumor progression by decreased vimentin levels and reinduction of E-cadherin expression; decreases Stat-3 activity	Burton et al. 2014
Muscadine grape skin extract	LNCaP, ARCaP-E	Antagonizes Snail-Cathepsin L-mediated invasion, migration and osteoclastogenesis	Burton et al. 2015
Genistein	LNCaP	Inhibits cell growth; induces apoptosis, decreases the transcriptional activation of PSA by both androgen-dependent and androgen-independent methods	Davis et al. 2002
Genistein in combination with calcitriol	DU145	Inhibits cell growth; directly inhibits CYP24 enzyme activity	Swami et al. 2005
Genistein	PC3-M	Inhibits metastasis; increases tumor levels of FAK, p38 MAPK, and HSP27	Lakshman et al. 2008
Quercetin	PC-3, DU145, LNCaP, TRAMP-C2, 22Rv1, CWR22Rv1, C4-2	Inhibits cell proliferation; induces apoptosis and cell cycle arrest; inhibits PI3K/AKT and IGF pathway; reverses EMT; suppresses angiogenesis; downregulates matrix metalloproteinase	Yang et al. 2015
Quercetin	Nude mice, SCID mice, SD rat	Inhibits xenograft tumor growth by inhibiting angiogenesis, proliferation, and HSP72; induces apoptosis; reduces wet prostate weight by inhibiting proliferation; reduces phosphor-MEK1/2 and phosphor-MAPK; increases p15, p21, and p27	Rajkumar et al. 2011

(*Continued*)

Table 3.3 (*Continued*) Preclinical Studies of Antioxidant Nutraceuticals in the Prevention of Prostate Cancer

Agent	*In Vitro/In Vivo* System	Effects and Mechanism	Reference
Quercetin	LNCaP, PC-3	Suppresses AR expression and activity; blocks JNK signaling pathway; induces association of a c-Jun/Sp1/AR protein complex	Yuan et al. 2010
Brazilian propolis extract	DU145, PC-3, RC-58T/h/SA#4	Inhibits cell growth	Li et al. 2007
Polish/Brazilian propolis	LNCaP	Induces apoptosis; upregulates TRAIL-R2	Szliszka et al. 2011 and 2013
Caffeic acid phenethyl ester from propolis	LNCaP, DU145, PC-3	Suppresses cell proliferation; inhibits tumor growth; inhibits p70S6K and Akt pathway	Chuu et al. 2012
Caffeic acid phenethyl ester/chemotherapeutic drugs	PC-3	Induces p21Cip1); reduces Akt signaling; inhibits growth; synergistic suppression effect when combined with vinblastine, paclitaxiol, or estramustine	Lin et al. 2012
Caffeic acid phenethyl ester from propolis	CRPC	Induces cell cycle arrest; inhibits growth; regulates Skp2, p53, p21, Cip1, and p27Kip1	Lin et al. 2015
Caffeic acid phenethyl ester with chemotherapeutic drugs	PC-3, DU-145, LNCaP	Synergistically enhances docetaxel and paclitaxel's antiproliferative and cytotoxic effect; induces alterations in ER-α and ER-β abundance	Tolba et al. 2013
Vitamin D/calcitriol	C57BL/6 $\times$ 129 mice, MKP-1$^{-/-}$ mice	Upregulates MKP-1; inhibits LPS-induced p38 activation and cytokine production in monocytes/macrophages	Zhang et al. 2012
Vitamin D/calcitriol	Primary cultures derived from normal human prostate and various prostate cancer cells	Targets COX-2, NF-κB and TGF-β; induces cell cycle arrest in G1, apoptosis, and differentiation; modulates growth factor signaling; inhibits invasion and metastasis	Welsh 2012
Vitamin D/calcitriol	*Nkx3.1; Pten* mutant mice	Delays the onset of HGPIN	Banach-Petrosky et al. 2006
Vitamin D/calcitriol	Various types of prostate cancer cells and xenograft mouse model	Increases differentiation and apoptosis; decreases proliferation, invasiveness, and metastasis	Chen and Holick 2003

Table 3.4 Clinical Studies of Antioxidant Nutraceuticals in the Prevention of Prostate Cancer

Agent	Cohort	Endpoint	Results	Reference
Saw palmetto	Patients with BPH	Objective and subjective signs of BPH	Improve the objective and subjective signs of BPH; fewer side effects	Carbin et al. 1990, Champault et al. 1984, Gerber et al. 2001
Saw palmetto	Men with risk of prostate cancer	Cancer registry	No association with risk of prostate cancer development	Bonnar-Pizzorno et al. 2006
Lycopene	Prostate cancer patients and control	Cancer incidence	Serum lycopene is inversely related to prostate cancer risk in U.S. blacks and whites	Vogt et al. 2002
Lycopene	Patients with HGPIN	PSA	Chemopreventive effect in the treatment of HGPIN with no toxicity and good patient tolerance	Mohanty et al. 2005
Lycopene	Patients with possible diagnosis of prostate cancer	Lycopene level and antioxidant biomarker	A higher level of lycopene found in the lycopene treatment group; however, no significant changes in the DNA oxidation product 8-oxo-deoxyguanosine and the lipid peroxidation product malondialdehyde	van Breemen et al. 2011
Lycopene	Patients with HGPIN	PSA and plasma lycopene levels	No significant change for PSA level; lycopene did not prevent the progression of HGPIN to prostate cancer	Mariani et al. 2014
Lycopene/tomato	Men in general population	Cancer incidence	Lycopene/tomato may have a modest effect in the prevention of prostate cancer	Chen et al. 2013, Dagnelie et al. 2004
Lycopene/tomato	Men in general population	Cancer incidence	Only a diet high in lycopene may be effective in prostate cancer prevention	Etminan et al. 2004
Lycopene	Prostate cancer patients and control	Cancer incidence	No associations of lycopene with prostate cancer risk	Kristal et al. 2011
Lycopene/tomato	Men with no history of prostate cancer	Cancer incidence	No associations of lycopene with prostate cancer risk	Kirsh et al. 2006
Lycopene	Patients with progressive hormone refractory prostate cancer	PSA	No positive effect in hormone-refractory prostate cancer	Schwenke et al. 2009

(*Continued*)

Table 3.4 (*Continued*) Clinical Studies of Antioxidant Nutraceuticals in the Prevention of Prostate Cancer

Agent	Cohort	Endpoint	Results	Reference
Lycopene alone or with soy isoflavones	Patients with prostate cancer	PSA	Stable PSA level, may delay progression of both hormone-refractory and hormone-sensitive prostate cancer	Vaishampayan et al. 2007
Lycopene	Men with biochemically relapsed prostate cancer	PSA	Safe, well-tolerated, no effect on PSA level	Clark et al. 2006
Lycopene	Patients with prostate cancer	Survival rate	Addition of lycopene prior to orchiecomy reduced the size of tumor compared to orchiectomy alone	Ansari and Gupta 2004
Lycopene	Patients with prostate cancer	PSA and other biomarkers	Lycopene may decrease the growth of prostate cancer	Kucuk et al. 2001
Polyphenon E	Men with HGPIN	Rate of progression to prostate cancer	No differences in the number of prostate cancer cases; no difference in adverse events between the study groups	Kumar et al. 2015
Green tea extract with quercetin	Patients scheduled for prostatectomy	Bioavailability enhancement	Ongoing	
Green tea extract	Patients with low-risk prostate cancer	PSA and other biomarkers	Ongoing	
Broccoli sprouts	Healthy volunteers	Pharmacokinetics of SFN and carcinogen	Inverse correlation between SFN treatment and carcinogen excretion and interindividual differences in the bioavailability of SFN	Kensler et al. 2005
Broccoli sprout extracts	Healthy volunteers	Safety, tolerance, and pharmacokinetics	No significant toxicity observed	Shapiro et al. 2006
Broccoli sprouts	Healthy volunteers	HDAC activity	HDAC activity significantly inhibited in peripheral blood mononuclear cells	Myzak et al. 2007
Broccoli-rich diet	HGPIN volunteers	Gene expression and GSTM1 genotype	Broccoli-rich diet-induced differential changes in the gene expression in prostate tissues between GSTM1 genotypes	Traka et al. 2008

(*Continued*)

Table 3.4 (*Continued*) Clinical Studies of Antioxidant Nutraceuticals in the Prevention of Prostate Cancer

Agent	Cohort	Endpoint	Results	Reference
SFN from broccoli sprout extracts	Patients with recurrent prostate cancer	PSA and safety	25% of patients achieve a 50% decline in PSA levels; no adverse effect	
SFN from broccoli sprout extracts	Healthy volunteers	HDAC inhibition and DNA methylation	Ongoing	
SFN from broccoli sprout extracts	Patients with low and intermediate prostate cancer	Global gene expression metabolic changes	Ongoing	
Muscadine grape skin extract	Patients with biochemically recurrent prostate cancer (BRPC	Safety, tolerability and PSA	Safe; exploratory review of lengthening in men with BRPC; no decline in PSA level	Paller et al. 2015
Muscadine plus grape skin extract	Patients with BPRC	Safety; PSA	Ongoing	
Isoflavones/ curcumin	Men with increased PSA but negative prostate biopsy	PSA	Combined treatment of soy isoflavones and curcumin could significantly decrease the serum PSA level	Ide et al. 2010
Soy/soy isoflavone	Patients with prostate cancer	PSA	PSA level will decrease when patients consume soy/soy isoflavone	Dalais et al. 2004, Hussain et al. 2003
Genistein	Patients with prostate cancer	PSA and biomarker	PSA level decreased; no effect on hormone	Lazarevic et al. 2011
Soy isoflavones	Men with increased PSA but negative prostate biopsy	PSA and cancer incidence	For patients aged 65 years or more, incidence of cancer in isoflavone group was significantly lower with no effect on PSA	Miyanaga et al. 2012
Soy protein	Men with HGPIN or low-grade prostate cancer	PSA and other biomarkers	A significantly lower rate of prostate cancer developed in the soy groups; no effect on PSA	Hamilton-Reeves et al. 2008
Soy isoflavone	Prostate cancer patients and control	Cancer incidence	Soy isoflavone may protect against prostate cancer	Nagata et al. 2007
Soy isoflavones	Patients with prostate cancer	Serum testosterone level and estrogen receptor status	No effect on testosterone level	

(*Continued*)

Table 3.4 (*Continued*) Clinical Studies of Antioxidant Nutraceuticals in the Prevention of Prostate Cancer

Agent	Cohort	Endpoint	Results	Reference
Quercetin	Patients with chronic prostatitis and healthy volunteers	Safety	No drug-related toxicity or side effects	Cialdella-Kam et al. 2013, Shoskes et al. 1999
Quercetin	Prostate cancer patients and control	Cancer incidence	Consumption of quercetin can reduce 27% risk for prostate cancer	McCann et al. 2005
Quercetin/genistein	Men with an increased PSA	PSA and cancer incidence	Ongoing	
Serum vitamin D level	Healthy volunteers	Cancer incidence	Low serum levels of vitamin D (25-hydroxyvitamin D associated with an increased risk for prostate cancer	Ahonen et al. 2000
Calcitriol	Patients with prostate cancer	Anti-cancer efficacy and PSA	No anti-cancer effect	Osborn et al. 1995
Calcitriol	Patients with advanced cancer	Maximum tolerated dose and pharmacokinetics	High calcitriol serum concentrations can be achieved, although with interindividual variability	Muindi et al. 2002
Vitamin D3	Patients with low-risk prostate cancer	PSA and progression	55% of subjects showed a decrease in the progression of prostate cancer	Marshall et al. 2012
Calcitriol plus docetaxel	Patients with androgen-independent prostate cancer	PSA	High dose of calcitriol treatment was associated with improved survival; No significant response for PSA	Beer et al. 2007
Vitamin D3	Patients with low-grade prostate cancer	PSA and pathology status	No significant changes in PSA levels and number of positive biopsy cores compared to the corresponding values assessed before enrollment	
Vitamin D and soy supplements	Patients with recurrent prostate cancer	PSA	No statistical analysis provided for number of participants showing a 50% reduction in PSA during treatment	

3.3.1 Saw palmetto

Saw palmetto (*Serenoa repens*) is a commonly used over-the-counter nutraceutical for the prevention and treatment of BPH and prostate cancer (Barnes et al. 2004). Saw palmetto inhibits 5-α-reductase and antagonizing the androgen receptors (Sultan et al. 1984). Multiple clinical trials have examined the effectiveness of saw palmetto in treating the symptoms of BPH. Some clinical trials have shown effectiveness (Champault et al. 1984, Carbin et al. 1990, Descotes et al. 1995, Gerber et al. 2001), whereas others have shown minimal or no improvements (Smith et al. 1986, Gerber et al. 1998, Marks et al. 2000, Willetts et al. 2003, Bent et al. 2006, Barry et al. 2011).

The American Urological Association (AUA) does not support the use of saw palmetto in the treatment of LUTS in BPH (McVary et al. 2011). This is due to the lack of controlled clinical trials to validate its effectiveness. A Cochrane systemic review of saw palmetto did not show a significant difference in the improvement in LUTS or maximal urinary flow rate (MacDonald et al. 2012), although the review concluded that the adverse reactions of saw palmetto appear to be mild.

In vitro studies of saw palmetto showed potential in the treatment of prostate cancer by inducing apoptosis and inflammatory response (Hostanska et al. 2007, Yang et al. 2007, Baron et al. 2009, Petrangeli et al. 2009, Silvestri et al. 2013). However, dietary supplements containing saw palmetto may radiosensitize normal prostate cells and thus should be discontinued in patients undergoing radiotherapy (Hasan et al. 2010). A prospective cohort study in more than 35,000 males did not show an association between the use of saw palmetto and the risk of prostate cancer (Bonnar-Pizzorno et al. 2006).

The multicenter PROCOMB trial examined the use of saw palmetto, lycopene, and selenium (SeR–Ly–Se) as one combination and in conjunction with tamsulosin (SeR–Ly–Se + tamsulosin) (Morgia et al. 2014). The regimen of SeR–Ly–Se + tamsulosin significantly improved International Prostate Symptom Score (IPSS) and urine volume (Qmax) compared to individual therapies of SeR–Ly–Se or tamsulosin.

3.3.2 Lycopene

Lycopene, a carotenoid contained in tomatoes (*Lycopersicum esculentum*), may lower risks of prostate cancer (Clinton et al. 1996, Gann et al. 1999, Lu et al. 2001). Lycopene is found in high levels in prostate tissue and may have chemoprotective effects. *In vitro* studies in cancer cell lines have shown that lycopene can inhibit cancer cell growth (Campbell et al. 2004) due to proapoptotic (Lee et al. 2011) or antioxidant actions (Zhang et al. 2010). Patients who have lowered serum levels of lycopene have a higher risk of prostate cancer (Vogt et al. 2002). The use of lycopene in the prevention

of prostate cancer has produced mixed clinical results. Patients with high-grade prostate intraepithelial neoplasia (HGPIN) showed lowered progression to prostate cancer when taking lycopene compared to the placebo (Mohanty et al. 2005). Oral lycopene was given to African–American veterans who were at risk for BPH or prostate cancer in a randomized, double-blind, controlled trial (van Breemen et al. 2011). Following 21 days of lycopene therapy, prostate biopsy revealed significantly higher levels of lycopene levels in the treatment group compared to the placebo group. However, clinical trials using lycopene supplementation did not prevent the progression of HGPIN to prostate cancer (Mariani et al. 2014).

Two meta-analyses showed that lycopene consumption may only have a modest effect in the prevention of prostate cancer (Dagnelie et al. 2004, Chen et al. 2013), whereas another meta-analysis showed that a diet high in lycopene may be effective (Etminan et al. 2004). The Prostate Cancer Prevention Trial and the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial did not support the use of lycopene (Kirsh et al. 2006, Kristal et al. 2011). Furthermore, there were no associations with reduced prostate cancer risk in patients exhibiting higher serum lycopene levels (Chang et al. 2005, Peters et al. 2007, Beilby et al. 2010). Lycopene administration did not demonstrate positive effects in hormone-refractory prostate cancer (Jatoi et al. 2007, Schwenke et al. 2009). In a phase-II clinical trial, patients taking lycopene alone or lycopene with soy isoflavones showed stabilized PSA levels, suggesting some beneficial effects in delaying disease progression (Vaishampayan et al. 2007). In another study, increasing lycopene intake was safe in patients, but did not improve PSA scores (Clark et al. 2006).

The addition of lycopene prior to orchiectomy reduced the size of primary and secondary tumors compared to orchiectomy alone (Ansari and Gupta 2004). A similar result was found in the supplementation of lycopene prior to radical prostatectomy (Kucuk et al. 2001).

3.3.3 Green tea

Green tea (*Camellia sinensis*) is one of the most extensively studied antioxidant nutraceuticals for cancer prevention. It contains a number of polyphenol catechchins, including the most abundant (−)-epigallocatechin-3-gallate (EGCG). The green tea polyphenols act on several targets in the pathways of prostate carcinogenesis and show potential in its prevention and therapy. The activities include antioxidation, inhibition of inflammation via NF-kB, cyclooxygenase 2 (COX-2), and insulin-like growth factor (IGF), and androgen blockage (Yang et al. 2009). More recently, tea polyphenols were found to inhibit class 1 HDAC and induce p53-dependent and p53-independent apoptosis in prostate cancer cells (Gupta et al. 2012a, Thakur et al. 2012). Many animal studies have demonstrated both the effectiveness and nontoxic nature of tea polyphenols in carcinogen-induced tumor models, xenograft

and transgenic adenocarcinoma of the mouse prostate (TRAMP) models, and healthy animals (Yang et al. 2009, Kim et al. 2014). Nanoformulation of EGCG improved its efficacy, possibly due to enhanced and sustained oral absorption of the compound (Khan et al. 2014).

In contrast to the consistently positive results in animal models, the effect of tea polyphenols for prostate cancer prevention and treatment from human studies are mixed (Wang et al. 2013a, Yuan 2013). Epidemiologic studies do not provide strong evidence for the chemopreventive effect of green tea intake against the development of prostate cancer. In a prospective cohort study with 49,920 men conducted in Japan, green tea consumption was found associated with a dose-dependent decrease in the risk of advanced prostate cancer, but not with localized cancer (Kurahashi et al. 2008). In another clinical study of 19,561 Japanese men, no association was found between green tea and prostate cancer risk (Kikuchi et al. 2006).

Several phase-II clinical trials have shown an efficacy of green tea extract against the progression of prostate premalignant lesions to malignant tumors (Wang et al. 2013a, Yuan 2013). A 1-year proof-of-principle double-blind, placebo-controlled study was conducted in 60 men with HGPIN who received 600 mg green tea catechins or the placebo daily. After 1 year, only 3% of catechins-treated men were diagnosed with prostate cancer, in comparison to 30% in the control group. However, there was no difference between the total PSA in the two groups, but catechins-treated men showed lower values. In addition, no significant side effects or adverse effects were noted in the catechins-treated group (Bettuzzi et al. 2006). A follow-up study after 2 years showed that the chemopreventive effect of catechins was long-lasting, even after the termination of drug administration (Brausi et al. 2008).

Most recently, a placebo-controlled, randomized clinical trial of Polyphenon E (PolyE), containing 400 mg EGCG per day was completed in 97 men with HGPIN and/or atypical small acinar proliferation (ASAP) for a 1-year period. The study found no differences in the number of prostate cancer cases. However, the cumulative rate of prostate cancer plus ASAP among men with HGPIN without ASAP at baseline was decreased in treated group. A decrease in serum PSA was also observed, whereas there was no difference in adverse events between the two study groups. The authors concluded that daily intake of the study agent for 1 year accumulated in plasma and was well tolerated but did not reduce the likelihood of prostate cancer in men with baseline HGPIN or ASAP (Kumar et al. 2015).

3.3.4 Broccoli

Isothiocyanates, which are converted from glucosinolates, are found in cruciferous vegetables such as broccoli (Clarke et al. 2008). Isothiocyanates, including sulforaphane (SFN) and phenethyl isothiocyanate (PEITC), exhibited *in vitro* and *in vivo* activities against prostate cancer with promising

results (Clarke et al. 2008). SFN from broccoli is an important and well-studied chemopreventive isothiocyanate. It may affect the progression of various types of cancers, including prostate cancer (Appendino and Bardelli 2010). SFN is a potent inhibitor of members of the cytochrome P-450 family (Fimognari et al. 2008b) and it can block cancer initiation via inhibiting phase-I enzymes that activate carcinogens and by inducing phase-II enzymes that detoxify carcinogens (Wang et al. 2013b). In addition, SFN targets the Nrf2 pathway to enhance cell-defense mechanisms against oxidative damage and promotes the removal of carcinogens (Juge et al. 2007). SFN induced the apoptosis of prostate cancer cell by activating caspases, ERK1/2, and Akt, and increasing the protein level of p53 and bax (Fimognari et al. 2008a). SFN can inhibit histone deacetylase (HDAC) activity in LNCaP and PC3 cells without cytotoxic effects to normal prostate epithelial cells (Ho et al. 2011). More recently, a cell-based study showed that exposure of prostate cancer cell lines (LNCaP, 22Rv1, C4-2, and PC-3) to SFN (2.5 and 5 μM) could downregulate CXCR4 expression (Sakao et al. 2015). It was also reported that PEITC could reduce the migration of PC-3 human prostate cancer cells by inactivating Akt and suppressing VEGF, epidermal growth factor (EGF) expression, and granulocyte colony-stimulating factor (G-CSF) secretion (Wu et al. 2009).

Epidemiological studies suggest that consumption of broccoli is inversely related to prostate cancer risk, particularly during the early stage (Cohen et al. 2000, Kolonel et al. 2000). To date, a few pilot and phase-I clinical trials have been conducted to evaluate the effect of SFN on cancer outcomes (**Table 3.4**). A study was performed in Qidong, China in a randomized, placebo-controlled trial to test whether drinking hot water infusions of 3-day-old broccoli sprouts, containing defined concentrations of glucosinolates, could alter the disposition of certain carcinogens (Kensler et al. 2005). An inverse association between the excretion of SFN metabolites and carcinogen–DNA adducts was observed. Moreover, it was found that there is a strong interindividual difference in the bioavailability of SFN metabolites. Shapiro et al. conducted a double-blind, placebo-controlled, randomized phase-I clinical study of sprout extracts containing either glucosinolates (principally glucoraphanin, the precursor of SFN) or isothiocyanates (principally SFN) in healthy volunteers (Shapiro et al. 2006). Three dose groups of broccoli sprout extracts, including 25 μmol of glucosinolate (the precursor of SFN, cohort A), 100 μmol of glucosinolate (cohort B), or 25 μmol of isothiocyanate (principally SFN, cohort C), were administered orally at 8-h intervals for 7 days (total 21 doses). The mean cumulative excretion of dithiocarbamates, the SFN metabolites, as a fraction of dose was very similar in cohorts A and B and much higher and more consistent in cohort C. No significant toxicity associated with any of the sprout extract ingestions was observed in the study.

Another study was conducted to validate the new mechanism of SFN on epigenetics in human subjects (Myzak et al. 2007). HDAC activity was significantly inhibited in peripheral blood mononuclear cells 3 and 6 h following

consumption of a single dose of 68 g broccoli sprouts. A more recent clinical study was conducted to determine the effect of dietary intervention of broccoli or peas (as negative control) on the changes of gene expression in prostate tissue in men diagnosed with HGPIN (Traka et al. 2008). Consuming Broccoli consumption was shown to interact with glutathione S-transferase μ1 (GSTM1) genotype on the TGF β1 and EGF signal pathways. For individuals consuming the pea-rich diet, there were no differences in gene expression between GSTM1 positive (with at least one allele) and null individuals (with the gene deletion), whereas significant differences between GSTM1 genotypes were found for subjects consuming the broccoli-rich diet. The broccoli-rich diet induced more changes of gene expression in prostate tissue than the pea-rich diet. This may be mediated through the chemical interaction of isothiocyanates with signaling peptides in the plasma. Currently, a number of clinical trials on SFN and broccoli sprout extracts are ongoing for their chemotherapeutic and chemoprevention effects (**Table 3.4**).

3.3.5 Grape skin

Resveratrol (trans-3,5,4′-trihydroxystilbene) is a compound found largely in red grape skins. It has broad-spectrum beneficial activities such as anti-infective, antioxidant, and cardioprotective functions, and its potential cancer preventive effects have been widely investigated in recent years (Khan et al. 2010, Huang et al. 2011). The underlying mechanisms for the chemoprevention effect of resveratrol include antioxidation, anti-inflammation, cell cycle and androgen receptor modulation, and epigenetic regulation (Aggarwal et al. 2004, Cimino et al. 2012, Wang et al. 2013b). It was reported that resveratrol could inhibit growth of LNCaP, DU145, and PC-3 cells, and it lowered both intracellular and secreted PSA in LNCaP cells (Hsieh and Wu 1999). An *in vivo* study using transgenic rats as a model for adenocarcinoma of prostate found that resveratrol could suppress prostate cancer growth (Seeni et al. 2008). The preclinical and clinical studies of resveratrol on several types of cancer were summarized in a recent review (Subramanian et al. 2010). However, pharmacokinetic studies showed that resveratrol has poor bioavailability (about 1%) due to extensive phase-II metabolism (glucuronidation and sulfation) as well as metabolism by gut bacterial enzymes. Oral consumption of 5 g resveratrol could only generate a peak plasma concentration of 500 ng/mL, which is much lower than the concentrations used in most *in vitro* experiments. Therefore, the challenges in drug formulation and bioavailability need to be solved to achieve effective concentrations in human subjects for chemoprevention (Scott et al. 2012). Presently, no clinical trials of resveratrol for prostate cancer prevention have been completed.

Muscadine grape is another type of grape distinct from the more common red grape and its main bioactive components are anthocyanins. Anthocyanins have strong antioxidant activity (Ichikawa et al. 2001) and show anti-tumor activities by inhibiting DNA synthesis (Singletary et al. 2003), suppressing

carcinogen-induced DNA adduct formation (Jung et al. 2006), and retarding blood vessel growth in some tumors (Hudson et al. 2007). Muscadine grape skin extract (MSKE), containing no resveratrol, has been found to inhibit cell growth and promote apoptosis in prostate cancer cells that represent different stages of prostate cancer progression without affecting normal epithelial cells (Hudson et al. 2007). In contrast to MSKE, resveratrol arrested cell growth at G1-S phase but did not induce apoptosis, which indicated that MSKE and resveratrol target distinct pathways to inhibit prostate cancer cell growth. MSKE could revert epithelial to mesenchymal transition (EMT) and ROS-mediated tumor progression in human prostate cancer cells (Burton et al. 2014). A recent study showed that MSKE could antagonize Snail-Cathepsin L-mediated invasion, migration and osteoclastogenesis in LNCaP and ARCaP-E prostate cancer cells (Burton et al. 2015). Therefore, MSKE may be an important source for further development of chemopreventive agents against prostate cancer. A cohort phase-I study found that 4,000 mg of muscadine grape skin was safe in men with biochemically recurrent prostate cancer. However, no decline in serum PSA from baseline was observed (Paller et al. 2015). Since 2011, the Department of Defense Prostate Cancer Consortium has initiated a randomized, multicenter, placebo-controlled phase-I/II study of two doses of *Muscadine Plus* (muscadine grape skin extract, which does not contain resveratrol) for men with biochemical recurrence of prostate cancer (NCT01317199) (**Table 3.4**).

3.3.6 Soy

Soy and soy-based products are rich in phytoestrogens, which are a group of phytochemicals with estrogen-like structures. Soy isoflavones, a class of phytoestrogens, have been suggested to have chemopreventive and anti-cancer activity (Adlercreutz 2002). The predominant and most biologically active isoflavones in soy products are genistein and daidzein, which can inhibit the growth and induce apoptosis of prostate cancer cells (Davis et al. 2002, Swami et al. 2005). Possible mechanisms include estrogen receptor modulation, tyrosine protein kinase inhibition, antioxidation, anti-angiogenesis, and inhibition of 5α-reductases (Wang et al. 2013b). *In vitro* and animal studies have yielded evidence in support of the chemopreventive effect of isoflavones in prostate cancer (see Bemis et al. 2006, Van Poppel and Tombal 2011 for extensive review). However, findings from epidemiologic studies indicate that there is a lack of association between isoflavones intake and prostate cancer risk (Table 3.4). Most trials employed serum PSA levels as an end point and showed inconsistent results (Mahmoud et al. 2014). In a double-blind clinical trial, men who received prostate biopsies, but were not found to have prostate cancer, were given a daily supplement containing isoflavones and curcumin or placebo (Ide et al. 2010). The PSA level was measured before and 6 months after treatment. It was found that the combined treatment of soy isoflavones and curcumin could significantly decrease serum PSA levels. Other results also demonstrated the ability of soy isoflavones to reduce serum PSA in subsets of men with localized prostate cancer with high PSA levels prior to therapy

(Hussain et al. 2003, Dalais et al. 2004, Lazarevic et al. 2011). In contrast, other trials showed that isoflavone supplements had no effect on serum PSA levels in healthy men (Adams et al. 2004, Miyanaga et al. 2012) or men with high risk of prostate cancer (Hamilton-Reeves et al. 2008). In a recent randomized, double-blind, placebo-controlled phase-II trial, isoflavone (60 mg/day) was orally given to healthy men between 50 and 75 years of age with rising PSA for 12 months (Miyanaga et al. 2012). The PSA level showed no significant difference before and after treatment, and there was no significant difference for the incidence of biopsy detectable prostate cancer between the isoflavone and placebo groups. However, for patients aged 65 years or more, the incidence of prostate cancer in the isoflavone group was significantly lower than that in the placebo group. In another study, men with HGPIN or low-grade prostate cancer were divided into three groups for receiving three different protein preparations: (1) soy protein (107 mg isoflavones/day), (2) alcohol-washed soy protein (<6 mg isoflavones/day), or (3) milk protein (no isoflavones/day) (Hamilton-Reeves et al. 2008). It was found that consumption of soy protein preparations did not alter any of the prostate cancer tumor markers, including PSA. However, there was a significantly lower rate of prostate cancer developed in the soy protein groups compared with the milk protein group. Other studies showed that dose-dependent serum isoflavone levels were associated with a decreased risk for prostate cancer (Van Poppel and Tombal 2011). A case-controlled study of Japanese men involving 200 patients and 200 age-matched controls showed that the odds ratio for the highest isoflavone intake category (≥89.9 mg/day) compared with the lowest isoflavone intake category (<30.5 mg/day) was 0.42 (95% CI = 0.24–0.72, $p < 0.01$), indicating isoflavones might be an effective dietary protective factor against prostate cancer (Nagata et al. 2007).

3.3.7 Quercetin

Quercetin is a natural flavonoid compound found in various fruits and vegetables, including onions, broccoli, apples, grapes, and soybeans (Wang et al. 2013b). Quercetin is well known for its antioxidant, anti-inflammatory, and immunomodulating activities. Recently, its anti-cancer effects have been widely investigated (Cimino et al. 2012). When applied *in vitro*, whether alone or in combination, quercetin greatly inhibited cell proliferation and induced cell apoptosis in various prostate cancer cells. When applied *in vivo*, quercetin could effectively inhibit prostate cancer cell xenograft tumor growth (Yang et al. 2015). Quercetin acts on prostate cancer *via* multiple mechanisms, including induction of cell cycle arrest, inhibition of PI3K/AKT and IGF signaling pathway, reversal of EMT, suppression of angiogenesis, and downregulation of the matrix metalloproteinases (Yang et al. 2015). In addition, quercetin was reported to downregulate AR function in prostate cancer cells (Yuan et al. 2010). The pharmacokinetics of quercetin in the human body was studied in 15 volunteers (Egert et al. 2008). After taken orally for 2 weeks, the plasma concentration of quercetin increased in a dose-dependent manner. However, similar to resveratrol, the main issue concerning quercetin utilization is its low

bioavailability. Quercetin is present in the glycosylated form, which can be easily metabolized by specific enzymes present in the gut and liver resulting in low blood concentrations (Nemeth et al. 2003). In epidemiologic studies, quercetin has been demonstrated to have a negative association with prostate cancer incidence. In two clinical studies, no drug-related toxicity or side effects were observed in chronic prostatitis patients taking quercetin (500 mg, twice daily) for a month or healthy adults taking 1000 mg daily for 3 months (Shoskes et al. 1999, Cialdella-Kam et al. 2013). The results of a case-controlled study involving 433 men with primary, histologically confirmed prostate cancer and 538 population-based controls in western New York showed that consumption of at least 24 mg of quercetin daily reduced 27% prostate cancer risk with an odd ratio of 0.64 (95% confidence interval [CI]): 0.44–0.92) (McCann et al. 2005). A clinical trial on prostate cancer prevention titled Prostate Cancer Prevention Trial with Quercetin and Genistein (QUERGEN) (NCT01538316) was initiated in 2012.

3.3.8 Propolis

Propolis (also known as bee putty or bee glue), a resinous substance collected by bees from various trees and shrubs, is enriched with salivary and enzymatic secretions (Pietta et al. 2002). The chemical composition of propolis is complex. The main phenolics found in propolis are phenolic acids and various flavonoids (Maciejewicz et al. 2001, Szliszka et al. 2013). It has a long history of being used as a folk medicine since 300 BC (Ghisalberti 1979). Propolis is extensively used in food or drinks to improve human health and prevent disease. Numerous studies have reported a broad spectrum of biological activities for propolis such as antibacterial, antifungal, antiviral (Kujumgiev et al. 1999), antioxidant (Russo et al. 2004), anti-inflammatory (Park and Kahng 1999, Borrelli et al. 2002), immunomodulatory (Bratter et al. 1999), and anticancer properties (Banskota et al. 2001). An *in vitro* study showed that the ethanol extracts of Brazilian propolis could significantly inhibit cell growth of metastasis-derived human prostate cancer cell line (DU145 and PC-3) and primary human prostate cancer-derived cell line (telomerase immortalized RC-58T/h/SA#4) (Li et al. 2007). The cytotoxic and apoptotic activities of Brazilian ethanolic extract of propolis on hormone-sensitive LNCaP cells were also observed (Szliszka et al. 2011 and 2013). Phenolic components contribute to the major cancer preventive and anti-tumor properties of propolis. Caffeic acid phenethyl ester (CAPE) is one of the major biologically active phenolic components extracted from propolis. Several studies reported its anti-cancer activity in multiple cancer models *in vitro* and *in vivo* (Hung et al. 2003, McEleny et al. 2004, Watabe et al. 2004, Chuu et al. 2012, Lin et al. 2012). CAPE was shown to suppress oxidative stress and inflammation that play crucial roles in the pathogenesis of prostate cancer (Sugar 2006, Battisti et al. 2011). CAPE is a well-known NF-κB inhibitor and a 5α-reductase inhibitor (Natarajan et al. 1996, Hiipakka et al. 2002). At concentrations of 50–80 μM, it could inhibit the activation of NF-κB, which regulates the expression of many

genes involved in inflammation and cancer. CAPE dose-dependent suppressed cell growth of androgen-dependent LNCaP 104-s and AR-negative PC-3 cells *in vitro* and *in vivo* by suppressing c-Myc and Akt-related protein signaling networks (Chuu et al. 2012, Lin et al. 2012). Lin et al. further investigated the possibility of using CAPE as an adjuvant therapeutic agent for treatment of patients with advanced prostate cancer (Lin et al. 2013a). After 96 h with CAPE treatment, the EC_{50} to cause growth inhibition in advanced human prostate cancer cell lines was approximately 0.7–18.7 μM (Lin et al. 2012). Oral administration of CAPE (10 mg/kg per day) for 6 weeks inhibited 50% in tumor growth of LNCaP xenografts, whereas IP injection 10 mg/kg of CAPE (10 mg/kg per day) for 5 weeks inhibited 33% in tumor growth of PC-3 xenografts. A recent study suggested that CAPE treatment might be a potential therapy for patients with castration-resistant prostate cancer (CRPC) (Lin et al. 2015). Treatment with CAPE could induce cell cycle arrest and growth inhibition in CRPC cells via regulation of Skp2, p53, $p21^{Cip1}$, and $p27^{kip1}$. The achievable concentration of CAPE in human serum is approximately 17 μM (Celli et al. 2007), whereas CAPE treatment at 10–20 μM can effectively suppress the survival and proliferation of CRPC cells; therefore, administration of CAPE is a possible therapeutic agent for CRPC. Furthermore, CAPE exhibited a chemopreventive effect. It has been found to sensitize cancer cells to chemotherapeutic drugs and radiation procedures (Akyol et al. 2012). Co-treatment of CAPE (2.5–20 μM) with chemotherapeutic drugs vinblastine, paclitaxel, or estramustine demonstrated synergistic suppression on PC-3 cells and may reduce the dosage of chemotherapy drug required (Lin et al. 2012). Tolba et al. (2013) reported that CAPE synergistically enhanced docetaxel and paclitaxel cytotoxicity in prostate cancer cells through augmentation of docetaxel and paclitaxel proapoptotic effects in addition to CAPE-induced alterations in ER-α and ER-β populations in PC-3 cells. Therefore, CAPE may enable lower doses of chemotherapy or radiotherapy to achieve a clinical response and lower the associated toxicities (Ozturk et al. 2012). Moreover, *in vitro* and *in vivo* usage of CAPE prevent the chemotherapy induced damage and side effects in experimental animals, which makes CAPE to be a promising protective agent in clinical trials during chemotherapy regimen (Garg et al. 2005, Akyol et al. 2012).

3.3.9 Vitamin D

Vitamin D, as a biologically inactive prohormone, is produced by enzymatic modification of cholesterol after exposure to ultraviolet B radiation in the skin (Favus and Langman 1986). Vitamin D is metabolized to 25-hydroxyvitamin D3 in the liver, then to 1α,25-dihydroxyvitamin D3 (calcitriol) mainly in the kidney before it exerts its functions (DeLuca 2004). Vitamin D receptor (VDR) is widely distributed in tissues, including the prostate gland, as well as in benign prostate hyperplasia and prostate cancer cells (Berger et al. 1988, Wang et al. 2012). Vitamin D may exert its anti-tumor effect through binding of the bioactive form (calcitriol) to VDR. Many studies showed that calcitriol inhibits proliferation, invasion, metastasis, and angiogenesis as well as increases

apoptosis and differentiation of several cancers cells (Shabahang et al. 1993, Simboli-Campbell et al. 1996, Wang and Studzinski 2001, Ylikomi et al. 2002, Chen and Holick 2003). Chronic inflammation has been implicated in the development and progression of prostate cancer (Sfanos and De Marzo 2012). It was reported that vitamin D or calcitriol exhibits anti-inflammatory actions by inhibiting proinflammatory cytokine production (Zhang et al. 2012) and targeting COX-2, NF-κB, and TGF-β (Welsh 2012). Many epidemiologic, cellular, and animal studies have shown compelling evidence for the benefit of vitamin D or calcitriol on prostate cancer prevention or chemotherapy (Swami et al. 2011, Welsh 2012, Giammanco et al. 2015). For example, a chemoprevention study in an animal model showed calcitriol delayed the onset of HGPIN (Banach-Petrosky et al. 2006). The clinical studies for the effect of vitamin D or calcitriol on prostate cancer are summarized in **Table 3.4**. A clinical study showed that UVR exposure has a significant protective effect in prostate cancer. Although the protective mechanism is unclear, it is likely that increased synthesis of vitamin D3 increases the concentration of calcitriol in the prostate (Luscombe et al. 2001). In a nested case-controlled study based on a 13-year follow-up of approximately 19,000 middle-aged men, decreased serum level of 25-hydroxyvitamin D was associated with an increased risk of early onset and more aggressive progression of prostate cancer (Ahonen et al. 2000). A phase-II dose-escalation study of calcitriol (daily dose of 0.5–1.5 μg) showed that only 2 of 14 prostate cancer patients displayed a 25% and 45% decrease of PSA levels, and calcitriol did not show any anti-cancer activity in this small-scale study. Hypercalcemia was the dose-limiting toxicity in this study (Osborn et al. 1995). A phase-I trial utilizing a combination of calcitriol and paclitaxel in advanced cancer patients did not show signs of additional toxicity. Very high doses of calcitriol up to 38 μg were administered for three consecutive days and achieving plasma concentrations of 600–1440 pg/mL (Muindi et al. 2002). In a recent open-label clinical trial, subjects with a diagnosis of low-risk prostate cancer under active surveillance were given vitamin D3 supplementation at 4000 international units per day for 1 year (Marshall et al. 2012). No adverse events associated with vitamin D3 supplementation were observed and PSA level showed no difference after treatment. However, 55% of subjects showed a decrease in the progression of prostate cancer with decreased Gleason scores compared to their biopsies a year before (Marshall et al. 2012). Two trials have evaluated the effects of vitamin D, calcitriol, or vitamin D metabolites, alone or in combination with chemotherapeutic agents, in men with prostate cancer, but the findings have been disappointing despite preclinical data strongly suggesting a benefit for the treatment of prostate cancer (Swami et al. 2011, Wang et al. 2013b). A few clinical trials are ongoing focusing on the effects of vitamin D on prostate cancer prevention. For example, in NCT00953225, the effect of vitamin D3 was examined on veteran subjects diagnosed with early stage, low-risk prostate cancer, who elected to have their disease monitored through active surveillance. Patients who were given vitamin D3 did not demonstrate significant changes on PSA levels and number of positive biopsy cores compared to baseline levels. More clinical investigations

are needed to determine the effect of vitamin D on prostate cancer chemoprevention for patients without clinical cancer.

3.3.10 Others

In addition to the above-mentioned examples, a number of other herbs and nutraceuticals have also been reported with potential antioxidant activity, such as β-carotene, α-tocopherol, rainbow trout, *ganoderma tsugae*, *picrorhiza kurroa*, flumbagin, and many flavonoids identified in herbal medicines (Kandaswami et al. 2005, Powolny and Singh 2008, Rajkumar et al. 2011, Dolara et al. 2012, Lin et al. 2013b, Patel 2014). Such compounds or raw extracts may have the chemopreventive effect of prostate cancer and promote the prostate health. However, most of the evidence was based on *in vitro* studies. Their definitive clinical effects need to be investigated.

3.4 Conclusion

Although many antioxidants in nutraceuticals showed *in vitro* benefits for prostate cancer prevention and treatment in preclinical model systems, their clinical outcomes remain controversial. In one recent study, high doses of lycopene, green tea catechins, and selenium in men harboring high-grade prostatic intraepithelial neoplasia and/or atypical small acinar proliferation was associated with a higher incidence of prostate cancer at rebiopsy and expression of microRNAs implicated in prostate cancer progression at molecular analysis (Gontero et al. 2015).

Several issues need to be addressed in using nutraceuticals for the prevention and treatment of human diseases. First of all, all preparations need to be standardized. This enables the comparability of results from different studies. Second, the bioavailability of most antioxidant nutraceuticals is limited. Phenolic compounds undergo extensive first-pass metabolism such as glucuronidation and sulfation, leading to poor absorption and low bioavailability. Novel formulation and prodrug approaches are urgently needed to effectively deliver the drug to the plasma and the target tissue, that is, the prostate. Third, a better understanding of the role of drug metabolism needs to be achieved. Some metabolites may be equally active as the parent nutraceutical, whereas some may be inactive. Finally, there is a molecular distinction between cancer prevention and cancer treatment. Some nutraceuticals may be effective for the prevention of prostate cancer; however, they may have opposite effect for the treatment of prostate cancer.

In conclusion, antioxidant nutraceuticals show great potential for prostate cancer prevention and treatment. However, more research work especially clinical trials need to be performed for the safe and effective use of antioxidant nutraceuticals against prostate cancer.

References

Abrams, P., C. Chapple, S. Khoury, C. Roehrborn, and J. de la Rosette. 2013. Evaluation and treatment of lower urinary tract symptoms in older men. *J Urol* 189 (1 Suppl):S93–S101.

Acharya, A., I. Das, D. Chandhok, and T. Saha. 2010. Redox regulation in cancer: A double-edged sword with therapeutic potential. *Oxid Med Cell Longev* 3 (1):23–34.

Adams, K. F., C. Chen, K. M. Newton, J. D. Potter, and J. W. Lampe. 2004. Soy isoflavones do not modulate prostate-specific antigen concentrations in older men in a randomized controlled trial. *Cancer Epidemiol Biomarkers Prev* 13 (4):644–648.

Adlercreutz, H. 2002. Phyto-oestrogens and cancer. *Lancet Oncol* 3 (6):364–373.

Aggarwal, B. B., A. Bhardwaj, R. S. Aggarwal, N. P. Seeram, S. Shishodia, and Y. Takada. 2004. Role of resveratrol in prevention and therapy of cancer: Preclinical and clinical studies. *Anti-cancer Res* 24 (5A):2783–2840.

Ahonen, M. H., L. Tenkanen, L. Teppo, M. Hakama, and P. Tuohimaa. 2000. Prostate cancer risk and prediagnostic serum 25-hydroxyvitamin D levels (Finland). *Cancer Causes Control* 11 (9):847–852.

Akyol, S., Z. Ginis, F. Armutcu, G. Ozturk, M. R. Yigitoglu, and O. Akyol. 2012. The potential usage of caffeic acid phenethyl ester (CAPE) against chemotherapy-induced and radiotherapy-induced toxicity. *Cell Biochem Funct* 30 (5):438–443.

Albertsen, P. C., J. A. Hanley, D. F. Gleason, and M. J. Barry. 1998. Competing risk analysis of men aged 55 to 74 years at diagnosis managed conservatively for clinically localized prostate cancer. *JAMA* 280 (11):975–980.

American Cancer Society. 2015. Cancer Facts and Figures. American Cancer Society, Atlanta, Georgia. http://oralcancerfoundation.org/wp-content/uploads/2016/03/Us_Cancer_Facts.pdf.

Amin, M. B., D. W. Lin, J. L. Gore, J. R. Srigley, H. Samaratunga, L. Egevad, M. Rubin et al. 2014. The critical role of the pathologist in determining eligibility for active surveillance as a management option in patients with prostate cancer: Consensus statement with recommendations supported by the College of American Pathologists, International Society of Urological Pathology, Association of Directors of Anatomic and Surgical Pathology, the New Zealand Society of Pathologists, and the Prostate Cancer Foundation. *Arch Pathol Lab Med* 138 (10):1387–1405.

Ansari, M. S. and N. P. Gupta. 2004. A comparison of lycopene and orchidectomy vs orchidectomy alone in the management of advanced prostate cancer. *BJU Int* 94 (4):678.

Appendino, G. and A. Bardelli. 2010. Broccoli, PTEN deletion and prostate cancer: Where is the link? *Mol Cancer* 9:308.

Attard, G., C. Parker, R. A. Eeles, F. Schröder, S. A. Tomlins, I. Tannock, C. G. Drake, and J. S. de Bono. 2015. Prostate cancer. *Lancet* 387 (10013):70–82.

Aune, D., D. A. N. Rosenblatt, D. S. Chan, A. R. Vieira, R. Vieira, D. C. Greenwood, L. J. Vatten, and T. Norat. 2015. Dairy products, calcium, and prostate cancer risk: A systematic review and meta-analysis of cohort studies. *Am J Clin Nutr* 101 (1):87–117.

Banach-Petrosky, W., X. Ouyang, H. Gao, K. Nader, Y. Ji, N. Suh, R. S. DiPaola, and C. Abate-Shen. 2006. Vitamin D inhibits the formation of prostatic intraepithelial neoplasia in Nkx3.1; Pten mutant mice. *Clin Cancer Res* 12 (19):5895–5901.

Banskota, A. H., Y. Tezuka, and S. Kadota. 2001. Recent progress in pharmacological research of propolis. *Phytother Res* 15 (7):561–571.

Barnes, P. M., E. Powell-Griner, K. McFann, and R. L. Nahin. 2004. Complementary and alternative medicine use among adults: United States, 2002. *Adv Data* 343:1–19.

Baron, A., M. Mancini, E. Caldwell, A. Cabrelle, P. Bernardi, and F. Pagano. 2009. Serenoa repens extract targets mitochondria and activates the intrinsic apoptotic pathway in human prostate cancer cells. *BJU Int* 103 (9):1275–1283.

Barry, M. J., S. Meleth, J. Y. Lee, K. J. Kreder, A. L. Avins, J. C. Nickel, C. G. Roehrborn et al. 2011. Effect of increasing doses of saw palmetto extract on lower urinary tract symptoms: A randomized trial. *Jama* 306 (12):1344–1351.

Bartsch, G., R. S. Rittmaster, and H. Klocker. 2002. Dihydrotestosterone and the concept of 5α-reductase inhibition in human benign prostatic hyperplasia. *World J Urol* 19 (6):413–425.

Battisti, V., L. D. Maders, M. D. Bagatini, L. G. Reetz, J. Chiesa, I. E. Battisti, J. F. Goncalves, M. M. Duarte, M. R. Schetinger, and V. M. Morsch. 2011. Oxidative stress and antioxidant status in prostate cancer patients: Relation to Gleason score, treatment and bone metastasis. *Biomed Pharmacother* 65 (7):516–524.

Beer, T. M., C. W. Ryan, P. M. Venner, D. P. Petrylak, G. S. Chatta, J. D. Ruether, C. H. Redfern et al. 2007. Double-blinded randomized study of high-dose calcitriol plus docetaxel compared with placebo plus docetaxel in androgen-independent prostate cancer: A report from the ASCENT Investigators. *J Clin Oncol* 25 (6):669–674.

Beilby, J., G. L. Ambrosini, E. Rossi, N. H. de Klerk, and A. W. Musk. 2010. Serum levels of folate, lycopene, beta-carotene, retinol and vitamin E and prostate cancer risk. *Eur J Clin Nutr* 64 (10):1235–1238.

Bemis, D. L., A. E. Katz, and R. Buttyan. 2006. Clinical trials of natural products as chemopreventive agents for prostate cancer. *Expert Opin Investig Drugs* 15 (10):1191–1200.

Bent, S., C. Kane, K. Shinohara, J. Neuhaus, E. S. Hudes, H. Goldberg, and A. L. Avins. 2006. Saw palmetto for benign prostatic hyperplasia. *N Engl J Med* 354 (6):557–566.

Berger, U., P. Wilson, R. A. McClelland, K. Colston, M. R. Haussler, J. W. Pike, and R. C. Coombes. 1988. Immunocytochemical detection of 1,25-dihydroxyvitamin D receptors in normal human tissues. *J Clin Endocrinol Metab* 67 (3):607–613.

Berry, S. J., D. S. Coffey, P. C. Walsh, and L. L. Ewing. 1984. The development of human benign prostatic hyperplasia with age. *J Urol* 132 (3):474–479.

Bettuzzi, S., M. Brausi, F. Rizzi, G. Castagnetti, G. Peracchia, and A. Corti. 2006. Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: A preliminary report from a one-year proof-of-principle study. *Cancer Res* 66 (2):1234–1240.

Bindoli, A. and M. P. Rigobello. 2013. Principles in redox signaling: From chemistry to functional significance. *Antioxid Redox Signal* 18 (13):1557–1593.

Bonnar-Pizzorno, R. M., A. J. Littman, M. Kestin, and E. White. 2006. Saw palmetto supplement use and prostate cancer risk. *Nutr Cancer* 55 (1):21–27.

Borrelli, F., P. Maffia, L. Pinto, A. Ianaro, A. Russo, F. Capasso, and A. Ialenti. 2002. Phytochemical compounds involved in the anti-inflammatory effect of propolis extract. *Fitoterapia* 73 (Suppl 1):S53–S63.

Boyle, P., C. Robertson, R. Manski, R. J. Padley, and C. G. Roehrborn. 2001. Meta-analysis of randomized trials of terazosin in the treatment of benign prostatic hyperplasia. *Urology* 58 (5):717–722.

Bramson, H. N., D. Hermann, K. W. Batchelor, F. W. Lee, M. K. James, and S. V. Frye. 1997. Unique preclinical characteristics of GG745, a potent dual inhibitor of 5AR. *J Pharmacol Exp Ther* 282 (3):1496–1502.

Bratter, C., M. Tregel, C. Liebenthal, and H. D. Volk. 1999. Prophylactic effectiveness of propolis for immunostimulation: a clinical pilot study. *Forsch Komplementarmed* 6 (5):256–260.

Brausi, M., F. Rizzi, and S. Bettuzzi. 2008. Chemoprevention of human prostate cancer by green tea catechins: Two years later. A follow-up update. *Eur Urol* 54 (2):472–473.

Burton, L. J., P. Barnett, B. Smith, R. S. Arnold, T. Hudson, K. Kundu, N. Murthy, and V. A. Odero-Marah. 2014. Muscadine grape skin extract reverts snail-mediated epithelial mesenchymal transition via superoxide species in human prostate cancer cells. *BMC Complement Altern Med* 14:97.

Burton, L. J., B. A. Smith, B. N. Smith, Q. Loyd, P. Nagappan, D. McKeithen, C. Wilder, M. Platt, T. Hudson, and V. A. Odero-Marah. 2015. Muscadine grape skin extract (MSKE) can antagonize Snail-cathepsin L-mediated invasion, migration and osteoclastogenesis in prostate and breast cancer cells. *Carcinogenesis* 36 (9):1019–1027.

Byersdorfer, C. A. 2014. The role of fatty acid oxidation in the metabolic reprograming of activated t-cells. *Front Immunol* 5:641.
Caine, M., S. Perlberg, and R. Gordon. 1975. The treatment of benign prostatic hypertrophy with flutamide (SCH: 13521): A placebo-controlled study. *J Urol* 114 (4):564–568.
Caine, M., A. Pfau, and S. Perlberg. 1976. The use of alpha-adrenergic blockers in benign prostatic obstruction. *Br J Urol* 48 (4):255–263.
Campbell, J. K., K. Canene-Adams, B. L. Lindshield, T. W. Boileau, S. K. Clinton, and J. W. Erdman, Jr. 2004. Tomato phytochemicals and prostate cancer risk. *J Nutr* 134 (12 Suppl):3486s–3492s.
Carbin, B. E., B. Larsson, and O. Lindahl. 1990. Treatment of benign prostatic hyperplasia with phytosterols. *Br J Urol* 66 (6):639–641.
Celli, N., L. K. Dragani, S. Murzilli, T. Pagliani, and A. Poggi. 2007. In vitro and in vivo stability of caffeic acid phenethyl ester, a bioactive compound of propolis. *J Agric Food Chem* 55 (9):3398–3407.
Champault, G., J. C. Patel, and A. M. Bonnard. 1984. A double-blind trial of an extract of the plant Serenoa repens in benign prostatic hyperplasia. *Br J Clin Pharmacol* 18 (3):461–462.
Chang, S., J. W. Erdman, Jr., S. K. Clinton, M. Vadiveloo, S. S. Strom, Y. Yamamura, C. M. Duphorne et al. 2005. Relationship between plasma carotenoids and prostate cancer. *Nutr Cancer* 53 (2):127–134.
Chen, J., Y. Song, and L. Zhang. 2013. Lycopene/tomato consumption and the risk of prostate cancer: A systematic review and meta-analysis of prospective studies. *J Nutr Sci Vitaminol* (*Tokyo*) 59 (3):213–223.
Chen, T. C. and M. F. Holick. 2003. Vitamin D and prostate cancer prevention and treatment. *Trends Endocrinol Metab* 14 (9):423–430.
Cheung, K. L. and A. N. Kong. 2010. Molecular targets of dietary phenethyl isothiocyanate and sulforaphane for cancer chemoprevention. *AAPS J* 12 (1):87–97.
Chignalia, A. Z., M. A. Oliveira, V. Debbas, R. O. Dull, F. R. Laurindo, R. M. Touyz, M. H. Carvalho, Z. B. Fortes, and R. C. Tostes. 2015. Testosterone induces leucocyte migration by NADPH oxidase-driven ROS- and COX2-dependent mechanisms. *Clin Sci* 129 (1):39–48.
Chuu, C. P., H. P. Lin, M. F. Ciaccio, J. M. Kokontis, R. J. Hause, R. A. Hiipakka, S. S. Liao, and R. B. Jones. 2012. Caffeic acid phenethyl ester suppresses the proliferation of human prostate cancer cells through inhibition of p70S6K and Akt signaling networks. *Cancer Prev Res* 5 (5):788–797.
Cialdella-Kam, L., D. C. Nieman, W. Sha, M. P. Meaney, A. M. Knab, and R. A. Shanely. 2013. Dose-response to 3 months of quercetin-containing supplements on metabolite and quercetin conjugate profile in adults. *Br J Nutr* 109 (11):1923–1933.
Cimino, S., G. Sortino, V. Favilla, T. Castelli, M. Madonia, S. Sansalone, G. I. Russo, and G. Morgia. 2012. Polyphenols: Key issues involved in chemoprevention of prostate cancer. *Oxid Med Cell Longev* 2012:632959.
Clark, P. E., M. C. Hall, L. S. Borden, Jr., A. A. Miller, J. J. Hu, W. R. Lee, D. Stindt et al. 2006. Phase I-II prospective dose-escalating trial of lycopene in patients with biochemical relapse of prostate cancer after definitive local therapy. *Urology* 67 (6):1257–1261.
Clarke, J. D., R. H. Dashwood, and E. Ho. 2008. Multi-targeted prevention of cancer by sulforaphane. *Cancer Lett* 269 (2):291–304.
Clinton, S. K., C. Emenhiser, S. J. Schwartz, D. G. Bostwick, A. W. Williams, B. J. Moore, and J. W. Erdman, Jr. 1996. *cis*-trans lycopene isomers, carotenoids, and retinol in the human prostate. *Cancer Epidemiol Biomarkers Prev* 5 (10):823–833.
Cohen, J. H., A. R. Kristal, and J. L. Stanford. 2000. Fruit and vegetable intakes and prostate cancer risk. *J Natl Cancer Inst* 92 (1):61–68.
D'Alessandro, A. and L. Zolla. 2011. The SODyssey: Superoxide dismutases from biochemistry, through proteomics, to oxidative stress, aging and nutraceuticals. *Expert Rev Proteomics* 8 (3):405–421.

Dagnelie, P. C., A. G. Schuurman, R. A. Goldbohm, and P. A. Van den Brandt. 2004. Diet, anthropometric measures and prostate cancer risk: A review of prospective cohort and intervention studies. *BJU Int* 93 (8):1139–1150.

Dalais, F. S., A. Meliala, N. Wattanapenpaiboon, M. Frydenberg, D. A. Suter, W. K. Thomson, and M. L. Wahlqvist. 2004. Effects of a diet rich in phytoestrogens on prostate-specific antigen and sex hormones in men diagnosed with prostate cancer. *Urology* 64 (3):510–515.

Davis, J. N., O. Kucuk, and F. H. Sarkar. 2002. Expression of prostate-specific antigen is transcriptionally regulated by genistein in prostate cancer cells. *Mol Carcinog* 34 (2):91–101.

DeLuca, H. F. 2004. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr* 80 (6 Suppl):1689S–1696S.

Descotes, J. L., J. J. Rambeaud, P. Deschaseaux, and G. Faure. 1995. Placebo-controlled evaluation of the efficacy and tolerability of Permixon® in benign prostatic hyperplasia after exclusion of placebo responders. *Clin Drug Investig* 9 (5):291–297.

Djavan, B. and M. Marberger. 1999. A meta-analysis on the efficacy and tolerability of alpha1-adrenoceptor antagonists in patients with lower urinary tract symptoms suggestive of benign prostatic obstruction. *Eur Urol* 36 (1):1–13.

Dolara, P., E. Bigagli, and A. Collins. 2012. Antioxidant vitamins and mineral supplementation, life span expansion and cancer incidence: A critical commentary. *Eur J Nutr* 51 (7):769–781.

Egert, S., S. Wolffram, A. Bosy-Westphal, C. Boesch-Saadatmandi, A. E. Wagner, J. Frank, G. Rimbach, and M. J. Mueller. 2008. Daily quercetin supplementation dose-dependently increases plasma quercetin concentrations in healthy humans. *J Nutr* 138 (9):1615–1621.

Eguchi, M., K. Monden, and N. Miwa. 2003. Role of MAPK phosphorylation in cytoprotection by pro-vitamin C against oxidative stress-induced injuries in cultured cardiomyoblasts and perfused rat heart. *J Cell Biochem* 90 (2):219–226.

Engel, J. B. and A. V. Schally. 2007. Drug Insight: Clinical use of agonists and antagonists of luteinizing-hormone-releasing hormone. *Nat Clin Pract Endocrinol Metab* 3 (2):157–167.

Etminan, M., B. Takkouche, and F. Caamano-Isorna. 2004. The role of tomato products and lycopene in the prevention of prostate cancer: A meta-analysis of observational studies. *Cancer Epidemiol Biomarkers Prev* 13 (3):340–345.

Ewing, C. M., A. M. Ray, E. M. Lange, K. A. Zuhlke, C. M. Robbins, W. D. Tembe, K. E. Wiley et al. 2012. Germline mutations in HOXB13 and prostate-cancer risk. *N Engl J Med* 366 (2):141–149.

Favus, M. J. and C. B. Langman. 1986. Evidence for calcium-dependent control of 1,25-dihydroxyvitamin D3 production by rat kidney proximal tubules. *J Biol Chem* 261 (24):11224–11229.

Feldman, B. J. and D. Feldman. 2001. The development of androgen-independent prostate cancer. *Nat Rev Cancer* 1 (1):34–45.

Fimognari, C., M. Lenzi, and P. Hrelia. 2008a. Chemoprevention of cancer by isothiocyanates and anthocyanins: Mechanisms of action and structure-activity relationship. *Curr Med Chem* 15 (5):440–447.

Fimognari, C., M. Lenzi, and P. Hrelia. 2008b. Interaction of the isothiocyanate sulforaphane with drug disposition and metabolism: Pharmacological and toxicological implications. *Curr Drug Metab* 9 (7):668–678.

Fox, C. J., P. S. Hammerman, and C. B. Thompson. 2005. Fuel feeds function: Energy metabolism and the T-cell response. *Nat Rev Immunol* 5 (11):844–852.

Gacci, M., V. Ficarra, A. Sebastianelli, G. Corona, S. Serni, S. F. Shariat, M. Maggi, F. Zattoni, M. Carini, and G. Novara. 2014. Impact of medical treatments for male lower urinary tract symptoms due to benign prostatic hyperplasia on ejaculatory function: A systematic review and meta-analysis. *J Sex Med* 11 (6):1554–1566.

Gann, P. H., J. Ma, E. Giovannucci, W. Willett, F. M. Sacks, C. H. Hennekens, and M. J. Stampfer. 1999. Lower prostate cancer risk in men with elevated plasma lycopene levels: Results of a prospective analysis. *Cancer Res* 59 (6):1225–1230.

Garg, A. K., T. A. Buchholz, and B. B. Aggarwal. 2005. Chemosensitization and radiosensitization of tumors by plant polyphenols. *Antioxid Redox Signal* 7 (11–12):1630–1647.

Gerber, G. S., D. Kuznetsov, B. C. Johnson, and J. D. Burstein. 2001. Randomized, double-blind, placebo-controlled trial of saw palmetto in men with lower urinary tract symptoms. *Urology* 58 (6):960–964; discussion 964–965.

Gerber, G. S., G. P. Zagaja, G. T. Bales, G. W. Chodak, and B. A. Contreras. 1998. Saw palmetto (*Serenoa repens*) in men with lower urinary tract symptoms: Effects on urodynamic parameters and voiding symptoms. *Urology* 51 (6):1003–1007.

Ghisalberti, E. L. 1979. Propolis: A review. *Bee World* 60 (2):59–84.

Giammanco, M., D. Di Majo, M. La Guardia, S. Aiello, M. Crescimannno, C. Flandina, F. M. Tumminello, and G. Leto. 2015. Vitamin D in cancer chemoprevention. *Pharm Biol* 53:1–36.

Giancaspero, T. A., E. Dipalo, A. Miccolis, E. Boles, M. Caselle, and M. Barile. 2014. Alteration of ROS homeostasis and decreased lifespan in *S. cerevisiae* elicited by deletion of the mitochondrial translocator FLX1. *BioMed Res Int* 2014:101286.

Goldgar, D. E., D. F. Easton, L. A. Cannon-Albright, and M. H. Skolnick. 1994. Systematic population-based assessment of cancer risk in first-degree relatives of cancer probands. *J Natl Cancer Inst* 86 (21):1600–1608.

Gontero, P., G. Marra, F. Soria, M. Oderda, A. Zitella, F. Baratta, G. Chiorino et al. 2015. A randomized double-blind placebo controlled phase I-II study on clinical and molecular effects of dietary supplements in men with precancerous prostatic lesions. Chemoprevention or "chemopromotion"? *Prostate* 75 (11):1177–1186.

Gormley, G. J., E. Stoner, R. C. Bruskewitz, J. Imperato-McGinley, P. C. Walsh, J. D. McConnell, G. L. Andriole et al. 2002. The effect of finasteride in men with benign prostatic hyperplasia. 1992. *J Urol* 167 (2 Pt 2):1102–1107; discussion 1108.

Grubisha, M. J. and D. B. DeFranco. 2013. Local endocrine, paracrine and redox signaling networks impact estrogen and androgen crosstalk in the prostate cancer microenvironment. *Steroids* 78 (6):538–541.

Gupta, K., V. S. Thakur, N. Bhaskaran, A. Nawab, M. A. Babcook, M. W. Jackson, and S. Gupta. 2012a. Green tea polyphenols induce p53-dependent and p53-independent apoptosis in prostate cancer cells through two distinct mechanisms. *PLoS One* 7 (12):e52572.

Gupta, S. C., D. Hevia, S. Patchva, B. Park, W. Koh, and B. B. Aggarwal. 2012b. Upsides and downsides of reactive oxygen species for cancer: The roles of reactive oxygen species in tumorigenesis, prevention, and therapy. *Antioxid Redox Signal* 16 (11):1295–1322.

Gupta-Elera, G., A. R. Garrett, R. A. Robison, and K. L. O'Neill. 2012. The role of oxidative stress in prostate cancer. *Eur J Cancer Prev* 21 (2):155–162.

Hamilton-Reeves, J. M., S. A. Rebello, W. Thomas, M. S. Kurzer, and J. W. Slaton. 2008. Effects of soy protein isolate consumption on prostate cancer biomarkers in men with HGPIN, ASAP, and low-grade prostate cancer. *Nutr Cancer* 60 (1):7–13.

Hasan, Y., D. Schoenherr, A. A. Martinez, G. D. Wilson, and B. Marples. 2010. Prostate-specific natural health products (dietary supplements) radiosensitize normal prostate cells. *Int J Radiat Oncol Biol Phys* 76 (3):896–904.

Hiipakka, R. A., H. Z. Zhang, W. Dai, Q. Dai, and S. Liao. 2002. Structure–activity relationships for inhibition of human 5α-reductases by polyphenols. *Biochem Pharmacol* 63 (6):1165–1176.

Ho, E., L. M. Beaver, D. E. Williams, and R. H. Dashwood. 2011. Dietary factors and epigenetic regulation for prostate cancer prevention. *Adv Nutr* 2 (6):497–510.

Hostanska, K., A. Suter, J. Melzer, and R. Saller. 2007. Evaluation of cell death caused by an ethanolic extract of *Serenoae repentis fructus* (Prostasan®) on human carcinoma cell lines. *Anti-cancer Res* 27 (2):873–881.

Howlader, N., A. M. Noone, M. Krapcho, J. Garshell, D. Miller, S. F. Altekruse, C. L. Kosary. et al. (Eds). 2015. SEER Cancer Statistics Review, 1975–2012, National Cancer Institute. http://seer.cancer.gov/csr/1975_2012/.

Hsieh, T. C. and J. M. Wu. 1999. Differential effects on growth, cell cycle arrest, and induction of apoptosis by resveratrol in human prostate cancer cell lines. *Exp Cell Res* 249 (1):109–115.

Hu, R., C. L. Saw, R. Yu, and A. N. Kong. 2010. Regulation of NF-E2-related factor 2 signaling for cancer chemoprevention: antioxidant coupled with antiinflammatory. *Antioxid Redox Signal* 13 (11):1679–1698.

Huang, J., C. Plass, and C. Gerhauser. 2011. Cancer chemoprevention by targeting the epigenome. *Curr Drug Targets* 12 (13):1925–1956.

Hudson, T. S., D. K. Hartle, S. D. Hursting, N. P. Nunez, T. T. Y. Wang, H. A. Young, P. Arany, and J. E. Green. 2007. Inhibition of prostate cancer growth by muscadine grape skin extract and resveratrol through distinct mechanisms. *Cancer Res* 67 (17):8396–8405.

Hung, M. W., M. S. Shiao, L. C. Tsai, G. G. Chang, and T. C. Chang. 2003. Apoptotic effect of caffeic acid phenethyl ester and its ester and amide analogues in human cervical cancer ME180 cells. *Anti-cancer Res* 23 (6C):4773–4780.

Hussain, M., M. Banerjee, F. H. Sarkar, Z. Djuric, M. N. Pollak, D. Doerge, J. Fontana et al. 2003. Soy isoflavones in the treatment of prostate cancer. *Nutr Cancer* 47 (2):111–117.

Ichikawa, H., T. Ichiyanagi, B. Xu, Y. Yoshii, M. Nakajima, and T. Konishi. 2001. Antioxidant activity of anthocyanin extract from purple black rice. *J Med Food* 4 (4):211–218.

Ide, H., S. Tokiwa, K. Sakamaki, K. Nishio, S. Isotani, S. Muto, T. Hama, H. Masuda, and S. Horie. 2010. Combined inhibitory effects of soy isoflavones and curcumin on the production of prostate-specific antigen. *Prostate* 70 (10):1127–1133.

Jatoi, A., P. Burch, D. Hillman, J. M. Vanyo, S. Dakhil, D. Nikcevich, K. Rowland et al. 2007. A tomato-based, lycopene-containing intervention for androgen-independent prostate cancer: Results of a Phase II study from the North Central Cancer Treatment Group. *Urology* 69 (2):289–294.

Jena, N. R. 2012. DNA damage by reactive species: Mechanisms, mutation and repair. *J Biosci* 37 (3):503–517.

Jiao, H. and S. Y. Wang. 2000. Correlation of antioxidant capacities to oxygen radical scavenging enzyme activities in blackberry. *J Agric Food Chem* 48 (11):5672–5676.

Johns, L. E. and R. S. Houlston. 2003. A systematic review and meta-analysis of familial prostate cancer risk. *BJU Int* 91 (9):789–794.

Juge, N., R. F. Mithen, and M. Traka. 2007. Molecular basis for chemoprevention by sulforaphane: A comprehensive review. *Cell Mol Life Sci* 64 (9):1105–1127.

Jung, K. J., M. A. Wallig, and K. W. Singletary. 2006. Purple grape juice inhibits 7,12-dimethylbenz[a]anthracene (DMBA)-induced rat mammary tumorigenesis and in vivo DMBA-DNA adduct formation. *Cancer Lett* 233 (2):279–288.

Kandaswami, C., L. T. Lee, P. P. Lee, J. J. Hwang, F. C. Ke, Y. T. Huang, and M. T. Lee. 2005. The anti-tumor activities of flavonoids. *In Vivo* 19 (5):895–909.

Kensler, T. W., J. G. Chen, P. A. Egner, J. W. Fahey, L. P. Jacobson, K. K. Stephenson, L. Ye et al. 2005. Effects of glucosinolate-rich broccoli sprouts on urinary levels of aflatoxin-DNA adducts and phenanthrene tetraols in a randomized clinical trial in He Zuo township, Qidong, People's Republic of China. *Cancer Epidemiol Biomarkers Prev* 14 (11 Pt 1):2605–2613.

Kermanizadeh, A., C. Chauché, D. M. Brown, S. Loft, and P. Møller. 2015. The role of intracellular redox imbalance in nanomaterial induced cellular damage and genotoxicity: A review. *Environ Mol Mutagen* 56 (2):111–124.

Khan, N., V. M. Adhami, and H. Mukhtar. 2010. Apoptosis by dietary agents for prevention and treatment of prostate cancer. *Endocr Relat Cancer* 17 (1):R39–R52.

Khan, N., D. J. Bharali, V. M. Adhami, I. A. Siddiqui, H. Cui, S. M. Shabana, S. A. Mousa, and H. Mukhtar. 2014. Oral administration of naturally occurring chitosan-based nanoformulated green tea polyphenol EGCG effectively inhibits prostate cancer cell growth in a xenograft model. *Carcinogenesis* 35 (2):415–423.

Khandrika, L., B. Kumar, S. Koul, P. Maroni, and H. K. Koul. 2009. Oxidative stress in prostate cancer. *Cancer Lett* 282 (2):125–136.
Khrapko, K. and D. Turnbull. 2014. Mitochondrial DNA mutations in aging. *Prog Mol Biol Transl Sci* 127:29–62.
Kikuchi, N., K. Ohmori, T. Shimazu, N. Nakaya, S. Kuriyama, Y. Nishino, Y. Tsubono, and I. Tsuji. 2006. No association between green tea and prostate cancer risk in Japanese men: The Ohsaki Cohort Study. *Br J Cancer* 95 (3):371–373.
Kim, S. J., E. Amankwah, S. Connors, H. Y. Park, M. Rincon, H. Cornnell, G. Chornokur et al. 2014. Safety and chemopreventive effect of Polyphenon E in preventing early and metastatic progression of prostate cancer in TRAMP mice. *Cancer Prev Res* (*Phila*) 7 (4):435–444.
Kirsh, V. A., S. T. Mayne, U. Peters, N. Chatterjee, M. F. Leitzmann, L. B. Dixon, D. A. Urban, E. D. Crawford, and R. B. Hayes. 2006. A prospective study of lycopene and tomato product intake and risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev* 15 (1):92–98.
Kobayashi, S., R. Tang, E. Shapiro, and H. Lepor. 1993. Characterization and localization of prostatic alpha 1 adrenoceptors using radioligand receptor binding on slide-mounted tissue section. *J Urol* 150 (6):2002–2006.
Kolonel, L. N., J. H. Hankin, A. S. Whittemore, A. H. Wu, R. P. Gallagher, L. R. Wilkens, E. M. John et al. 2000. Vegetables, fruits, legumes and prostate cancer: A multiethnic case-control study. *Cancer Epidemiol Biomarkers Prev* 9 (8):795–804.
Kristal, A. R., C. Till, E. A. Platz, X. Song, I. B. King, M. L. Neuhouser, C. B. Ambrosone, and I. M. Thompson. 2011. Serum lycopene concentration and prostate cancer risk: Results from the prostate cancer prevention trial. *Cancer Epidemiol Biomarkers Prev* 20 (4):638–646.
Kucuk, O., F. H. Sarkar, W. Sakr, Z. Djuric, M. N. Pollak, F. Khachik, Y. W. Li et al. 2001. Phase II randomized clinical trial of lycopene supplementation before radical prostatectomy. *Cancer Epidemiol Biomarkers Prev* 10 (8):861–868.
Kujumgiev, A., I. Tsvetkova, Y. Serkedjieva, V. Bankova, R. Christov, and S. Popov. 1999. Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. *J Ethnopharmacol* 64 (3):235–240.
Kumar, N. B., J. Pow-Sang, K. M. Egan, P. E. Spiess, S. Dickinson, R. Salup, M. Helal et al. 2015. Randomized, placebo-controlled trial of green tea catechins for prostate cancer prevention. *Cancer Prev Res* (*Phila*) 8 (10):879–887.
Kurahashi, N., S. Sasazuki, M. Iwasaki, M. Inoue, and S. Tsugane. 2008. Green tea consumption and prostate cancer risk in Japanese men: A prospective study. *Am J Epidemiol* 167 (1):71–77.
Lakshman, M., L. Xu, V. Ananthanarayanan, J. Cooper, C. H. Takimoto, I. Helenowski, J. C. Pelling, and R. C. Bergan. 2008. Dietary genistein inhibits metastasis of human prostate cancer in mice. *Cancer Res* 68 (6):2024–2032.
Lazarevic, B., G. Boezelijn, L. M. Diep, K. Kvernrod, O. Ogren, H. Ramberg, A. Moen et al. 2011. Efficacy and safety of short-term genistein intervention in patients with localized prostate cancer prior to radical prostatectomy: A randomized, placebo-controlled, double-blind Phase 2 clinical trial. *Nutr Cancer* 63 (6):889–898.
Le, H., J. T. Arnold, K. K. McFann, and M. R. Blackman. 2006. DHT and testosterone, but not DHEA or E2, differentially modulate IGF-I, IGFBP-2, and IGFBP-3 in human prostatic stromal cells. *Am J Physiol Endocrinol Metab* 290 (5):E952–E960.
Lee, C., J. M. Kozlowski, and J. T. Grayhack. 1997. Intrinsic and extrinsic factors controlling benign prostatic growth. *Prostate* 31 (2):131–138.
Lee, J. H., T. O. Khor, L. Shu, Z. Y. Su, F. Fuentes, and A. N. Kong. 2013. Dietary phytochemicals and cancer prevention: Nrf2 signaling, epigenetics, and cell death mechanisms in blocking cancer initiation and progression. *Pharmacol Ther* 137 (2):153–171.
Lee, J. S. and Y. J. Surh. 2005. Nrf2 as a novel molecular target for chemoprevention. *Cancer Lett* 224 (2):171–184.

Lee, S. T., P. F. Wong, S. C. Cheah, and M. R. Mustafa. 2011. Alpha-tomatine induces apoptosis and inhibits nuclear factor-kappa B activation on human prostatic adenocarcinoma PC-3 cells. *PLoS One* 6 (4):e18915.

Lepor, H., D. I. Gup, M. Baumann, and E. Shapiro. 1988. Laboratory assessment of terazosin and alpha-1 blockade in prostatic hyperplasia. *Urology* 32 (6 Suppl):21–26.

Li, H. Z., A. Kapur, J. X. Yang, S. Srivastava, D. G. McLeod, J. F. Paredes-Guzman, A. Daugsch, Y. K. Park, and J. S. Rhim. 2007. Antiproliferation of human prostate cancer cells by ethanolic extracts of Brazilian propolis and its botanical origin. *Int J Oncol* 31 (3):601–606.

Lin, H., J. P. Lu, P. Laflamme, S. Qiao, B. Shayegan, I. Bryskin, L. Monardo, B. C. Wilson, G. Singh, and J. H. Pinthus. 2010. Inter-related in vitro effects of androgens, fatty acids and oxidative stress in prostate cancer: A mechanistic model supporting prevention strategies. *Int J Oncol* 37 (4):761–766.

Lin, H. P., S. S. Jiang, and C. P. Chuu. 2012. Caffeic acid phenethyl ester causes p21(Cip1) induction, Akt signaling reduction, and growth inhibition in PC-3 human prostate cancer cells. *PLoS One* 7 (2):e31286.

Lin, H. P., C. Y. Lin, C. Huo, P. H. Hsiao, L. C. Su, S. S. Jiang, T. M. Chan et al. 2015. Caffeic acid phenethyl ester induced cell cycle arrest and growth inhibition in androgen-independent prostate cancer cells via regulation of Skp2, p53, p21Cip1 and p27Kip1. *Oncotarget* 6 (9):6684–6707.

Lin, H. P., C. Y. Lin, C. C. Liu, L. C. Su, C. Huo, Y. Y. Kuo, J. C. Tseng, J. M. Hsu, C. K. Chen, and C. P. Chuu. 2013a. Caffeic acid phenethyl ester as a potential treatment for advanced prostate cancer targeting Akt signaling. *Int J Mol Sci* 14 (3):5264–5283.

Lin, K. W., Y. T. Chen, S. C. Yang, B. L. Wei, C. F. Hung, and C. N. Lin. 2013b. Xanthine oxidase inhibitory lanostanoids from *Ganoderma tsugae*. *Fitoterapia* 89:231–238.

Liu, W., Z. Xu, T. Yang, Y. Deng, B. Xu, S. Feng, and Y. Li. 2014. The protective role of tea polyphenols against methylmercury-induced neurotoxic effects in rat cerebral cortex via inhibition of oxidative stress. *Free Radic Res* 48 (8):849–863.

Lu, Q. Y., J. C. Hung, D. Heber, V. L. Go, V. E. Reuter, C. Cordon-Cardo, H. I. Scher, J. R. Marshall, and Z. F. Zhang. 2001. Inverse associations between plasma lycopene and other carotenoids and prostate cancer. *Cancer Epidemiol Biomarkers Prev* 10 (7):749–756.

Luscombe, C. J., A. A. Fryer, M. E. French, S. Liu, M. F. Saxby, P. W. Jones, and R. C. Strange. 2001. Exposure to ultraviolet radiation: Association with susceptibility and age at presentation with prostate cancer. *The Lancet* 358 (9282):641–642.

Ma, Q. and X. He. 2012. Molecular basis of electrophilic and oxidative defense: Promises and perils of Nrf2. *Pharmacol Rev* 64 (4):1055–1081.

MacDonald, R., J. W. Tacklind, I. Rutks, and T. J. Wilt. 2012. Serenoa repens monotherapy for benign prostatic hyperplasia (BPH): An updated Cochrane systematic review. *BJU Int* 109 (12):1756–1761.

Maciejewicz, W., M. Daniewski, K. Bal, and W. Markowski. 2001. GC-MS identification of the flavonoid aglycones isolated from propolis. *Chromatographia* 53 (5–6):343–346.

Mahmoud, A. M., W. Yang, and M. C. Bosland. 2014. Soy isoflavones and prostate cancer: A review of molecular mechanisms. *J Steroid Biochem Mol Biol* 140 (0):116–132.

Mancuso, C., T. E. Bates, D. A. Butterfield, S. Calafato, C. Cornelius, A. De Lorenzo, A. T. Dinkova Kostova, and V. Calabrese. 2007. Natural antioxidants in Alzheimer's disease. *Expert Opin Investig Drugs* 16 (12):1921–1931.

Manda, G., G. Isvoranu, M. V. Comanescu, A. Manea, B. D. Butuner, and K. S. Korkmaz. 2015. The redox biology network in cancer pathophysiology and therapeutics. *Redox Biol* 5:347–357.

Mariani, S., L. Lionetto, M. Cavallari, A. Tubaro, D. Rasio, C. De Nunzio, G. M. Hong, M. Borro, and M. Simmaco. 2014. Low prostate concentration of lycopene is associated with development of prostate cancer in patients with high-grade prostatic intraepithelial neoplasia. *Int J Mol Sci* 15 (1):1433–1440.

Marks, L. S., A. W. Partin, J. I. Epstein, V. E. Tyler, I. Simon, M. L. Macairan, T. L. Chan et al. 2000. Effects of a saw palmetto herbal blend in men with symptomatic benign prostatic hyperplasia. *J Urol* 163 (5):1451–1456.

Marshall, D. T., S. J. Savage, E. Garrett-Mayer, T. E. Keane, B. W. Hollis, R. L. Horst, L. H. Ambrose, M. S. Kindy, and S. Gattoni-Celli. 2012. Vitamin D3 supplementation at 4000 international units per day for one year results in a decrease of positive cores at repeat biopsy in subjects with low-risk prostate cancer under active surveillance. *J Clin Endocrinol Metab* 97 (7):2315–2324.

Maynard, S., S. H. Schurman, C. Harboe, N. C. de Souza-Pinto, and V. A. Bohr. 2009. Base excision repair of oxidative DNA damage and association with cancer and aging. *Carcinogenesis* 30 (1):2–10.

McCann, S. E., C. B. Ambrosone, K. B. Moysich, J. Brasure, J. R. Marshall, J. L. Freudenheim, G. S. Wilkinson, and S. Graham. 2005. Intakes of selected nutrients, foods, and phytochemicals and prostate cancer risk in western New York. *Nutr Cancer* 53 (1):33–41.

McEleny, K., R. Coffey, C. Morrissey, K. Williamson, U. Zangemeister-Wittke, J. M. Fitzpatrick, and R. W. G. Watson. 2004. An antisense oligonucleotide to cIAP-1 sensitizes prostate cancer cells to fas and TNFα mediated apoptosis. *Prostate* 59 (4):419–425.

McVary, K. T., C. G. Roehrborn, A. L. Avins, M. J. Barry, R. C. Bruskewitz, R. F. Donnell, H. E. Foster et al. 2011. Update on AUA guideline on the management of benign prostatic hyperplasia. *J Urol* 185 (5):1793–1803.

Mehraein-Ghomi, F., S. J. Kegel, D. R. Church, J. S. Schmidt, Q. R. Reuter, E. L. Saphner, H. S. Basu, and G. Wilding. 2014. Targeting androgen receptor and JunD interaction for prevention of prostate cancer progression. *Prostate* 74 (7):792–803.

Minelli, A., I. Bellezza, C. Conte, and Z. Culig. 2009. Oxidative stress-related aging: A role for prostate cancer? *Biochimica et Biophysica Acta* 1795 (2):83–91.

Miyanaga, N., H. Akaza, S. Hinotsu, T. Fujioka, S. Naito, M. Namiki, S. Takahashi et al. 2012. Prostate cancer chemoprevention study: An investigative randomized control study using purified isoflavones in men with rising prostate-specific antigen. *Cancer Sci* 103 (1):125–130.

Mohanty, N. K., S. Saxena, U. P. Singh, N. K. Goyal, and R. P. Arora. 2005. Lycopene as a chemopreventive agent in the treatment of high-grade prostate intraepithelial neoplasia. *Urol Oncol* 23 (6):383–385.

Morgia, G., G. I. Russo, S. Voce, F. Palmieri, M. Gentile, A. Giannantoni, F. Blefari et al. 2014. Serenoa repens, lycopene and selenium versus tamsulosin for the treatment of LUTS/BPH. An Italian multicenter double-blinded randomized study between single or combination therapy (PROCOMB trial). *Prostate* 74 (15):1471–1480.

Muindi, J. R., Y. Peng, D. M. Potter, P. A. Hershberger, J. S. Tauch, M. J. Capozzoli, M. J. Egorin, C. S. Johnson, and D. L. Trump. 2002. Pharmacokinetics of high-dose oral calcitriol: Results from a phase 1 trial of calcitriol and paclitaxel. *Clin Pharmacol Ther* 72 (6):648–659.

Myzak, M. C., P. Tong, W. M. Dashwood, R. H. Dashwood, and E. Ho. 2007. Sulforaphane retards the growth of human PC-3 xenografts and inhibits HDAC activity in human subjects. *Exp Biol Med* (*Maywood*) 232 (2):227–234.

Nagata, Y., T. Sonoda, M. Mori, N. Miyanaga, K. Okumura, K. Goto, S. Naito et al. 2007. Dietary isoflavones may protect against prostate cancer in Japanese men. *J Nutr* 137 (8):1974–1979.

Natarajan, K., S. Singh, T. R. Burke, D. Grunberger, and B. B. Aggarwal. 1996. Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-kappa B. *Proc Natl Acad Sci USA* 93 (17):9090–9095.

National Cancer Institute. 2015. Cancer Stat Fact Sheets: Prostate cancer. National Cancer Institute, Bethesda, Maryland. https://seer.cancer.gov/statfacts/html/prost.html.

Nemeth, K., G. W. Plumb, J. G. Berrin, N. Juge, R. Jacob, H. Y. Naim, G. Williamson, D. M. Swallow, and P. A. Kroon. 2003. Deglycosylation by small intestinal epithelial cell beta-glucosidases is a critical step in the absorption and metabolism of dietary flavonoid glycosides in humans. *Eur J Nutr* 42 (1):29–42.

Oberley, T. D. 2002. Oxidative damage and cancer. *Am J Pathol* 160 (2):403–408.
Odin, A. P. 1997. Vitamins as antimutagens: Advantages and some possible mechanisms of antimutagenic action. *Mutat Res* 386 (1):39–67.
Osborn, J. L., G. G. Schwartz, D. C. Smith, R. Bahnson, R. Day, and D. L. Trump. 1995. Phase II trial of oral 1,25-dihydroxyvitamin D (calcitriol) in hormone refractory prostate cancer. *Urol Onc Semi Ori* 1 (5):195–198.
Ozturk, G., Z. Ginis, S. Akyol, G. Erden, A. Gurel, and O. Akyol. 2012. The Anti-cancer mechanism of caffeic acid phenethyl ester (CAPE): review of melanomas, lung and prostate cancers. *Eur Rev Med Pharmacol Sci* 16 (15):2064–2068.
Paller, C. J., M. A. Rudek, X. C. Zhou, W. D. Wagner, T. S. Hudson, N. Anders, H. J. Hammers et al. 2015. A phase I study of muscadine grape skin extract in men with biochemically recurrent prostate cancer: Safety, tolerability, and dose determination. *Prostate* 75 (14):1518–1525.
Park, E. H. and J. H. Kahng. 1999. Suppressive effects of propolis in rat adjuvant arthritis. *Arch Pharm Res* 22 (6):554–558.
Parker, C. 2004. Active surveillance: Towards a new paradigm in the management of early prostate cancer. *Lancet Oncol* 5 (2):101–106.
Patel, V. H. 2014. Nutrition and prostate cancer: An overview. *Expert Rev Anti-cancer Ther* 14 (11):1295–1304.
Peters, U., M. F. Leitzmann, N. Chatterjee, Y. Wang, D. Albanes, E. P. Gelmann, M. D. Friesen, E. Riboli, and R. B. Hayes. 2007. Serum lycopene, other carotenoids, and prostate cancer risk: A nested case-control study in the prostate, lung, colorectal, and ovarian cancer screening trial. *Cancer Epidemiol Biomarkers Prev* 16 (5):962–968.
Petrangeli, E., L. Lenti, B. Buchetti, P. Chinzari, P. Sale, L. Salvatori, L. Ravenna et al. 2009. Lipido-sterolic extract of *Serenoa repens* (LSESr, Permixon) treatment affects human prostate cancer cell membrane organization. *J Cell Physiol* 219 (1):69–76.
Phaniendra, A., D. B. Jestadi, and L. Periyasamy. 2015. Free radicals: Properties, sources, targets, and their implication in various diseases. *Indian J Clin Biochem* 30 (1):11–26.
Pietta, P. G., C. Gardana, and A. M. Pietta. 2002. Analytical methods for quality control of propolis. *Fitoterapia* 73 (Suppl 1):S7–S20.
Pinto, M. and C. T. Moraes. 2015. Mechanisms linking mtDNA damage and aging. *Free Radic Biol Med* 85:250–258.
Powolny, A. A. and S. V. Singh. 2008. Plumbagin-induced apoptosis in human prostate cancer cells is associated with modulation of cellular redox status and generation of reactive oxygen species. *Pharm Res* 25 (9):2171–2180.
Rajkumar, V., G. Guha, and R. A. Kumar. 2011. Antioxidant and anti-neoplastic activities of Picrorhiza kurroa extracts. *Food Chem Toxicol* 49 (2):363–369.
Rand, H. W. 1895. V. A contribution to the surgery of the hypertrophied prostate. *Ann Surg* 22 (2):217–225.
Renschler, M. F. 2004. The emerging role of reactive oxygen species in cancer therapy. *Eur J Cancer* 40 (13):1934–1940.
Roehrborn, C. G., P. Boyle, J. C. Nickel, K. Hoefner, and G. Andriole. 2002. Efficacy and safety of a dual inhibitor of 5-alpha-reductase types 1 and 2 (dutasteride) in men with benign prostatic hyperplasia. *Urology* 60 (3):434–441.
Rubin, M. A., N. R. Mucci, J. Figurski, A. Fecko, K. J. Pienta, and M. L. Day. 2001. E-cadherin expression in prostate cancer: A broad survey using high-density tissue microarray technology. *Hum Pathol* 32 (7):690–697.
Russo, A., V. Cardile, F. Sanchez, N. Troncoso, A. Vanella, and J. A. Garbarino. 2004. Chilean propolis: Antioxidant activity and antiproliferative action in human tumor cell lines. *Life Sci* 76 (5):545–558.
Ryan, C. J. and D. J. Tindall. 2011. Androgen receptor rediscovered: the new biology and targeting the androgen receptor therapeutically. *J Clin Oncol* 29 (27):3651–3658.

Sakao, K., A. R. Vyas, S. R. Chinni, A. I. Amjad, R. Parikh, and S. V. Singh. 2015. CXCR4 is a novel target of cancer chemopreventative isothiocyanates in prostate cancer cells. *Cancer Prev Res* 8 (5):365–374.

Salisbury, D. and U. Bronas. 2015. Reactive oxygen and nitrogen species: Impact on endothelial dysfunction. *Nurs Res* 64 (1):53–66.

Sauer, H., M. Wartenberg, and J. Hescheler. 2001. Reactive oxygen species as intracellular messengers during cell growth and differentiation. *Cell Physiol Biochem* 11 (4):173–186.

Schally, A. V., A. M. Comaru-Schally, A. Nagy, M. Kovacs, K. Szepeshazi, A. Plonowski, J. L. Varga, and G. Halmos. 2001. Hypothalamic hormones and cancer. *Front Neuroendocrinol* 22 (4):248–291.

Schroder, F. H., J. Hugosson, M. J. Roobol, T. L. Tammela, S. Ciatto, V. Nelen, M. Kwiatkowski et al. 2009. Screening and prostate-cancer mortality in a randomized European study. *N Engl J Med* 360 (13):1320–1328.

Schroder, F. H., J. Hugosson, M. J. Roobol, T. L. Tammela, S. Ciatto, V. Nelen, M. Kwiatkowski et al. 2012. Prostate-cancer mortality at 11 years of follow-up. *N Engl J Med* 366 (11):981–990.

Schwenke, C., B. Ubrig, P. Thurmann, C. Eggersmann, and S. Roth. 2009. Lycopene for advanced hormone refractory prostate cancer: A prospective, open phase II pilot study. *J Urol* 181 (3):1098–1103.

Scott, E., W. P. Steward, A. J. Gescher, and K. Brown. 2012. Resveratrol in human cancer chemoprevention—choosing the 'right' dose. *Mol Nutr Food Res* 56 (1):7–13.

Scott, T. L., S. Rangaswamy, C. A. Wicker, and T. Izumi. 2014. Repair of oxidative DNA damage and cancer: Recent progress in DNA base excision repair. *Antioxid Redox Signal* 20 (4):708–726.

Seeni, A., S. Takahashi, K. Takeshita, M. Tang, S. Sugiura, S. Y. Sato, and T. Shirai. 2008. Suppression of prostate cancer growth by resveratrol in the transgenic rat for adenocarcinoma of prostate (TRAP) model. *Asian Pac J Cancer Prev* 9 (1):7–14.

Sfanos, K. S. and A. M. De Marzo. 2012. Prostate cancer and inflammation: The evidence. *Histopathology* 60 (1):199–215.

Shabahang, M., R. R. Buras, F. Davoodi, L. M. Schumaker, R. J. Nauta, and S. R. T. Evans. 1993. 1,25-Dihydroxyvitamin-D3 receptor as a marker of human colon-carcinoma cell-line differentiation and growth-inhibition. *Cancer Res* 53 (16):3712–3718.

Shapiro, T. A., J. W. Fahey, A. T. Dinkova-Kostova, W. D. Holtzclaw, K. K. Stephenson, K. L. Wade, L. Ye, and P. Talalay. 2006. Safety, tolerance, and metabolism of broccoli sprout glucosinolates and isothiocyanates: A clinical phase I study. *Nutr Cancer* 55 (1):53–62.

Shen, Y., L. Zhong, S. Markwell, and D. Cao. 2010. Thiol-disulfide exchanges modulate aldo-keto reductase family 1 member B10 activity and sensitivity to inhibitors. *Biochimie* 92 (5):530–537.

Shiota, M., A. Yokomizo, and S. Naito. 2011. Oxidative stress and androgen receptor signaling in the development and progression of castration-resistant prostate cancer. *Free Radic Biol Med* 51 (7):1320–1328.

Shoskes, D. A., S. I. Zeitlin, A. Shahed, and J. Rajfer. 1999. Quercetin in men with category III chronic prostatitis: A preliminary prospective, double-blind, placebo-controlled trial. *Urology* 54 (6):960–963.

Siegel, R., D. Naishadham, and A. Jemal. 2013. Cancer statistics, 2013. *CA Cancer J Clin* 63 (1):11–30.

Silvestri, I., S. Cattarino, A. Agliano, C. Nicolazzo, S. Scarpa, S. Salciccia, L. Frati, V. Gentile, and A. Sciarra. 2013. Effect of Serenoa repens (Permixon(R)) on the expression of inflammation-related genes: Analysis in primary cell cultures of human prostate carcinoma. *J Inflamm* (*Lond*) 10:11.

Simboli-Campbell, M., C. J. Narvaez, M. Tenniswood, and J. Welsh. 1996. 1,25-Dihydroxyvitamin D3 induces morphological and biochemical markers of apoptosis in MCF-7 breast cancer cells. *J Steroid Biochem Mol Biol* 58 (4):367–376.

Singh, V. P., S. Singh, J. Kumar, and S. M. Prasad. 2015. Hydrogen sulfide alleviates toxic effects of arsenate in pea seedlings through up-regulation of the ascorbate-glutathione cycle: Possible involvement of nitric oxide. *J Plant Physiol* 181:20–29.

Singletary, K. W., M. J. Stansbury, M. Giusti, R. B. Van Breemen, M. Wallig, and A. Rimando. 2003. Inhibition of rat mammary tumorigenesis by concord grape juice constituents. *J Agric Food Chem* 51 (25):7280–7286.

Slimen, I. B., T. Najar, A. Ghram, H. Dabbebi, M. Ben Mrad, and M. Abdrabbah. 2014. Reactive oxygen species, heat stress and oxidative-induced mitochondrial damage. A review. *Int J Hyperthermia* 30 (7):513–523.

Smith, H. R., A. Memon, C. J. Smart, and K. Dewbury. 1986. The value of permixon in benign prostatic hypertrophy. *Br J Urol* 58 (1):36–40.

Stangelberger, A., M. Waldert, and B. Djavan. 2008. Prostate cancer in elderly men. *Rev Urol* 10 (2):111–119.

Subramanian, L., S. Youssef, S. Bhattacharya, J. Kenealey, A. S. Polans, and P. R. van Ginkel. 2010. "Resveratrol: Challenges in translation to the clinic—a critical discussion. *Clin Cancer Res* 16 (24):5942–5948.

Sugar, L. M. 2006. Inflammation and prostate cancer. *Can J Urol* 13 (Suppl 1):46–47.

Sultan, C., A. Terraza, C. Devillier, E. Carilla, M. Briley, C. Loire, and B. Descomps. 1984. Inhibition of androgen metabolism and binding by a liposterolic extract of "Serenoa repens B" in human foreskin fibroblasts. *J Steroid Biochem* 20 (1):515–519.

Suzuki, H., T. Ueda, T. Ichikawa, and H. Ito. 2003. Androgen receptor involvement in the progression of prostate cancer. *Endocr Relat Cancer* 10 (2):209–216.

Swami, S., A. V. Krishnan, and D. Feldman. 2011. Vitamin D metabolism and action in the prostate: implications for health and disease. *Mol Cell Endocrinol* 347 (1–2):61–69.

Swami, S., A. V. Krishnan, D. M. Peehl, and D. Feldman. 2005. Genistein potentiates the growth inhibitory effects of 1,25-dihydroxyvitamin D3 in DU145 human prostate cancer cells: Role of the direct inhibition of CYP24 enzyme activity. *Mol Cell Endocrinol* 241 (1–2):49–61.

Szliszka, E., A. Sokol-Letowska, A. Z. Kucharska, D. Jaworska, Z. P. Czuba, and W. Krol. 2013. Ethanolic extract of polish propolis: Chemical composition and TRAIL-R2 death receptor targeting apoptotic activity against prostate cancer cells. *Evid Based Complement Alternat Med* 2013 (2013):12.

Szliszka, E., G. Zydowicz, B. Janoszka, C. Dobosz, G. Kowalczyk-Ziomek, and W. Krol. 2011. Ethanolic extract of Brazilian green propolis sensitizes prostate cancer cells to TRAIL-induced apoptosis. *Int J Oncol* 38 (4):941953.

Sönmez, A. Y., S. Bilen, G. Alak, O. Hisar, T. Yanık, and G. Biswas. 2015. Growth performance and antioxidant enzyme activities in rainbow trout (*Oncorhynchus mykiss*) juveniles fed diets supplemented with sage, mint and thyme oils. *Fish Physiol Biochem* 41 (1):165–175.

Thakur, V. S., K. Gupta, and S. Gupta. 2012. Green tea polyphenols causes cell cycle arrest and apoptosis in prostate cancer cells by suppressing class I histone deacetylases. *Carcinogenesis* 33 (2):377–384.

Thompson, I. M., P. J. Goodman, C. M. Tangen, M. S. Lucia, G. J. Miller, L. G. Ford, M. M. Lieber et al. 2003. The influence of finasteride on the development of prostate cancer. *N Engl J Med* 349 (3):215–224.

Thompson, I. M., Jr., P. J. Goodman, C. M. Tangen, H. L. Parnes, L. M. Minasian, P. A. Godley, M. S. Lucia, and L. G. Ford. 2013. Long-term survival of participants in the prostate cancer prevention trial. *N Engl J Med* 369 (7):603–610.

Thompson, I., J. B. Thrasher, G. Aus, A. L. Burnett, E. D. Canby-Hagino, M. S. Cookson, A. V. D'Amico et al. 2007. Guideline for the management of clinically localized prostate cancer: 2007 update. *J Urol* 177 (6):2106–2131.

Tolba, M. F., A. Esmat, A. M. Al-Abd, S. S. Azab, A. E. Khalifa, H. A. Mosli, S. Z. Abdel-Rahman, and A. B. Abdel-Naim. 2013. Caffeic acid phenethyl ester synergistically enhances docetaxel and paclitaxel cytotoxicity in prostate cancer cells. *IUBMB Life* 65 (8):716–729.

Tolis, G., D. Ackman, A. Stellos, A. Mehta, F. Labrie, A. T. Fazekas, A. M. Comaru-Schally, and A. V. Schally. 1982. Tumor growth inhibition in patients with prostatic carcinoma treated with luteinizing hormone-releasing hormone agonists. *Proc Natl Acad Sci USA* 79 (5):1658–1662.

Traka, M., A. V. Gasper, A. Melchini, J. R. Bacon, P. W. Needs, V. Frost, A. Chantry et al. 2008. Broccoli consumption interacts with GSTM1 to perturb oncogenic signalling pathways in the prostate. *PLoS One* 3 (7):e2568.

Vaishampayan, U., M. Hussain, M. Banerjee, S. Seren, F. H. Sarkar, J. Fontana, J. D. Forman et al. 2007. Lycopene and soy isoflavones in the treatment of prostate cancer. *Nutr Cancer* 59 (1):1–7.

van Breemen, R. B., R. Sharifi, M. Viana, N. Pajkovic, D. Zhu, L. Yuan, Y. Yang, P. E. Bowen, and M. Stacewicz-Sapuntzakis. 2011. Antioxidant effects of lycopene in African American men with prostate cancer or benign prostate hyperplasia: A randomized, controlled trial. *Cancer Prev Res* (*Phila*) 4 (5):711–718.

Van Poppel, H. and B. Tombal. 2011. Chemoprevention of prostate cancer with nutrients and supplements. *Cancer Manag Res* 3:91–100.

Veeramani, S., Y. W. Chou, F. C. Lin, S. Muniyan, F. F. Lin, S. Kumar, Y. Xie, S. M. Lele, Y. Tu, and M. F. Lin. 2012. Reactive oxygen species induced by p66Shc longevity protein mediate nongenomic androgen action via tyrosine phosphorylation signaling to enhance tumorigenicity of prostate cancer cells. *Free Radic Biol Med* 53 (1):95–108.

Vickers, A. J., D. Ulmert, D. D. Sjoberg, C. J. Bennette, T. Bjork, A. Gerdtsson, J. Manjer et al. 2013. Strategy for detection of prostate cancer based on relation between prostate specific antigen at age 40–55 and long term risk of metastasis: Case-control study. *BMJ* 346:f2023.

Vogt, T. M., S. T. Mayne, B. I. Graubard, C. A. Swanson, A. L. Sowell, J. B. Schoenberg, G. M. Swanson et al. 2002. Serum lycopene, other serum carotenoids, and risk of prostate cancer in US Blacks and Whites. *Am J Epidemiol* 155 (11):1023–1032.

Walden, P. D., C. Gerardi, and H. Lepor. 1999. Localization and expression of the alpha1A-1, alpha1B and alpha1D-adrenoceptors in hyperplastic and non-hyperplastic human prostate. *J Urol* 161 (2):635–640.

Walsh, P. C. 2010. Chemoprevention of prostate cancer. *N Engl J Med* 362 (13):1237–1238.

Wang, X. N. and G. P. Studzinski. 2001. Activation of extracellular signal-regulated kinases (ERKs) defines the first phase of 1,25-dihydroxyvitamin D-3-induced differentiation of HL60 cells. *J Cell Biochem* 80 (4):471–482.

Wang, Y. J., J. G. Zhu, and H. F. DeLuca. 2012. Where is the vitamin D receptor? *Arch Biochem Biophys* 523 (1):123–133.

Wang, Z., J. Fan, M. Liu, S. Yeung, A. Chang, M. S. Chow, D. Pon, and Y. Huang. 2013a. Nutraceuticals for prostate cancer chemoprevention: From molecular mechanisms to clinical application. *Expert Opin Investig Drugs* 22 (12):1613–1626.

Wang, Z. J., J. Fan, M. Liu, S. Yeung, A. Chang, M. S. S. Chow, D. Pon, and Y. Huang. 2013b. Nutraceuticals for prostate cancer chemoprevention: From molecular mechanisms to clinical application. *Expert Opin Investig Drugs* 22 (12):1613–1626.

Watabe, M., K. Hishikawa, A. Takayanagi, N. Shimizu, and T. Nakaki. 2004. Caffeic acid phenethyl ester induces apoptosis by inhibition of NFkappaB and activation of Fas in human breast cancer MCF-7 cells. *J Biol Chem* 279 (7):6017–6026.

Welsh, J. 2012. Cellular and molecular effects of vitamin D on carcinogenesis. *Arch Biochem Biophys* 523 (1):107–114.

Weydert, C. J. and J. J. Cullen. 2010. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. *Nat Protoc* 5 (1):51–66.

Willetts, K. E., M. S. Clements, S. Champion, S. Ehsman, and J. A. Eden. 2003. Serenoa repens extract for benign prostate hyperplasia: A randomized controlled trial. *BJU Int* 92 (3):267–270.

Wilt, T. J., R. Macdonald, K. Hagerty, P. Schellhammer, J. Tacklind, M. R. Somerfield, and B. S. Kramer. 2010. 5-alpha-Reductase inhibitors for prostate cancer chemoprevention: An updated Cochrane systematic review. *BJU Int* 106 (10):1444–1451.

Wu, X., Q. H. Zhou, and K. Xu. 2009. Are isothiocyanates potential anti-cancer drugs? *Acta Pharmacologica Sinica* 30 (5):501–512.

Yang, C. S., X. Wang, G. Lu, and S. C. Picinich. 2009. Cancer prevention by tea: Animal studies, molecular mechanisms and human relevance. *Nat Rev Cancer* 9 (6):429–439.

Yang, F., L. Song, H. Wang, J. Wang, Z. Xu, and N. Xing. 2015. Quercetin in prostate cancer: Chemotherapeutic and chemopreventive effects, mechanisms and clinical application potential (Review). *Oncol Rep* 33 (6):2659–2668.

Yang, Y., T. Ikezoe, Z. Zheng, H. Taguchi, H. P. Koeffler, and W. G. Zhu. 2007. Saw Palmetto induces growth arrest and apoptosis of androgen-dependent prostate cancer LNCaP cells via inactivation of STAT 3 and androgen receptor signaling. *Int J Oncol* 31 (3):593–600.

Yeh, S., M. Y. Tsai, Q. Xu, X. M. Mu, H. Lardy, K. E. Huang, H. Lin et al. 2002. Generation and characterization of androgen receptor knockout (ARKO) mice: an in vivo model for the study of androgen functions in selective tissues. *Proc Natl Acad Sci USA* 99 (21):13498–13503.

Ylikomi, T., I. Laaksi, Y. R. Lou, P. Martikainen, S. Miettinen, P. Pennanen, S. Purmonen, H. Syvala, A. Vienonen, and P. Tuohimaa. 2002. Antiproliferative action of vitamin D. *Vitam Horm* 64:357–406.

Yuan, H., C. Y. Young, Y. Tian, Z. Liu, M. Zhang, and H. Lou. 2010. Suppression of the androgen receptor function by quercetin through protein-protein interactions of Sp1, c-Jun, and the androgen receptor in human prostate cancer cells. *Mol Cell Biochem* 339 (1–2):253–262.

Yuan, J. M. 2013. Cancer prevention by green tea: Evidence from epidemiologic studies. *Am J Clin Nutr* 98 (6 Suppl):1676S–1681S.

Yusuf, M. A., T. Chuang, G. J. Bhat, and K. S. Srivenugopal. 2010. Cys-141 glutathionylation of human p53: Studies using specific polyclonal antibodies in cancer samples and cell lines. *Free Radic Biol Med* 49 (5):908–917.

Zhang, X., Q. Wang, B. Neil, and X. Chen. 2010. Effect of lycopene on androgen receptor and prostate-specific antigen velocity. *Chin Med J* (*Engl*) 123 (16):2231–2236.

Zhang, Y., D. Y. Leung, B. N. Richers, Y. Liu, L. K. Remigio, D. W. Riches, and E. Goleva. 2012. Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. *J Immunol* 188 (5):2127–2135.

4

Natural Antioxidants in General Cancer Prevention

Avipsha Sarkar and Shampa Sen

Contents

4.1 Introduction

Pharmaceutical plus *nutrition* combines to form nutraceutical. Nutraceuticals are known to be portions of certain food items or food as a whole, which participate in altering and managing physiological functions of human beings and keeps them healthy. The recent fitness trends, as well as the contemporary population, are the prime reasons that lead to the enhancement of nutraceutical market. Nutraceuticals used in food merchandise are characterized as probiotics, polyunsaturated fatty acids, and dietary fibers along with antioxidants and prebiotics. It also includes various usual plant-derived foods.

Table 4.1 Dietary Fat Linked to Diseases

	Total or Saturated Fat	Antioxidants
Cancer	−	+
Cataract	−	+
Immune function	−	+
Obesity	−	+
Birth outcomes	−	−
Diabetes	−	+

Source: Kramer, K. et al., *Nutaceuticals in Health and Disease Prevention*, Marcel Dekkar, New York, 2001.
Note: The "+" sign denotes that antioxidants work positively on the diseases and the "−" sign denotes the negative effects of total and saturated fats.

These facilitate to fight against major health difficulties of recent times, including cancer, obesity, cardiovascular diseases, diabetes, osteoporosis along with arthritis and cholesterol, and so on. Summing up, a new epoch of medication and well-being has been opened up by nutraceutical, which therefore now includes food sector within the research-based zone (Das et al. 2011). There has been an increase in the popularity of both nutraceuticals and functional foods in the global market. In 2011, the worldwide market of nutraceuticals reached US$142.1 billion dollars, which is likely to reach US$204.8 billion dollars by 2017 (Albany 2013). **Table 4.1** illustrates the dietary factors linked to diseases and the role of antioxidants to combat these dreaded diseases. The "+" sign denotes that antioxidants work positively on the diseases and the "−" sign denotes the negative effects of total and saturated fats.

4.1.1 Causes of chronic disease: Cancer

Most of the common chronic disorders can be accredited to a catalog of principal risk issues and according to the *Centers for Disease Control and Prevention*, the universal risk factors to which most of the common people have been exposed to include the following:

- Disproportionate alcohol use
- Tobacco exploitation with exposure to passive smoking
- Nutrition high in sodium and saturated fats
- Physical idleness
- High blood pressure
- Diets low in fruits and vegetables
- Obesity (high body mass index)

Chronic diseases can be averted in most cases. The mechanistic properties that form a link between well-being and diet require more research but the available observations endow with adequately strong and credible basis to rationalize taking action. The civic health advance for initial prevention is reasonably priced,

expenditure efficient, as well as sustainable compared to medial management for those who have already developed the disease, which makes it more suitable to deal with the unremitting disease outbreak globally (Choi et al. 2001).

The U.S. financial system has suffered a yearly loss of US$70 billion in remedial and production costs due to unremitting disorders that are related to diet, which includes stroke, arteriosclerosis, heart disorders, without even including the premature deaths allied with these infirmities (Frazão 1999). Diet, with a specific mention to food constituents having a role in influencing the health has been addressed and recognized officially by the U.S. government in the conference on *Food, Nutrition and Health* in 1969 organized by The White House. Keeping the health benefits in mind, the middle of the 1980s saw food processors such as Kellogg and National Cancer Institute collaboratively started marketing certain foods mainly those rich in fiber that prevents colorectal cancer.

4.2 Antioxidant nutraceuticals

A list of diseases are claimed to have been managed and inhibited by antioxidants. In a broader sense, coronary disease can be inhibited by antioxidant combinations present in red wine and other valuable assistance as a blend in cosmetics. Other well-recognized information suggests that aspirin and anti-inflammatory nonsteroidal pills inhibit colon cancer (Giovannucci et al. 1995). Similarly, α-tocopherol has beneficial properties that lead to cardioprotection (Stampfer et al. 1993). Uncertainty lies in the mechanistic behavior of how these antioxidants work. Recently, it was more evident that antioxidants also contain other significant properties, including gene expression, signal transduction, as well as certain enzymes, phosphatase and kinase activation. **Figure 4.1** illustrates in brief the functions of antioxidants.

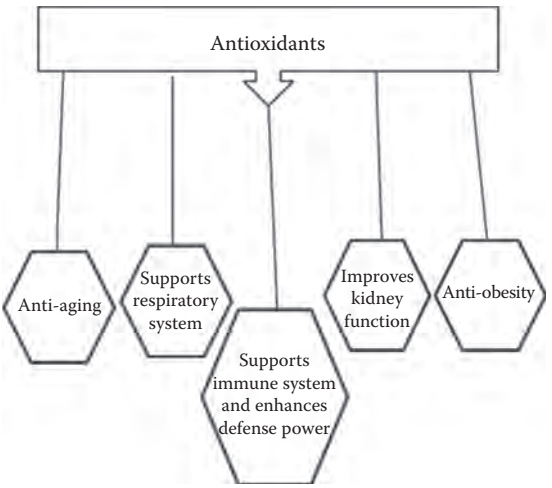


Figure 4.1 *Functions of antioxidant nutraceuticals.*

Table 4.2 Anti-cancer Antioxidants and Their Source

Serial no.	Natural Antioxidants	Source
1.	Vitamin C	Red algae, brown algae, citrus foods
2.	Vitamin E	Brown algae, green algae
3.	Beta-carotene	Red, green, and brown algae, cyanobacteria
4.	Curcumin	Turmeric
5.	Lycopene	Tomatoes are the main sources, gac, watermelon, autumn olive, pink guava, papaya, wolfberry

Source: Shebis, Y. et al., *Food and Nutrition Sciences*, 4, 643, 2013.

4.2.1 Natural antioxidants

Natural antioxidants can be found in large amounts within aquatic algae that has negative effects on the pathogenesis of various disorders with comparatively less costly extraction techniques (Lordan et al. 2011). These antioxidants play a pivotal role against diseases and aging by inhibiting oxidative damage in the cells (Kim et al. 2006; Karawita et al. 2007). Recent explorations have revealed some prospective novel sources that have elevated free radical quenching property, which includes industrially produced *Pheodactylum tricornutum, Botryococcus braunii, Himanthalia elongate, Neochlorisoleo abundans, Chondrus crispus, Isochrysis* sp. *Gayralia oxysperma, Undaria pinnatifida, Chlorella vulgaris*, and more (Kelman et al. 2004; Plaza et al. 2008; Goiris et al. 2012). Antioxidant suppliers such as plums, strawberries, grapes, broccoli, blueberries, and spinach have been included in the diet due to their enhanced scavenging capacity (Grossman 1994; Cao et al. 1998). Moreover, distinct antioxidants, novel antioxidant (NAO), have been discovered in the aqueous extracts of spinach leaf. NAO consists of flavonoids and *p*-coumaric acid derivatives that help to inhibit prostate cancer (Grossman et al. 2011). **Table 4.2** discusses the natural antioxidants against cancer and their source.

4.3 Functions of antioxidant nutraceuticals

Antioxidant nutraceuticals are utilized for combating diseases such as cancer, diabetes, aging, and other cardiovascular diseases without much side effects. The efficiency of these has led to the extensive research involving this class of nutraceuticals and it is observed that they are quite efficient in managing a number of diseases.

4.3.1 Anti-aging

Recently, the relationship between reactive oxygen species (ROS) and difficulties of aging has been observed; and hence, nutraceuticals were considered as a requisite to prevent and manage aging along with the endorsement of healthy aging. ROS plays a pivotal role in diseases such as cancer, hyperoxia, hepatitis, arthritis, dermatitis, and few others. Probable negative effects of

ROS are nullified by the body with the help of enzymes such as glutathione peroxidase along with glutathione catalase and superoxide dismutase. Recently, the nutrition patrons across the globe have made the nutraceuticals a top drift in the food business (Lee et al. 2006).

4.3.2 Anti-cardiovascular

Oxidative alterations of low-density lipoprotein (LDL) have proved to play a decisive role in the advancement of atherosclerosis (Steinberg et al. 1989; Steinberg 1992; Aviram 1993). The LDL uptake by the macrophages is heightened due to LDL oxidation, which in turn promotes the development of foam cells as well as fatty streaks (Steinberg et al. 1989). Carotid atherosclerosis is linked to an augmented vulnerability to LDL oxidation (Salonen et al. 1992). A few studies say that vitamin E intake has a contrasting relationship with coronary heart disorder, (Rimm et al. 1993; Stampfer et al. 1993), whereas few others say that vitamin C (Enstrom et al. 1992) and provitamin A carotenoids (Gaziano 1995) may have a protective action.

4.3.3 Anti-diabetes

It is evident that recently plant-derived food polyphenols have proved to be exceptional in terms of managing various phases of type 2 diabetes mellitus. There has been a prospective efficiency of polyphenols that also includes flavonoids, lignin, phenolic acids, as well as polymeric lignin on disorders that involve metabolism and those that are provoked by diabetes. *Emblica officinalis* (EB) along with *Terminalia bellerica* (TB) bear fruits consisting of high amount of bioactives and work against bacteria, viruses, and inflammation. The above-mentioned plants are the key components of triphala, a formulation which is used in ayurveda and is suggested to people (*E. officinalis, T. bellerica, and Terminaliachebula*) (Naik et al. 2005).

4.3.4 Anti-obesity

A high occurrence of diseases such as obesity as well as type 2 Diabetes has been observed globally. Fucoxanthin which is a carotenoid obtained from brown seaweeds have been described as antidiabetic and also possess anti-obesity properties. It is proved to provoke uncoupling protein 1 (UCP1), which is a proton carrier within the white adipose tissue (WAT) mitochondria of the abdomen by the nutrigenomics experimentation. By managing the secretion of cytokines from WAT the insulin resistance is made better by fucoxanthin, which also lowers blood glucose level (Miyashita et al. 2011).

4.3.5 Immune functions

Recently, there has been an enhancement in the occurrence of bacteria that are multidrug resistant (MDR) and there have been other complications associated with the utilization of antibiotics. These have led to an increased interest

in flora having antimicrobial activities. Bacterial species such as *Escherichia coli* 0517: H7 along with *Acinetobacter* sp. produce verocytotoxin. This is a toxin that has extra chemotherapeutic challenges because the toxin level increases when resisted with antibiotics in the growth medium. Certain medicinal herbs have antioxidant activities along with other mechanistic properties that are completely new and are yet to be studied. Once the mechanism is known, it can be utilized to manage the verocytotoxin producers that are MDR (Doughari et al. 2009).

4.4 Antioxidants against cancer

Certain nutraceuticals are compared for their efficacy in inhibiting skin cancer caused by croton oil and by initiation of dimethylbenz[a]anthracene (DMBA). The nutraceuticals included spinach leaves from New Zealand, sugar beet roots along with turmeric and cucumber. The nutraceuticals were applied on the skin approximately 1 hour before applying croton coil, which proved to be most effective among other techniques that were applied. Certain events, including late commencement of tumors, a decline in the skin tumor occurrence percentage, and the rate of multiplication was also diminished in comparison to the control (tumor initiated with DMBA and croton oil without application of nutraceuticals) (Villaseñor et al. 2002). Among all nutraceuticals that were examined, turmeric was found to be the most effective because it reduced incidence of the tumor by 30%, lowered skin tumors by 87.2%, and also held up the initiation of skin tumor by 5 weeks in contrast to the positive control, which was treated with croton oil and DMBA to initiate tumor formation. The most compelling is turmeric because the typical number of tumor formation was found to be statistically dissimilar, that is, the number of tumors decreased at $\alpha = 0.01$ with respect to the positive control (Villaseñor et al. 2002).

In the United States, 29% mortality is due to lung cancer, which is considered as the most important reason for death (Wingo et al. 1995). Novel approaches should be tried and implemented in order to inhibit lung cancer among people who has experienced an exposure to asbestos or people who have smoking habit. It was observed that 29% and 25% men and women, respectively, fall in the age group between 45 and 64 years who smoke at present (Centers for Disease Control and Prevention 1991) and about 40% and 20% men and women belonging to this group had a past history of smoking (Schoenborn and Boyd 1987). The observations show that about 4000–6000 people die due to lung cancer every year, which is accredited to asbestos exposure (Nicholson et al. 1982; Omenn et al. 1986).

Vitamin A along with beta-carotene has gained interest from the research fraternity due to its potential of inhibiting lung cancer, which is based on laboratory experimentations and the corresponding observations (Peto et al. 1981; Greenwald 1993; Omenn et al. 1994; Lippman et al. 2009). Some of the

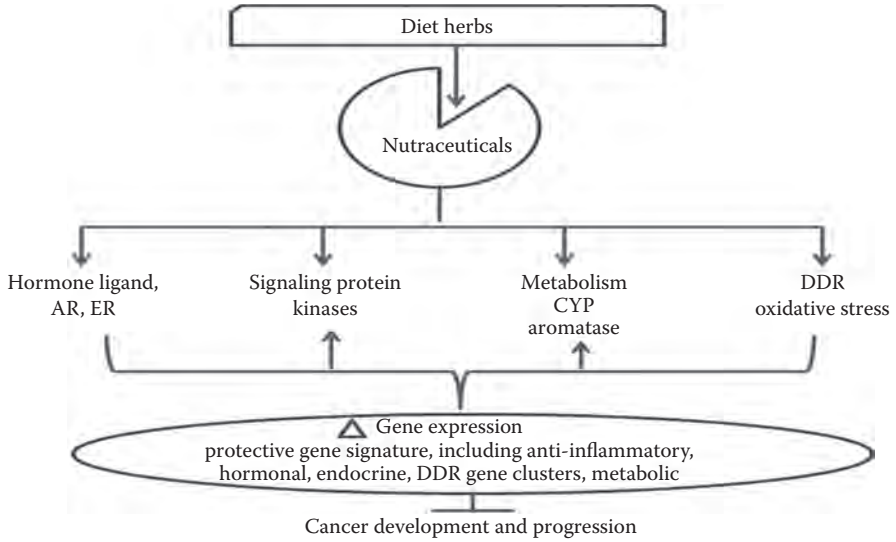


Figure 4.2 *Nutraceuticals against cancer.*

recent trials include beta-carotene as well as retinol efficacy trial (CARET) to measure the efficiency of chemoprevention as well as security of beta-carotene along with other linked agents (Buring and Hennekens 1992; The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group 1994; Hennekens et al. 1996; Omenn et al. 1996a). **Figure 4.2** illustrates how nutraceuticals are effective against cancer.

4.5 Natural antioxidants combating cancer

There are certain natural antioxidants obtained from food or algal products that protect against cancer. These include beta-carotene, vitamin C, and vitamin E.

4.5.1 Beta-carotene

Beta-carotene is a carotenoid derivative obtained from plants that has anti-cancer properties due to their pro-retinol action (Peto et al. 1981; Hennekens 1986). Numerous explorations have shown the inversely proportional relationship of cancer and intake of fruits and vegetables containing elevated amounts of beta-carotene (Peto et al. 1981; Willet and MacMahon 1984; Hennekens 1986; Wald 1987; Ziegler 1989). The mechanistic properties explaining their anti-cancer activity include quenching of reactive species such as organic and peroxide free radicals along with singlet oxygen (Peto et al. 1981; Burton and Ingold 1984; Krinsky 1989; Mathews 1989).

4.5.2 Vitamin C

Cisplatin is a known anti-cancer agent that produces toxicity in the liver (hepatotoxicity) but its main side effect is that it completely impairs the functions of the kidney (Meyer and Madias 1994; Liu et al. 1998). The drug generates ROS that includes hydroxyl radicals as well as superoxide species (Masuda et al. 1994; Baliga et al. 1998; Matsushima 1998) and also hinders the enzymatic activity of the antioxidant enzymes in the kidney (Appenroth et al. 1997). Lusania et al. in 2000 studied the effect of vitamin C and revealed that this may inhibit lipid peroxidation caused by cisplatin or depletion in glutathione. It also lowered the glomerular filtration rate and when pretreatment was done with vitamin C there was no increase in the serum creatinine level. The scientists also revealed that the free radicals produced due to cisplatin are either completely stopped or may be quenched before these radicals reached the kidney cells disrupting its function.

4.5.3 Vitamin E

Studies in the previous decade suggest that vitamin E consists of certain varieties that have anti-cancer properties (Sylvester and Theriault 2003; Friderich 2004). The vitamin E varieties of tocopherols and tocotrienols individually have different potentials in tumor growth inhibition as well as apoptosis induction in the neoplastic epithelial cells of the mammary glands (McIntyre et al. 2000a, b). Scientific explorations have also proved that synthetic anti-cancer drugs combined with natural antioxidants have a more potent effect. For example, tamoxifen, when combined with tocotrienols, had a more potent effect than tamoxifen alone (Guthrie et al. 1997). The anti-cancer property of this vitamin E derivative may not be only due to its antioxidant nature but also depends on the activities such as antiproliferation, enhancing the immunity, anti-angiogenesis, and stimulating apoptosis.

Explorations have indicated that tocotrienols can act on several types of cancer cells and cause apoptosis by both intrinsic as well as extrinsic mechanisms. In the extrinsic method, death receptors are stimulated (Park et al. 2010) and caspase-8 is activated, which in turn activates caspase-3 (Shah and Sylvester 2004). Tocotrienols also work by inhibiting the expression of Vasoactive Intestinal Growth Factor (VEGF) (Shibata et al. 2008; Weng-Yew et al. 2009) as well as its receptors (Miyazawa et al. 2008; Nakagawa 2009; Shibata et al. 2009), thereby suppressing angiogenesis. These vitamin E derivatives have also drawn attention due to their interaction with estrogen receptors when studied in breast cancer cells (Guthrie et al. 1997; Comitato et al. 2010). Studies reveal that compared to a- or b-tocotrienols, both d- and g-derivatives show enhanced anti-cancer activity (Kamat et al. 1997; Nesaretnam et al. 1998; Constantinou et al. 2009; Wu and Ng 2010). The anti-cancer properties of vitamin E derivative, tocotrienols, are discussed in **Table 4.3**.

Table 4.3 Anti-cancer Properties of Vitamin E Derivative: Tocotrienols

Cancer Type	Result	Reference
In Vitro Studies (Cancer Cell Line)		
Human breast cancer cell lines	Inhibited proliferation	Nesaretnam et al. 1995; Nesaretnam et al. 1998; Guthrie et al. 1997
Prostate cancer cells	Inhibited proliferation	Conte 2004; Srivastava and Gupta 2006
Human and murine tumor cells	Induced cell-cycle arrest in the G-1 phase and apoptosis in the G-1 phase	Yu et al. 1999
Neoplastic mammary epithelial cells	Inhibited proliferation-induced apoptosis	McIntyre et al. 2000a, b
Estrogen-independent MDA–MB–231 cells	Modulated 46 out of 1200 genes and affected cell homeostasis	Nesaretnam et al. 2004
Human colorectal adenocarcinoma cells DLD-1	Downregulation of telomerase activity	Eitsuka et al. 2006
Hepatocellular carcinoma HepG2 cells	Antiproliferative effect	Wada et al. 2005
In Vivo Studies		
Human colorectal cancer cells	Anti-angiogenesis	Nakagawa et al. 2007
Liver and lung cancer	Antiproliferative effect	Wada et al. 2005

4.5.4 Curcumin

The rhizome of *Curcuma longa*, a plant belonging to the family of ginger contains a spice known as turmeric. Turmeric, in turn, consists of curcuminoids that are fat-soluble polyphenols responsible for the color of turmeric. The main curcuminoid in turmeric is curcumin whose chemical name is diferuloylmethane ($C_{21}H_{20}O_6$) and is the major active component. The plant grows in nature throughout India and also in other tropical nations, predominantly in Southeast Asia. This spice obtained has been an important constituent of ayurvedic medication in India from 1900 BCE. The anti-cancer properties were studied in a MDR variety of breast cancer cell lines (MCF-7R) and were found to be significant in both MCF-7 and MCF-7R cell lines (Labbozzetta et al. 2009). According to Labbozzetta et al. (2009), curcumin might work in breast carcinoma through ER-dependent and independent ways to apply its anti-cancer effects and thereby act as an MDR reversal drug intervened by a transporter. The other cell lines where curcumin showed significant effects were bladder carcinoma (Tian et al. 2008) and prostate cancer cell lines (Dorai et al. 2001; Aggarwal 2008). *In vivo* studies showed that *in situ* apoptosis can be stimulated by curcumin, which in turn can inhibit the progression of bladder cancer.

Scientists have also revealed that curcumin can be used as ointments against oral (oral cavity), skin, and breast cancer (Kuttan et al. 1987). They found

that curcumin as an ointment caused a significant relief in the symptoms of patients with cancers that are external.

4.5.5 Lycopene

Though there have been many studies reporting the anti-cancer properties of lycopene, a tomato carotenoid, the exact mechanism remain unknown. This carotenoid inhibits the progression of breast and endometrial cancer in humans by arresting the cell cycle in G1 phase, which makes it more significant as a defensive agent for cancer. Zhang et al. in 1997 reported that lycopene is a potential agent that lowers the risk of breast carcinoma, whereas Giovannucci et al. in 1995 reported against prostate cancer and Michaud et al. in 2000 against lung carcinoma. Lycopene also works significantly against other types of malignancies, including mammary, endometrial (Levy et al. 1995), along with leukemic carcinoma cells (Amir et al. 1999). All the studies showed that lycopene worked by inhibiting cancer expansion by delaying the transfer of cell cycle from G1 to S phase.

4.6 Conclusion

Technical explorations and the fast speed of recent advancements have opened some ways to identify the anti-cancer properties as well as the mechanisms of the natural antioxidants. However, the exact pathways followed by the vitamin E derivatives are still unknown, and hence scientists are working on it throughout the globe. Hence, a closer look is required to understand the exact function of these antioxidants in the system for both their antioxidant and non-antioxidant properties.

Antioxidant nutraceuticals have proved to have many functions, some of which are critical to the human body. Research has been conducted on antioxidant nutraceuticals to understand their mechanistic properties. These are quite beneficial to the human body as a whole and in supplement form as well. They help the body without causing many side effects, which is another reason why the antioxidant nutraceuticals are preferred over synthetic drugs.

References

Aggarwal, B. B. 2008. Prostate cancer and curcumin: Add spice to your life. *Cancer Biol Ther* 7:1427–1435.

Albany, N. Y. 2013. Transparency market research. *Nutraceuticals World*.

Amir, H., M. Karas, J. Giat, M. Danilenko, R. Levy, T. Yermiahu, J. Levy, and Y. Sharoni. 1999. Lycopene and 1,25-dihydroxyvitamin D3 cooperate in the inhibition of cell cycle progression and induction of differentiation in HL-60 leukemic cells. *Nutr Cancer* 33:105–112.

Antunes, L. M. G., J. D. Darin, and M. D. Bianchi. 2000. Protective effects of vitamin Cagainstcisplatin-induced nephrotoxicity and lipid peroxidation in adult rats: Adose dependent study. *Pharmacol Res* 41:405–411.

Appenroth, D., S. Frob, L. Kersten, E. K. Splinter, and K. Winnefeld. 1997. Protective effects of vitamin E and C on cisplatin nephrotoxicity in developing rats. *Arch Toxicol* 71:677–683.

Aviram, M. 1993. Modified forms of low density lipoprotein and atherosclerosis. *Atherosclerosis* 98:1–9.

Baliga, R., Z. Zhang, M. Baliga, N. Ueda, and S. Shah. 1998. *In vitro* and *in vivo* evidence suggesting a role for iron in cisplatin-induced nephrotoxicity. *Kidney Int* 53:394–401.

Buring, J. E. and C. H. Hennekens. 1992. The Women's health study: Summary of the study design. *J Myocard Ischemia* 4:27–29.

Burton, G. W. and K. U. Ingold. 1984. Beta-carotene: An unusual type of lipid antioxidant. *Science* 224:7569–7573.

Cao, G. H., R. M. Russell, N. Lischner, and R. L. Prior. 1998. Serum antioxidant capacity is increased by consumption of strawberries, spinach, red wine or vitamin C in elderly women. *J Nutr* 128 (12):2383–2390.

Centers for Disease Control and Prevention 1991. Cigarette smoking among adults—United States. *MMWR Morb Mortal Wkly Rep* 42:230–233.

Choi, B. C. K., R. Bonita, and D. V. McQueen. 2001. The need for global risk factor surveillance. *J Epidemiol Community Health* 55 (6):370.

Comitato, R., G. Leoni, R. Canali, R. Ambra, K. Nesaretnam, and F. Virgili. 2010. Tocotrienols activity in MCF-7 breast cancer cells: Involvement of ERbeta signal transduction. *Mol Nutr Food Res* 64:327–332.

Constantinou, C., J. A. Hyatt, P. S. Vraka, A. Papas, K. A. Papas, C. Neophytou, V. Hadjivassiliou, and A. I. Constantinou. 2009. Induction of caspase-independent programmed cell death by vitamin Enatural homologs and synthetic derivatives. *Nutr Cancer* 61:864–874.

Conte, C., A. Floridi, C. Aisa, M. Pirrodi, A. Floridi, and F. Galli. 2004. Tocotrienol metabolism and antiproliferative effect in prostatecancer cells. *Ann N Y Acad Sci* 1031:391–394.

Das, L., E. Bhaumik, U. Raychaudhuri, and R. Chakraborty. 2012. Role of nutraceuticals in human health. *J Food Sci Technol* 49 (2):173–183.

Dorai, T., Y. C. Cao, B. Dorai, R. Buttyan, and A. E. Katz. 2001. Therapeutic potential of curcumin in human prostate cancer. III. Curcumin inhibits proliferation, induces apoptosis, and inhibits angiogenesis of LNCaP prostate cancer cells in vivo. *Prostate* 47:293–303.

Doughari, J. H., S. Human, S. Bennade and P. A. Ndakidemi. 2009. Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. *J Med Plants Res* 3 (11):839–848.

Eitsuka, T., K. Nakagawa, and T. Miyazawa. 2006. Down-regulation of telomerase activity in DLD-1 human colorectal adenocarcinoma cells by tocotrienol. *BBRC* 348:170–175.

Enstrom, J. E., L. E. Kanim, and M. A. Klein. 1992. Vitamin C intake and mortality among a sample of the United States population. *Epidemiology* 3:194–202.

Frazão, E. 1999. America's eating habits: Changes and consequences. *Agric Inf Bull* (750):494.

Friderich, M. J. 2004. To "E" or not to "E", vitamin E's role in health and disease is the question. *Jama* 292:671–673.

Gaziano, J. M., J. E. Manson, L. G. Branch, G. A. Colditz, W. C. Willett, and J. E. Buring. 1995. A prospective study of consumption of carotenoids in fruits and vegetables and decreased cardiovascular mortality in the elderly. *Ann Epidemiol* 5:255–260.

Giovannucci, E., A. Ascherio, E. B. Rimm, M. J. Stampfer, G. A. Colditz, and W. C. Willett. 1995. Intake of carotenoids and retino in relation to risk of prostate cancer. *J Natl Canc Inst* 87:1767–1776.

Goiris, K., K. Muylaert, I. Fraeye, I. Foubert, J. De Brabanter, and L. De Cooman. 2012. Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. *J Appl Phycol* 24 (6):1477–1486.

Greenwald, P. 1993. NCI cancer prevention and control research. *Prev Med* 22:642–660.
Grossman, S., S. Dovrat, and M. Bergman. 2011. Natural antioxidants: Just free radical scavengers or much more? *Trends Cancer Res* 7:57–73.
Grossman, S., R. Reznik, T. Tamari, and M. Albeck. 1994. New plant water soluble antioxidant (NAO) from spinach. In: Asada K. and T. Toshikawa. (Eds.). *Frontiers of Reactive Oxygen Species in Biology and Medicine*. Excerpta medica international congress series: Elsevier, pp. 57–73.
Guthrie, N., A. Gapor, A. F. Chambers, and K. K. Carroll. 1997. Inhibition of proliferation of estrogen receptor-negative MDA-MB-435 and positive MCF-7 human breast cancer cells by palm oil tocotrienol and tamoxifen, alone and in combination. *J Nutr* 127:544S–548S.
Hennekens, C. H., J. E. Buring, J. E. Manson, M. Stampfer, B. Rosner, N. R. Cook, C. Belanger. et al. 1996. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *N Engl J Med* 334:1145–1149.
Hennekens, C. H., S. L. Mayrent, and W. Willett. 1986. Vitamin A, carotenoids and retinoids. *Cancer* 58 (8):1837–1841.
Kamat, J. P., H. D. Sarma, T. P. Devasagayam, K. Nesaretnam, and Y. Basiron. 1997. Tocotrienols from palm oil as effective inhibitors of protein oxidation and lipid peroxidation in rat liver microsomes. *Mol Cell Biochem* 170:131–137.
Karawita, R., M. Senevirathne, Y. Athukorala, A. Affan, Y. J. Lee, S. K. Kim, J. B. Lee, and Y. J. Jeon. 2007. Protective effect of enzymatic extracts from microalgae against DNA damage induced by H_2O_2. *Mar Biotechnol* 9 (4):479–490.
Kelman, D., E. K. Posner, K. J. McDermid, N. K. Tabandera, P. R. Wright, and A. D. Wright. 2012. Antioxidant activity of Hawaiian marine algae. *Mar Drugs* 10 (2):403–416.
Kim, K. N., S. J. Heo, C. B. Song, J. Lee, M. S. Heo, I. K. Yeo, K. A. Kang, J. W. Hyun, and Y. J. Jeon. 2006. Protective effect of ecklonia cava enzymatic extracts on hydrogen peroxide-induced cell damage. *Process Biochem* 41 (12):2393–2401.
Kramer, K., P. P. Hoppe, and L. Packer. 2001. *Nutaceuticals in Health and Disease Prevention*. New York: Marcel Dekkar.
Krinsky, N. I. 1989. Carotenoids as chemopreventive agents. *Prev Med* 18:592–602.
Kuttan, R., P. C. Sudheeran, and C. D. Josph. 1987. Turmeric and curcumin as topical agents in cancer therapy. *Tumori* 73:29–31.
Labbozzetta, M., M. Notarbartolo, P. Poma, A. Maurici, L. Inguglia, P. Marchetti, M. Rizzi. et al. 2009. Curcumin as a possible lead compound against hormone-independent, multidrug-resistant breast cancer. *Ann NY Acad Sci* 1155:278–283.
Lee J., N. Koo, and D. B. Min. 2006. Reactive oxygen species, aging, and antioxidative nutraceuticals. *Compr Rev Food Sci Food Saf* 3 (1):21–33.
Levy, J., E. Bosin, B. Feldman, Y. Giat, A. Miinster, M. Danilenko, and Y. Sharoni. 1995. Lycopene is a more potent inhibitor of human cancer cell proliferation than either α-carotene or β-carotene. *Nutr. Cancer* 24:257–267.
Lippman, S. M. 2009. In vitro antioxidant and inhibitory potential of *Terminalia bellerica* and *Emblica officinalis* fruits against LDL oxidation and key enzymes linked to type 2 diabetes. *J Med Plants Res* 3 (11):839–848.
Liu, J., Y. Liu, S. S. M. Habeebu, and C. D. Klaassen. 1998. Metal-lothionein MT null mice are sensitive to cisplatin-induced hepatotoxicity. *Toxicol Appl Pharmacol* 149:24–31.
Lordan, S., R. P. Ross, and C. Stanton. 2011. Marine bioactives as functional food ingredients: Potential to reduce the incidence of chronic diseases. *Mar Drugs* 9 (6):1056–1100.
Masuda, H., T. Tanaka, and U. T. Takahama. 1994. Cisplatin generates superoxide anion by interaction with DNA ina cell-free system. *Biochem Biophys Res Commun* 203:1175–1180.
Mathews-Roth, M. M. 1989. *Beta-carotene, Canthaxanthin and Phytoene. Nutrition and Cancer Prevention: Investigating the Role of Micronutrients*. New York: Marcel Dekker, pp. 273–290.

Matsushima, H., K. Yonemura, K. Ohishi, and A. Hishida. 1998. The role of oxygen free radicals in cisplatin-inducedacute renal failure in rats. *J Lab Clin Med* 131:518–526.
McIntyre, B. S., K. P. Briski, A. Gapor, and P. W. Sylvester. 2000a. Antiproliferative and apoptotic effects of tocopherols and tocotrienols on preneoplastic and neoplastic mouse mammary epithelial cells. *Proc Soc Exp Biol Med* 224:292–301.
McIntyre, B. S., K. P. Briski, M. A. Tirmenstein, A. M. W. Fariss, A. Gapor, and P. W. Sylvester. 2000b. Antiproliferative and apoptotic effects of tocopherols and tocotrienols on normal mouse mammary epithelial cells. *Lipids* 35:171–180.
Meyer, K. B. and M. E. Madias. 1994. Cisplatin nephrotoxicity. *Miner Electrolyte Metab* 20:201–213.
Michaud, D. S., D. Feskanich, E. B. Rimm, G. A. Colditz, F. E. Speizer, W. C. Willett, and E. Giovannucci. 2000. Intake of specific carotenoids and risk of lung cancer in 2 prospective US cohorts. *Am J Clin Nutr* 72:990–997.
Miyashita, K., S. Nishikawa, F. Beppu, T. Tsukui, M. Abe, and M. Hosokawa. 2011. The allenic carotenoid fucoxanthin, a novel marine nutraceutical from brown seaweeds. *J Sci Food Agr* 91 (7):1166–1174.
Miyazawa, T., A. Shibata, P. Sookwong, Y. Kawakami, T. Eitsuka, A. Asai, S. Oikawa, and K. Nakagawa. 2009. Antiangiogenic and anticancer potential of unsaturated vitamin E (tocotrienol). *J Nutr Biochem* 20 (2):79–86.
Naik, G. H., K. I. Priyadarsini, R. G. Bhagirathi, B. Mishra, M. P. Mishra, M. M. Banavalikar, and H. Mohan. 2005. In vitro antioxidant studies and free radical reactions of triphala, an ayurvedic formulation and its constituents. *Phytother Res* 19 (7):582–586.
Nakagawa, K., A. Shibata, A. Yamashita, A. T. Tsuzuki, J. Kariya, S. Oikawa, and T. Miyazawa. 2007. In vivo angiogenesis is suppressed by unsaturated vitamin E. *J Nutr* 137:1938–1943.
Nesaretnam, K., R. Ambra, K. R. Selvaduray, A. Radhakrishnan, R. Canali, and F. Virgili. 2004. Tocotrienol-rich fraction from palm oil and gene expression in human breast cancer cells. *Ann N Y Acad Sci* 1031:143–157.
Nesaretnam, K., N. Guthrie, A. F. Chambers, and K. K. Carroll. 1995. Effect of tocotrienol on the growth of a human breast cancer cell line in culture. *Lipids* 30:1139–1143.
Nesaretnam, K., R. Stephen, R. Dils, and P. Darbre. 1998. Tocotrienols inhibit the growth of human breast cancer cells irrespective of estrogen receptor status. *Lipids* 33:461–469.
Nicholson, W. J., G. Perkel, and I. J. Selikoff. 1982. Occupational exposure to asbestos: Population at risk and projected mortality—1980–2030. *Am J Ind Med* 3:259–311.
Omenn, G. S., G. Goodman, M. Thornquist, J. Grizzle, L. Rosenstock, S. Barnhart, J. Balmes, M. G. Cherniack, M. R. Cullen, A. Glass, and J. Keogh. 1994. The β-Carotene and Retinol Efficacy Trial (CARET) for chemoprevention of lung cancer in high risk populations: Smokers and asbestos-exposed workers. *Cancer Res* 54 (7):2038s–2043s.
Omenn, G. S., G. Goodman, M. Thornquist, S. Barnhart, J. Balmes, M. Cherniack, M. Cullen. et al. 1996a. Chemoprevention of lung cancer: The beta-Carotene and Retinol Efficacy Trial (CARET) in high-risk smokers and asbestos-exposed workers. *IARC Sci Publ* 136:67–85.
Omenn, G. S., G. E. Goodman, M. D. Thornquist, J. Balmes, M. R. Cullen, A. Glass, J. P. Keogh. et al. 1996b. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med* 334:1150–1155.
Omenn, G. S., J. Merchant, E. Boatman, J. M. Dement, M. Kuschner, W. Nicholson, J. Peto, and L. Rosenstock. 1986. Contribution of environmental fibers to respiratory cancer. *Environ Health Perspect* 70:51–56.
Park, S. K., B. G. Sanders, and K. Kline. 2010. Tocotrienols induce apoptosis in breast cancer cell lines via an endoplasmic reticulum stress-dependent increase in extrinsic death receptor signaling. *Breast Cancer Res Treat* 124 (2):361–375.
Peto, R., R. Doll, J. D. Buckley, and M. B. Sporn. 1981. Can dietary beta-carotene materially reduce human cancer rates? *Nature* 290:201–208.

Plaza, M., A. Cifuentes, and E. Ibánez. 2008. In the search of new functional food ingredients from algae. *Trends Food Sci Tech* 19 (1):31–39.
Rimm, E. B., M. J. Stampfer, A. Ascherio, E. Giovannucci, G. A. Colditz, and W. C. Willett. 1993. Vitamin E consumption and the risk of coronary heart disease in men. *N Engl J Med* 328:1450–1456.
Salonen, J. T., S. Yla-Herttuala, R. Yamamoto, H. Korpela, R. Salonen, K. Nyyssonen, S. Butler, W. Palinski, and J. L. Witztum. 1992. Autoantibody against oxidised LDL and progression of carotid atherosclerosis. *Lancet* 339:883–887.
Schoenborn, C. A. and G. M. Boyd. 1987. Smoking and other tobacco use: United States. *Vital Health Stat* 10 (169):89–1597.
Shah, S. and P. W. Sylvester. 2004. Tocotrienol-induced caspase-8 activation is unrelated to death receptor apoptotic signaling in neoplastic mammary epithelial cells. *Exp Biol Med* 229 (8):745–755.
Shibata, A., K. Nakagawa, P. Sookwong, T. Tsuzuki, S. Oikawa, and T. Miyazawa. 2008. Tumor anti-angiogenic effect and mechanism of action of δ-tocotrienol. *Biochem Pharmacol* 76 (3):330–339.
Shibata, A., K. Nakagawa, P. Sookwong, T. Tsuduki, S. Oikawa, and T. Miyazawa. 2009. δ-Tocotrienol suppresses VEGF induced angiogenesis whereas α-tocopherol does not. *J Agric Food Chem* 57 (18):8696–8704.
Shebis, Y., D. Iluz, Y. Kinel-Tahan, Z. Dubinsky, and Y. Yehoshua. 2013. Natural antioxidants: Function and sources. *Food and Nutrition Sciences*. 4 (6):643.
Srivastava, J. K. and S. Gupta. 2006. Tocotrienol-rich fraction of palm oil induces cell cycle arrest and apoptosis selectively inhuman prostate cancer cells. *Biochem Biophys Res Commun* 346:447–453.
Stampfer, M. J., C. H. Hennekens, J. E. Manson, G. A. Colditz, B. Rosner, and W. C. Willett. 1993. Vitamin E consumption and the risk of coronary disease in women. *N Engl J Med* 328:1444–1449.
Steinberg, D. 1992. Antioxidants in the prevention of human atherosclerosis: Summary of the proceedings of a National Heart, Lung, and Blood Institute workshop: Bethesda, Maryland. *Circulation* 85:2337–2344.
Steinberg, D., S. Parthasarathy, T. E. Carew, J. C. Khoo, and J. L. Witztum 1989. Beyond cholesterol: Modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 320:915–924.
Sylvester, P. W. and A. Theriault. 2003. A role of tocotrienols in the prevention of cardiovascular disease and breast cancer. *Curr Top Nutraceutical Res* 1:121–136.
The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. 1994. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med* 330:1029–1035.
Tian, B., Z. Wang, Y. Zhao, D. Wang, Y. Li, L. Ma, X. Li. et al. 2008. Effects of curcumin on bladder cancer cells and development of urothelial tumors in a rat bladder carcinogenesis model. *Cancer Lett* 264:299–308.
Villaseñor I. M., M. K. Simon, and A. M. Villanueva. 2002. Comparative potencies of nutraceuticals in chemically induced skin tumor prevention. *Nutr Cancer* 44 (1):66–70.
Wada, S., Y. Satomi, M. Murakoshi, N. Noguchi, T. Yoshikawa, and H. Nishino. 2005. Tumor suppressive effects of tocotrienol in vivo and in vitro. *Cancer Lett* 229:181–191.
Wald, N. 1987. Retinol, beta-carotene and cancer. *Cancer Surv* 6:635–651.
Weng-Yew, W., K. R. Selvaduray, C. H. Ming, and K. Nesaretnam. 2009. Suppression of tumor growth by palm tocotrienols via the attenuation of angiogenesis. *Nutr Cancer*, 61 (3):367–373.
Willet, W. C. and B. MacMahon. 1984. Diet and cancer-an overview. *N Engl J Med* 310:633–638.
Wingo, P. A., T. Tong, and S. Bolden. 1995. Cancer statistics. CA *Cancer J Clin* 45:8–30.
Wu, S. J. and L. T. Ng. 2010. Tocotrienols inhibited growth and induced apoptosis in human HeLa cells through the cell cycle signaling pathway. *Integr Cancer Ther* 9:66–72.

Yu, W., M. Simmons-Menchaca, A. Gapor, B. G. Sanders, and K. Klinr. 1999. Induction of apoptosis in human breast cancer cells by tocopherols and tocotrienols. *Nutr Cancer* 33:26–33.

Zhang, S. M., G. Tang, R. M. Russell, K. A. Mayzel, M. J. Stampfer, W. C. Willett, and D. J. Hunter. 1997. Measurement of retinoids and carotenoids in breast adipose tissue and a comparison of concentrations in breast cancer cases and control subjects. *Am J Clin Nutr* 66:626–632.

Ziegler, R. G. 1989. A review of epidemiologic evidence that carotenoids reduce the risk of cancer. *J Nutr* 119:116–122.

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

5

Antioxidant Nutraceuticals, Alzheimer's Disease, and Dementia

Nandini Doshi, Sarvadaman Pathak, and Kiran Patil

Contents

The essential features of dementia are acquired and persistently compromise multiple cognitive domains which interfere with daily activities. Dementia is broadly classified into cortical and subcortical dementia. In a subcortical dementia there is impaired information processing, whereas in cortical dementia, there is domain-specific deficits such as aphasia, apraxia, and agnosia. Alzheimer's disease (AD) is the most common type of dementia, although there are other types of dementia, which are present such as frontotemporal dementia Parkinsonian dementia, dementia with Lewy bodies, progressive supranuclear palsy, dementia associated with drugs, dementia due to infections, and vascular dementia. AD in neurodegenerative disorder featuring gradually progressing cognitive and functional deficits as well as behavioral changes is associated with accumulation of amyloid and tau deposition in the brain. Cognitive symptoms of AD most commonly include deficits in short-term memory, executive and visuospatial dysfunction, and praxis. AD is the most common neurodegenerative disorder and the sixth most common cause of disease in the United States. Although there is increasing evidence that AD pathology starts in brain in midlife, the first clinical symptoms usually observed were after the age of 65.

In 2012, the World Health Organization declared dementia as a public health priority. AD prevalence is rapidly increasing in large part because of the

proportion of people who are 65 years and older is increasing than any other age sector of the population worldwide. Although age is the greatest risk factor for development of AD, it is not sufficient to cause AD. Other risk factors include presence of apolipoproteins gene alleles (APOE4), low educational and occupational attainment, prior history of traumatic brain injuries, cardiovascular risk factors as well as family history of dementia. Memory impairment is the most important feature of AD. In one nationally representative U.S. data, the Aging demographics and memory study (ADAMS) estimated that in the United States 14% of people who are 71 years and older have dementia. AD dementia accounted for 70% of the dementia cases across the age spectrum in this cohort. The number of cases of dementia in 2010 was estimated at 35.6 million worldwide. For diagnosing dementia the prominent neuropsychiatric symptoms are cognitive dysfunction, which is commonly seen in all types of dementia, although maybe accompanying other disorders to varying degrees. Neuropsychiatric assessment plays an important role in differential diagnosis between dementia and primary psychiatric disturbances such as major depression. There are battery of tests used to assess the cognitive ability of the patient, including mini-mental status assessment, wherein cognitive abilities such as language, executive functions language fluency is assessed. In addition, laboratory evaluation of vitamin B12, thyroid function test, and other blood works such as screening for syphilis as well as genetic testing for apolipoprotein and cerebral spinal fluid analysis for beta-amyloid and tau and phospho-tau does give additional information about the disease process.

The American academy of Neurology dementia practice guidelines recommended a noncontrast computed tomography as well as magnetic resonance imaging scan in most cases of dementia. Although the yield of such testing is relatively low for detecting structural brain lesion such as central nervous system neoplasm or subdural hematoma, it does give an idea about any structural asymmetry as well as temporoparietal abnormalities in the AD frontal anterior temporal abnormalities in frontotemporal dementia, temporal parietal–occipital abnormalities in dementia with Lewy bodies. Functional brain imaging with single-photon emission CT (SPECT) and positron emission tomography (PET) have identified disease-specific patterns for several dementias.

Treatment: The clinical management of patients with dementia involves multiple steps, initial diagnostic in prognostication as well as common shining counseling of all the patients about the date to do activities as well as the appropriate medication. Patient has coexisting behavioral and non-neurological conditions that need to be optimally managed; there is a need of coordination of care from the physician's nurse practitioner, social workers, and the institution where the patient has an advanced dementia. Two classes of medication have been approved: the first one is acetylcholine esterase inhibitors and the second one is NMDA receptor antagonist memantine. Three medicines have been currently approved and marketed in the United States: (1) Donepezil, (2) rivastigmine, and (3) galantamine. Glutamate receptor modulator, memantine, has low-to-moderate affinity for N-methyl-D-aspartic acid (NMDA) receptor

and is used as an add-on treatment for dementia. Memantine is approved by U.S. FDA for moderate-to-severe Alzheimer dementia in the United States. Cholinesterase inhibitors and memantine are frequently prescribed off-label for mild cognitive impairment, but in recent meta-analysis they have not been demonstrated. In addition to the first-line treatment, there is lot of medication use for behavioral symptoms with selective serotonin reuptake inhibitors, which is commonly used for patients with agitation or disruptive behavior. The new medications are rate typical antipsychotic medications such as choir 13 risperdal on olanzapine are often used in low doses with careful titration. The drugs currently available (i.e., anticholinesterase and memantine) are symptomatic and can only temporarily slow down AD symptoms (Aisen 2010, Vellas and Aisen 2010). Because of their symptomatic action, they are intended only for patients with mild-to-severe AD. One of the main AD challenges over the coming decade lies in the finding of a curative drug that could modify the neurodegenerative process (Aisen 2010, Vellas and Aisen 2010).

5.1 Vitamin B12

Vitamin B12 is an important vitamin also known as cobalamin and it is a water-soluble vitamin and plays a role in normal functioning of the brain. Vitamin B12 has been studied extensively and has a role in dementia, and vitamin B12 is an important component for normal neuronal functioning. The common cause of vitamin B12 deficiency is due to low intakes, malabsorption, certain intestinal disorders, low presence of binding proteins, and use of certain medications. In addition, vitamin B12 is only found in animal products. The deficiency of vitamin B12 is commonly seen in people with vegetarian diet as well as in elderly population with low intake of animal products. Serum vitamin B12 has a wide range or normal lab values, with a low reference range of 200 to 220 pg/mL. Methylmalonic acid (MMA) is a substance produced in the body when there is a deficiency of vitamin B12, and so MMA concentration is considered to be a sensitive and specific indicator of vitamin B12 deficiency. MMA level will increase when vitamin B12 levels are less than 400 pg/mL. For patients with vitamin B12 levels less than 400 pg/mL, begin daily supplement with vitamin B12 at 1000–2000 mcg per day. Vitamin B12 may be given parenterally, nasally, or orally. Adequate treatment may be provided by any of these routes.

The functional deficiency of B12 can be detected by measuring levels of homocysteine (Hcy) and MMA. Hyperhomocystenemia has been associated with B vitamin deficiencies of folate, B6, and B12 due to their role as cofactors in the metabolism of Hcy. Vitamin B12 is also a cofactor in other methylation reactions. In particular, vitamin B12 deficiency has been shown to result in cognitive impairment (Moore et al. 2012). Although whether the cognitive impairment is a direct result of B12 deficiency or whether it is associated with subsequent hyperhomocystenemia is not clear. The probable mechanism for this observation could be linked to methylation of myelin basic protein.

Myelin basic protein is methylated on an arginine group and a defect in methylation could produce an unstable protein, leading to neurological disorders (Sponne et al. 2000). Increased levels of Hcy have been documented to produce changes in the structure and function of cerebral blood vessels along with oxidative stress, which plays a key role in cerebral vascular dysfunction (Dayal et al. 2005). Several animal models have shown the role of HHcy in cerebrovascular pathology, cognitive decline, and learning disabilities (Pirchl et al. 2010). The authors observed short- and long-term hyperhomocystenemia differentially affecting the spatial memory as tested in a partially baited eight-arm radial maze. Hyperhomocysteinemia (HHcy) significantly reduced the number of choline acetyltransferase (ChAT)-positive neurons in the basal nucleus of Meynert and ChAT-positive axons in the cortex only after short-term but not long-term treatment, whereas acetylcholine levels in the cortex were decreased at both the time points. Shah and Singh (2007) reported that HHcy induced via administration of L-methionine in rodents has been reported to produce a significant degree of VaD. Other studies have shown intracerebral Hcy injections in rodent brains to produce AD-like symptoms (Ataie et al. 2010, Kamat et al. 2013). The experiments done by Ataie et al. showed that curcumin antioxidant and neuroprotective properties of the polyphenolic antioxidant compound, Curcumin against Hcy neurotoxicity were investigated. Curcumin (5 and 50 mg/kg) was injected intraperitoneally once daily for a period of 10 days beginning 5 days prior to Hcy (0.2 μmol/μL) intrahippocampal injection in rats. Biochemical and behavioral studies, including passive avoidance learning and locomotor activity tests were studied 24 h after the last curcumin or its vehicle injection. We detected malondialdehyde (MDA) and superoxide anion (SOA) in rats' hippocampi. Results indicated that Hcy could induce lipid peroxidation and increase MDA and SOA levels in rats' hippocampi. In addition, Hcy impaired memory retention in passive avoidance learning test. However, curcumin treatment decreased MDA and SOA levels significantly as well as improved learning and memory in rats. Histopathological analysis also indicated that Hcy could decrease hippocampus cell count and curcumin inhibited this toxic effect. These results suggest that Hcy may induce lipid peroxidation in rats' hippocampi and polyphenol treatment (Curcumin) improved learning and memory deficits by protecting the nervous system against Hcy toxicity.

Hcy has been associated with dementia. High total plasma homocysteine (tHcy) has been associated with cognitive impairment and dementia (Bell et al. 1992, Clark et al. 1998), although it is unclear whether this link is causal. This is important because tHcy can be lowered by about 20% with oral supplementation of specific B vitamins marking it as a potentially modifiable risk factor. Hcy is a risk factor for AD. In the first report on the VITACOG trial, we showed that Hcy-lowering treatment with B vitamins slows the rate of brain atrophy in mild cognitive impairment (MCI).

The experiments done by Zhang et al. (2008) wherein Hcy was injected via vena caudalis injection for 2 weeks caused AD-like tau hyperphosphorylation at multiple sites in rat brain hippocampus. The proposed mechanism is Hcy inhibited

the activity of protein phosphatase 2A (PP2A) with a simultaneously increased Leu(309)-demethylation and Tyr(307)-phosphorylation of PP2A catalytic subunit (PP2A(C)). PP2A(C) Leu(309)-demethylation was positively correlated with its Tyr(307)-phosphorylation; and the abnormally modified PP2A(C) was incompetent in binding to its regulatory subunit (PP2A(B)). Hcy also activated methylesterase, which stimulates demethylation of PP2A(C). In hippocampal slices of the Hcy-injected rats and AD patients, the demethylated but not the methylated PP2A(C) was colocalized with the hyperphosphorylated tau. A simultaneous supplement of folate and vitamin B12 partially restored the plasma Hcy level, and thus significantly antagonized the Hcy-induced tau hyperphosphorylation and PP2A inactivation and the activity-related modifications of PP2A(C). These results suggest that Hcy may be an upstream effector to induce AD-like tau hyperphosphorylation through inactivating PP2A.

In an intervention trial done by de Jager et al. (2012), B vitamins appears to slow cognitive and clinical decline in people with MCI, in particular in those with elevated Hcy. B vitamins stabilized executive function (CLOX) relative to placebo ($P = 0.015$). There was significant benefit of B vitamin treatment among participants with baseline Hcy above the median (11.3 μmol/L) in global cognition (Mini Mental State Examination, $P < 0.001$), episodic memory (Hopkins Verbal Learning Test-delayed recall, $P = 0.001$), and semantic memory (category fluency, $P = 0.037$). Clinical benefit occurred in the B vitamin group for those in the upper quartile of Hcy at the baseline in global clinical dementia rating score ($P = 0.02$) and IQCODE score ($P = 0.01$).

5.2 Vitamin D

The term vitamin D describes a group of fat-soluble secosterols that encourage the regulation of serum calcium and phosphate levels and, consequently, bone growth and mineralization. Its two predominant forms, vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) achieve biological activation on two consecutive hydroxylations in the body—first in the liver to form calcifediol and then in the kidney to form biologically active hormone calcitriol. Although vitamin D2 is generally man-made and then mixed into foods, whereas vitamin D3 can be formed in the skin during exposure to sunlight or consumed through animal-based food products, both generating identical metabolic reactions and can also be ingested through dietary supplements (Institute of Medicine [IOM] 2011). Various dietary sources of vitamin D are noted in **Table 5.1**.

On reaching biological activation, vitamin D's metabolite calcitriol binds to the nuclear hormone receptor vitamin D receptor (VDR). The consequent conformational change in the receptor facilitates formation of a heterodimeric complex with retinoid X receptor (RXR) to act on Vitamin D response element (VDREs), specific sequences in the genome, and in turn, activate or suppress transcription of target genes (Bikle 2014, Christakos 2016). The widespread expression of VDR proteins and the enzymes required for the

Table 5.1 Examples of Food Sources Containing Vitamin D

Food	International Units	µg	Daily Value[a] (%)
Mushrooms, portabella, exposed to ultraviolet light, one cup (86 grams)	976	24.4	162
Salmon, pink, canned, three ounces (85 grams)	465	11.6	77.5
Fish oil, cod liver, one tsp (4.5 grams)	450	11.2	75
Sardines, Atlantic, canned in oil three ounces (85 grams)	164	4.1	27
Milk, nonfat, with added vitamins A and D, one cup (245 grams)	115	2.9	19
Orange juice from concentrate, with added calcium and vitamin D, one cup (249 grams)	100	2.5	16.7
Tuna white, canned in water three ounces (85 grams)	68	1.7	11.3
Beef liver 100 grams	49	1.2	8.2
One large egg (vitamin D found in yolk)	37	0.9	6.2

Source: Institute of Medicine 2011, National Institutes of Health 2016, U.S. Department of Agriculture 2016.

[a] Percent daily value calculated according to the 600 IU/day recommended dietary allowance (RDA) suggested by the U.S. Food and Drug Administration for children and adults over the age of four.

two-step hydroxylation of vitamin D into calcitriol in the brain, especially in regions associated with learning and memory, indicates that along with regulating calcium homeostasis and bone mineralization, vitamin D may influence cognitive function and dementia (Jones 1998, Garcion 2002, Eyles 2005, Buell 2008).

A multitude of evidence suggests that vitamin D possesses neuroprotective properties. For instance, multiple studies indicate that it regulates synthesis of neurotrophins, growth factors that enable the development and function of neurons (Garcion et al. 2002). Moreover, vitamin D also influences calcium homeostasis and synaptic transmission in the brain. An increase in L-type voltage-sensitive Ca^{2+} channels (L-VSCC) is seen during hippocampal aging (Landfield et al. 1998). Through downregulating hippocampal-VSCC expression, vitamin D offers neuroprotective benefits against age-related neurodegenerative conditions (Brewer et al. 2001). Likewise, a study performed by Latimer and colleagues demonstrated that increased vitamin D also upregulated pathways and genes involved in synaptic transmission that thus strengthened synaptic function in the hippocampus of aging rats (Latimer et al. 2014).

Vitamin D affects several mechanisms of AD pathogenesis, including the production, clearance, phagocytosis, and enzymatic degradation of Aβ peptides, as well as tau phosphorylation (Keeney and Butterfield 2015). Vitamin D3-enriched diets in rodents had shown a significant reduction of brain Aβ

burden along with improved cognitive performances (Yu et al. 2011, Briones and Darwish 2012, Durk et al. 2014). This is in line with the observation that vitamin D deficiency strengthens the spatial learning deficits of rats after intracerebroventricular Aβ42-injection.

The influence of vitamin D on the cerebral Aβ levels might be based on an increased brain-to-blood efflux transport of the peptide at the blood–brain barrier and a stimulation of microglial Aβ phagocytosis (Masoumi et al. 2009, Ito et al. 2011, Briones and Darwish 2012). An elevation of classical Aβ-degrading enzyme neprilysin (NEP) in the hippocampus, as well as a reduction in β-secretase amyloid cleaving enzyme (BACE1) level, has been shown in the brain tissue of aged rats after dietary supplementation of vitamin D3 (Briones and Darwish 2012). In addition, a significantly increased protein level and activity of BACE1 combined with a decreased expression and activity of NEP in the brain tissue of vitamin D-deficient mice was found (Grimm et al. 2014). An impact of vitamin D on Aβ degradation is further strengthened by the transcriptional upregulation of several Aβ-degrading enzymes, and hence total Aβ degradation in neuroblastoma cells treated with 25-$(OH)D_3$ (Grimm et al. 2014). In another study, 1α,25-$(OH)_2D_3$ has been found to inhibit APP promoter activity in a neuroblastoma cell line, indicating Aβ secretion to be reduced in the presence of this vitamin D metabolite due to a decreased gene expression of its precursor protein (Wang et al. 2012). In addition, vitamin D might affect tau phosphorylation, as previously published by Cheng et al. In this study, the combination of vitamin D and resveratrol has been found to reverse the Aβ25-35-induced cytotoxicity and tau phosphorylation in SH–SY5Y neuroblastoma cells treated with 25-$(OH)D_3$ (Grimm et al. 2014). In another study, 1α,25-$(OH)_2D_3$ has been found to inhibit APP promoter activity in a neuroblastoma cell line, indicating Aβ secretion to be reduced in the presence of this vitamin D metabolite due to a decreased gene expression of its precursor protein (Wang et al. 2012). In addition, vitamin D might affect tau phosphorylation, as previously published by Cheng et al. In this study the combination of vitamin D and resveratrol has been found to reverse the Aβ25-35-induced cytotoxicity and tau phosphorylation in SH–SY5Y cells (Cheng et al.).

5.3 Folic acid

Folate is reduced to dihydrofolate (DHF) and subsequently to tetrahydrofolate (THF), serving as a single carbon donor in the form of 5-methyl THF. Consequently, 5-methyl THF feeds into the one-carbon metabolism cycle by donating its methyl group to Hcy converting it to methionine. Folates are vitamins essential to the development of the central nervous system. Insufficient folate activity at the time of conception and early pregnancy can result in congenital neural tube defects. Folate acts through one-carbon metabolism to support the methylation of multiple substrates, including DNA. Folic acid upregulated DNA methylation levels in N2a-APP cells and AD transgenic

mouse brains. In addition, functional network analysis of folic acid-induced DMGs in these AD models revealed subnetworks composed of 24 focus genes in the janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathway and 12 focus genes in the long-term depression (LTD) signaling pathway (Li et al. 2016). In addition, the experiments showed folic acid treatment prevented memory impairment and Na(+), K(+)–ATPase inhibition in the striatum and cortex in adult rats that suffered neonatal hypoxia–ischemia (HI) (Carletti et al. 2016). Elevated plasma Hcy is one of the primary consequences of folate deficiency. Furthermore, older people with low folate status are at a higher risk of cognitive impairment, dementia, and AD (Riggs et al. 1996), and it has been postulated that the effect of folate deficiency on brain function is mediated by Hcy. However, several studies found that the association between low folate status and cognitive impairment, dementia, or AD remains significant after controlling for confounding by Hcy, thus suggesting that folate may affect the brain function through mechanisms not directly related to hyperhomocysteinemia (Riggs et al. 1996, Clarke et al. 1998, Wang et al. 2001, Quadri et al. 2004). Ebly et al. (1998) found that there was a higher percentage of subjects with dementia in the lowest quartile of serum folate (≤10 nmol/L) compared with the highest quartile (>14 nmol/L). Moreover, the mean 3MSE score for the subjects in the lowest quartile of serum folate was significantly lower than for subjects in the highest quartile.

5.4 Vitamin A

Vitamin A and its derivatives, the retinoids, are involved in several important cellular working in the brain, including neuronal differentiation, neurotransmitter release, and long-term potentiation. They have antioxidative properties and also helps in regulating gene expression by interacting with the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs) acting as transcription factors (Lane and Bailey 2005, Sodhi and Singh 2013).

Significantly lowered serum and plasma concentrations of vitamin A and the provitamin A β-carotene have been observed in AD patients (Jimenez-Jimenez et al. 1999, Rinaldi et al. 2003). In addition, enhanced β-carotene plasma levels have been found to be associated with better cognitive performances in the elderly (Perrig et al. 1997). Vitamin A deficiency results in an enhanced Aβ deposition in the brain tissue and in cerebral blood vessels of adult rats (Corcoran et al. 2004). In a recent study Aβ production was also shown to be increased in the brain parenchyma of a hypovitaminosis mouse model along with a reduction of sAPPα content indicating a shift from non-amyloidogenic to amyloidogenic APP processing (Reinhardt et al. 2016). In a double-transgenic AD mouse model, a robust decrease of cerebral Aβ accumulation along with improved cognitive functions was observed after treatment with all-trans retinoic acid over eight weeks (Ding et al. 2008). Similar effects on cognition and Aβ plaque load have been observed in mice with

streptozotocin-induced dementia after supplementation with all-trans retinoic acid. These animals also display a restored acetylcholinesterase activity, attenuated oxidative alterations, and a reduced content of myeloperoxidase, a marker of inflammation (Sodhi and Singh 2014). In line with this, treatment of APP/tau-double transgenic mice with a RARα agonist leads to the reduction of Aβ plaque load and enhanced cognitive performances. RARα signaling improves cognition in the Tg2576 mice; it has an anti-inflammatory effect and promotes Aβ clearance by increasing insulin-degrading enzyme and neprilysin activity in both microglia and neurons. In addition, RARα signaling prevents tau phosphorylation (Goncalves et al. 2013). In addition to the cerebral Aβ content, tau pathology and, thus, a further pathological hallmark of AD might be affected by vitamin A. Previously fewer and smaller tau aggregates have been observed in the brain tissues of APP/PS1/tau-transgenic mice supplemented with all-trans retinoic acid. This effect might result from a downregulation of the cyclin-dependent kinase 5 (Cdk5) and the glycogen synthase kinase 3β (GSK3β). In agreement with these data, a prevented tau phosphorylation has been demonstrated in APP/tau-double transgenic mice medicated with a RARα agonist, as well as in APP/PS1 transgenic mice treated with retinoic acid (Ding et al. 2008, Goncalves et al. 2013). Although the role of vitamin A is shown in animal models, there are no trials analyzing the efficacy of vitamin A supplementation on the progression of AD in humans.

5.5 Oxidative stress and role of vitamin C, ascorbic acid, vitamin E, and coenzyme Q10

Oxidative stress plays a major role in the neurodegeneration by causing DNA damage and increased protein and lipid oxidation. The role of antioxidative pathways sometimes is not adequate. In addition, oxidative protein damage contributes to agent-dependent accumulation of disturbance in mitochondria or protein aggregates. Vitamin C is extremely important as an antioxidant owing to its ability to neutralize oxygen and nitrogen-based radicals, and because it also recycles both vitamin E and tetrahydrobiopterin, which respectively have key antioxidant and enzyme cofactor functions in brain. Furthermore, vitamin C is a cofactor in a number of hydroxylation reactions where it protects hydroxylase enzymes by reducing Fe^{3+} and Cu^{2+} at their active sites, such as in the synthesis of collagen, carnitine, and norepinephrine and also in the regulation of the gene HIF1-α 9 (Harrison and May 2009).

The current U.S. RDA for vitamin C is 75 mg/day in adult females, 90 mg/day in males, with amounts up to 125 mg/day recommended for pregnant or lactating women (Monsen 2000). Oxidative stress is important in the pathogenesis of AD. The supplementation of the primary antioxidant system helps to suppress the initiation of oxidative stress, which has been tested in animal models and has shown promising results.

The brain contains high levels of oxidizable lipids that must be protected by antioxidants. Low concentrations of vitamin E, quantitatively the major lipophilic antioxidant in the brain, are frequently observed in cerebrospinal fluid (CSF) of AD patients, suggesting that supplementation with vitamin E might delay the development of AD. In a placebo-controlled trial, vitamin E (2000 IU/day, 2 years) slowed (−53%) functional deterioration in patients with moderate AD (Sano et al. 1997). In addition, vitamin E and vitamin C supplementation, in combination, has been associated with reduced prevalence (−78%) and incidence (−64%) of AD in elderly population (Zandi et al. 2004). These results are consistent with the ability of the supplementation with vitamin E (400 IU/day, 1 month) to increase its level in CSF (123%) and plasma (145%) of AD patients and, in combination, with vitamin C (1000 g/day), to decrease the susceptibility of CSF lipoproteins (up to −32%) to *in vitro* oxidation (Kontush et al. 2001). In addition, vitamin E supplementation is given early on reduced lipid peroxidation and amyloid deposition in a transgenic mice model of AD (Sung et al. 2004).

Vitamin E has been reported to improve cognitive function in elderly individuals (Ortega et al. 2002). It is known that the soluble Aβ oligomers cause cognitive loss and synaptic dysfunction in AD patients. The treatment with vitamin C for six months attenuated Aβ oligomer formation, restored the reduced synaptophysin level, and mitigated the memory behavioral decline in an AD mouse model (Murakami et al. 2011). More recent research showed that vitamins C and E supplementation mitigated the melamine-induced impairment of hippocampal synaptic plasticity (An and Zhang 2014). The potential role of DHA and EPA in the prevention of cognitive decline, including the decline associated with AD, has attracted major interest over the past 20 years. Recent research showed that *n*-3 fatty acids supplementation ameliorated memory deficits, which increased the serum total antioxidant capacity (Abd Allah et al. 2014).

Dietary supplementation with coenzyme Q10 (CoQ10) could be a pharmacological way to enhance antioxidant defenses in mitochondria. CoQ10 is an important cofactor in the mitochondrial electron transport chain and has well-characterized antioxidant properties in mitochondria and lipid membranes. CoQ10 protects neuronal cells in culture from oxidative insults (McCarthy et al. 2004, Somayajulu et al. 2005). Orally administered CoQ10 reduced neuronal degeneration and increased survival in toxin-induced and transgenic animal models of Parkinson's and Huntington's diseases (Ferrante et al. 2002, Matthews et al. 1998). Concurrent administration of CoQ10 and α-tocopherol improved learning in aged mice (McDonald et al. 2005), and CoQ10 reduced amyloid pathology in presenilin mouse models of AD (Yang et al. 2008). The supplementation of CoQ10 on pathology and cognition in the Tg19959 mouse model of AD studies and noted decreased brain oxidative stress, Aβ42 levels, and plaque burden, and improved cognitive performance (Dumont et al. 2011).

Dementia is an irreversible neurodegenerative disorder that affects over 5 million Americans. Although we are now able to diagnose dementia in early

and even in three symptomatic stays is where we are still lacking preventive medication that can alter its course, so the use of antioxidants becomes very important in treating dementia and prolonging the disease process without having much of side effects, which are commonly seen with medication such as donepezil, rivastigmine, or memantine.

References

Abd Allah, E. S., A. M. Gomaa, and M. M. Sayed. 2014. The effect of omega-3 on cognition in hypothyroid adult male rats. *Acta Physiologica Hungarica* 101 (3):362–376. doi:10.1556/aphysiol.101.2014.3.11.

Aisen, P. S. 2010. Pre-dementia Alzheimer's trials: Overview. *J Nutr Health Aging* 14:294.

An, L. and T. Zhang. 2014. Vitamins C and E reverse melamine-induced deficits in spatial cognition and hippocampal synaptic plasticity in rats. *Neurotoxicology* 44:132–139. doi:10.1016/j.neuro.2014.06.009.

Ataie, A., M. Sabetkasaei, A. Haghparast, A. H. Moghaddam, and B. Kazeminejad. 2010. Neuroprotective effects of the polyphenolic antioxidant agent, Curcumin, against homocysteine-induced cognitive impairment and oxidative stress in the rat. *Pharmacol Biochem Behav* 96:378–385.

Bell, I. R., J. S. Edman, J. Selhub, F. D. Morrow, D. W. Marby, H. L. Kayne, and J. O. Cole. 1992. Plasma homocysteine in vascular disease and in nonvascular dementia of depressed elderly people. *Acta Psychiatr Scand* 86:386–390.

Brewer, L. D., V. Thibault, K. C. Chen, M. C. Langub, P. W. Landfield, and N. M. Porter. 2001. Vitamin D hormone confers neuroprotection in parallel with downregulation of L-type calcium channel expression in hippocampal neurons. *J Neurosci* 21 (1):98–108.

Briones, T. L. and Darwish, H. 2012. Vitamin D mitigates age-related cognitive decline through the modulation of pro-inflammatory state and decrease in amyloid burden. *J Neuroinflamm* 9: 244.

Carletti, J. V., B. F. Deniz, J. J. Rojas, P. M. Miguel, J. Kolling, E. B. Scherer, A. T. de Souza Wyse, C. A. Netto, and L. O. Pereira. 2016. Folic acid can contribute to memory deficit and Na+, K+- ATPase failure in the hippocampus of adolescent rats submitted to hypoxia-ischemia. *CNS Neurol Disord Drug Targets* 15 (1):64–72.

Cheng, C. M., V. Tseng, J. Wang, D. Wang, L. Matyakhina, and C. A. Bondy. 2005. Tau is hyperphosphorylated in the insulin-like growth factor-I null brain. *Endocrinology* 146 (12):5086–5091.

Clarke, R., A. D. Smith, K. A. Jobst, H. Refsum, L. Sutton, and P. M. Ueland. 1998. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. *Arch Neurol* 55:1449–1455.

Corcoran, J. P., P. L. So, and M. Maden. 2004. Disruption of the retinoid signalling pathway causes a deposition of amyloid β in the adult rat brain. *Eur J Neurosci* 20:896–902.

Dayal, S., A. M. Devlin, R. B. McCaw, M. L. Liu, E. Arning, T. Bottiglieri, B. Shane, F. M. Faraci, and S. R. Lentz. 2005. Cerebral vascular dysfunction in methionine synthase deficient mice. *Circulation* 112:737–744.

de Jager, C. A., A. Oulhaj, R. Jacoby, H. Refsum, and A. D. Smith. 2012. Cognitive and clinical outcomes of homocysteine-lowering B-vitamin treatment in mild cognitive impairment: A randomized controlled trial. *Int. J Geriatr Psychiatry* 27 (6):592–600.

Ding, Y., A. Qiao, Z. Wang, J. S. Goodwin, E. S. Lee, M. L. Block, M. Allsbrook, M. P. McDonald, and G. H. Fan. 2008. Retinoic acid attenuates β-amyloid deposition and rescues memory deficits in an Alzheimer's disease transgenic mouse model. *J Neurosci* 28:11622–11634.

Dumont, M., K. Kipiani, F. Yu, E. Wille, M. Katz, N. Y. Calingasan, G. K. Gouras, M. T. Lin, and M. F. Beal. 2011. Coenzyme Q10 decreases amyloid pathology and improves behavior in a transgenic mouse model of Alzheimer's disease. *J Alzheimers Dis* 27 (1):211–223.

Durk, M. R., K. Han, E. C. Chow, R. Ahrens, J. T. Henderson, P. E. Fraser, and K. S. Pang. 2014. 1α,25-dihydroxyvitamin D3 reduces cerebral amyloid-β accumulation and improves cognition in mouse models of Alzheimer's disease. *J Neurosci* 34:7091–7101.

Ebly, E. M., J. P. Schaefer, N. R. Campbell, and D. B. Hogan. 1998. Folate status, vascular disease and cognition in elderly Canadians. *Age Ageing* 27:485–491.

Ferrante, R. J., O. A. Andreassen, A. Dedeoglu, K. L. Ferrante, B. G. Jenkins, S. M. Hersch, and M. F. Beal. 2002. Therapeutic effects of coenzyme Q10 and remacemide in transgenic mouse models of Huntington's disease. *J Neurosci* 22:1592–1599.

Garcion, E., N. Wion-Barbot, C. N. Montero-Menei, F. Berger, and D. Wion. 2002. New clues about vitamin D functions in the nervous system. *Trends Endocrinol Metab* 13 (3):100–105.

Goncalves, M. B., Clarke, E., Hobbs, C., Malmqvist, T., Deacon, R., Jack, J., and Corcoran, J. P. 2013. Amyloid β inhibits retinoic acid synthesis exacerbating Alzheimer disease pathology which can be attenuated by an retinoic acid receptor α agonist. *Eur J Neurosci* 37: 1182–1192.

Grimm, M. O., J. Lehmann, J. Mett, V. C. Zimmer, S. Grosgen, C. P. Stahlmann, B. Hundsdorfer et al. 2014. Impact of vitamin D on amyloid precursor protein processing and amyloid-β peptide degradation in Alzheimer's disease. *Neurodegener Di* 13:75–81.

Harrison, F. E. and J. M. May. 2009. Vitamin C function in the brain: Vital role of the ascorbate transporter SVCT2. *Free Radic Biol Med* 46:719–730.

Ito, S., S. Ohtsuki, Y. Nezu, Y. Koitabashi, S. Murata, and T. Terasaki. 2011. 1α,25-Dihydroxyvitamin D3 enhances cerebral clearance of human amyloid-β peptide(1–40) from mouse brain across the blood-brain barrier. *Fluids Barriers CNS* 8:20.

Jimenez-Jimenez, F. J., J. A. Molina, F. de Bustos, M. Orti-Pareja, J. Benito-Leon, A. Tallon-Barranco, T. Gasalla, J. Porta, and J. Arenas. 1999. Serum levels of β-carotene, α-carotene and vitamin A in patients with Alzheimer's disease. *Eur J Neurol* 6:495–497.

Kamat, P. K., A. Kalani, S. Givvimani, P. B. Sathnur, S. C. Tyagi, and N. Tyagi. 2013. Hydrogen sulfide attenuates neurodegeneration and neurovascular dysfunction induced by intracerebral-administered homocysteine in mice. *Neuroscience* 252:302–319.

Keeney, J. T. and D. A. Butterfield. 2015. Vitamin D deficiency and Alzheimer disease: Common links. *Neurobiol Dis* 84:84–98.

Kontush, A., U. Mann, S. Arlt, A. Ujeyl, C. Lührs, T. Müller-Thomsen, and U. Beisiegel. 2001. Influence of vitamin E and C supplementation on lipoprotein oxidation in patients with Alzheimer's disease. *Free Radic Biol Med* 31 (3):345–354.

Landfield, P. W. and L. Cadwallader-Neal. 1998. Long-term treatment with calcitriol ($1,25(OH)_2$ vit D_3) retards a biomarker of hippocampal aging in rats. *Neurobiol Aging* 19 (5):469–477.

Lane, M. A. and S. J. Bailey. 2005. Role of retinoid signalling in the adult brain. *Prog Neurobiol* 75:275–293. doi:10.1016/j.pneurobio.2005.03.002.

Latimer, C. S., L. D. Brewer, J. L. Searcy, K. C. Chen, J. Popović, S. D. Kraner, O. Thibault, E. M. Blalock, P. W. Landfield, and N. M. Porter. 2014. Vitamin D prevents cognitive decline and enhances hippocampal synaptic function in aging rats. *Proc Natl Acad Sci USA* 111 (41):E4359–E4366. doi:10.1073/pnas.1404477111.

Li, W., H. Liu, M. Yu, X. Zhang, Y. Zhang, H. Liu, J. X. Wilson, and G. Huang. 2016. Folic acid alters methylation profile of JAK-STAT and long-term depression signaling pathways in Alzheimer's disease models. *Mol Neurobiol* 53 (9):6548–6556.

Masoumi, A., B. Goldenson, S. Ghirmai, H. Avagyan, J. Zaghi, K. Abel, X. Zheng, A. Espinosa-Jeffrey et al. 2009. 1α,25-Dihydroxyvitamin D3 interacts with curcuminoids to stimulate amyloid-β clearance by macrophages of Alzheimer's disease patients. *J Alzheimer's Dis* 17:703–717.

Matthews, R. T., L. Yang, S. Browne, M. Baik, and M. F. Beal. 1998. Coenzyme Q10 administration increases brain mitochondrial concentrations and exerts neuroprotective effects. *Proc Natl Acad Sci USA* 95:8892–8897.

McCarthy, S., M. Somayajulu, M. Sikorska, H. Borowy-Borowski, and S. Pandey. 2004. Paraquat induces oxidative stress and neuronal cell death; neuroprotection by water-soluble Coenzyme Q10. *Toxicol Appl Pharmacol* 201:21–31.

McDonald, S. R., R. S. Sohal, and M. J. Forster. 2005. Concurrent administration of coenzyme Q10 and alpha-tocopherol improves learning in aged mice. *Free Radic Biol Med* 38:729–736.

Monsen, E. R. 2000. Dietary reference intakes for the antioxidant nutrients: Vitamin C, vitamin E, selenium, and carotenoids. *J Am Diet Assoc* 100:637–640.

Moore, E., A. Mander, D. Ames, R. Carne, K. Sanders, and D. Watters. 2012. Cognitive impairment and vitamin B12: A review. *Int Psychogeriatr* 24:541–556.

Murakami, K., N. Murata, Y. Ozawa, N. Kinoshita, K. Irie, T. Shirasawa, and T. Shimizu. 2011. Vitamin C restores behavioral deficits and amyloid-β oligomerization without affecting plaque formation in a mouse model of alzheimer's disease. *J Alzheimers Dis* 26 (1):7–18. doi:10.3233/jad-2011-101971.

Ortega, R. M., A. M. Requejo, A. M. López-Sobaler, P. Andrés, B. Navia, J. M. Perea, and F. Robles. 2002. Cognitive function in elderly people is influenced by vitamin E status. *J. Nutr* 132 (7):2065–2068.

Perrig, W. J., P. Perrig, and H. B. Stahelin. 1997. The relation between antioxidants and memory performance in the old and very old. *J Am Geriatr Soc* 45:718–724.

Pirchl, M., C. Ullrich, and C. Humpel. 2010. Differential effects of short- and long-term hyperhomocysteinaemia on cholinergic neurons, spatial memory and microbleedings *in vivo* in rats. *Eur J Neurosci* 32:1516–1527.

Quadri, P., C. Fragiacomo, R. Pezzati, E. Zanda, G. Forloni, M. Tettamanti, and U. Lucca. 2004. Homocysteine, folate, and vitamin B-12 in mild cognitive impairment, Alzheimer disease, and vascular dementia. *Am J Clin Nutr* 80:114–122.

Reinhardt, S., M. O. Grimm, C. Stahlmann, T. Hartmann, K. Shudo, T. Tomita, and K. Endres. 2016. Rescue of hypovitaminosis a induces non-amyloidogenic amyloid precursor protein (APP) processing. *Curr Alzheimer Res* 13:1277–1289.

Riggs, K. M., A. Spiro, K. Tucker, and D. Rush. 1996. Relations of vitamin B-12, vitamin B-6, folate, and homocysteine to cognitive performance in the Normative Aging Study. *Am J Clin Nutr* 63:306–314.

Rinaldi, P., M. C. Polidori, A. Metastasio, E. Mariani, P. Mattioli, A. Cherubini, M. Catani, R. Cecchetti, U. Senin, and P. Mecocci. 2003. Plasma antioxidants are similarly depleted in mild cognitive impairment and in Alzheimer's disease. *Neurobiol Aging* 24:915–919.

Sano, M., C. Ernesto, R. G. Thomas, M. R. Klauber, K. Schafer, M. Grundman, P. Woodbury et al. 1997. A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. The Alzheimer's disease cooperative study. *N Engl J Med* 336:1216–1222.

Shah, D. I. and M. Singh. 2007. Possible role of Akt to improve vascular endothelial dysfunction in diabetic and hyperhomocysteinemic rats. *Mol Cell Biochem* 295:65–74.

Sodhi, R. K. and N. Singh. 2013. All-trans retinoic acid rescues memory deficits and neuropathological changes in mouse model of streptozotocin-induced dementia of Alzheimer's type. *Prog Neuro-Psychopharmacol Biol Psychiatry* 40:38–46.

Sodhi, R. K. and N. Singh. 2014. Retinoids as potential targets for Alzheimer's disease. *Pharmacol Biochem Behav* 120:117–123. doi:10.1016/j.pbb.2014.02.016.

Somayajulu, M., S. McCarthy, M. Hung, M. Sikorska, H. Borowy-Borowski, and S. Pandey. 2005. Role of mitochondria in neuronal cell death induced by oxidative stress; neuroprotection by Coenzyme Q10. *Neurobiol Dis* 18:618–627.

Sponne, I. E., D. Gaire, S. P. Stabler, S. Droesch, F. M. Barbé, R. H. Allen, D. A. Lambert, and J. P. Nicolas. 2000. Inhibition of vitamin B12 metabolism by OH-cobalamin c-lactam in rat oligodendrocytes in culture a model for studying neuropathy due to vitamin B12 deficiency. *Neurosci Lett* 3:191–194.

Sung, S., Y. Yao, K. Uryu, H. Yang, V. M. Lee, J. Q. Trojanowski, and D. Praticò. 2004. Early vitamin E supplementation in young but not aged mice reduces Abeta levels and amyloid deposition in a transgenic model of Alzheimer's disease. *FASEB J* 18 (2):323–325.

Vellas, B. and P. S. Aisen. 2010. Early Alzheimer's trials: New developments. *J Nutr Health Aging* 14:293.

Wang, H. X., A. Wahlin, H. Basun, J. Fastbom, B. Winblad, and L. Fratiglioni. 2001. Vitamin B(12) and folate in relation to the development of Alzheimer's disease. *Neurology* 56:1188–1194.

Wang, L., K. Hara, J. M. van Baaren, J. C. Price, G. W. Beecham, P. J. Gallins, P. L. Whitehead et al. 2012. Vitamin D receptor and Alzheimer's disease: A genetic and functional study. *Neurobiol Aging* 33:1841–1849.

Yang, X., Y. Yang, G. Li, J. Wang, and E. S. Yang. 2008. Coenzyme Q10 attenuates beta-amyloid pathology in the aged transgenic mice with Alzheimer presenilin 1 mutation. *J Mol Neurosci* 34:165–171.

Yu, J., M. Gattoni-Celli, H. Zhu, N. R. Bhat, K. Sambamurti, S. Gattoni-Celli, and M. S. Kindy 2011. Vitamin D3-enriched diet correlates with a decrease of amyloid plaques in the brain of AβPP transgenic mice. *J Alzheimer's Dis* 25:295–307.

Zandi, P. P., J. C. Anthony, A. S. Khachaturian, S. V. Stone, D. Gustafson, J. T. Tschanz, M. C. Norton, K. A. Welsh-Bohmer, and J. C. Breitner. 2004. Reduced risk of Alzheimer disease in users of antioxidant vitamin supplements: The Cache County Study. *Arch Neurol* 61 (1):82–88.

Zhang, C. E., Q. Tian, W. Wei, J. H. Peng, G. P. Liu, X. W. Zhou, Q. Wang, D. W. Wang, and J. Z. Wang. 2008. Homocysteine induces tau phosphorylation by inactivating protein phosphatase 2A in rat hippocampus. *Neurobiol Aging* 29 (11):1654–1665.

6

Antioxidant Nutraceuticals and Parkinson's Disease

Adryan Perez, Andrew Tran, and Chuanhai Cao

Contents

6.1 Introduction

Parkinson's disease (PD) was established as a medical condition many years after James Parkinson noted a resemblance in cases among his older patients in his 1817 publication *An Essay on the Shaking Palsy* (Shulman et al. 2011). It is currently the second most common neurodegenerative disorder and is expected to impose an increasing social and economic toll on societies as population age (de Lau and Breteler 2006). PD is characterized as a progressive neurodegenerative movement disorder that results from the death of dopaminergic neurons in the substantia nigra (Moore et al. 2005). The development of PD is associated with a long prodromal phase between the appearance of symptoms and the time of clinical diagnoses. Clinical detection

of PD is based on the detection of two of the four cardinal signs associated with the disease, including resting tremor, bradykinesia, rigidity, and postural imbalance (de Lau and Breteler 2006). In addition to the physical symptoms, PD patients commonly display nonmotor symptoms such as depression, lack of motivation, passivity, and dementia (Fahn 2003). After the clinical diagnoses of the disease patients live an average life of 15 years before death occurs, which could be due to the higher prevalence of PD among older age groups (Lees et al. 2009).

Presently, there is no known cure for PD, and drugs used to treat it are focused on suppressing symptoms instead of preventing the onset or the progression of the disease. Modern treatments, including dopamine replacement therapy have led to a significant improvement in both the quality of life and life expectancy of patients but have been unable to eradicate the significant disabilities accompanied with the disease progression. These dopamine replacement therapies have been found to cause levodopa-induced dyskinesias (LIDs) followed by chronic treatment (Niccolini et al. 2015). However, given the wide applications of nutraceuticals and their significance in new drug discoveries demonstrate that nutraceuticals could play a critical role in providing a mechanism for the prevention and treatment of PD.

The introduction of modern medicine has extended the average lifespan of the population. The increase in life expectancy has made age-related disorders such as PD and Alzheimer's disease (AD) increasingly prevalent in the society. The etiology of PD is commonly attributed to the increased prevalence of reactive oxygen species (ROS) found in older age groups. ROS are produced by the human body in order to undergo normal physiological processes, including critical roles in the immune system, stimulation of growth factors, the development of an inflammatory response, and apoptosis (Zuo and Motherwell 2013). Oxidative stress has been associated with the abnormal accumulation of endogenous protein alpha-synuclein forming the hallmark inclusions of PD termed Lewy bodies (LBs), which lead to mitochondrial modifications eventually causing neuronal cell death (Hsu et al. 2000). Currently, vast amounts of antioxidant nutraceuticals on the market have been shown to ameliorate oxidative stress and inhibit alpha-synuclein aggregation, potentially serving a primary or secondary role in the treatment of PD.

The term *nutraceutical* is a combination of the terms *nutrition* and *pharmaceutical* and refers to the food products that may provide therapeutic benefit. Nutraceuticals encompass a wide variety of products ranging from isolated nutrients, dietary supplements, and herbal products to processed foods such as cereals, soups, and beverages. Although nutraceuticals consist of a wide range of products, the implementation of antioxidant nutraceuticals is applicable in prevention and treatment of PD due to their ability to provide neuroprotection through a wide range of proposed mechanisms, such as scavenging of free radicals and ROS, chelation of the iron, modulation of cell-signaling pathways, and inhibition of inflammation (Chao et al. 2012).

The Section 6.2 through 6.4 present modern treatments, discuss a proposed mechanism for the pathogenesis of PD, and present various antioxidant nutraceuticals presumed to play a beneficial role in the prevention and treatment of the neurodegenerative disease.

6.2 Modern treatments

A variety of modern treatments have been developed to combat PD (**Table 6.1**). Although these treatments provide temporary benefits in treating PD, they do not provide a concrete way of preventing and curing the disorder. Current research has unveiled other compounds, specifically antioxidant nutraceuticals, with therapeutic properties that have the potential to treat and prevent the onset of PD. The benefit of using these nutraceutical treatments is their potential ability to provide a safer way to combat the disease without many of the accompanying side effects of current treatments.

Table 6.1 Modern Treatments

Modern Treatment	Treatment Mechanism
Levodopa	Levodopa crosses the blood–brain barrier and enters dopaminergic neurons. There it is metabolized into dopamine by the enzyme aromatic L-amino acid decarboxylase and replaces the depleted endogenous neurotransmitter (Lopez et al. 2001). This method of treatment remains to be the most effective in treating symptoms of Parkinson's disease, but chronic use has been associated with involuntary movement disorders such as dyskinesia (Sweet and McDowell 1975, Lesser et al. 1979).
Dopamine agonists	Dopamine agonists treat Parkinson's symptoms by mimicking the neurotransmitter dopamine and acting directly on dopamine receptors (Quinn 1995). Dopamine agonists can be added to levodopa regimens to reduce the dosage of the latter and to delay the onset of induced dyskinesia (Fischer 1995, Oertel and Quinn 1997, Kondo 2002).
Monoamine oxidase B (MAO-B) inhibitors	MAO-B is a naturally occurring enzyme found in the human brain that breaks down a variety of chemicals, including dopamine (Saura et al. 1994, Youdim and Weinstock 2004, Mallajosyula et al. 2009). Two MAO-B inhibitors have been approved by the FDA: (1) selegiline and (2) rasagiline (Heinonen and Myllylä 1998, Fernandez and Chen 2007). These inhibitors are used to block the breakdown of dopamine in the brain, making it more readily available.
Catechol-O-methyltransferase (COMT) inhibitors	COMT inhibitors can also be used with other methods of treatment, including levodopa, where it was found to increase the amount of levodopa to enter the brain, and consequently lower the dosages of the drug and its associated effects (Männistö and Kaakkola 1990, Lamberti et al. 2005).
Anticholinergics	Anticholinergics possess anti-Parkinsonian characteristics by counteracting an imbalance between striatal dopamine and acetylcholine levels from the denervation of dopaminergic neurons exhibited in Parkinson's disease (Cooper et al. 1992). However, clinical studies have suggested that the use of anticholinergics have a greater risk of unfavorable side effects such as blurred vision, nausea, and glaucoma (Katzenschlager et al. 2003, Diederich et al. 2005).

(*Continued*)

Table 6.1 (*Continued*) Modern Treatments

Modern Treatment	Treatment Mechanism
Amantadine	The symptoms of Parkinson's disease have been shown to be relieved in amantadine treatments (Crosby et al. 2003). Studies have supported that amantadine possesses *N*-methyl-D-aspartate (NMDA) antagonistic qualities by blocking NMDA receptor aids in relieving levodopa-induced dyskinesias in Parkinson's disease (Verhagen Metman et al. 1998, Metman et al. 1999).
Deep Brain Stimulation (DBS)	DBS is a surgical procedure used to implant a neurostimulator that delivers electrical stimulation to specific areas of the brain that control movement to treat symptoms of Parkinson's disease. The device blocks irregular nerve signals that are the cause of tremors and other PD symptoms (Woods et al. 2002, Benabid et al. 2009). Studies have shown DBS to improve the symptoms and quality of life of patients with early and advanced stages of Parkinson's disease (Jahanshahi et al. 2000, Rodriguez-Oroz et al. 2005, Hacker et al. 2015, Liu et al. 2015).

6.3 Pathogenesis of Parkinson's disease

PD is one of the most common neurodegenerative disorders (Bertram and Tanzi 2005). The disease is characterized by the loss of dopaminergic neurons in the substantia nigra region of the brain and the presence of insoluble α-synuclein fibrils known as LBs (Gibb and Lees 1988, Spillantini et al. 1997, Eriksen et al. 2005). In normal brain tissue, dopaminergic neurons produce a key neurotransmitter, dopamine. This neurotransmitter is involved in many neurological processes, including relaying chemical messages to and from the substantia nigra to other parts of the brain that control muscle and movement (Double and Crocker 1995, González-Hernández and Rodríguez 2000). Denervation results in the loss of dopaminergic neurons and overall loss of control of body movement and the motor symptoms typically experienced by those afflicted with PD include tremors and shakes (1983, Tomer et al. 1993).

The etiology and pathogenesis of PD are still unknown, and some speculations point to an underlying genetic cause (Polymeropoulos et al. 1997, Piccini et al. 1999, Mouradian 2002, Bras and Singleton 2009, Lesage and Brice 2009). Other hypotheses state that toxins in the environment may play a role in PD pathogenesis (Klawans et al. 1982, Kuopio et al. 1999, Sohn et al. 2000). However, in many of the most accepted hypotheses, oxidative stress is believed to be involved in the development of PD. Oxidation is the process in which free radical molecules attempt to replace a missing electron. This is usually accomplished by the free radical reacting with another species (Kumar et al. 2012). In a healthy brain, these radicals are formed, and there are mechanisms by which they are eliminated. PD models have shown that these mechanisms malfunction or produce too many free radicals. The excess free radicals oxidize with other species in the brain and lead to tissue damage (Ebadi et al. 1996, Jenner 1996, Kumar et al. 2012).

Chemical species with unpaired electrons, referred to as ROS exist in normal, healthy brains. Major ROS include hydrogen peroxide (H_2O_2), the superoxide anion (O_2^-), and the hydroxyl radical (OH). ROS are created as by-products of many metabolic processes, regulated cellular processes, including cell growth and proliferation, and generated as a defensive mechanism against foreign pathogens (Rhee 1999, Thannickal and Fanburg 2000). Aside from the various benefits from ROS, the compounds can also cause a variety of negative side effects. ROS can react nonspecifically with biological molecules, including RNA, DNA, and proteins. The consistent attack on DNA by ROS can lead to DNA damage and mutations (Lindahl and Nyberg 1972, Kitagawa et al. 2004). ROS interactions with enzymes can also lead to conformational changes in the protein structure and result in the loss of function (Finkel 2003).

There have been studies that suggest a correlation between the physiological processes of aging and oxidative stress from ROS. Through the process of aging, the frequency of oxidation by ROS increases, whereas the repair processes slow down (Lovell et al. 1997, Leutner et al. 2001). It has become apparent that free radicals participate in the process of aging, and aging is a major risk factor in the development of PD. Although there are other considerable risk factors for the pathogenesis of PD, oxidative stress from oxidation by ROS remains to be a major factor. Analysis of postmortem brain samples has shown to display enhanced levels of oxidized proteins, nucleic acids, and lipids in those afflicted with PD (Dexter et al. 1986, Fahn and Cohen 1992). The evidence suggests that oxidative stress may be implicated in the initiation of PD pathology.

PD pathology is commonly correlated with an imbalance present in the immune system due to aging. As the body ages, the immune system loses its ability to maintain a critical balance between the innate and adaptive immune system leading to the aggregation of the endogenous protein, alpha synuclein. Once the protein aggregates to form the higher order oligomer the immune system is unable to clear the excess protein leading to disease pathology. The basis for disease prevention is reliant on the ability of compounds such as antioxidant nutraceuticals to assist the immune system in maintaining the critical balance necessary to remain in its disease-free state.

6.4 Antioxidant nutraceuticals

Natural antioxidants are capable of providing beneficial neuroprotective effects by inhibiting the formation of free radicals and interrupting free radical propagation by one or more several mechanisms, including scavenging species that initiate peroxidation, chelating metal ions such that they are unable to generate reactive species or decompose lipid peroxides, quenching superoxide radicals preventing the formation of peroxides, breaking the autoxidative chain reaction, and/or reducing localized O_2 concentrations (Brewer 2011). The application of antioxidant nutraceuticals in PD may play a critical role in the treatment and prevention due to the finding of low levels of endogenous

antioxidants, increased reactive species, augmented dopamine oxidation, and high levels of iron in brains from PD patients (Sutachan et al. 2012).

Antioxidant nutraceuticals can be categorized into distinct groups according to their chemical structures such as flavonoid polyphenols, non-flavonoid polyphenols, phenolic acids or phenolic diterpenes, and organosulfur compounds (Kelsey et al. 2010). Each different structure may potentially function through different mechanisms of action to provide potential neuroprotective effects for the prevention and treatment of PD. Additional noteworthy compounds have also shown alleviating effects in the treatment and prevention of PD, including tea, coffee, and melatonin.

6.4.1 Flavonoid polyphenols

6.4.1.1 Epigallocatechin 3-gallate

Epigallocatechin 3-gallate (EGCG) (**Figure 6.1**) is one of the most well-known nutraceuticals and is ascribed as the positive neuroprotective effects correlated with green tea consumption as it is the most abundant catechin found in green tea (Weinreb et al.). The prolific polyphenol content displayed in the structure of EGCG is crucial to its antioxidative properties as it has extensive electron delocalization allowing for radical stabilization. Given their advantageous structure, EGCG and other tea polyphenols have been prevalent research topics due to their potential therapeutic benefits found both *in vivo* and *in vitro*, including radical scavenging, iron chelating, anti-inflammatory, anticarcinogenic, and anti-angiogenic actions (Levites et al. 2001). In regards to PD, numerous epidemiological studies have correlated regular tea consumption with a reduced risk of PD development (Ng et al. 2008, Tan et al. 2008).

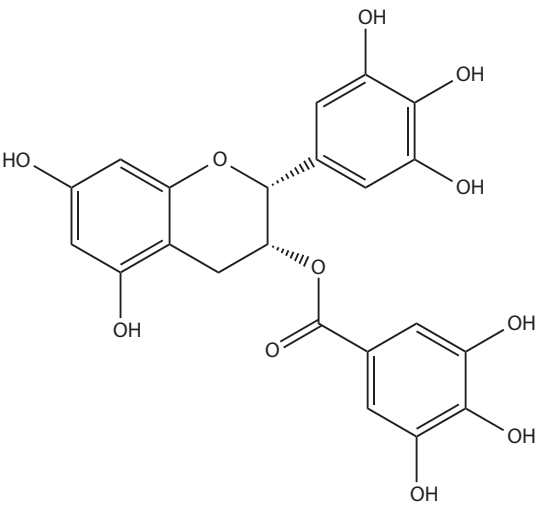


Figure 6.1 *Chemical structure of epigallocatechin 3-gallate (EGCG).*

A proposed mechanism for EGCG is the elimination of oxidative stress through its capability to penetrate the blood–brain barrier (BBB) where it can act in the substantia nigra as both an ROS scavenger and an iron chelator to clear the redox active ferrous iron (Chao et al. 2012).

In addition to the elimination of oxidative stress, polyphenolic compounds such as EGCG have been found to exhibit inhibitory activity on alpha synuclein aggregation and disaggregation of preformed alpha synuclein oligomers, thus demonstrating potential therapeutic uses for the treatment of PD (Caruana et al. 2011).

6.4.1.2 Quercetin

Quercetin (**Figure 6.2**) is a flavonoid polyphenol found in many fruits, vegetables, leaves, and grains. Properties of quercetin include antioxidant activity, anti-inflammatory activity, and the ability to cross the BBB. Quercetin has demonstrated ROS scavenging ability enabling it to inhibit lipid peroxidation and the ability to protect primary cultured hippocampal cells from potassium cyanide (KCN)-induced oxidative stress by attenuating ROS generation and Ca^{2+} influx and maintaining higher GSH levels (Pandey et al. 2012).

A research study combined quercetin with an iron chelator to treat 6-hydroxydopamine (6-OHDA)-induced neurotoxicity in the striatum of rats and found them to provide significant neuroprotective effects by preventing dopaminergic neuronal loss and maintaining the striatal dopamine level (Haleagrahara et al. 2013). In another investigation, treatment with quercetin was found to protect mesencephalic cultures from injury by *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridinium hydrochloride (MPP+)-induced toxicity (Mercer et al. 2005). Although these studies have found many beneficial effects of quercetin in the treatment of neurodegeneration, there is a need to replicate these effects in additional human trials.

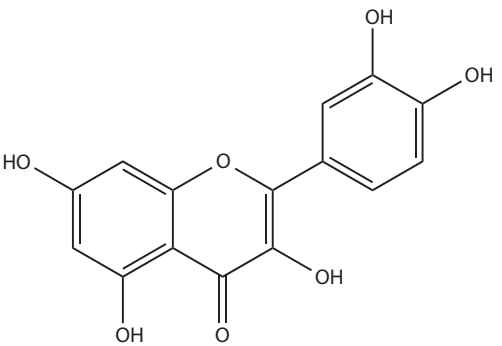


Figure 6.2 *Chemical structure of quercetin.*

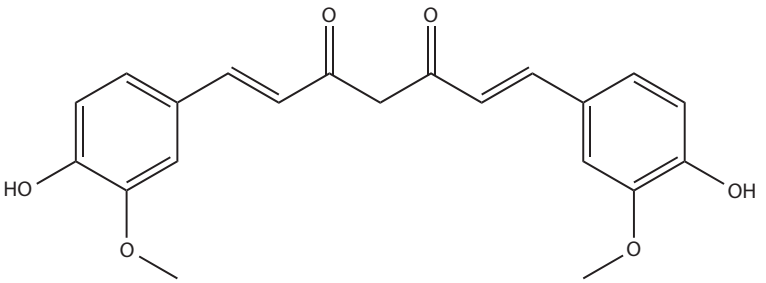


Figure 6.3 *Chemical structure of curcumin.*

6.4.2 Non-flavonoid polyphenols

6.4.2.1 Curcumin

Curcumin (**Figure 6.3**) is the main curcuminoid found in turmeric (a common Indian spice) and is often identified by its characteristic bright-yellow appearance. A multitude of studies have associated curcumin with antioxidant, anti-inflammatory, and anticancerous properties (Vallianou et al. 2015). It has been proposed to provide neuroprotection in a variety of neurodegenerative disorders; however, its mechanism of action remains a topic of continued research.

In addition to its many beneficial properties such as reducing oxidative stress, curcumin has also been associated with effects more specific to PD. An investigation on curcumin found it to improve motor behavior in alpha synuclein transgenic mice (Spinelli et al. 2015). Other studies on the effects of curcumin on PD have revealed its ability to inhibit dopaminergic neuronal loss in PD MPTP mouse models by suppressing mitochondria dysfunction and apoptosis (Pan et al. 2012). A study made *in vitro* found curcumin to bind strongly to alpha synuclein in the hydrophobic nonamyloid-β component region, thus preventing protein aggregation (Ahmad and Lapidus 2012). This study demonstrates the potential of curcumin to be used for prevention or the reduction of PD progression.

6.4.2.2 Resveratrol

Resveratrol (**Figure 6.4**) is a molecule of the stilbene family produced by some spermatophytes, such as grapevines, in response to injury (Frémont 2000). Due to the antioxidant capabilities of resveratrol, it may be able to impose a vast amount of beneficial effects to its consumer. Resveratrol has shown promising results *in vitro* with models of epilepsy, AD, PD, Huntington's disease, amyotrophic lateral sclerosis, and nerve injury (Rocha-González et al. 2008).

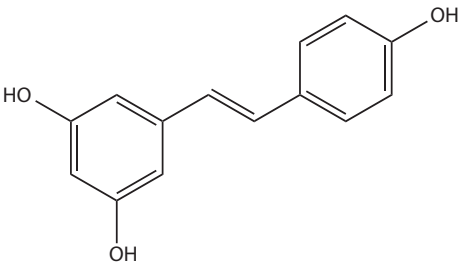


Figure 6.4 *Chemical structure of resveratrol.*

It is known that ROS perform an important role as secondary messengers in the process of cell signaling (Forman 2010). Nonetheless, the accumulation of extraneous ROS is associated with aging and many neurodegenerative disorders. Other than its antioxidant properties, resveratrol has also been reported to affect a wide range of signaling transduction pathways in both *in vitro* and *in vivo* models demonstrating its ability to modulate cellular growth and differentiation, apoptosis, angiogenesis, and metastasis (Tellone et al. 2015). However, the exact mechanism by which resveratrol exerts its effects is yet to be elucidated and does not allow for definitive conclusions to be made regarding the efficacy of resveratrol on PD.

6.4.3 Phenolic acids

6.4.3.1 Rosmarinic acid

Rosmarinic acid (**Figure 6.5**) is one of the most abundant antioxidant compounds found in rosemary along with carnosic acid (Kelsey et al. 2010). Rosmarinic acid and its derivatives have been attributed to a wide range of promising biological activities, such as improvement of cognitive performance, prevention of the development of AD, cardioprotective effects, reduction of the severity of kidney disease, and cancer chemoprevention (Bulgakov et al. 2011).

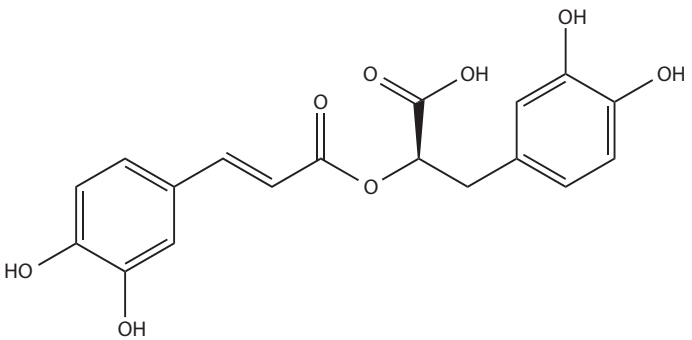


Figure 6.5 *Chemical structure of rosmarinic acid.*

In regards to PD, research conducted *in vitro* in three different models of neuronal death, including oxidative stress, excitotoxicity, and oxygen–glucose deprivation/reoxygenation has demonstrated the ability of rosmarinic acid to protect neurons from injury (Fallarini et al. 2009). Experiments have tested the effects of rosmarinic acid and have found that through its antioxidation properties it is capable of exerting its protective effects against 6-OHDA-induced neurotoxicity (Ren et al. 2009). A study has also concluded that rosmarinic acid can potentially play a neuroprotective role by demonstrating the ability to ameliorate mitochondrial dysfunction against MPP(+)-induced cell apoptosis (Du et al. 2010). The results of these experiments indicate that rosmarinic acid may potentially be used alone or in conjunction with other treatments for the prevention or treatment of PD.

6.4.3.2 Carnosic acid

Carnosic acid is derived from rosemary and possesses antioxidative and antimicrobial properties, which have been increasingly exploited within the food and cosmetic industries (Birtić et al. 2015). Similar to rosmarinic it has been attributed with the ability to protect against various neurodegenerative disorders, including PD.

A recent work investigated the behavioral activity and neuroprotective effects of carnosic acid on the 6-OHDA-induced rat model. The study observed improved locomotor activity and reduced apomorphine-caused rotation as well as significant protection against lipid peroxidation and GSH reduction in rats treated with carnosic acid (Wu et al. 2015). A similar study suggests carnosic acid may be a promising candidate for PD treatment due to its capability to prevent 6-OHDA-induced apoptosis through an increase in Glutathione S-transferase P (GSTP) expression via activation of the PI3K/Akt/NF-κB pathway (Lin et al. 2014). Although many studies have demonstrated the potential of carnosic acid, more work needs to be conducted to elucidate the exact mechanism of action in order to combine it with other treatments and maximize its potential efficacy (**Figure 6.6**).

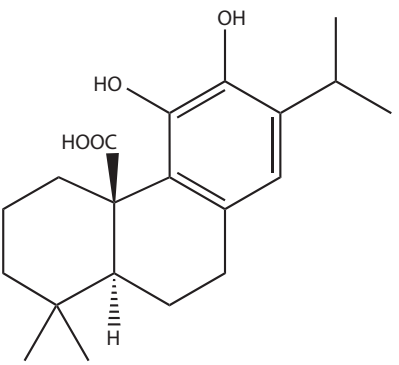


Figure 6.6 *Chemical structure of carnosic acid.*

6.5 Coffee

Coffee is one of the most frequently consumed caffeinated beverages in the world and has been shown to stimulate the central nervous system as well as exert positive effects on long-term memory (Mejia and Ramirez-Mares 2014). In addition to PD, the use of coffee has shown therapeutic benefits in the prevention and treatment in neurodegenerative disorders such as in AD (Arendash and Cao 2010). Given its wide use worldwide it has been extensively studied for its potential benefits.

Caffeine is considered the main active therapeutic component in coffee and has been shown to exert protective effects in PD by keeping the BBB intact, thus helping to regulate and protect the microenvironment of the brain (Chen et al. 2010). The BBB is considered an important element in the prevention of PD, as its dysfunction is deemed as a causative mechanism in disease development (Kortekaas et al. 2005). In 6-OHDA-lesioned rats, caffeine increased dopamine contents, reversed the decrease in striatal dopamine, and decreased the number of immunopositive cells for Histone deacetylases (HDAC) and proinflammatory cytokines TNF-α and IL-1β (Machado-Filho et al. 2014). The broad effects of caffeine demonstrate its potential use in prevention and treatment of PD alone or in conjunction with current therapies.

Coffee contains many other components that are capable of exerting positive neuroprotective effects, including eicosanoyl-5-hydroxytryptamide (EHT). EHT treatment in the MPTP model of PD has displayed many beneficial effects relevant to PD, including dose-dependent preservation of nigral dopaminergic neurons, attenuation of neuroinflammatory response, and modulation of methylation and PP2A (Lee et al. 2013).

6.6 Melatonin

Melatonin (**Figure 6.7**) is the major product secreted by the pineal gland, is an antioxidant and an effective protector of mitochondrial bioenergetic function

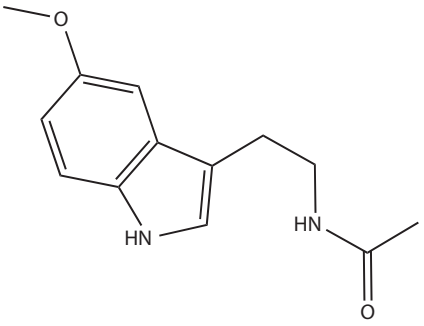


Figure 6.7 *Chemical structure of melatonin.*

(Cardinali et al. 2013). It is secreted during darkness and is involved in the regulation of circadian rhythms, sleep homeostasis, retinal neuromodulation, and vasomotor responses (Pandi-Perumal et al. 2013). It has been proposed that melatonin regulation may play an important role in the treatment and prevention of PD. Given its natural origin, it has become increasingly popular both in the treatment of PD and its use as a sleep aid.

In the MPTP-induced neurotoxicity model of PD, melatonin displayed neuroprotective effects through reduced aggregation of SNCA/α-synuclein (Su et al. 2015). Melatonin has also been linked with treating PD pathology through neuronal and mitochondrial protection in a study treating chronic Parkinson's mice with 5 mg/kg/day via intraperitoneal injection over a span of 18 weeks (Patki and Lau 2011). These results indicate the potential for melatonin to act as a powerful antioxidant by relieving oxidative stress and reducing the progression of idiopathic PD.

6.7 Conclusion

Antioxidant nutraceuticals have great potential in contributing to the treatment of PD. Due to the role oxidative stress is presumed to play in disease pathogenesis, their proposed ability to reduce oxidative stress makes them attractive compounds for future studies. In contrast to modern pharmaceuticals, which are used after disease onset to slow disease pathogenesis and reduce symptoms, antioxidant nutraceuticals may be used throughout the human lifespan to prevent disease emergence altogether. This approach is becoming more appealing as medical professionals and patients are shifting focus to methods of disease prevention. As further research is conducted, it is expected that antioxidant nutraceuticals would become critical factors in the treatment and prevention of PD.

References

Ahmad, B. and L. J. Lapidus. 2012. Curcumin prevents aggregation in α-synuclein by increasing reconfiguration rate. *J Biol Chem* 287 (12):9193–9199. doi:10.1074/jbc.M111.325548.

Arendash, G. W. and C. Cao. 2010. Caffeine and coffee as therapeutics against Alzheimer's disease. *J Alzheimers Dis* 20 (Suppl 1):S117–S126. doi:10.3233/JAD-2010-091249.

Benabid, A. L., S. Chabardes, J. Mitrofanis, and P. Pollak. 2009. Deep brain stimulation of the subthalamic nucleus for the treatment of Parkinson's disease. *Lancet Neurol* 8 (1):67–81. doi:10.1016/S1474-4422(08)70291-6.

Bertram, L. and R. E. Tanzi. 2005. The genetic epidemiology of neurodegenerative disease. *J Clin Invest* 115 (6):1449–1457. doi:10.1172/JCI24761.

Birtić, S., P. Dussort, F. X. Pierre, A. C. Bily, and M. Roller. 2015. Carnosic acid. *Phytochemistry* 115:9–19. doi:10.1016/j.phytochem.2014.12.026.

Bras, J. M. and A. Singleton. 2009. Genetic susceptibility in Parkinson's disease. *Biochim Biophys Acta* 1792 (7):597–603. doi:10.1016/j.bbadis.2008.11.008.

Brewer, M. S. 2011. Natural antioxidants: Sources, compounds, mechanisms of action, and potential applications. *Compr Rev Food Sci Food Saf* 10 (4):221–247. doi:10.1111/j.1541-4337.2011.00156.x.

Bulgakov, V. P., Y. V. Inyushkina, and S. A. Fedoreyev. 2011. Rosmarinic acid and its derivatives: Biotechnology and applications. *Crit Rev Biotechnol* 32 (3):203–217. doi:10.3109/07388551.2011.596804.

Cardinali, D. P., E. S. Pagano, P. A. Bernasconi, R. Reynoso, and P. Scacchi. 2013. Melatonin and mitochondrial dysfunction in the central nervous system. *Horm Behav* 63 (2): 322–330. doi:10.1016/j.yhbeh.2012.02.020.

Caruana, M., T. Högen, J. Levin, A. Hillmer, A. Giese, and N. Vassallo. 2011. Inhibition and disaggregation of α-synuclein oligomers by natural polyphenolic compounds. *FEBS Lett* 585 (8):1113–1120. doi:10.1016/j.febslet.2011.03.046.

Chao, J., Y. Leung, M. Wang, and R. C. Chang. 2012. Nutraceuticals and their preventive or potential therapeutic value in Parkinson's disease. *Nutr Rev* 70 (7):373–386.

Chen, X., O. Ghribi, and J. D. Geiger. 2010. Caffeine protects against disruptions of the blood-brain barrier in animal models of Alzheimer's and Parkinson's disease. *J Alzheimer's Dis* 20 (Suppl 1):S127–S141. doi:10.3233/JAD-2010-1376.

Cooper, J. A., H. J. Sagar, S. M. Doherty, N. Jordan, P. Tidswell, and E. V. Sullivan. 1992. Different effects of dopaminergic and anticholinergic therapies on cognitive and motor function in Parkinson's disease. A follow-up study of untreated patients. *Brain* 115 (Pt 6):1701–1725.

Crosby, N. J., K. H. Deane, and C. E. Clarke. 2003. Amantadine for dyskinesia in Parkinson's disease. *Cochrane Database Syst Rev* (2):CD003467. doi:10.1002/14651858.CD003467.

de Lau, L. M. and M. M. Breteler. 2006. Epidemiology of Parkinson's disease. *Lancet Neurol* 5 (6):525–535. doi:10.1016/S1474-4422(06)70471-9.

de Mejia, E. G. and M. V. Ramirez-Mares. 2014. Impact of caffeine and coffee on our health. *Trends Endocrinol Metab* 25 (10):489–492. doi:10.1016/j.tem.2014.07.003.

Dexter, D., C. Carter, F. Agid, Y. Agid, A. J. Lees, P. Jenner, and C. D. Marsden. 1986. Lipid peroxidation as cause of nigral cell death in Parkinson's disease. *Lancet* 2 (8507):639–640.

Diederich, N. J., C. G. Goetz, and G. T. Stebbins. 2005. Repeated visual hallucinations in Parkinson's disease as disturbed external/internal perceptions: Focused review and a new integrative model. *Mov Disord* 20 (2):130–140. doi:10.1002/mds.20308.

Double, K. L. and A. D. Crocker. 1995. Dopamine receptors in the substantia nigra are involved in the regulation of muscle tone. *Proc Natl Acad Sci USA* 92 (5):1669–1673.

Du, T., L. Li, N. Song, J. Xie, and H. Jiang. 2010. Rosmarinic acid antagonized 1-methyl-4-phenylpyridinium (MPP+)-induced neurotoxicity in MES23.5 dopaminergic cells. *Int J Toxicol* 29 (6):625–633.

Ebadi, M., S. K. Srinivasan, and M. D. Baxi. 1996. Oxidative stress and antioxidant therapy in Parkinson's disease. *Prog Neurobiol* 48 (1):1–19.

Eriksen, J. L., Z. Wszolek, and L. Petrucelli. 2005. Molecular pathogenesis of Parkinson disease. *Arch Neurol* 62 (3):353–357. doi:10.1001/archneur.62.3.353.

Fahn, S. and G. Cohen. 1992. The oxidant stress hypothesis in Parkinson's disease: Vidence supporting it. *Ann Neurol* 32 (6):804–812. doi:10.1002/ana.410320616.

Fahn, S. 2003. Description of Parkinson's disease as a clinical syndrome. *Ann N Y Acad Sci* 991 (1):1–14. doi:10.1111/j.1749-6632.2003.tb07458.x.

Fallarini, S., G. Miglio, T. Paoletti, A. Minassi, A. Amoruso, C. Bardelli, S. Brunelleschi, and G. Lombardi. 2009. Clovamide and rosmarinic acid induce neuroprotective effects in in vitro models of neuronal death. *Br J Pharmacol* 157 (6):1072–1084. doi:10.1111/j.1476-5381.2009.00213.x.

Fernandez, H. H. and J. J. Chen. 2007. Monoamine oxidase-B inhibition in the treatment of Parkinson's disease. *Pharmacotherapy* 27 (12 Pt 2):174S–185S. doi:10.1592/phco.27.12part2.174S.

Finkel, T. 2003. Oxidant signals and oxidative stress. *Curr Opin Cell Biol* 15 (2):247–254.

Fischer, P. A. 1995. Treatment strategies in Parkinson's disease after a quarter century experiences with L-DOPA therapy. *J Neural Transm Suppl* 46:381–389.

Forman, H. J. 2010. Reactive oxygen species and α,β-unsaturated aldehydes as second messengers in signal transduction. *Ann N Y Acad Sci* 1203:35–44. doi:10.1111/j.1749-6632.2010.05551.x.

Frémont, L. 2000. Biological effects of resveratrol. *Life Sci* 66 (8):663–673. doi:10.1016/S0024-3205(99)00410-5.

Gibb, W. R., and A. J. Lees. 1988. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. *J Neurol Neurosurg Psychiatry* 51 (6):745–752.

González-Hernández, T. and M. Rodríguez. 2000. Compartmental organization and chemical profile of dopaminergic and GABAergic neurons in the substantia nigra of the rat. *J Comp Neurol* 421 (1):107–135.

Hacker, M. L., J. Tonascia, M. Turchan, A. Currie, L. Heusinkveld, P. E. Konrad, T. L. Davis et al. 2015. Deep brain stimulation may reduce the relative risk of clinically important worsening in early stage Parkinson's disease. *Parkinsonism Relat Disord* 21 (10):1177–1183. doi:10.1016/j.parkreldis.2015.08.008.

Haleagrahara, N., C. J. Siew, and K. Ponnusamy. 2013. Effect of quercetin and desferrioxamine on 6-hydroxydopamine (6-OHDA) induced neurotoxicity in striatum of rats. *J Toxicol Sci* 38 (1):25–33. doi:10.2131/jts.38.25.

Heinonen, E. H. and V. Myllylä. 1998. Safety of selegiline (deprenyl) in the treatment of Parkinson's disease. *Drug Saf* 19 (1):11–22.

Hsu, L. J., Y. Sagara, A. Arroyo, E. Rockenstein, A. Sisk, M. Mallory, J. Wong, T. Takenouchi, M. Hashimoto, and E. Masliah. 2000. α-Synuclein promotes mitochondrial deficit and oxidative stress. *Am J Pathol* 157 (2):401–410.

Jahanshahi, M., C. M. Ardouin, R. G. Brown, J. C. Rothwell, J. Obeso, A. Albanese, M. C. Rodriguez-Oroz et al. 2000. The impact of deep brain stimulation on executive function in Parkinson's disease. *Brain* 123 (Pt 6):1142–1154.

Jenner, P. 1996. Oxidative stress in Parkinson's disease and other neurodegenerative disorders. *Pathol Biol* (*Paris*) 44 (1):57–64.

Katzenschlager, R., C. Sampaio, J. Costa, and A. Lees. 2003. Anticholinergics for symptomatic management of Parkinson's disease. *Cochrane Database Syst Rev* (2):CD003735. doi:10.1002/14651858.CD003735.

Kelsey, N. A., H. M. Wilkins, and D. A. Linseman. 2010. Nutraceutical antioxidants as novel neuroprotective agents. *Molecules* 15 (11):7792–7814.

Kitagawa, Y., K. Suzuki, A. Yoneda, and T. Watanabe. 2004. Effects of oxygen concentration and antioxidants on the in vitro developmental ability, production of reactive oxygen species (ROS), and DNA fragmentation in porcine embryos. *Theriogenology* 62 (7):1186–1197. doi:10.1016/j.theriogenology.2004.01.011.

Klawans, H. L., R. W. Stein, C. M. Tanner, and C. G. Goetz. 1982. A pure parkinsonian syndrome following acute carbon monoxide intoxication. *Arch Neurol* 39 (5):302–304.

Kondo, T. 2002. Initial therapy for Parkinson's disease: Levodopa vs. dopamine receptor agonists. *J Neurol* 249 (Suppl 2):II25–II29. doi:10.1007/s00415-002-1205-3.

Kortekaas, R., K. L. Leenders, J. C. H. van Oostrom, W. Vaalburg, J. Bart, A. T. M. Willemsen, and N. H. Hendrikse. 2005. Blood–brain barrier dysfunction in parkinsonian midbrain in vivo. *Ann Neurol* 57 (2):176–179. doi:10.1002/ana.20369.

Kumar, H., H. W. Lim, S. V. More, B. W. Kim, S. Koppula, I. S. Kim, and D. K. Choi. 2012. The role of free radicals in the aging brain and Parkinson's Disease: Convergence and parallelism. *Int J Mol Sci* 13 (8):10478–10504. doi:10.3390/ijms130810478.

Kuopio, A. M., R. J. Marttila, H. Helenius, and U. K. Rinne. 1999. Environmental risk factors in Parkinson's disease. *Mov Disord* 14 (6):928–939.

Lamberti, P., S. Zoccolella, G. Iliceto, E. Armenise, A. Fraddosio, M. de Mari, and P. Livrea. 2005. Effects of levodopa and COMT inhibitors on plasma homocysteine in Parkinson's disease patients. *Mov Disord* 20 (1):69–72. doi:10.1002/mds.20261.

Lee, K. W., J. Y. Im, J. M. Woo, H. Grosso, Y. S. Kim, A. C. Cristovao, P. K. Sonsalla et al. 2013. Neuroprotective and anti-inflammatory properties of a coffee component in the MPTP model of Parkinson's disease. *Neurotherapeutics* 10 (1):143–153. doi:10.1007/s13311-012-0165-2.

Lees, A. J., J. Hardy, and T. Revesz. 2009. Parkinson's disease. *The Lancet* 373 (9680): 2055–2066. doi:10.1016/S0140-6736(09)60492-X.

Lesage, S. and A. Brice. 2009. Parkinson's disease: From monogenic forms to genetic susceptibility factors. *Hum Mol Genet* 18 (R1):R48–R59. doi:10.1093/hmg/ddp012.

Lesser, R. P., S. Fahn, S. R. Snider, L. J. Cote, W. P. Isgreen, and R. E. Barrett. 1979. Analysis of the clinical problems in parkinsonism and the complications of long-term levodopa therapy. *Neurology* 29 (9 Pt 1):1253–1260.

Leutner, S., A. Eckert, and W. E. Müller. 2001. ROS generation, lipid peroxidation and antioxidant enzyme activities in the aging brain. *J Neural Transm* 108 (8–9):955–967.

Levites, Y., O. Weinreb, G. Maor, M. B. H. Youdim, and S. Mandel. 2001. Green tea polyphenol (–)-epigallocatechin-3-gallate prevents N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced dopaminergic neurodegeneration. *J Neurochem* 78 (5):1073–1082. doi:10. 1046/j.1471-4159.2001.00490.x.

Lin, C. Y., J. H. Chen, R. H. Fu, and C. W. Tsai. 2014. Induction of Pi form of glutathione S-transferase by carnosic acid is mediated through PI3K/Akt/NF-κB pathway and protects against neurotoxicity. *Chem Res Toxicol* 27 (11):1958–1966. doi:10.1021/tx5003063.

Lindahl, T. and B. Nyberg. 1972. Rate of depurination of native deoxyribonucleic acid. *Biochemistry* 11 (19):3610–3618.

Liu, H. G., K. Zhang, A. C. Yang, and J. G. Zhang. 2015. Deep brain stimulation of the subthalamic and pedunculopontine nucleus in a patient with Parkinson's disease. *J Korean Neurosurg Soc* 57 (4):303–306. doi:10.3340/jkns.2015.57.4.303.

Lopez, A., A. Muñoz, M. J. Guerra, and J. L. Labandeira-Garcia. 2001. Mechanisms of the effects of exogenous levodopa on the dopamine-denervated striatum. *Neuroscience* 103 (3):639–651.

Lovell, M. A., W. D. Ehmann, M. P. Mattson, and W. R. Markesbery. 1997. Elevated 4-hydroxynonenal in ventricular fluid in Alzheimer's disease. *Neurobiol Aging* 18 (5):457–461.

Machado-Filho, J. A., A. O. Correia, A. B. A. Montenegro, M. E. P. Nobre, G. S. Cerqueira, K. R. T. Neves, M. da Graça Naffah-Mazzacoratti et al. 2014. Caffeine neuroprotective effects on 6-OHDA-lesioned rats are mediated by several factors, including pro-inflammatory cytokines and histone deacetylase inhibitions. *Behav Brain Res* 264:116–125. doi:10.1016/j.bbr.2014.01.051.

Mallajosyula, J. K., S. J. Chinta, S. Rajagopalan, D. G. Nicholls, and J. K. Andersen. 2009. Metabolic control analysis in a cellular model of elevated MAO-B: Relevance to Parkinson's disease. *Neurotox Res* 16 (3):186–193. doi:10.1007/s12640-009-9032-2.

Mejia, E. G. and M. V. Ramirez-Mares. 2014. Impact of caffeine and coffee on our health. *Trends Endocrinol Metab* 25 (10):489–492. doi:10.1016/j.tem.2014.07.003.

Mercer, L. D., B. L. Kelly, M. K. Horne, and P. M. Beart. 2005. Dietary polyphenols protect dopamine neurons from oxidative insults and apoptosis: Investigations in primary rat mesencephalic cultures. *Biochem Pharmacol* 69 (2):339–345. doi:10.1016/j.bcp.2004.09.018.

Metman, L. V., P. Del Dotto, K. LePoole, S. Konitsiotis, J. Fang, and T. N. Chase. 1999. Amantadine for levodopa-induced dyskinesias: A 1-year follow-up study. *Arch Neurol* 56 (11):1383–1386.

Metman, L. V., P. Del Dotto, P. van den Munckhof, J. Fang, M. M. Mouradian, and T. N. Chase. 1998. Amantadine as treatment for dyskinesias and motor fluctuations in Parkinson's disease. *Neurology* 50 (5):1323–1326.

Moore, D. J., A. B. West, V. L. Dawson, and T. M. Dawson. 2005. Molecular pathophysiology of parkinson's disease. *Annu Rev Neurosci* 28 (1):57–87. doi:10.1146/annurev.neuro.28.061604.135718.

Mortimer, J. A., F. J. Pirozzolo, E. C. Hansch, and D. D. Webster. 1982. Relationship of motor symptoms to intellectual deficits in Parkinson disease. *Neurology* 32 (2):133–137. doi:10.1212/WNL.32.2.133.

Mouradian, M. M. 2002. Recent advances in the genetics and pathogenesis of Parkinson disease. *Neurology* 58 (2):179–185.

Männistö, P. T. and S. Kaakkola. 1990. Rationale for selective COMT inhibitors as adjuncts in the drug treatment of Parkinson's disease. *J Pharmacol Toxicol* 66 (5):317–323.

Ng, T. P., L. Feng, M. Niti, E. H. Kua, and K. B. Yap. 2008. Tea consumption and cognitive impairment and decline in older Chinese adults. *Am J Clin Nutr* 88 (1):224–231.

Niccolini, F., L. Rocchi, and M. Politis. 2015. Molecular imaging of levodopa-induced dyskinesias. *Cell Mol Life Sci* 72 (11):2107–2117. doi:10.1007/s00018-015-1854-x.

Oertel, W. H. and N. P. Quinn. 1997. Parkinson's disease: Drug therapy. *Baillieres Clin Neurol* 6 (1):89–108.

Pan, J., H. Li, J. F. Ma, Y. Y. Tan, Q. Xiao, J. Q. Ding, and S. D. Chen. 2012. Curcumin inhibition of JNKs prevents dopaminergic neuronal loss in a mouse model of Parkinson's disease through suppressing mitochondria dysfunction. *Transl Neurodegener* 1:16–16. doi:10.1186/2047-9158-1-16.

Pandey, A. K., R. Patnaik, D. F. Muresanu, A. Sharma, and H. S. Sharma. 2012. Quercetin in hypoxia-induced oxidative stress: Novel target for neuroprotection. *Int Rev Neurobiol* 102:107–146.

Pandi-Perumal, S. R., A. S. BaHammam, G. M. Brown, D. W. Spence, V. K. Bharti, C. Kaur, R. Hardeland, and D. P. Cardinali. 2013. Melatonin antioxidative defense: Therapeutical implications for aging and neurodegenerative processes. *Neurotox Res* 23 (3):267–300. doi:10.1007/s12640-012-9337-4.

Patki, G. and Y. S. Lau. 2011. Melatonin protects against neurobehavioral and mitochondrial deficits in a chronic mouse model of Parkinson's disease. *Pharmacol Biochem Behav* 99 (4):704–711. doi:10.1016/j.pbb.2011.06.026.

Piccini, P., D. J. Burn, R. Ceravolo, D. Maraganore, and D. J. Brooks. 1999. The role of inheritance in sporadic Parkinson's disease: Evidence from a longitudinal study of dopaminergic function in twins. *Ann Neurol* 45 (5):577–582.

Polymeropoulos, M. H., C. Lavedan, E. Leroy, S. E. Ide, A. Dehejia, A. Dutra, B. Pike et al. 1997. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 276 (5321):2045–2047.

Quinn, N. 1995. Drug treatment of Parkinson's disease. *BMJ* 310 (6979):575–579.

Ren, P., H. Jiang, R. Li, J. Wang, N. Song, H. M. Xu, and J. X. Xie. 2009. Rosmarinic acid inhibits 6-OHDA-induced neurotoxicity by anti-oxidation in MES23.5 cells. *J Mol Neurosci* 39 (1–2):220–225. doi:10.1007/s12031-009-9182-y.

Rhee, S. G. 1999. Redox signaling: Hydrogen peroxide as intracellular messenger. *Exp Mol Med* 31 (2):53–59. doi:10.1038/emm.1999.9.

Rocha-González, H. I., M. Ambriz-Tututi, and V. Granados-Soto. 2008. Resveratrol: A natural compound with pharmacological potential in neurodegenerative diseases. *CNS Neurosci Ther* 14 (3):234–247. doi:10.1111/j.1755-5949.2008.00045.x.

Rodriguez-Oroz, M. C., J. A. Obeso, A. E. Lang, J. L. Houeto, P. Pollak, S. Rehncrona, J. Kulisevsky et al. 2005. Bilateral deep brain stimulation in Parkinson's disease: A multicentre study with 4 years follow-up. *Brain* 128 (Pt 10):2240–2249. doi:10.1093/brain/awh571.

Saura, J., J. M. Luque, A. M. Cesura, M. Da Prada, V. Chan-Palay, G. Huber, J. Löffler, and J. G. Richards. 1994. Increased monoamine oxidase B activity in plaque-associated astrocytes of Alzheimer brains revealed by quantitative enzyme radioautography. *Neuroscience* 62 (1):15–30.

Shulman, J. M., P. L. De Jager, and M. B. Feany. 2011. Parkinson's disease: Genetics and pathogenesis. *Annu Rev Pathol-Mech* 6 (1):193–222. doi:10.1146/annurev-pathol-011110-130242.

Sohn, Y. H., Y. Jeong, H. S. Kim, J. H. Im, and J. S. Kim. 2000. The brain lesion responsible for parkinsonism after carbon monoxide poisoning. *Arch Neurol* 57 (8):1214–1218.

Spillantini, M. G., M. L. Schmidt, V. M. Lee, J. Q. Trojanowski, R. Jakes, and M. Goedert. 1997. Alpha-synuclein in Lewy bodies. *Nature* 388 (6645):839–840. doi:10.1038/42166.

Spinelli, K. J., V. R. Osterberg, C. K. Meshul, A. Soumyanath, and V. K. Unni. 2015. Curcumin treatment improves motor behavior in α-synuclein transgenic mice. *PLoS One* 10 (6):e0128510. doi:10.1371/journal.pone.0128510.

Su, L. Y., H. Li, L. Lv, Y. M. Feng, G. D. Li, R. Luo, H. J. Zhou et al. 2015. Melatonin attenuates MPTP-induced neurotoxicity via preventing CDK5-mediated autophagy and SNCA/α-synuclein aggregation. *Autophagy* 11 (10):1745–1759. doi:10.1080/15548627.2015.1082020.

Sutachan, J. J., Z. Casas, S. L. Albarracin, B. R. Stab, I. Samudio, J. Gonzalez, L. Morales, and G. E. Barreto. 2012. Cellular and molecular mechanisms of antioxidants in Parkinson's disease. *Nutr Neurosci* 15 (3):120–126. doi:10.1179/1476830511Y.0000000033.

Sweet, R. D. and F. H. McDowell. 1975. Five years' treatment of Parkinson's disease with levodopa. Therapeutic results and survival of 100 patients. *Ann Inter Med* 83 (4):456–463.

Tan, L. C., W. P. Koh, J. M. Yuan, R. Wang, W. L. Au, J. H. Tan, E. K. Tan, and M. C. Yu. 2008. Differential effects of black versus green tea on risk of Parkinson's disease in the Singapore Chinese health study. *Am J Epidemiol* 167 (5):553–560. doi:10.1093/aje/kwm338.

Tellone, E., A. Galtieri, A. Russo, B. Giardina, and S. Ficarra. 2015. Resveratrol: A focus on several neurodegenerative diseases. *Oxid Med Cell Longe* 2015:392169. doi:10.1155/2015/392169.

Thannickal, V. J. and B. L. Fanburg. 2000. Reactive oxygen species in cell signaling. *Am J Physiol Lung Cell Mol Physiol* 279 (6):L1005–L1028.

Tomer, R., B. E. Levin, and W. J. Weiner. 1993. Side of onset of motor symptoms influences cognition in Parkinson's disease. *Ann Neurol* 34 (4):579–584. doi:10.1002/ana.410340412.

Vallianou, N. G., A. Evangelopoulos, N. Schizas, and C. Kazazis. 2015. Potential anticancer properties and mechanisms of action of curcumin. *Anticancer Res* 35 (2):645–651.

Weinreb, O., S. Mandel, T. Amit, and M. B. Youdim. 2009. Neuro protective molecular mechanisms of (-)-epigallocatechin-3-gallate: A reflective outcome of its antioxidant, iron chelating and neuritogenic properties. *Genes Nut* 4 (4):283–296.

Woods, S. P., J. A. Fields, and A. I. Tröster. 2002. Neuropsychological sequelae of subthalamic nucleus deep brain stimulation in Parkinson's disease: A critical review. *Neuropsychol Rev* 12 (2):111–126.

Wu, C. R., C. W. Tsai, S. W. Chang, C. Y. Lin, and L. C. Huang. 2015. Carnosic acid protects against 6-hydroxydopamine-induced neurotoxicity in in vivo and in vitro model of Parkinson's disease: Involvement of antioxidative enzymes induction. *Chemico-Biological Interactions* 225:40–46. doi:10.1016/j.cbi.2014.11.011.

Youdim, M. B. and M. Weinstock. 2004. Therapeutic applications of selective and non-selective inhibitors of monoamine oxidase A and B that do not cause significant tyramine potentiation. *Neurotoxicology* 25 (1–2):243–250. doi:10.1016/S0161-813X(03)00103-7.

Zuo, L. and M. S. Motherwell. 2013. The impact of reactive oxygen species and genetic mitochondrial mutations in Parkinson's disease. *Gene* 532 (1):18–23. doi:10.1016/j.gene.2013.07.085.

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

7

Nutraceutical or Food-Based Antioxidants for Depression

Oyinlola Adeyanju, Thomas Towers, and Thea Moore

Contents

7.1 Introduction

Depression is one of the most prevalent and disabling disorders in the United States (U.S.) and worldwide (Hasin and Grant 2015). Depression typically has an onset in the late 20's and has a huge disease burden during peak working years. This being the case, it has commonly been asked if there is a way to prevent and/or treat major depression in a manner other than prescription medications. Furthermore, there have been many inquiries into the role of diet and phytochemicals in prevention and management of depression. It has been hypothesized that activation of the immune-inflammatory process, increase in the metabolism of monoamines, and abnormalities in lipid compounds may cause overproduction of reactive oxygen species (ROS), which leads to the oxidative stress playing a role in the pathophysiology of depressive disorders (Bilici et al. 2001, Moylan et al. 2014, Maes et al. 2011). Although it is noted that oxidative stress is closely linked to the inflammatory process and proinflammatory cytokines are produced in response to oxidative stress (Black et al. 2015), this chapter will examine the evidence regarding the role of oxidative stress in depression, thereby indicating a potential role for antioxidants in treating depression. This chapter will examine free radicals produced by

the body and the role of antioxidants in the oxidative stress equation. There has been some controversy regarding the best way to measure the amount of oxidative stress that has occurred or is occurring physiologically. The evidence base for oxidative stress markers and how these have been utilized to provide data and insight into the role of oxidative stress in depression will be examined. Finally, this chapter will review the evidence for antioxidants from dietary supplements and/or food sources as therapy in the treatment of depression.

7.2 Incidence and prevalence of depression

Depression is one of the most common mental illnesses in the United States and worldwide. According to the World Health Organization (WHO), depression occurs in an estimated 350 million people worldwide. It is a mood disorder that affects all ages, with more women affected than men (Reynolds et al. 2015). In 2013, Substance Abuse and Mental Health Services Administration (SAMHSA) published 12-month prevalence data on various aspects of depression, based on National Survey on Drug Use and Health Project by the SAMHSA it was estimated that 43.8 million adults aged 18 or older in the United States had various mental illnesses in the past year (SAMHSA, 2014). This represents 18.5% of all adults in the United States. The majority of the adults were between the ages of 26–49 years (21.5%) old, followed by ages 18–25 (19.4%) and ages 50 or older (15.3%). A comprehensive national survey of 12,312 adults aged 55 and older revealed that major depression was the most prevalent mood disorder (5.6% out of 6.8%) within the past year (Reynolds et al. 2015). The National Epidemiology Survey on Alcohol and Related Conditions also found that major depressive disorder (MDD) was more prevalent than other affective disorders within the past 12 months (5.3%) and over the lifetime (13.2%) (Hasin and Grant 2015).

It was also estimated that 10.1 million adults (4.3%) and 1.9 million youths (7.7%) had at least one major depressive episode (MDE) with severe impairment in the previous year. Severe impairment was defined as interference in home management, school, work, close relationships with family, and social life. SAMHSA also estimated that women had higher rates of MDD than men (6.9% versus 3.6%) in 2013 (Hasin and Grant 2015).

Depression is an underdiagnosed and undertreated illness, particularly in primary care settings (Mihrshahi et al. 2015). This creates significant social and economic burden for the individual and population. It is the leading cause of disability globally. Mental health treatment spending accounted for 6.2% ($100 billion) of all healthcare spending in the United States in 2003 (Mark et al. 2007). WHO estimates the global cost of mental illness at about $2.5 trillion (two-thirds in indirect costs) in 2010, with a projected increase to more than $6 trillion by 2030 (Bloom et al. 2011). By 2020, depression is predicted to be one of the top three leading causes of disability-adjusted life years (DALYs) in developing countries, developed countries, and worldwide (Murray and Lopez 1997).

However, WHO has observed that mental health conditions have been the leading cause of DALYs worldwide since 2011, accounting for 37% loss of healthy life years from non-communicable diseases (Bloom et al. 2011). This is detrimental to the economy because depression is chronic with high risk of recurrence. It affects productivity and may increase the risk of suicide, especially in undertreated or untreated patients. In 2013, SAMSHA estimated 9.3 million adults (3.9%) aged 18 or older had serious thoughts of suicide, in which 2.7 million (1.1%) made suicide plans and 1.3 million (0.6%) attempted suicide within the past year (SAMHSA, 2014). Prevalence of suicide thoughts was highest among adults aged 18–25 (7.4%) followed by ages 26–49 (4%) and ages 50 or older (2.7%). It has been hypothesized that oxidative and nitrosative stress, as well as lowered antioxidant levels, play a role in the pathophysiology of suicidal behavior (Vargas et al. 2013).

Deaths from non-communicable diseases are expected to increase from 59% in 2002 to 69% in 2030 (Mathers and Loncar 2006). This demonstrates the need for effective treatment options at a lower cost. Although there are multiple known, effective treatments for depression, fewer than half of those affected obtain treatment. Treatment that is available through food sources and/or dietary supplements may increase access and utilization.

7.3 Role of oxidative and nitrosative stress in pathophysiology of depression

Several studies support the role of oxidative stress in many diseases and disorders such as diabetes, atherosclerosis, and cognitive impairment as well as neuropsychiatric disorders such as schizophrenia, bipolar disorder, and major depression (Pandya et al. 2013, Palta et al. 2014). Multiple studies have indicated that depression is associated with an increased oxidative stress and a decrease in antioxidant defenses (Maes et al. 2011, Chung et al. 2013, Pandya et al. 2013, Moylan et al. 2014, Palta et al. 2014). Oxidative stress may be defined as an imbalance or lack of homeostasis between free radicals (oxidants) and antioxidants. In oxidative stress, the damaging effects of free radicals to lipids, proteins, and DNA outweigh the protective effects of antioxidants (Cumurcu et al. 2009, Maes et al. 2011, Moylan et al. 2014). This imbalance leads to cellular damage of lipids, proteins, and DNA and disruption of redox signaling and control, which eventually may lead to cell death and apoptosis (**Figure 7.1**) (Moylan et al. 2014).

7.4 Free radicals

The majority of the free radicals formed in the body are ROS and reactive nitrogen species (RNS) (Pandya et al. 2013). Examples of ROS/RNS include peroxynitrite, superoxides, peroxides, and nitric oxides (NO) and are produced

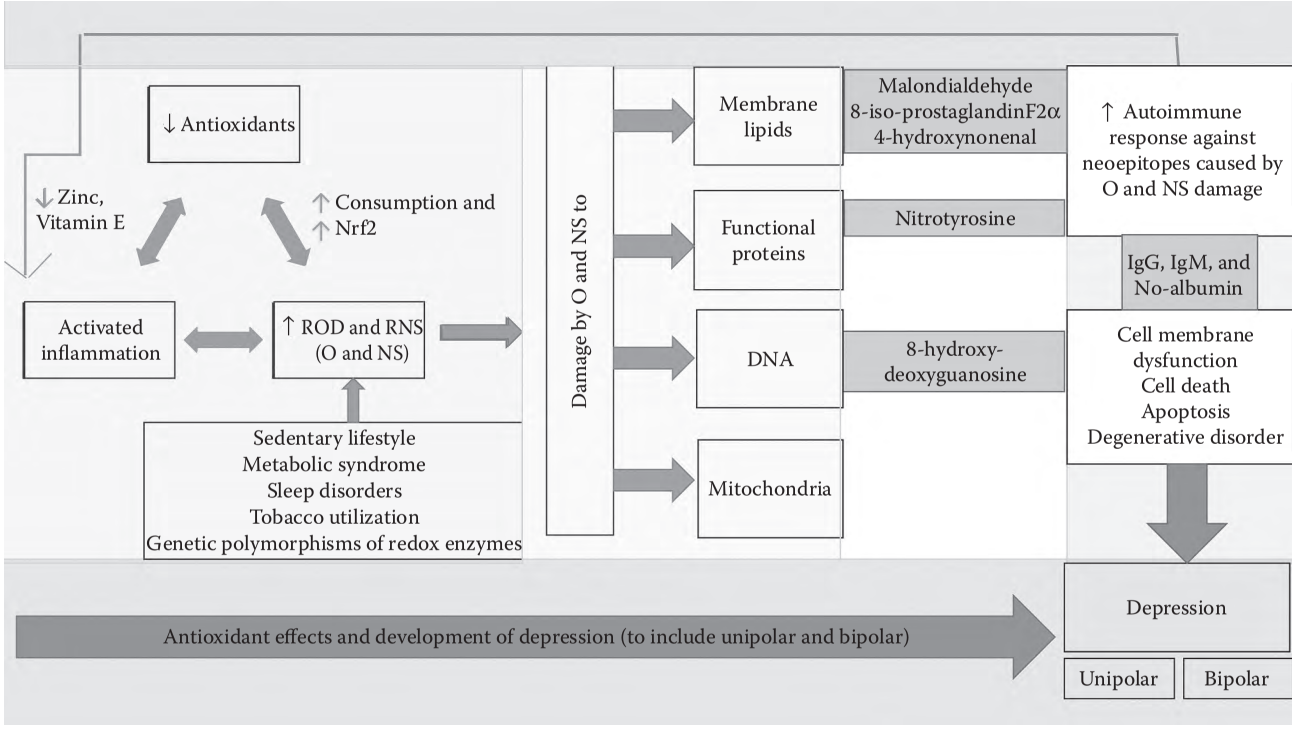


Figure 7.1 *This figure illustrates the inflammatory, oxidative, and nitrosative stress (O&NS) pathways causing degenerative progression. Contributing factors that may lead to increased O&NS are also illustrated. Lower levels of key antioxidants and increased O&NS will activate nuclear factor erythroid 2-related factor (NrF2) leading to an increase in the endogenous antioxidants. Damage caused by O&NS to membrane lipids and functional proteins can result in an (auto) immune response against the neoepitopes. The following assays have been employed to measure depression: membrane lipids (malondialdehyde, 8-iso-prostaglandinF2α, 4-hydroxynonenal), functional proteins (nitrotyrosine), and DNA (8-hydroxy-deoxyguanosine).*

during typical cellular processes (Maes et al. 2011, Moylan et al. 2014). Free radicals interact with proteins, fatty acids, and DNA to perform a multitude of roles in regulating cellular function. Free radicals are generated during inflammatory and mitochondrial metabolic processes. ROS and RNS consist of radicals and other highly reactive oxygen/nitrogen factors that interact with numerous substrates, including fatty acids, proteins, mitochondria, and DNA (Maes et al. 2011). An excess of ROS/RNS (free radicals) leads to damage of lipids, proteins, and DNA when interacting with these substrates. Excessive ROS has been linked to alterations in structural components of the cell membrane, which then results in reduced membrane microviscosity as well as neurotransmitter dysfunction and may ultimately lead to cell death (Maes et al. 2011, Chang et al. 2015).

7.5 Antioxidants

Under normal circumstances, the antioxidant defense system consisting of antioxidants, antioxidant enzymes, and proteins aids in regulating the amount of ROS and RNS produced and removed. Antioxidants play a role in balancing oxidative and nitrosative reactions by removing ROS and RNS through scavenging radicals and decreasing the production of ROS and RNS. Examples of nonenzymatic scavenger antioxidants include coenzyme Q10, glutathione, vitamin C, and vitamin E. Enzymatic antioxidants neutralize ROS such as peroxides and superoxides. Superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione reductase (GR), and glutathione-S-transferase (GST) are examples of antioxidant enzymes. Other nonenzymatic antioxidants include reduced glutathione, *N*-acetyl-cysteine (NAC), uric acid, carotenoids, flavonoids, ubiquinol, and zinc (Pandya et al. 2013). Proteins that function as antioxidants include albumin, transferrin, haptoglobin, and ceruloplasmin. These proteins bind ROS and RNS, thus protecting the tissues against potential damage from ROS and RNS. Oxidative and nitrosative stress results when there is an imbalance between the amount of ROS/RNS present and antioxidant defenses. Elevated levels of ROS/RNS may result from lowered antioxidant concentrations in the body and/or lowered activities of antioxidant enzymes. Excess free radicals cause significant damage to macromolecules such as DNA, proteins, and lipids (lipid peroxidation). The oxidation of lipids by ROS, lipid peroxidation of polyunsaturated fatty acids (PUFAs) leads to the production of reactive compounds such as croton aldehyde, malondialdehyde (MDA), and 4-hydroxyalkenals (Pandya et al. 2013). The oxidative damage to membrane lipids and DNA, as well as nitrosative damage to proteins, may lead to cellular dysfunction through damage to the cell wall, mitochondria, DNA, and functional proteins, which eventually would result in cell death and apoptosis (Maes et al. 2011). ROS and RNS are primarily generated within the mitochondrion; therefore, oxidative stress has the potential to damage mitochondrial defense systems, which would further perpetuate the oxidative and nitrosative stress. Due to high metabolic rate, increased amount of PUFA, which are prone to oxidation, lower antioxidant enzyme activity and antioxidant levels, organs

such as the brain are more susceptible to deleterious effects of oxidative and nitrosative stress (Kodydkova et al. 2009, Maes et al. 2011).

Often, the focus is on the detrimental effects of free radicals. It is worth noting that ROS are required for a number of biological reactions with protective functions. ROS play an important role in cytochrome P450-dependent detoxification reactions. ROS are also key mediators of apoptosis. Caution is recommended with the use of antioxidants because excessive antioxidant use could interfere with some of the protective functions of ROS.

7.6 Nutrient antioxidants

In addition to vitamins C, D, and minerals such as zinc or selenium antioxidants mentioned in **Table 7.1**, there are hundreds of other nutrient antioxidants. Commonly studied nutrient antioxidants include polyphenols,

Table 7.1 List of Free Radicals, Antioxidants, Oxidative Stress Markers, and Antioxidant Status Markers

Free Radicals	Antioxidants	Oxidative Stress Markers	Antioxidant Status Markers
Reactive oxygen species (ROS) • Superoxide anion • Hydroxy radical • Hydrogen peroxide Reactive nitrogen species (RNS) • Nitric oxide • Nitrogen dioxide • Peroxynitrite	Enzymatic Adenosine deaminase (ADA) Superoxide dismutase (SOD) Catalase (CAT) Copper Glutathione peroxidase (GPx) Glutathione reductase (GR) Glutathione-S-transferase (GST) Paraoxonase 1 (PON1) Nonenzymatic albumin Reduced glutathione (GSH) Coenzyme Q10 High-density lipoprotein (HDL) cholesterol Provitamin A Vitamin C Vitamin E *N*-acetyl-cysteine (NAC) Uric acid Carotenoids Flavonoids Selenium Ubiquinol Zinc	Malondialdehyde (MDA) Nitric oxide (NO) 8-F2-isoprostanes 8-hydroxy-2-deoxyguanosine (8-OHdG) Total oxidative species (TOS) Xanthine oxidase (XO) Protein carbonyl Thiobarbituric acid reactive substances (TBARS)	Total antioxidant capacity (T-AOC/TOC) Total antioxidant status (TAS) Superoxide dismutase (SOD) Ascorbic acid GPx Bilirubin Vitamin C Vitamin E Oxygen radical absorbance capacity (ORAC) Ferric ion reducing antioxidant power (FRAP) Trolox equivalence antioxidant capacity (TEAC)

Source: Pandya, C. D. et al., *Prog. Neuropsychopharmacol Biol. Psychiatry*, 46, 214–223, 2013; Cumurcu, B. E. et al., *Psychiatry Clin. Neurosci.*, 63, 639–645, 2009; Moylan, S. et al., *Neurosci. Biobehav. Rev.*, 45, 46–62, 2014; Liu, T., *PLoS One.*, 10, e0138904, 2015a; Kodydkova, J. et al., *Clin. Biochem.*, 42, 1368–1374, 2009.

Table 7.2 Food Sources for Dietary Antioxidants

Dietary Antioxidants	Food Sources
Carotenoids *α-carotene* *β-carotene* *β-cryptoxanthin* *lutein* *lycopene* *zeaxanthin*	Apricots, asparagus, beets, broccoli, cantaloupe, carrots, corn, green peppers, kale, mango, turnip, and collard greens, nectarines, peaches, pink grapefruit, pumpkin, squash, spinach, sweet potato, tangerines, tomatoes, and watermelon
Polyphenols *Epigallocatechin 3-gallate (EGCG)* *Flavonoids* *Phenolic acids* *lignans* *resveratrol* *stilbenes*	Red wine, cloves, peppermint, cocoa powder, dark chocolate, green teas
Retinol	Egg yolks, liver, fish oil, whole milk, and butter[a]
Selenium	Brazil nuts, tuna, beef, poultry, fortified breads, and other grain products
Vitamin C	Berries, broccoli, Brussels sprouts, cantaloupe, cauliflower, grapefruit, honeydew, kale, kiwi, mango, nectarine, orange, papaya, snow peas, sweet potato, strawberries, tomatoes, and red, green, or yellow peppers
Vitamin E	Broccoli (boiled), avocado, chard, mustard and turnip greens, mangoes, nuts, papaya, pumpkin, red peppers, spinach (boiled), and sunflower seeds
Zinc	Beans, nuts, whole grains, some fortified cereals, and dairy products

Source: WebMD, WebMD Antioxidants-Topic Overview. Accessed 12/11/15. http://www.webmd.com/food-recipes/antioxidants-topic-overview, 2015; Perez-Jimenez, J. et al., *Eur. J. Clin. Nutr.,* 64, S112–S120, 2010.

[a] Plants can synthesize carotenoids but cannot convert them to retinoids; this process occurs in the human body.

which encompass compounds such as flavonoids, anthocyanins, and epigallocatechin 3-gallate (EGCG). Polyphenols are the most abundant source of antioxidants in foods (Perez-Jimenez et al. 2010, Scalbert et al. 2005). These may be found in foods such as berries, grapes, red wine, and green tea. See **Table 7.2** for common food sources of dietary antioxidants.

7.7 Antioxidant capacity, oxidative stress markers, and depression

One of the greatest challenges in research and investigations of the association between oxidative stress and depression is determining the most accurate and appropriate method to measure oxidative stress. In these investigations, multiple methods have been utilized to assess antioxidant levels and

oxidative stress. Once the antioxidant and oxidative stress levels have been determined, the next challenge is figuring out how these levels relate to physical and clinical symptoms.

In 2007, the United States Department of Agriculture (USDA) released the first database of antioxidant activity for 277 selected foods using oxygen radical absorbance capacity (ORAC) methodology. The ORAC assay measures the degree of inhibition of peroxy-radical–induced oxidation by the chemicals or compounds of interest in a chemical milieu. It measures the value as Trolox equivalents and includes both inhibition time and the extent of inhibition of oxidation. This has been the assay of choice used to measure the antioxidant activity of foods. The ORAC assay is considered by some to be a preferable method because of its biological relevance to the *in vivo* antioxidant efficacy. This methodology was designed to give an estimate of the collective antioxidant power of a variety of components in a given food instead of focusing on individual or minimal components. Prior and colleagues developed a method that measures both hydrophilic (H–ORAC) and lipophilic ORAC (L–ORAC) for water-soluble and fat-soluble antioxidant compounds, respectively (Prior et al. 2003).

Other measures of antioxidant capacity (AC) include ferric ion reducing antioxidant power (FRAP) and Trolox equivalence antioxidant capacity (TEAC) assays (Prior et al. 2005, Carlsen et al. 2010). These assays are based on discrete underlying mechanisms that use differing radical or oxidant sources and therefore generate distinct values and cannot be compared directly. The Folin assay is considered a crude measure of total antioxidant content. The Folin assay is commonly used to roughly estimate the total polyphenol content in foods. This test is not specific for polyphenols however, since the Folin reagent also reacts to other food constituents. Therefore, it is recommended that the Folin assay be regarded as a global antioxidant assay, comparable to other redox assays such as the ORAC (Perez-Jimenez et al. 2010). Since antioxidant compounds with dissimilar chemical structures interact with different free radical sources, the relationship between any two AC methods will be quite low if considered across all foods. Thus, it is not possible to develop a mathematical relationship between two methods across a wide spectrum of foods. Similar to the content of any food component, AC values will vary due to a wide array of factors, such as the manner of cultivation, growing conditions, harvesting, food processing and preparation, sampling, and analytical procedures. Again, this presents a challenge when attempting to make comparisons of antioxidant capacity of a variety of foods and dietary supplements. This is also true when evaluating results from multiple trials or studies that utilize a wide variety of oxidative stress markers and/or antioxidant capacity measures.

In 2012, the USDA Nutrient Data laboratory removed the ORAC database from its website due to issues of improper and misleading use stating, “ORAC values are routinely misused by food and dietary supplement manufacturing companies to promote their products and by consumers to guide their food

and dietary supplement choices" (U.S. Department of Agriculture USDA). They went on to state "The values indicating antioxidant capacity have no relevance to the effects of specific bioactive compounds, including polyphenols on human health." This statement from the USDA refers to the challenges that presented when attempting to demonstrate the relationship between antioxidant activity and expression of chronic disease.

Recent studies examining oxidative product levels in peripheral, blood, red blood cells (RBC), mononuclear cells, urine, cerebrospinal fluid, and postmortem brains indicate that these levels were abnormal as compared to nondepressed patients. A meta-analysis of oxidative stress markers in depression indicates that serum total antioxidant capacity (TAC), paraoxonase, and antioxidant levels are lower, whereas serum free radical and oxidative damage product levels are higher in depressed patients as compared to controls (Liu et al. 2015a).

Some studies have looked at the levels of oxidative stress markers, including oxidative products of lipid peroxidation such as MDA, 8-F2-isoprostane. Others have looked at markers of oxidative damage to DNA, RNA, and protein such as 8-hydroxy-2-deoxyguanosine (8-OHdG), 8-oxo-7, 8-dihydroguanosine, and protein carbonyl, respectively. One systematic review and meta-analysis examined the association of 8-OHdG or F2-isoprostanes with elevated depressive symptoms, MDD or bipolar disorder (BD) (Black et al. 2015). The authors concluded that both oxidative stress markers, 8-OHdG and F2-isoprostanes are increased in subjects with depression (i.e., MDD, BD, and depressive symptoms) compared to controls. The effect sizes ranged from small to moderate range. Subgroup analyses of the 8-OHdG studies indicated that there was some variation in results based on the type of biological specimen and/or laboratory method for oxidative stress measurement. The findings for F2-isoprostanes had less variation and did not differ when analyzed by the type of depression, biological specimen, or laboratory method.

The increase in these two oxidative stress markers indicates increased damage to DNA and lipids. These findings are consistent with other studies such as the investigation by Chung and colleagues that tested the hypothesis that urinary F2 isoprostanes as a robust marker of oxidative stress are increased in patients with depression and are associated with symptom response and treatment. The study found that increased F2-isoprostane excretion was significantly higher in patients with depression as compared to controls, even after adjusting for age, sex, and BMI (Chung et al. 2013). Similarly, in the meta-analysis by Palta et al. 23 studies were included in which there were 12 different oxidative stress markers reported and 7 different depression measures (Palta et al. 2014). Overall there was a clear association between oxidative stress and depression, but the results were somewhat heterogeneous. For example, some of the oxidative stress markers exhibited stronger associations with depression than others (malondialdehyde and 8-oxo-2-deoxyguanosine). Most frequently, MDA and NO were reported as oxidative stress markers. There was

also significant variability in measurement of antioxidant stress markers, with SOD and glutathione peroxidase being examined most commonly. It is important to note that there were several different measures of depression, including Hamilton Depression Rating Scale, Geriatric Depression Scale, and Beck Depression Inventory (BDI) (**Table 7.3**).

Most recently, a meta-analysis was done by Jimenez-Fernandez and colleagues to investigate the role of oxidative stress and antioxidants in depression (Jimenez-Fernandez et al. 2015). These authors elaborated on significant limitations with the Palta meta-analysis. Although the results of the Palta meta-analysis are interesting and promising, multiple methodological flaws complicate the interpretation of these results. Effect sizes were pooled across the 12 reported oxidative stress markers. This may be problematic because information is lost regarding the individual markers. Only selected markers were available across a number of studies; therefore, the total mean values were not complete and not equally weighted. Lack of information about the individual markers makes it difficult to identify potential targets for the development of specific drugs (Jimenez-Fernandez et al. 2015).

The association of oxidative stress and depression has been a major focus of much research. Some of the heterogeneity of studies in systematic reviews and meta-analyses has been due to investigators pooling results from studies of bipolar depression with results from studies with unipolar depression. This is problematic since the difference in symptoms of the two disorders may lead to inaccurate estimations or conclusions regarding the effect of antioxidants on symptom response (Jimenez-Fernandez et al. 2015). This is discussed further later in this chapter. Summary of key trials evaluating the association of antioxidants and unipolar depression may be seen in Table 7.3.

In order to address some of these limitations, Jimenez-Fernandez and colleagues performed a meta-analysis that included studies in patients with unipolar depression comparing antioxidant or oxidative stress markers with those in healthy controls before and after antidepressant medication treatment. This meta-analysis examined individual stress markers and endogenous antioxidants in this patient population. There were two main findings of the meta-analysis: (1) several indicators of oxidative stress were abnormal in depressed patients compared to healthy control subjects. This included significantly elevated levels of MDA (final product of lipid degradation), significantly lower levels of the antioxidants zinc and uric acid, and upregulated levels of the enzymatic antioxidant SOD. (2) Antidepressant treatment was effective in improving some of these abnormalities. For example, MDA levels decreased significantly following treatment with antidepressants, the nonenzymatic antioxidants uric acid and zinc increased significantly, and there was significant decrease in the enzymatic antioxidant SOD. After treatment the levels of MDA, uric acid, and zinc were comparable to the levels in healthy individuals. Antidepressant treatment did not fully normalize levels of SOD to that of healthy controls.

Table 7.3 Notable References Examining Association of Oxidative Stress/Antioxidant Activity with Depression

Source	Study Design	Depression Measure	Oxidative Stress Markers or Antioxidants	Results	Limitations
(Palta et al. 2014)	Meta-analysis of 23 observational studies with 4980 participants reporting on association between depression and oxidative stress	Beck Depression Inventory (1) CESD (1) DSM-IV criteria (16) Geriatric Depression Scale (1) Hamilton Depression Rating Scale (2) PHQ-9 (1) Zung Self-rating Depression Scale (1)	8-OHdG 8-iso-PGF2α F2a-isoprostanes GGT LOOH MDA NO OxLDL Peroxides TOS	Depression was associated with increased oxidative stress and lower antioxidant status. Effect size relating high oxidative stress to depression was stronger than effect size relating low antioxidant status to depression.	High heterogeneity between studies. Sample sizes ranging from 30 to 3000. Seven different measures of depression used, 12 measurements of oxidative stress status.
(Jimenez-Fernandez et al. 2015)	Meta-analysis of studies comparing oxidative stress markers and antioxidants in depressed patients as compared to healthy controls and depressed patients before and after antidepressant treatment	DSM-IV criteria Hamilton Depression Rating Scale	*Oxidative stress markers* MDA Total nitrites *Antioxidants* Uric acid Zinc *Enzymatic antioxidants* SOD CAT GPX	Individuals with depression have elevated levels of the oxidative stress indicator, MDA, and lower levels of the antioxidants zinc and uric acid and upregulated levels of SOD as compared to healthy controls. Antidepressant treatment decreased MDA levels, SOD levels. Uric acid and zinc levels increased significantly.	Small number of studies and participants. Heterogeneity of studies as it relates to illness duration and severity. Studies did not match patients and controls and/or failed to control for confounding factors such as tobacco/substance use, BMI, and diet.

(*Continued*)

Table 7.3 (*Continued*) Notable References Examining Association of Oxidative Stress/Antioxidant Activity with Depression

Source	Study Design	Depression Measure	Oxidative Stress Markers or Antioxidants	Results	Limitations
(Beydoun et al. 2013)	Epidemiological study using national data on U.S. adults (N = 1798) to examine relationship of elevated depressive symptoms with antioxidant status	Patient Health Questionnaire (PHQ)	*Antioxidants* Retinol, retinyl esters Carotenoids: α-carotene, β-carotene, β-cryptoxanthin, lutein+zeaxanthin, total lycopene and total carotenoids Vitamin E Vitamin C	There is an inverse relationship between serum carotenoids and depressive symptoms with some dose response. There was insufficient evidence to support the role of antioxidants, in general, and carotenoids, specifically, for prevention of depression or depressive symptoms. This result was not attenuated by dietary or supplemental intake.	Estimates of dietary supplement use and antidepressants were crude. Multiple antioxidants were not addressed or included in this study. Other lifestyle and health-related factors may have played a role.

These findings and those of previous studies would indicate that oxidative stress has a role in the expression of depression. Although it is difficult to draw any conclusion about the role of specific antidepressants in addressing or treating oxidative stress, the findings would support that medications, supplements, or foods that impact antioxidant status may have a role in the treatment of depression. Therefore, future studies might address the impact of nutrient antioxidants or antioxidants in dietary supplements and how these might be involved in the treatment of depression and/or depressive symptoms. More recently, investigators are increasingly examining the role of diet and dietary supplements in depression and more specifically, the association between dietary antioxidants and depressive symptoms.

There are several studies that have examined the role of diet in depression. Specifically, multiple studies have looked at associations between fruit and vegetable consumption and depressive symptoms (Payne et al. 2012, Beydoun et al. 2013, Mihrshahi et al. 2015, Liu et al. 2015b, Kingsbury et al. 2016). Findings in these studies point to an inverse relationship between fruit and vegetable consumption and depressive symptoms. The studies also suggest that the association may be more complex than originally thought because the significance of the relationship is attenuated by variables such as tobacco smoking and physical activity. Many of the studies have not taken these confounding variables into account or have accounted for and measured them in different ways. A key question that remains after reviewing these studies is whether or not the antioxidants are responsible for the effect of decreased depressive symptoms or other components of fruits and vegetables such as vitamins, minerals, and/or fiber without antioxidant properties are responsible for the decrease in depressive symptoms.

Another limitation in determining associations between nutrient sources of antioxidants and depressive symptoms is identifying the best or most appropriate method of quantifying the exposure to nutrient antioxidants. Studies of antioxidant nutrients typically have estimated the intake of nutrients from food by the use of (1) questionnaires or food-record techniques and/or (2) biochemical (laboratory) measures such as use of biomarkers as discussed earlier in this chapter.

Beydoun and colleagues are one of the first groups to use national data from the National Health and Nutrition Examination Surveys (NHANES) to examine the relationship of increased depressive symptoms with antioxidant status (Beydoun et al. 2013). The questionnaire section of the NHANES 2005–2006 included the Patient Health Questionnaire, which is a reliable and valid tool for measuring depression. Serum concentrations of key antioxidants were measured using HPLC with photodiode array detection. Total dietary intakes of alcohol and selected antioxidants (alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein + zeaxanthin, lycopene, total carotenoids, vitamin C, and vitamin E) were evaluated. Utilization of a national health survey allowed investigators to account for and/or control for variables such as smoking,

physical activity, other dietary components, and use of antidepressant medication. These variables were major confounders in previous studies.

Key findings include the following: (1) inverse relationship between the total serum carotenoid level and increased depressive symptoms; (2) dose-response relationship when total carotenoids were expressed as quartiles but not significant with other antioxidant levels; and (3) among the carotenoids, beta-carotene (both sexes) and lutein + zeaxanthin (among women, after controlling for dietary intake and supplement use) had an inverse association with elevated depressive symptoms among adults in the United States. Finally, the authors did not find a strong association between depressive symptoms and dietary carotenoids. Furthermore, they conclude that depressive symptoms caused by external stressors trigger oxidative stress, which then causes reduced total concentrations of total carotenoids in serum that is independent of dietary intake of carotenoids.

Payne and colleagues examined the associations between clinically diagnosed depression and intakes of fruits, vegetables, and antioxidants in older adults. Antioxidants assessed included vitamin C, lutein, and beta-cryptoxanthin. Antioxidants, fruits, and vegetables intake were assessed in 278 (144 with depression, 134 without depression) elderly patients through the use of Block 1998 food frequency questionnaire.

Key findings in this study include the following: (1) lower intakes of fruits, vegetables, vitamin C from food and beta-cryptoxanthin were seen in older adults with depression compared to older adults without depression and (2) interestingly, only food sources of antioxidants were inversely associated with depression. This could indicate that form and delivery of antioxidants are crucial and that other components of fruits and vegetables may have an association with depression.

Additional small studies have been done to examine individual antioxidants and association with depression. Niu et al. analyzed a cross-sectional survey of 986 individuals, 70 years and older, community dwelling Japanese individuals (Niu et al. 2013). Specifically, the investigators examined the relationship between vegetables, including tomatoes/tomato products as a major source of lycopene, and depressive symptoms. Lycopene, as the most powerful antioxidant among the carotenoids, seems a reasonable antioxidant to investigate. Dietary intake was assessed by a self-administered dietary intake questionnaire, whereas depressive symptoms were evaluated with the use of the 30-item Geriatric Depression Scale. The authors concluded that a tomato-rich diet is independently related to lower prevalence of depressive symptoms. No relationship was observed between other vegetables and depressive symptoms. Similarly, a small study examining vitamin D supplementation and the effects on the BDI and markers of oxidative stress found beneficial effects of vitamin D supplements on BDI and oxidative stress (Sepehrmanesh et al. 2015).

The typical diet provides more than 25,000 bioactive compounds, which may impact a number of processes relating to chronic illnesses (Carlsen et al. 2010). Therefore, many investigators have hypothesized that measurement of only one

or a few antioxidants will yield limited results. For this reason, more studies are attempting to investigate total antioxidant capacities or more extensive and thorough ways to measure the antioxidant load, in general. Researchers might then have the ability to recommend a varied diet that is high in a number of antioxidant-rich foods. As mentioned previously, the challenge here is the variety of laboratory methods available to measure and assess antioxidant capacity of foods. The previously widely accepted ORAC method was retired by the USDA due to misuse and misleading claims by marketers regarding ORAC values of supplements and other food items marketed for antioxidant properties. Nevertheless, there are other widely accepted methods to measure the antioxidant capacity of foods. If we were to recommend antioxidants or an antioxidant-rich diet, the next question is: What should be included in the diet? What are the best food sources of antioxidants? Numerous lists have been compiled ranking the antioxidant capacity of a variety of foods. The top 20 antioxidant-rich foods as adapted from a list in a recent paper published in the *European Journal of Clinical Nutrition (ECJN)* are given in **Table 7.4** (Perez-Jimenez et al. 2010).

Table 7.4 Antioxidant Content of Top 20 Foods

Rank	Antioxidant Food Source	Antioxidant Content (mg/serving)
1	Globe artichoke heads	1918
2	Black beans	1216
3	Blackberries	821
4	Blueberries	678
5	Currants	646
6	Chestnuts	524
7	Coffee	507
8	Strawberries	480
9	Plums	349
10	Dark chocolate	316
11	Pecans	272
12	Red wine	269
13	Sweet cherries	249
14	Apples	221
15	Raspberries	213
16	Tea	204 (black tea) 121 (green tea)
17	Hazelnuts	192
18	Green beans	185
19	Spinach	170
20	Beer	160

Source: Perez-Jimenez, J. et al., *Eur. J. Clin. Nutr.*, 64, S112–S120, 2010.

There is increasingly more research being conducted to determine the relationship between oxidative stress and depression. Current evidence would indicate that there is an inverse association between antioxidant status and depression. Recent studies have found that this relationship is less strong when factoring in confounding variables such as physical activity and cigarette smoking. There is some evidence that consumption of diets rich in fruits and vegetables also has an inverse relationship with depressive symptoms. Future research is needed to determine causality and temporal relationship. Are persons with depression more likely to have a poor diet and consume fewer fruits, vegetables, and antioxidant-rich foods, or are people who have low consumption of fruits, vegetables, and foods high in antioxidants more likely to become depressed?

References

Beydoun, M. A., H. A. Beydoun, A. Boueiz, M. R. Shroff, and A. B. Zonderman. 2013. Antioxidant status and its association with elevated depressive symptoms among US adults: National health and nutrition examination surveys 2005–2006. *Br J Nutr* 109 (9):1714–1729. doi:10.1017/s0007114512003467.

Bilici, M., H. Efe, M. A. Koroglu, H. A. Uydu, M. Bekaroglu, and O. Deger. 2001. Antioxidative enzyme activities and lipid peroxidation in major depression: Alterations by antidepressant treatments. *J Affect Disord* 64 (1):43–51.

Black, C. N., M. Bot, P. G. Scheffer, P. Cuijpers, and B. W. Penninx. 2015. Is depression associated with increased oxidative stress? A systematic review and meta-analysis. *Psychoneuroendocrinology* 51:164–175. doi:10.1016/j.psyneuen.2014.09.025.

Bloom, D. E., E. T. Cafiero, and E. Jane-Llopis. 2011. *The Global Economic Burden of Noncommunicable Diseases*. Geneva, Switzerland: World Economic Forum.

Carlsen, M. H., B. L. Halvorsen, K. Holte, S. K. Bohn, S. Dragland, L. Sampson, C. Willey et al. 2010. The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. *Nutr J* 9:3. doi:10.1186/1475-2891-9-3.

Chang, C. C., C. T. Lee, T. H. Lan, P. C. Ju, Y. H. Hsieh, and T. J. Lai. 2015. Effects of antidepressant treatment on total antioxidant capacity and free radical levels in patients with major depressive disorder. *Psychiatry Res* 230 (2):575–580. doi:10.1016/j.psychres.2015.10.006.

Chung, C. P., D. Schmidt, C. M. Stein, J. D. Morrow, and R. M. Salomon. 2013. Increased oxidative stress in patients with depression and its relationship to treatment. *Psychiatry Res* 206 (2–3):213–216. doi:10.1016/j.psychres.2012.10.018.

Cumurcu, B. E., H. Ozyurt, I. Etikan, S. Demir, and R. Karlidag. 2009. Total antioxidant capacity and total oxidant status in patients with major depression: Impact of antidepressant treatment. *Psychiatry Clin Neurosci* 63 (5):639–645. doi:10.1111/j.1440-1819.2009.02004.x.

Hasin, D. S. and B. F. Grant. 2015. The national epidemiologic survey on alcohol and related conditions (NESARC) waves 1 and 2: Review and summary of findings. *Soc Psychiatry Psychiatr Epidemiol* 50 (11):1609–1640. doi:10.1007/s00127-015-1088-0.

Jimenez-Fernandez, S., M. Gurpegui, F. Diaz-Atienza, L. Perez-Costillas, M. Gerstenberg, and C. U. Correll. 2015. Oxidative stress and antioxidant parameters in patients with major depressive disorder compared to healthy controls before and after antidepressant treatment: Results from a meta-analysis. *J Clin Psychiatry* 76 (12):1658–1667. doi:10.4088/JCP.14r09179.

Kingsbury, M., G. Dupuis, F. Jacka, M. H. Roy-Gagnon, S. E. McMartin, and I. Colman. 2016. Associations between fruit and vegetable consumption and depressive symptoms: Evidence from a national canadian longitudinal survey. *J Epidemiol Community Health* 70 (2):155–161. doi:10.1136/jech-2015-205858.

Kodydkova, J., L. Vavrova, M. Zeman, R. Jirak, J. Macasek, B. Stankova, E. Tvrzicka, and A. Zak. 2009. Antioxidative enzymes and increased oxidative stress in depressive women. *Clin Biochem* 42 (13–14):1368–1374. doi:10.1016/j.clinbiochem.2009.06.006.

Liu, T., S. Zhong, X. Liao, J. Chen, T. He, S. Lai, and Y. Jia. 2015a. A meta-analysis of oxidative stress markers in depression. *PLoS One* 10 (10):e0138904. doi:10.1371/journal.pone.0138904.

Liu, X., Y. Yan, F. Li, and D. Zhang. 2015b. Fruit and vegetable consumption and the risk of depression: A meta-analysis. *Nutrition* doi:10.1016/j.nut.2015.09.009.

Maes, M., P. Galecki, Y. S. Chang, and M. Berk. 2011. A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro) degenerative processes in that illness. *Prog Neuropsychopharmacol Biol Psychiatry* 35 (3):676–692. doi:10.1016/j.pnpbp.2010.05.004.

Mark, T. L., K. R. Levit, R. M. Coffey, and D. R. McKusick. 2007. *National Expenditures for Mental Health Services and Substance Abuse Treatment, 1993–2003 SAMHSA Publication No. SMA 07-4227.* Rockville, MD: Substance Abuse and Mental Health Services Administration.

Mathers, C. D. and D. Loncar. 2006. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med* 3 (11):e442. doi:10.1371/journal.pmed.0030442.

Mihrshahi, S., A. J. Dobson, and G. D. Mishra. 2015. Fruit and vegetable consumption and prevalence and incidence of depressive symptoms in mid-age women: Results from the Australian longitudinal study on women's health. *Eur J Clin Nutr* 69 (5):585–591. doi:10.1038/ejcn.2014.222.

Moylan, S., M. Berk, O. M. Dean, Y. Samuni, L. J. Williams, A. O'Neil, A. C. Hayley et al. 2014. Oxidative & nitrosative stress in depression: Why so much stress?. *Neurosci Biobehav Rev* 45:46–62. doi:10.1016/j.neubiorev.2014.05.007.

Murray, C. J. and A. D. Lopez. 1997. Alternative projections of mortality and disability by cause 1990–2020: Global burden of disease study. *Lancet* 349 (9064):1498–1504. doi:10.1016/s0140-6736(96)07492-2.

Niu, K., H. Guo, M. Kakizaki, Y. Cui, K. Ohmori-Matsuda, L. Guan, A. Hozawa et al. 2013. A tomato-rich diet is related to depressive symptoms among an elderly population aged 70 years and over: A population-based, cross-sectional analysis. *J Affect Disord* 144 (1–2):165–170. doi:10.1016/j.jad.2012.04.040.

Palta, P., L. J. Samuel, E. R. Miller, 3rd, and S. L. Szanton. 2014. Depression and oxidative stress: Results from a meta-analysis of observational studies. *Psychosom Med* 76 (1):12–19. doi:10.1097/psy.0000000000000009.

Pandya, C. D., K. R. Howell, and A. Pillai. 2013. Antioxidants as potential therapeutics for neuropsychiatric disorders. *Prog Neuropsychopharmacol Biol Psychiatry* 46:214–223. doi:10.1016/j.pnpbp.2012.10.017.

Payne, M. E., S. E. Steck, R. R. George, and D. C. Steffens. 2012. Fruit, vegetable, and antioxidant intakes are lower in older adults with depression. *J Acad Nutr Diet* 112 (12):2022–2027. doi:10.1016/j.jand.2012.08.026.

Perez-Jimenez, J., V. Neveu, F. Vos, and A. Scalbert. 2010. Identification of the 100 richest dietary sources of polyphenols: An application of the phenol-explorer database. *Eur J Clin Nutr* 64 (Suppl 3):S112–S120. doi:10.1038/ejcn.2010.221.

Prior, R. L., H. Hoang, L. Gu, X. Wu, M. Bacchiocca, L. Howard, M. Hampsch-Woodill, D. Huang, B. Ou, and R. Jacob. 2003. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC(FL))) of plasma and other biological and food samples. *J Agric Food Chem* 51 (11):3273–3279. doi:10.1021/jf0262256.

Prior, R. L., X. Wu, and K. Schaich. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J Agric Food Chem* 53 (10):4290–4302. doi:10.1021/jf0502698.

Reynolds, K., R. H. Pietrzak, R. El-Gabalawy, C. S. Mackenzie, and J. Sareen. 2015. Prevalence of psychiatric disorders in U.S. older adults: Findings from a nationally representative survey. *World Psychiatry* 14 (1):74–81. doi:10.1002/wps.20193.

SAMHSA, Substance Abuse and Mental Health Services Administration. 2014. *Results from the 2013 National Survey on Drug Use and Health: Summary of National Findings, NSDUH Series H-48, HHS Publication NO. (SMA) 14-4863*. Rockville, MD: Substance Abuse and Mental Health Services Administration. Accessed 10/2/16. http://www.samhsa.gov/data/sites/default/files/NSDUHresultsPDFWHTML2013/Web/NSDUHresults2013.pdf.

Scalbert, A., I. T. Johnson, and M. Saltmarsh. 2005. Polyphenols: Antioxidants and beyond. *Am J Clin Nutr* 81 (1 Suppl):215s–217s.

Sepehrmanesh, Z., F. Kolahdooz, F. Abedi, N. Mazroii, A. Assarian, Z. Asemi, and A. Esmaillzadeh. 2015. Vitamin D supplementation affects the beck depression inventory, insulin resistance, and biomarkers of oxidative stress in patients with major depressive disorder: A randomized, controlled clinical trial. *J Nutr*. doi:10.3945/jn.115.218883.

U.S. Department of Agriculture USDA, Agricultural Research Service. *Oxygen Radical Absorbance Capacity (ORAC) of Selected Foods, Release 2. Nutrient Data laboratory Home Page*. Accessed 1/2/16. http://www.ars.usda.gov/Services/docs.htm?docid=15866.

Vargas, H. O., S. O. V. Nunes, M. Pizzo de Castro, C. C. Bortolasci, D. S. Barbosa, H. K. Morimoto et al. 2013. Oxidative stress and lowered total antioxidant status are associated with a history of suicide attempts. *J Affect Disord* 150 (3):923–930. doi:10.1016/j.jad.2013.05.016.

WebMD. 2015. WebMD Antioxidants-Topic Overview. Accessed 12/11/15. http://www.webmd.com/food-recipes/antioxidants-topic-overview.

8

Antioxidant Nutraceuticals and Preeclampsia

Monika M. Wahi

Contents

8.1 Introduction

A modern discussion of the application of antioxidant nutraceuticals to the prevention of preeclampsia must begin with a discussion of the modern definition and understanding of the etiology of preeclampsia. Currently, the American College of Obstetricians and Gynecologists (ACOG), National Heart, Lung, and Blood Institute (NHLBI), and National High Blood Pressure Education Program (NHBPEP) all agree on the definition of preeclampsia as "a hypertensive, multi-system disorder of pregnancy that significantly contributes to maternal and fetal/neonatal morbidity and mortality" (Bell 2010). Clinically, preeclampsia is a syndrome defined by the onset of hypertension and proteinuria at 20 weeks of gestation in previously healthy women, and if left untreated, can lead to a convulsive state known as eclampsia (Noris et al. 2005). Preeclampsia is the leading cause of both maternal and fetal morbidity and mortality, and a worldwide incidence of over 8 million cases has been estimated (Noris et al. 2005).

Although preeclampsia is a current public health issue, it also has allegedly been mentioned as far back as in ancient Egyptian, Chinese, and Indian writings (Chesley 1984), and has been described in ancient Greece in relation to the balance of four humors believed to cause illness and disease at the time (Bell 2010). For this reason, although currently "the etiology of pre-eclampsia remains unknown" (Bell 2010), preeclampsia has a rich history of thought behind its etiology and mechanisms, as well as documented experience with treatments and preventive measures. In these, nutraceuticals have inevitably played a role.

This chapter will review the state of the literature on antioxidant nutraceuticals for the prevention of preeclampsia. First, a historical understanding of preeclampsia will be reviewed, along with historical dietary interventions. Second, in order to explain the hypothesized mechanism behind why antioxidant nutraceuticals could prevent preeclampsia, the topics of oxidative stress, free radicals, and how antioxidants address oxidative stress are discussed. In addition, reactive oxygen species (ROS) and oxidative damage are described. Third, how supplementary and dietary antioxidants have been studied as potential preventive measures for preeclampsia will be reviewed, and findings will be summarized. Finally, specific antioxidant nutraceuticals that have been hypothesized to have antihypertensive properties will be discussed in the context of their potential protective role in preeclampsia.

8.2 Preeclampsia throughout history

In her historical overview of preeclampsia, Mandy Bell reviews various conceptions of preeclampsia throughout time, and these are summarized in **Table 8.1** (Bell 2010).

Table 8.1 Conceptions of Preeclampsia Throughout Time[a]

Period	Geographic Location	Presumed Etiology	Treatments
5th and 4th centuries	Greece	Imbalance of humors, fluid retention, *wandering womb.*	Altered diets, purging, bloodletting. Pregnancy, lactation, and menstruation thought to improve condition.
400–1700 CE	Greece, Egypt, Italy, Arabia, France	Religious/spiritual causes.	Earlier in the period, charms, amulets, faith healing, miracles, and prayers were used. Later, phlebotomies were used.
18th and 19th centuries	Europe, United States	Issues with blood flow, excess stimulus to the spinal center, bloodletting, variations in weather, irritation of uterus, and nearby anatomy, and toxemia from maternal and fetal wastes.	Earlier in period, bloodletting, opiates, warm baths, cold water on face, hastening delivery. In response to the *toxin* theory, later in period, restriction of consumption of meat, and prescribed diets of fruits, vegetables, and milk products.
20th century	International	Parasitic worm theory (which was disproven). Current dominant theory: endothelial disorder where ischemic placental releases damaging factor(s) into maternal circulation causing endothelial dysfunction.	Magnesium sulfate and antihypertensives to prevent or manage convulsions and acute hypertension, respectively. Choices with respect to delivery (e.g., cesarean section).

[a] Bell, M. J., *JOGNN/NAACOG.,* 39, 510–518.

A reflection on the history of thought on preeclampsia suggests that even today, the etiology of preeclampsia remains elusive. However, themes do exist in beliefs of purported mechanisms, and how those beliefs have led to experimental preventive measures and treatments. First, generally, preeclampsia has been seen as a cardiovascular disorder, and therefore, both historically and modernly, treatments have aimed at reducing hypertension and restoring healthy circulation. Next, preeclampsia has been seen as a result of an overload of some *toxins*, and this has led to the prescriptions of diets as both prevention and treatment measures.

Although dietary intervention in ancient times for preeclampsia was restricting meats in favor of vegetables, fruits, and milk products, modern attention has been given to specific nutrients and their possible role in preeclampsia. Roberts and colleagues reviewed current knowledge on nutrient involvement in preeclampsia in 2003 (Roberts et al. 2003). They observed that studies to date had focused on the following topics with respect to roles of energy intake and diet composition, role of dietary lipids, and roles of specific micronutrients

(such as calcium, sodium, magnesium, iron, and folate) (Roberts et al. 2003) in contributing to preeclampsia. They also described how nutrients chosen for study were intended to intervene on hypothesized mechanisms behind preeclampsia, such as endothelial dysfunction, the inflammatory response, insulin resistance, and oxidative stress (Roberts et al. 2003).

8.3 Oxidative stress

Oxidative stress, in particular, has been singled out as a focus in preeclampsia, because it is believed to be at the root cause of many chronic diseases (Lobo et al. 2010). Among the nutrients studied for the prevention of preeclampsia are those that are regarded as antioxidants. The reason antioxidants have been studied for the prevention and treatment of preeclampsia is that they are hypothesized to reduce the oxidative stress believed to be an important mechanism in preeclampsia (as well as cardiovascular disorder and other disorders) (Rumbold et al. 2008). Oxidative stress can be characterized as an overproduction of free radicals (e.g., high levels of oxidative damage), and the hypothesis is that oxidative stress can deplete the body's stores of antioxidants, increasing a woman's risk of preeclampsia (Rumbold et al. 2008). The following sections (Sections 8.3.1 through 8.3.3) describe oxidative stress, oxidative damage, and how antioxidants address oxidative stress and damage. This section ends with a review of synthetic versus natural antioxidants (Section 8.3.4).

8.3.1 Oxidative stress, free radicals, and antioxidants

An antioxidant has been defined as any substance that interferes with the reaction of any substance with dioxygen, or in other words, hinders a free radical reaction, a free radical being of any species that contains one or more unpaired electrons (Powell 2000). Antioxidants have been called "free radical scavengers that trap or decompose existing free radicals" and/or enzymes that inhibit peroxidase reactions that are part of producing free radicals (Rumbold et al. 2008). The normal oxygen atom has an outer electron orbit that includes four pairs of electrons (Finkel and Holbrook 2000) (**Figure 8.1**).

When one of these pairs loses an electron, it becomes a *free radical*. This free radical has a powerful attraction to the paired electrons in other normal oxygen atoms (**Figure 8.2**), as well as other stable atoms and free radicals, and this creates a feedback loop, increasing the number of free radicals (Finkel and Holbrook 2000) (**Figure 8.3**).

This chain reaction involving normal oxygen atoms and free radicals is the *oxidative stress* felt to be responsible for preeclampsia. How antioxidants relieve the oxidative stress is by donating free electrons to free radicals, thus completing the electron pairs and rendering the atom a normal oxygen atom again (**Figure 8.4**).

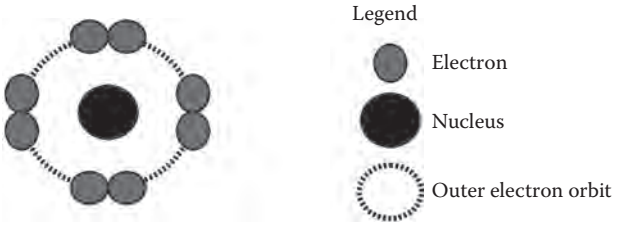


Figure 8.1 *Normal oxygen atom. Four pairs of electrons orbit around the nucleus.*

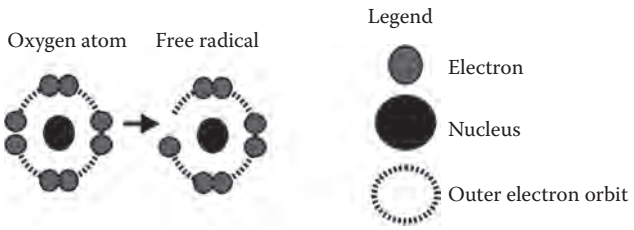


Figure 8.2 *Influence of free radical on normal oxygen atom. On the right, a free radical, or an oxygen atom that has lost an electron in one of its pairs, exerts a strong influence on a normal oxygen atom, on the left.*

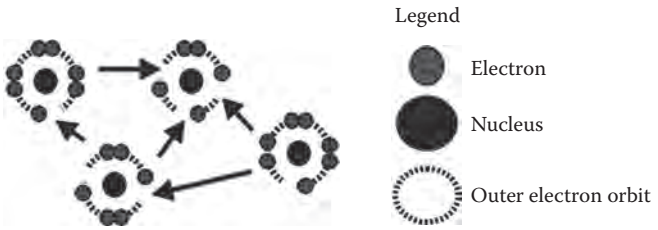


Figure 8.3 *Chain reaction that increases free radicals. As more stable oxygen atoms become free radicals, a chain reaction occurs that increases the number of free radicals and reduces the number of stable oxygen atoms.*

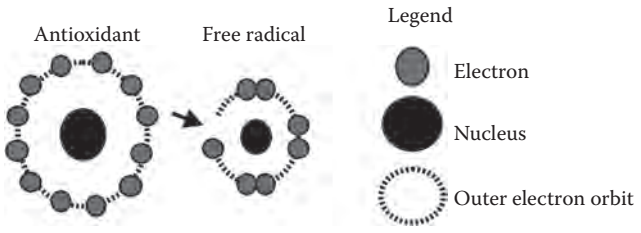


Figure 8.4 *How antioxidants reduce free radicals. Antioxidants have electrons available to donate to the oxygen atom to stabilize the atom.*

8.3.2 Reactive oxygen species and oxidative damage

In 2010, Lobo and colleagues published a comprehensive review of free radicals and antioxidants and how they relate to the concept of functional foods as far as impacting human health (Lobo et al. 2010). Free radicals are considered ROS, which simply means that the atom is missing at least one electron and is therefore *hungry* to fill this spot, thus impacting other atoms around it (as shown in Figure 8.3) (Lobo et al. 2010). ROS are either derived from normal essential metabolic processes, or from external sources such as X-rays and air pollutants (Lobo et al. 2010). The authors list 14 generated sources of free radicals, including metabolic processes such as inflammation and phagocytosis to external sources such as cigarette smoke and certain pesticides (Lobo et al. 2010).

The authors also go on to describe *oxidative damage*, which refers to modification of biomolecules as a result of oxidative stress (Lobo et al. 2010). First, proteins can suffer from oxidative damage, which can affect signal transduction, enzyme stability, and proteolysis susceptibility (Lobo et al. 2010). Next, lipids can undergo damage through peroxidation, and producing a lipid radical that can cause a chain reaction in the production of lipid radicals (see Figure 8.3) (Lobo et al. 2010). Lipids form cell membranes and their peroxidation forms compounds that are markers that can be measured in an assay (Lobo et al. 2010). Lipid peroxidation has been implicated in neurogenerative diseases, ischemic reperfusion injury, and diabetes (Lovell et al. 1995, Lobo et al. 2010). Finally, experiments have found evidence that DNA and RNA are susceptible to oxidative damage, especially reported in studies of aging and cancer (Lobo et al. 2010).

8.3.3 How antioxidants address oxidative stress and damage

Antioxidants work along three lines of defense (Lobo et al. 2010). In the first line of defense, preventive antioxidants prevent the formation of free radicals; in the second, antioxidants scavenge the active radicals to suppress chain initiation and or break the chain propagation reactions (as seen in Figure 8.3); and in the third, antioxidants conduct *clean-up* by removing oxidatively modified proteins (Lobo et al. 2010). Just as there are different types of ROS, there are different types of antioxidants. Some are considered enzymatic, such as superoxide dismutase, catalase, and glutathione systems, and some of considered nonenzymatic, such as ascorbic acid (Vitamin C), glutathione, melatonin, tocopherols, and tocotrienols (Vitamin E), and uric acid (5). See Chapter 15 for a discussion on free radicals and the role of antioxidants for better healthcare.

8.3.4 Synthetic versus natural antioxidants

Synthetic and natural food antioxidants are used routinely as part of food and medicine production, because including antioxidants in foods and medicines containing oils and fats will prevent the foods against oxidation

(Lobo et al. 2010). As an example, the compounds butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are synthetic antioxidants, which have been widely used in the food, cosmetics, and therapeutic industries (Lobo et al. 2010). Unfortunately, synthetic antioxidants such as BHT and BHA are highly volatile and unstable molecules at elevated temperature, and this may be why synthetic antioxidants have been found to be carcinogenic, and why legislation has come about on the use of food additives (Lobo et al. 2010). Hence, "consumer preferences have shifted the attention of manufacturers from synthetic to natural antioxidants" (Papas 1999, Lobo et al. 2010).

8.4 Supplemental and dietary antioxidants and preeclampsia

More recent observations in the literature that women with preeclampsia have decreased plasma and placental concentrations of antioxidants comprise evidence in favor of the hypothesis that preeclampsia is caused by oxidative stress (Rumbold et al. 2008). However, the current consensus is that the safety of pregnant women taking antioxidant supplements is unknown, and in nonpregnant women, there have been inconsistent findings about both the benefits and harms of taking supplement antioxidants (Rumbold et al. 2008). The following section (Section 8.4.1) first reviews what is known about taking supplemental antioxidants for the prevention of preeclampsia. In the next section (Section 8.4.2), findings regarding dietary antioxidants to prevent preeclampsia will be reviewed.

8.4.1 Supplemental antioxidants to prevent preeclampsia

Though the safety of pregnant women taking antioxidant supplements is unknown, many antioxidants have been studied for their potential for preventing preeclampsia (**Table 8.2**). Taking supplemental nutrients to combat the oxidative stress believed to be behind preeclampsia would be a preventive treatment reasonable to study, and the vehicle of administration of these nutrients that has been studied has been mainly oral supplementation (Rumbold et al. 2008, Hofmeyr et al. 2014). In addition, supplements containing nutrients with antioxidant properties or otherwise felt to have a role in combating oxidative stress have been studied (Trumbo and Ellwood 2007, Hofmeyr et al. 2014). This body of research largely consists of clinical trials where pregnant women believed to be at a risk for preeclampsia were randomized to either take a specified vitamin supplement or placebo (Sharma et al. 2003, Poston et al. 2006, Hobson et al. 2013) or, instead of placebo, nothing (Marya et al. 1987, De-Regil et al. 2012). The literature also includes reviews that have been or are being conducted of the findings of these trials (Mahomed et al. 2007, Trumbo and Ellwood 2007, Rumbold et al. 2008, De-Regil et al. 2012, Hofmeyr et al. 2014, Hobson et al. 2015) (Table 8.2).

As depicted in Table 8.2, the results of research on using supplementation with antioxidants for the prevention of preeclampsia have been overwhelmingly

Table 8.2 Antioxidant and Related Supplements Studied and Their Findings Relating to Prevention of Preeclampsia

Nutrient Supplement Studied	Antioxidant Status	Role in Prevention of Eclampsia
Calcium	It is not an antioxidant itself, but has a role in the production of antioxidants (Gandhi and Abramov 2012).	"Calcium supplementation (≥1 g/day) is associated with a significant reduction in the risk of preeclampsia, particularly for women with low calcium diets" (Hofmeyr et al. 2014).
Lycopene	Lycopene is an antioxidant (Sharma et al. 2003).	"…lycopene reduce[d] the development of pre-eclampsia" in primigravida women in one study (Sharma et al. 2003), but another found no difference, and increased rates of preterm labor and low birth weight babies in the lycopene group (Banerjee, et al. 2009).
Melatonin	Melatonin is a molecule with antioxidant properties that exists in animals and edible plants (Reiter and Tan 2002).	Unknown; currently being tested in clinical trials (Hobson et al. 2013), and Cochrane review is in preparation (Hobson et al. 2015).
Vitamin B	Vitamin B12 is a supplement with antioxidant properties (Birch et al. 2009), as is vitamin B9 (folic acid) (Medicine, National Library of 2015).	Although folic acid deficiency is implicated in preeclampsia (Ray and Laskin 1999), supplementation with B vitamins has not shown to prevent preeclampsia.
Vitamin C	Vitamin supplement with antioxidant properties (Talaulikar and Manyonda 2011).	"Vitamin C given orally…does not achieve sustained serum levels that might be required for effective antioxidant activity," which may explain its failure in clinical trials in diseases associated with oxidative stress, including preeclampsia (Talaulikar and Manyonda 2011). Concomitant supplementation with vitamin E is not advised as babies were more likely to be born with low birth weight (Rumbold et al. 2008, Poston et al. 2006).
Vitamin D	Vitamin D is a membrane antioxidant (Wiseman 1993).	In a summary of the only trial identified in a review of vitamin D supplementation with preeclampsia as an endpoint, "women who received 1200 IU Vitamin D along with 375 mg of elemental calcium per day were as likely to develop pre-eclampsia as women who received no supplementation" (De-Regil et al. 2012, Marya et al. 1987).

(*Continued*)

Table 8.2 (*Continued*) Antioxidant and Related Supplements Studied and Their Findings Relating to Prevention of Preeclampsia

Nutrient Supplement Studied	Antioxidant Status	Role in Prevention of Eclampsia
Vitamin E	Vitamin E supplement with antioxidant properties (Talaulikar and Manyonda 2011).	Supplementation with vitamin C and vitamin E, both concomitant or individually, does not prevent preeclampsia in women at risk (Rumbold et al. 2008, Poston et al. 2006).
Zinc	Zinc is a supplement with antioxidant properties (Powell 2000).	Aside from a 14% relative reduction in preterm birth compared to placebo, "there was no convincing evidence that zinc supplementation during pregnancy results in other useful or important benefits" (Mahomed et al. 2007).

disappointing. So disappointing, in fact, that in 2011, Talaulikar and Manyonda called the belief that vitamin C is an antioxidant supplement important to women's health "a myth in need of burial" (Talaulikar and Manyonda 2011). They also stated that they "wish to make a case that the massive and expensive clinical trials of vitamins C and E should cease" at present (Talaulikar and Manyonda 2011).

On the brighter side, one set of positive findings was with respect to calcium supplementation and prevention of preeclampsia. Calcium is not an antioxidant itself but has a role in the production of antioxidants (Gandhi and Abramov 2012). As recently as 2007, Trumbo and Ellwood from the United States Food and Drug Administration stated in their report in *Nutrition Reviews*, "The relationship between [supplemental] calcium and risk of pregnancy-induced hypertension and preeclampsia is highly unlikely" (Trumbo and Ellwood 2007). This consensus was reversed as a result of a 2014 Cochrane review that found that, "Calcium supplementation (≥1 g/day) is associated with a significant reduction in the risk of pre-eclampsia, particularly for women with low calcium diets," but cautioned that this effect may be overestimated due to bias from a number of sources (Hofmeyr et al. 2014). The Cochrane review noted that the World Health Organization (WHO) had been recommending calcium supplementation with 1.5–2 g elemental calcium daily for pregnant women with low dietary calcium since their 2011 report (Hofmeyr et al. 2014, Organization, World Health 2011), and the aim of the review was to examine the evidence associated with low-dose (<1 g/day) versus high-dose (≥1 g/day) calcium supplementation in high-risk pregnant women for the prevention of preeclampsia (Hofmeyr et al. 2014).

Although Talaulikar and Manyonda pointed out the opportunity cost of wastefully investing in repetitive trials of antioxidants and preeclampsia when the preponderance of evidence shows no benefit (Talaulikar and Manyonda 2011), the

results of the VIP trial, which compared a regimen of supplemental vitamin C and vitamin E (combined) with placebo in women at a high risk for preeclampsia, actually found harm (Poston et al. 2006). They reported, "Concomitant supplementation with Vitamin C and vitamin E does not prevent pre-eclampsia in women at risk, but does increase the rate of babies born with a low birthweight. As such, use of these high-dose antioxidants is not justified in pregnancy" (Poston et al. 2006). Hence, Talaulikar and Manyonda conclude, "Vitamin C supplementation to stave off pre-eclampsia, cancer and other diseases is a 'nutraceutical' industry-driven myth which should be abandoned" (Talaulikar and Manyonda 2011). (Chapter 11 in this book discusses the treatment of cardiovascular disease with both nutraceutical and supplemental antioxidants).

8.4.2 Diet and preeclampsia

Although it is important to acknowledge the preponderance of evidence that a pregnant woman taking nutrient supplements of antioxidants does not put her at lower risk for developing preeclampsia unless she is nutritionally deficient (Ray and Laskin 1999, Poston et al. 2006, Mahomed et al. 2007, Rumbold et al. 2008, Talaulikar and Manyonda 2011), and may even be harmful if she is not (Poston et al. 2006, Rumbold et al. 2008), it is still possible that dietary intake of antioxidants may play a positive protective role in the prevention of preeclampsia. This is because inverse associations have been found between high levels of dietary nutrients and risk of preeclampsia.

For example, in Ray and Laskin's systematic review, they noted that there is general agreement across several studies of folate deficiency among probable risk factors for placenta-mediated diseases (Ray and Laskin 1999). In a review of zinc supplementation to prevent negative birth outcomes, which concluded zinc supplementation had no effect on preventing eclampsia, the authors noted that the 14% relative reduction they observed for preterm birth for zinc compared to placebo was in the group of studies they reviewed focusing on low-income women (Mahomed et al. 2007). Hence, they suspected that these women were actually suffering from poor nutrition, which could have included a zinc deficiency, and that is why this effect was seen (Mahomed et al. 2007).

Supplementation is easier to study because it is easier to measure. Because there are different types of dietary antioxidants that come from different sources, studies of dietary antioxidants have approached measurement differently. This section will summarize several studies that have measured dietary antioxidants and preeclampsia in a variety of ways.

8.4.3 Oxidative damage and antioxidant power

Not only have associations been found between having a particular nutritional deficiency and risk for preeclampsia, but certain diets have been found to be more protective against preeclampsia. In 2005, Scholl and colleagues reported

on a prospective study of 307 pregnant women in Camden, New Jersey, in the United States (Scholl et al. 2005). Scholl's group hypothesized that "maternal factors may contribute to an imbalance between pro-oxidant and antioxidant forces that provides an environment for free radical generation" as described earlier, and state that therefore, the maternal intake of antioxidants and polyunsaturated fats, which contribute to neutralizing free radicals should be studied (Scholl et al. 2005).

To study this, they enrolled participants who were pregnant women and followed them from entry into care throughout their pregnancy outcomes (Scholl et al. 2005). They measured urinary isoprostane excretion (a marker of oxidative damage to lipids) and antioxidant power (a measure of global antioxidant levels) (Scholl et al. 2005). These measurements were to better understand the levels of oxidative stress (prooxidant) versus levels of dietary antioxidants (Scholl et al. 2005). This study found that "risk of preeclampsia was increased 5-fold with higher urinary isoprostane excretion and decreased 3-fold with higher total antioxidant power" (Scholl et al. 2005), suggesting that maternal diet is important in the prevention of preeclampsia.

8.4.4 Food frequency questionnaire: U.S. studies

A similar study that measured dietary antioxidants using a different approach enrolled 1718 women from the United States who were pregnant and followed them until birth to study the outcomes of gestational hypertension and preeclampsia (Oken et al. 2007). Authors hypothesized that higher levels of dietary *n*-3 fatty acids or fish oils would be protective against preeclampsia, but based on their analysis of the antioxidants that had been the focus of supplementation studies, as they wanted to analyze any associations between magnesium, folate, and vitamins C, D, and E from both foods and supplements and the outcome of preeclampsia (Oken et al. 2007). They used a food frequency questionnaire (FFQ) coupled with a nutrient composition database to estimate antioxidant intake; this measure was thought to be valid because estimated antioxidant intake correlated with measured blood levels (Oken et al. 2007). The authors did find a somewhat lower risk of preeclampsia associated with a higher intake of the elongated *n*-3 fatty acids docosahexaenoic acid and eicosapentaenoic acids and fish, but did not find associations with any of the other antioxidants studied (Oken et al. 2007).

8.4.5 Food frequency questionnaire: Non-U.S. studies

More recently, cross-sectional data from India's third National Family Health Survey were analyzed (Agrawal et al. 2015). Diet was measured by FFQ, and the authors found that women who did not report symptoms of preeclampsia during their most recent pregnancy were more likely to have a "diversified dietary intake and iron and folic acid supplementation in pregnancy" compared to those

who reported symptoms (Agrawal et al. 2015). Next, a cohort study was conducted in Tehran city, Iran, which followed 1033 pregnant women and measured their diet with an FFQ (Mokhlesi et al. 2015). Results showed that in the 20 women who developed preeclampsia, the average intake of dietary antioxidants, including zinc, vitamin A, C, E were statistically significantly lower than the non-preeclampsia group (Mokhlesi et al. 2015). A study of a Norwegian cohort found that women whose pattern of consumption was characterized by processed meat, salty snacks, and sweet drinks were at an increased risk, and those whose pattern of consumption was characterized by vegetables, plant foods, and vegetable oils were at decreased risk for preeclampsia (Brantsæter et al. 2009).

8.4.6 Dietary polyphenols

In a comprehensive 2015 review, Ly and colleagues examined available evidence of the effects of dietary polyphenols (also called phenolics) on reproductive health and early development (Ly et al. 2015). Polyphenols are the most abundant dietary antioxidants and are found in fruits, vegetables, seeds, nuts, chocolate, wine, coffee, and tea (Ly et al. 2015). Unfortunately, they review no studies focusing on dietary polyphenols and preeclampsia, saying that, "Studies that provide more precise individual data concerning intake of specific classes of polyphenols during pregnancy are required" (Ly et al. 2015). They also state that studies of polyphenol consumption on overall human reproductive health are limited and inconclusive (Ly et al. 2015). In addition, see Chapter 7 of this book for a discussion of the role of nutraceuticals with polyphenols in atherosclerosis.

8.4.7 Angiotensin-converting enzyme inhibitors

Angiotensin-converting enzyme (ACE) inhibitors have been demonstrated to have antioxidant properties (Chopra et al. 1992), and are approved as pharmaceuticals for prescription in the treatment of hypertension (Münzel and Keaney 2001). Many foods have naturally occurring ACE inhibitors, such as milk and dairy products, eggs, meat, fish, seafood, and plants (Ngo et al. 2012, Iwaniak et al. 2014). Unlike polyphenols, specific studies that focus on dietary ACE inhibitors for the prevention of preeclampsia have not been reported.

8.4.8 The role of fiber in absorbing dietary antioxidants

In 2011, Palafox-Carlos and colleagues published a summary of what is known about the role of dietary fiber in the bioaccesibility and bioavailability of fruit and vegetable antioxidants (Palafox-Carlos et al. 2011). The authors point out that the bioaccessibility and the bioavailability of antioxidants from fruits and vegetables differ by each compound, and "the most abundant antioxidants in ingested fruit are not necessarily those leading to the highest concentrations of active metabolites in target tissues" (Palafox-Carlos et al. 2011). The limited bioavailability of antioxidants in fruits and vegetables is determined by

their low bioaccessibility in the small intestine due to interactions with antioxidants and the indigestible polysaccharides present on cell walls (Palafox-Carlos et al. 2011). The authors call for studies on the role of dietary fiber, the indigestible cell wall component of plant material, as a "control-released system of bioactive compounds" (Palafox-Carlos et al. 2011). They also point out that dietary fiber interferes with adequate absorption of antioxidants, and therefore, studies that could better establish the timing of fiber flow through the digestive system could be useful (Palafox-Carlos et al. 2011).

8.4.9 Intake of antioxidants and preeclampsia

To summarize, the current consensus in the literature is that antioxidant supplementation is not useful in preventing preeclampsia in those without nutritional deficiencies, and may even be harmful. However, even in those without nutritional deficiencies, it seems that enhanced dietary intake of antioxidants could provide some protection against preeclampsia. Nevertheless, particular *preeclampsia diets* have not been studied and are not currently being recommended. One notable study of a community of vegans in 1987 found that of 775 vegan mothers, only one met the clinical criteria for preeclampsia, prompting the authors to speculate "it is possible that a vegan diet could alleviate most, if not all, of the signs and symptoms of preeclampsia" (Carter et al. 1987).

8.5 Antioxidant nutraceuticals and preeclampsia

The term *nutraceutical* was coined in 1989 by Dr. Stephen DeFelice, Chairman of the Foundation for Innovation in Medicine (Rajasekaran et al. 2008, Lobo et al. 2010). *Nutraceutical* is a marketing term that does not have a regulatory definition, but is generally considered "any substance that may be considered a food or part of a food and provides medical or health benefits, encompassing, prevention and treatment of diseases" (Rajasekaran et al. 2008). Another term for nutraceuticals is *functional foods*.

The idea of a functional food is as old as the ancient writings on preeclampsia. Hippocrates, who is credited with promoting the four humor theory mentioned earlier, also was credited with the saying, "Let food be your medicine and medicine be your food" (Rajasekaran et al. 2008). Hence, the idea of using diet for the prevention and treatment of preeclampsia is not a new idea, either. However, with the newer term *nutraceutical*, it is possible to take the view that rather than generically promoting a good diet, considering the actual function of different healthy foods in relationship to the particular condition of focus is indicated. This consideration should theoretically lead to healthy foods being differentially recommended depending on their purported function with respect to certain conditions.

Section 8.5.1 will first explain how the antihypertensive action of antioxidants is the focus behind the selection of nutraceuticals for study of the

prevention of preeclampsia, because it is the mechanism being targeted behind preeclampsia. This section will also review the limits to nutraceutical claims that can be made about their effects on preeclampsia in different international locations. Finally, what is known regarding antioxidant nutraceuticals hypothesized to have an impact on preventing preeclampsia either directly or by way of their antihypertensive properties will be reviewed.

8.5.1 Antioxidants as antihypertensives in the causal pathway to preeclampsia

Although the current consensus is that preeclampsia is likely a result of high levels of oxidative stress, where prooxidant processes overwhelm antioxidant processes, it is important to recognize that oxidative stress has been strongly linked to other serious conditions, including cancer and atherosclerosis (Lobo et al. 2010). Oxidative stress has specifically been linked to hypertension (including gestational hypertension) that can lead to stroke (see Chapter 6), coronary heart disease (see Chapter 19), and myocardial infarction (Chen et al. 2009). Therefore, recently, the focus in the nutraceutical literature has been on functional foods that have an effect on cardiovascular and cerebrovascular outcomes as a whole, theoretically because they contain high levels of antioxidants (Alissa and Ferns 2012).

8.5.2 Antioxidant nutraceutical claims about preeclampsia

Regulations about claims of links between functional foods and protection against preeclampsia are different in different countries. In her 2007 review, Johanna Dwyer observed that the U.S. Food and Drug Administration (FDA) allows food or nutraceutical health claims in applications for approval in the intervening of the association between pregnancy-induced hypertension and preeclampsia (Dwyer 2007).

It is notable that this is not a worldwide consensus. In a 2013 report by the Canadian Agricultural Innovation and Regulation Network (CAIRN), it was observed that only in the United States was there any health claim associated with preeclampsia and nutraceuticals that could be made to regulators; no comparable qualified health claims for food or nutraceuticals were found to be approved by regulators in the following countries: Canada, the European Union (EU), UK, Sweden, Russia, Australia, New Zealand, Japan, Brazil, Republic of Korea, China, Taiwan, Singapore, Hong Kong, India, Thailand, Malaysia, and the Philippines (Malla et al. 2013).

It must be disclaimed that many of these countries, such as Hong Kong, India, and Thailand, do not permit any health claims for nutraceuticals, but in places such as Russia, China, and Singapore where health claims for nutraceuticals are permitted, preeclampsia does not show up on the list of approved

indications (Malla et al. 2013). In Taiwan, the Health Food Control Act says that health claims must be proven for health or disease risk, but does not state specifics (Malla et al. 2013).

8.5.3 Nutraceutical antioxidants as antihypertensives to prevent preeclampsia

As described earlier, the mechanism of focus when studying nutraceuticals as functional foods for preventing preeclampsia is for their action preventing gestational hypertension, presumably by way of their antioxidant properties. Nutraceuticals having antioxidant properties that have been hypothesized to be antihypertensives that could be used to prevent preeclampsia include foods common around the world, such as fish and garlic (Chen et al. 2009), as well as foods that are typically easily accessible only in India or China, such as medicinal plants and vegetables native to those areas that are not found elsewhere in the world (Lobo et al. 2010).

Although many supplemental nutrients, dietary nutrients, and functional foods have been studied for their broader effects on cardiovascular disease, few human studies of nutraceuticals have included the outcomes of preeclampsia (Carter et al. 1987, Oken et al. 2007, Brantsæter et al. 2009, Brantsæter et al. 2011), or even specifically hypertension (Fugh-Berman 2000, Hooper et al. 2008, Pérez-Jiménez and Saura-Calixto 2008, Savica et al. 2010, Alissa and Ferns 2012, Grassi and Ferri 2012). Further, many papers that purport to present findings about *nutraceuticals* in actuality contain findings associated with supplemental nutrients or specific dietary nutrients (Fugh-Berman 2000, Alissa and Ferns 2012, Cicero and Borghi 2013), not functional foods.

Ultimately, there are few specific antioxidant foods or herbs that have been tested with respect to the specific outcomes of preeclampsia or hypertension. **Table 8.3** provides a selection of antioxidant nutraceuticals for which some type of evidence was available currently in the scientific literature with respect to preeclampsia or hypertension.

As seen in Table 8.3, while certain antioxidant nutraceuticals, such as cocoa, fish, and fish oil, show some consistency in findings, the overall lack of studies prevents the ability to make evidence-based recommendations for antioxidant nutraceuticals to include in a pregnancy diet to prevent gestational hypertension or preeclampsia.

8.5.4 Recommending antioxidant nutraceuticals to prevent preeclampsia

A strategic challenge for studying antioxidant nutraceuticals as a preventive intervention for preeclampsia is agreeing on what is actually being studied, both in terms of the intervention as well as in terms of the outcome. Studying *diets* is not studying individual nutraceuticals, nor is analyzing intake of *supplements*.

Table 8.3 Nutraceuticals Hypothesized to Act as Antihypertensives, Prevent Preeclampsia, or Both

Functional Food/ Nutraceutical	Type of Evidence	Association with Preeclampsia (and/or Hypertension)
Cocoa	Cohort studies and clinical trials	"Thus, all the earlier data indicate that the ingestion of cocoa-rich and therefore flavonoid-rich chocolate promotes a reduction in blood pressure" (Alissa and Ferns 2012, Grassi and Ferri 2012, Hooper et al. 2008).
Coffee	Clinical trials	Hypotensive effects of green coffee bean extract as well as roasted coffee have been demonstrated in numerous studies (Savica et al. 2010).
Fish and fish oil	Cohort studies	Researchers observed a somewhat reduced risk of preeclampsia with intake of fish. Researchers also measured elongated *n*-3 fatty acid intake and found a somewhat reduced risk associated with this (Oken et al. 2007). Fish oil has been shown to reduce blood pressure (Fugh-Berman 2000).
Fruits and vegetables	Cohort studies	Vegan diet associated with very low rate of preeclampsia (Carter et al. 1987). Norwegian women who had a diet high in vegetables, plant foods, and vegetable oils were at decreased risk for preeclampsia (Brantsæter et al. 2009).
Garlic	Clinical trials	"Garlic may reduce blood pressure, but the effect is mild and there is a relative dearth of data in hypertensive patients" (Fugh-Berman 2000).
Grapes/wine	Expert opinion	"In animal and human studies, grape products have been shown to produce hypotensive, hypolipidaemic, and anti-atherosclerotic effects, and also to improve antioxidant status as measured in terms of plasma antioxidant capacity, oxidation biomarkers, antioxidant compounds or antioxidant enzymes" (Alissa and Ferns 2012, Pérez-Jiménez and Saura-Calixto 2008).
Green or oolong tea	Cohort studies	Studies have reported that chronic, moderate consumers of green or oolong tea are less likely to develop hypertension, but the overall literature is inconsistent (Savica et al. 2010).
Marine organisms	Laboratory studies	"Currently, many natural ACE inhibitory peptides have been isolated from different food proteins such as cod frame, pollack skin, sea bream scales, yellowtail bone and scales, yellow sole frame, tuna frame and clam, krill, mussel, oyster, and shrimp" (Ngo et al. 2012).
Probiotic milk and probiotic yogurt	Cohort studies	Researchers found a strong protective association for severe preeclampsia, and concluded, "regular consumption of milk-based probiotics could be associated with lower risk of preeclampsia in primiparous women" (Brantsæter et al. 2011).[a]
Soy foods	Meta-analysis	Soy foods were associated with reduction in the systolic and diastolic blood pressure that was close to being statistically significant (Hooper et al. 2008).

[a] See Chapter 7 for a discussion of antioxidant nutraceuticals with probiotic application.

In addition, since antioxidant nutraceuticals must be considered as components being entered into a metabolic system, details about the nature and timing of fiber intake, as well as the type of antioxidants nutraceuticals being consumed become important when developing a preeclampsia prevention diet. In the case of studies using FFQs, instead of analyzing the data by the association of relative component nutrients in diets (which will include supplements) on outcomes, it would be more informative to select out individual antioxidant nutraceuticals, such as cocoa, fish, and coffee, and to analyze intake of those specific substances as they relate to outcomes such as preeclampsia and hypertension.

Next, studying antioxidant nutraceuticals to reduce hypertension in pregnant women may reveal different results than studying the same intervention in the general population, and studies that focus on pregnant women must also include the specific birth outcome of preeclampsia, and not only other birth outcomes (such as low birth weight). Until these strategic challenges are solved, it is not possible to provide evidence-based recommendations for antioxidant nutraceuticals to be included in a preeclampsia diet, because the necessary evidence is not available.

8.6 Conclusion

In conclusion, preeclampsia has been currently characterized by an imbalance between prooxidant and antioxidant processes, leading to hypertension and eclampsia. Hence, increasing antioxidant nutrient intake has been studied as the prevention of preeclampsia and gestational hypertension, but studies on supplemental antioxidant nutrients have not demonstrated reduced risk of preeclampsia except in nutritionally deficient women and may even cause harm. Relationships between levels of dietary antioxidants and preeclampsia have shown some protective association, but throughout the literature, findings have been inconsistent.

Few studies have focused specifically on intake of antioxidant nutraceuticals, and specifically on the outcomes of preeclampsia or gestational hypertension. In those that have been studied and been found to have positive effects, such as fish, fish oil, garlic, green tea, and soy products, the effects are mild, and the results are inconsistent between studies. For this reason, at this time, it is not possible to recommend intake of any specific antioxidant nutraceuticals to pregnant women for the prevention of preeclampsia or gestational hypertension.

References

Agrawal, S., J. Fledderjohann, S. Vellakkal, and D. Stuckler. 2015. Adequately diversified dietary intake and iron and folic acid supplementation during pregnancy is associated with reduced occurrence of symptoms suggestive of pre-eclampsia or eclampsia in Indian women. *PLoS One* 10 (3):e0119120. doi: 10.1371/journal.pone.0119120.

Alissa, E. M. and G. A. Ferns. 2012. Functional foods and nutraceuticals in the primary prevention of cardiovascular diseases. *J Nutr Metab* 2012:569486. doi: 10.1155/2012/569486.

Banerjee, S., S. Jeyaseelan, and R. Guleria. 2009. Trial of lycopene to prevent pre-eclampsia in healthy primigravidas: Results show some adverse effects. *J Obstet Gynaecol Res* 35 (3):477–482. doi: 10.1111/j.1447-0756.2008.00983.x.

Bell, M. J. 2010. A historical overview of preeclampsia-eclampsia. *JOGNN/NAACOG* 39 (5):510–518. doi: 10.1111/j.1552-6909.2010.01172.x.

Birch, C. S., N. E. Brasch, A. McCaddon, and J. H. H. Williams. 2009. A novel role for vitamin B_{12}: Cobalamins are intracellular antioxidants in vitro. *Free Radic Biol Med* 47 (2):184–188. doi: 10.1016/j.freeradbiomed.2009.04.023.

Brantsæter, A. L., M. Haugen, S. O. Samuelsen, H. Torjusen, L. Trogstad, J. Alexander, P. Magnus, and H. M. Meltzer. 2009. A dietary pattern characterized by high intake of vegetables, fruits, and vegetable oils is associated with reduced risk of preeclampsia in nulliparous pregnant Norwegian women. *J Nutr* 139 (6): 1162–1168.

Brantsæter, A. L., R. Myhre, M. Haugen, S. Myking, V. Sengpiel, P. Magnus, B. Jacobsson, and H. M. Meltzer. 2011. Intake of probiotic food and risk of preeclampsia in primiparous women: The Norwegian mother and child cohort study. *Am J Epidemiol* 174 (7):807–815. doi: 10.1093/aje/kwr168.

Carter, J. P., T. Furman, and H. R. Hutcheson. 1987. Preeclampsia and reproductive performance in a community of vegans. *The South Med J* 145 (7):163–167.

Chen, Z.-Y., C. Peng, R. Jiao, Y. M. Wong, N. Yang, and Y. Huang. 2009. Anti-hypertensive nutraceuticals and functional foods. *J Agric Food Chem* 57 (11):4485–4499. doi: 10.1021/jf900803r.

Chesley, L. C. 1984. History and epidemiology of preeclampsia—Eclampsia. *Clin Obstet Gynecol* 27 (4):801–820.

Chopra, M., H. Beswick, M. Clapperton, H. J. Dargie, W. E. Smith, and J. McMurray. 1992. Antioxidant effects of angiotensin-converting enzyme (ACE) inhibitors: Free radical and oxidant scavenging are sulfhydryl dependent, but lipid peroxidation is inhibited by both sulfhydryl- and nonsulfhydryl-containing ACE inhibitors. *J Cardiovasc Pharmacol* 19 (3):330–340.

Cicero, A. F. G. and C. Borghi. 2013. Evidence of clinically relevant efficacy for dietary supplements and nutraceuticals. *Curr Hypertens Rep* 15 (3):260–267. doi: 10.1007/s11906-013-0333-8.

De-Regil, L. M., C. Palacios, A. Ansary, R. Kulier, and J. P. Peña-Rosas. 2012. Vitamin D supplementation for women during pregnancy. *Cochrane Database Syst Rev* 2:CD008873–CD008873. doi: 10.1002/14651858.CD008873.pub2.

Dwyer, J. T. 2007. Do functional components in foods have a role in helping to solve current health issues? *J Nutr* 137 (11):2489S–2492S.

Finkel, T. and N. J. Holbrook. 2000. Oxidants, oxidative stress and the biology of ageing. *Nature* 408 (6809):239–247.

Fugh-Berman, A. 2000. Herbs and dietary supplements in the prevention and treatment of cardiovascular disease. *Prev Cardiol* 3 (1):24–32. doi: 10.1111/j.1520-037X.2000.80355.x.

Gandhi, S. and A. Y. Abramov. 2012. Mechanism of oxidative stress in neurodegeneration. *Oxid Med Cell Longev* 2012:428010. doi: 10.1155/2012/428010.

Grassi, D. and C. Ferri. 2012. Cocoa, chocolate and hypertension. In: *Chocolate and Health*, Milano, Italy: Springer Milan, pp. 115–125.

Hobson, S. R., R. Lim, E. E. Gardiner, N. O. Alers, and E. M. Wallace. 2013. Phase I pilot clinical trial of antenatal maternally administered melatonin to decrease the level of oxidative stress in human pregnancies affected by pre-eclampsia (PAMPR): Study protocol. *BMM J Open* 3 (9):e003788. doi: 10.1136/bmjopen-2013-003788.

Hobson, S. R., J. C. Mockler, R. Lim, N. O. Alers, S. L. Miller, and E. M. Wallace. 2015. Melatonin for preventing pre-eclampsia. *Cochrane Database Syst Rev* (5). doi: 10.1002/14651858.CD011657.

Hofmeyr, G. J., T. A. Lawrie, Á. N. Atallah, L. Duley, and M. R. Torloni. 2014. Calcium supplementation during pregnancy for preventing hypertensive disorders and related problems. *Cochrane Database Syst Rev* (6). doi: 10.1002/14651858.CD001059.pub4.

Hooper, L., P. A. Kroon, E. B. Rimm, J. S. Cohn, I. Harvey, K. A. Le Cornu, J. J. Ryder, W. L. Hall, and A. Cassidy. 2008. Flavonoids, flavonoid-rich foods, and cardiovascular risk: A meta-analysis of randomized controlled trials. *Am J Clin Nutr* 88 (1):38–50.

Iwaniak, A., P. Minkiewicz, and M. Darewicz. 2014. Food-originating ACE inhibitors, including antihypertensive peptides, as preventive food components in blood pressure reduction. *Compr Rev Food Sci Food Saf* 13 (2):114–134. doi: 10.1111/1541-4337.12051.

Lobo, V., A. Patil, A. Phatak, and N. Chandra. 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev* 4 (8):118–126. doi: 10.4103/0973-7847.70902.

Lovell, M. A., W. D. Ehmann, S. M. Butler, and W. R. Markesbery. 1995. Elevated thiobarbituric acid-reactive substances and antioxidant enzyme activity in the brain in Alzheimer's disease. *Neurology* 45 (8):1594–1601.

Ly, C., J. Yockell-Lelièvre, Z. M. Ferraro, J. T. Arnason, J. Ferrier, and A. Gruslin. 2015. The effects of dietary polyphenols on reproductive health and early development. *Hum Reprod Update* 21 (2):228–248.

Mahomed, K., Z. Bhutta, and P. Middleton. 2007. *Zinc Supplementation for Improving Pregnancy and Infant Outcome*. Oxford, UK: The Cochrane Library.

Malla, S., J. Hobbs, and K. S. Erick. 2013. Functional foods and natural health products regulations in Canada and around the world: Nutrition labels and health claims. *CAIRN*.

Marya, R. K., S. Rathee, and M. Manrow. 1987. Effect of calcium and vitamin D supplementation on toxaemia of pregnancy. *Gynecol Obstet Invest* 24 (1):38–42.

National Library of Medicine. 2015. Folic acid. Medline Plus.

Mokhlesi, S., S. Mohebi, P. Khoshdani, and L. M. Banaem. 2015. Survey the relationship between diet and occurrence of preeclampsia in the pregnant women of Tehran city. *Arch Hyg Sci* 3 (4):152–159.

Münzel, T. and J. F. Keaney. 2001. Are ACE inhibitors a "Magic bullet" against oxidative stress? *Circulation* 104 (13):1571–1574.

Ngo, D.-H., T.-S. Vo, D.-N. Ngo, I. Wijesekara, and S.-K. Kim. 2012. Biological activities and potential health benefits of bioactive peptides derived from marine organisms. *Int J Biol Macromol* 51 (4):378–383. doi: 10.1016/j.ijbiomac.2012.06.001.

Noris, M., N. Perico, and G. Remuzzi. 2005. Mechanisms of disease: Pre-eclampsia. *Nat Clin Pract Nephrol* 1 (2):98–114.

Oken, E., Y. Ning, S. L. Rifas-Shiman, J. W. Rich-Edwards, S. F. Olsen, and M. W. Gillman. 2007. Diet during pregnancy and risk of preeclampsia or gestational hypertension. *Ann Epidemiol* 17 (9):663–668. doi: 10.1016/j.annepidem.2007.03.003.

Organization, World Health. 2011. *WHO Recommendations for Prevention and Treatment of Pre-eclampsia and Eclampsia*. Geneva, Switzerland: WHO Press.

Palafox-Carlos, H., J. F. Ayala-Zavala, and G. A. González-Aguilar. 2011. The role of dietary fiber in the bioaccessibility and bioavailability of fruit and vegetable antioxidants. *J Food Sci* 76 (1):R6–R15. doi: 10.1111/j.1750-3841.2010.01957.x.

Papas, A. M. 1999. Diet and antioxidant status. *Food Chem Toxicol* 37 (9–10):999–1007. doi: 10.1016/S0278-6915(99)00088-5.

Pérez-Jiménez, J. and F. Saura-Calixto. 2008. Grape products and cardiovascular disease risk factors. *Nutr Res Rev* 21 (2):158–173.

Poston, L., A. L. Briley, P. T. Seed, F. J. Kelly, and A. H. Shennan. 2006. Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia (VIP trial): Randomised placebo-controlled trial. *The Lancet* 367 (9517):1145–1154. doi: 10.1016/S0140-6736(06)68433-X.

Powell, S. R. 2000. The antioxidant properties of zinc. *J Nutr* 130 (5):1447S–1454S.

Rajasekaran, A., G. Sivagnanam, and R. Xavier. 2008. Nutraceuticals as therapeutic agents: A review. *Res J Pharm Technol* 1 (4):328–340.

Ray, J. G. and C. A. Laskin. 1999. Folic acid and homocyst(e)ine metabolic defects and the risk of placental abruption, pre-eclampsia and spontaneous pregnancy loss: A systematic review. *Placenta* 20 (7):519–529. doi: 10.1053/plac.1999.0417.

Reiter, R. J. and D.-X. Tan. 2002. Melatonin. *Ann N Y Acad Sci* 957 (1):341–344. doi: 10.1111/j.1749-6632.2002.tb02938.x.

Roberts, J. M., J. L. Balk, L. M. Bodnar, J. M. Belizán, E. Bergel, and A. Martinez. 2003. Nutrient involvement in preeclampsia. *J Nutr* 133 (5):1684S–1692S.

Rumbold, A., L. Duley, C. A. Crowther, and R. R. Haslam. 2008. Antioxidants for preventing pre-eclampsia. *Cochrane Database Syst Rev* (1). doi: 10.1002/14651858.CD004227.pub3.

Savica, V., G. Bellinghieri, and J. D. Kopple. 2010. The effect of nutrition on blood pressure. *Ann Rev Nutr* 30 (1):365–401. doi: 10.1146/annurev-nutr-010510-103954.

Scholl, T. O., M. Leskiw, X. Chen, M. Sims, and T. P. Stein. 2005. Oxidative stress, diet, and the etiology of preeclampsia. *Am J Clin Nutr* 81 (6):1390–1396.

Sharma, J. B., A. Kumar, A. Kumar, M. Malhotra, R. Arora, S. Prasad, and S. Batra. 2003. Effect of lycopene on pre-eclampsia and intra-uterine growth retardation in primigravidas. *Int J Gynaecol Obstet* 81 (3):257–262. doi: 10.1016/S0020-7292(03)00040-7.

Talaulikar, V. S. and I. T. Manyonda. 2011. Vitamin C as an antioxidant supplement in women's health: A myth in need of urgent burial. *Eur J Obstet Gynecol Reprod Biol* 157 (1):10–13. doi: 10.1016/j.ejogrb.2011.03.017.

Trumbo, P. R. and K. C. Ellwood. 2007. Supplemental calcium and risk reduction of hypertension, pregnancy-induced hypertension, and preeclampsia: An evidence-based review by the US Food and Drug Administration. *Nutr Rev* 65 (2):78–87.

Wiseman, H. 1993. Vitamin D is a membrane antioxidant ability to inhibit iron-dependent lipid peroxidation in liposomes compared to cholesterol, ergosterol and tamoxifen and relevance to anticancer action. *FEBS Lett* 326 (1–3):285–288. doi: 10.1016/0014-5793(93)81809-E.

9

Antioxidant Nutraceuticals with Probiotic Applications

Arpita Das and Runu Chakraborty

Contents

9.1 Introduction

Free radicals disrupt the smooth functioning of the cells of our body, attacking key structural components; therefore, lipids and proteins in the cell membrane, and even DNA enzymes are responsible for the operation and refurbishment of the cell. Free radicals are a major cause of many degenerative diseases, such as atherosclerosis, cancer, cardiovascular diseases, inflammatory bowel diseases, skin aging, old age dementia, arthritis, and cancer, all of which are now the main cause of death in our society. Epidemiological data and randomized clinical trials provide ample indications that antioxidants play a fundamental role in the prevention of cancer and cardiovascular diseases (Shklar 1998; Surh 1999; Kris-Etherton et al. 2002; Ferrari and Torres 2003). Antioxidants are compounds whose primary function is to protect our body from the molecules known as free radicals (among others) that cause oxidative stress. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals that start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and

inhibit other oxidation reactions. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols (Sies 1997). Antioxidants terminate the chain reactions by removing free radical intermediates and inhibit other oxidation reactions by neutralizing free radicals. However, in the process, antioxidants are themselves oxidized. Thus, there is a constant need to replenish antioxidant resources as one antioxidant molecule can only react with a single free radical (Halliwell and Gutteridge 1990). Consequently, it is essential to search and develop natural nontoxic antioxidants to protect the human body from free radicals and to slowdown the progress of many chronic diseases. Due to changes in consumer perception toward food as sources of therapeutic value, there is a spurt in the market of food-based ingredients and supplements, which provide antioxidant. Antioxidants are grouped into three: (1) primary or natural antioxidants, (2) secondary or synthetic antioxidants, and (3) antioxidant from microbial origin.

9.1.1 Primary or natural antioxidants

They are the chain-breaking antioxidants, which react with lipid radicals and convert them into more stable products. Antioxidants of this group are mainly phenolic in structure and include the following (Hurrell 2003):

1. *Antioxidants minerals*: These are cofactor of antioxidant enzymes. Their absence will definitely affect the metabolism of many macromolecules such as carbohydrates. Examples include selenium, copper, iron, zinc, and manganese.
2. *Antioxidants vitamins*: It is needed for most of the body's metabolic functions. It includes vitamin C, vitamin E, and vitamin B.
3. *Phytochemicals*: These are phenolic compounds that are neither vitamins nor minerals.

9.1.2 Secondary or synthetic antioxidants

These are phenolic compounds that perform the function of capturing free radicals and stopping the chain reactions, the compounds include the following (Hurrell 2003):

1. Butylated hydroxylanisole (BHA)
2. Butylated hydroxyrotoluene (BHT)
3. Propyl gallate (PG) and metal chelating agent (EDTA)
4. Tertiary butyl hydroquinone (TBHQ)
5. Nordihydro guaretic acid (NDGA)

9.1.3 Antioxidant from microbial origin

Probiotics have antioxidant activity, which improves the function of biochemical reaction in a biological system (Afify et al. 2012). Probiotics are defined

as selected, viable microbial dietary supplements that, when introduced in sufficient quantities, beneficially affect human organism through their effects in the intestinal tract (Zubillaga et al. 2001; Holzapfel and Schillinger 2002). In addition, FAO/WHO has adopted the definition of probiotics as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO 2002). There are a large number of probiotics currently used and available in dairy fermented food, especially in yogurts. Lactic acid bacteria constitute a diverse group of organisms providing considerable benefits to mankind, some as natural inhabitants of the intestinal tract and others as fermentative lactic acid bacteria used in food industry, imparting flavor, texture, and possessing preservative properties.

9.2 Probiotic as a rich source of antioxidant

Reactive oxygen metabolites (ROM), generated through normal reactions within the body during respiration in aerobic organisms, can cause damage in proteins, mutations in DNA, oxidation of membrane phospholipids, and modification in low-density lipoproteins (Firuzi et al. 2011). To neutralize the oxidant molecules, the human body synthesizes antioxidant enzymes and molecules that, together with the antioxidants contained in food, form the biological antioxidant barrier. However, in certain circumstances, the defense system fails to protect the body against oxidative stress; consequently, the possibility of increasing antioxidant defenses is considered important in the maintenance of human health and disease prevention (Serafini and Del Rio 2004).

The projected market value of antioxidants to grow to US$238.5 million by 2018, at the CAGR of 4.5% (Feed antioxidants 2014). There is a renewed interest in the search of new sources of antioxidants, which can be safely used in food. In this trend, a novel pioneering approach is represented by the development of probiotics exerting antioxidant activity and counteracting the oxidative stress in the host due to their long tradition of safe use, along with potential therapeutic benefits; role of probiotics as an antioxidant is being fanatically investigated. Probiotics are known for their health beneficial effects and have established as dietary adjuncts. In particular, besides the long history of consumption of lactic acid bacteria, probiotic strains belonging to the genera *Lactobacillus* and *Bifidobacterium* have been reported to have a range of health-promoting features (Rossi and Amaretti 2010). Probiotics have established their efficiency as dietary factors, which can regulate gastrointestinal functions thereby imparting health benefits to consumers. Improvement of lactose intolerance, prevention of different forms of diarrhea and urogenital infections, cholesterol reduction, reduction of atopic diseases, and modulation of the immune system are some of the functions attributed to probiotics (Andersson et al. 2001; Chapman et al. 2011). The physiological effects related to probiotic bacteria include the reduction of

gut pH, production of some digestive enzymes and vitamins, production of antibacterial substances, for example, organic acids, bacteriocins, hydrogen peroxide, diacetyl, acetaldehyde, lactoperoxidase system, lactones and other unidentified substances, reconstruction of normal intestinal microflora after disorders caused by diarrheas, antibiotic therapy and radiotherapy, reduction of cholesterol level in the blood, stimulation of immune functions, suppression of bacterial infections, removal of carcinogens, improvement of calcium absorption as well as the reduction of fecal enzyme activity (Ouwehand et al. 1999; Zubillaga et al. 2001; Holzapfel and Schilling 2002).

Lactobacilli are the major source of probiotics and are usually explained as gram-positive bacteria, devoid of cytochromes and preferring anaerobic conditions, but are aerotolerant, fastidious, strictly fermentative, and produce lactic acid as the main product (Stiles and Holzapfel 1997). The competitive exclusion of pathogens and reduction in number as well as metabolic activities of harmful organisms by probiotics has been demonstrated *in vitro* (Mishra and Prasad 2005).

9.3 It must be safe and extra beneficial

From the safety point of view, the probiotic microorganisms should not be pathogenic, have no connection with diarrheagenic bacteria and no ability to transfer antibiotic resistance genes and to be able to maintain genetic stability. To be recognized as functional food components, they should demonstrate the following properties: acid and bile stability, resistance to digestive enzymes, adhesion to the intestinal surface, antagonistic activity against human pathogens, anticarcinogenic and antimutagenic activities, cholesterol-lowering effects, stimulation of the immune system without inflammatory effects, enhancement of bowel motility, maintenance of mucosal integrity, improvement of bioavailability of food compounds, and production of vitamins and enzymes (Ouwehand et al. 1999). Various species and strains of lactobacilli, that is, *L. acidophilus, L. casei, L. rhamnosus,* and *L. helveticus*, are considered as successful probiotics and different variants have been available in the market for human consumption. *Bifidobacterium*, even though not grouped with lactic acid bacteria, is another genus with probiotic functions. *B. lactis* (*B. animalis* ssp. *lactis*) is a very commonly used probiotic, although it is not a normal inhabitant of the human gastrointestinal tract. *B. longum* ssp. *longum* (and ssp. *infantis*) and *B. breve* are mainly used in supplements (Vasiljevic et al. 2008).

Among probiotics' beneficial effects, some authors have reported the protection against oxidative stress and the capability to decrease the risk of accumulation of ROM (Kaizu et al. 1993; Kullisar et al. 2002; Martarelli et al. 2011). The antioxidant mechanisms of probiotics could be assigned to ROS scavenging, metal ion chelation, enzyme inhibition, and to the reduction activity and inhibition of ascorbate autoxidation. Probiotic metabolic activities may have an antioxidant

effect via the scavenging of oxidant compounds or the prevention of their generation in the intestine (Lin and Yen 1999; Talwalkar and Kailasapathy 2003; Azcárate-Peril et al. 2011). Most lactic acid bacteria have scavenging systems for oxygen-free radicals. Some lactobacilli possess antioxidant activity and are able to decrease the risk of accumulation of ROS during ingestion of food (Kaizu et al. 1993). Production of bioactive peptides has been considered an effective mode of antioxidative activity in food containing probiotic bacteria. Peptides obtained from hydrolyzed food proteins have been shown to possess antioxidative activities, which can impart protection against the peroxidation of lipids or fatty acids (Saiga et al. 2003).

9.4 Application of probiotics as nutraceuticals

Nowadays consumer's perception toward functional foods, including probiotics has changed. The presence of probiotics in commercial food products has been very well accepted and has been known for number of health benefits. As a result of that, industries worldwide are concentrating on applications of probiotics in food products as well as constructing a new era of *probiotic health* food. Foods are considered as better delivery vehicles for probiotic organisms than others due to the role played by the food matrix in maintaining the viability of organisms during the transition through the gastrointestinal tract.

Studies have shown that selected strains of *lactobacilli* and *bifidobacteria* present antioxidative properties (Lin and Yen 1999; Wang et al. 2006; Zanoni et al. 2008; Spyropoulos et al. 2011) and can be used to prepare probiotic and fermented dairy products that improve total antioxidant status and decrease markers of oxidative stress in healthy people (Naruszewicz et al. 2002; Songisepp et al. 2005; Virtanen et al. 2007).

The probiotic potential and antioxidant property of *Enterococcus durans* LAB18s, a strain capable of selenium bioaccumulation, was investigated by Pieniz et al. (2014). *E. durans* LAB18s showed resistance to acid conditions, showing ability to survive in the presence of simulated gastric juice at pH 3. This bacterium also survived in the presence of simulated intestinal juice with or without bile salts, and did not show hemolytic activity. The antioxidant activity of culture supernatant and intracellular extract of *E. durans* LAB18s was analyzed by 2,2 azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods and it has the ability to scavenge both radicals. Both culture supernatant and intracellular extract showed high antioxidant activity when analyzed by thiobarbituric acid reactive substances (TBARS) method. *E. durans* LAB18s could be useful as a source of dietary selenium supplementation.

The consumption of probiotic yogurt improved fasting blood glucose and antioxidant status in type 2 diabetic patients. These results suggest that probiotic yogurt is a promising agent for diabetes management (Ejtahed et al. 2012).

Leek (*Allium ampeloprasum var. porrum*) is one of Belgium's most important outdoor vegetables, mainly cultivated for its white shaft. Fermentation of leek offers opportunities in view of biomass valorization and product diversification. A study on influence of three starter culture strains (*Lactobacillus plantarum* IMDO 788, *Lactobacillus sakei* IMDO 1358, and *Leuconostoc mesenteroides* IMDO 1347) on the metabolite kinetics of leek fermentation and antioxidant properties of leek was performed by Wouters et al. (2013). The antioxidant capacity of fermented leek samples, as measured with the oxygen radical absorbance capacity assay, increased when starter cultures were used.

Illupapalayam et al. (2014) demonstrated a study on the development of new type of novel probiotic yogurts containing spices with acceptable sensory properties, therapeutic levels of probiotics, and with beneficial antioxidant capacity. Eight types of yogurts with added spice oleoresins (cardamom, cinnamon, and nutmeg) and probiotics (*Lactobacillus acidophilus* strain 5 [LA5], or *Bifidobacterium animalis* ssp. *lactis* [Bb12]) were produced. During storage, significant increase in survival of both *B. animalis* ssp. *lactis* (Bb12) and *L. acidophilus* (LA5) was found without affecting the fermentation kinetics and these also provide strong antioxidant activity. Probiotic organisms had a statistically significant effect on the proteolytic activity and enhanced the generation of peptides with potential antioxidant and antimutagenic properties with good correlation between proteolytic and antioxidant or antimutagenic activities (Sah et al. 2014).

Free radical scavenging activity of *L. plantarum* and *L. mesenteroides* ranges from 14.7% to 50.8% (v/v) after 24 to 72 h fermentation, respectively, as determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (Abubakr et al. 2012).

Detection of antioxidative activity from cell-free extract of 100 strains of lactic acid bacteria by using TBARS and oxygen radical absorbance capacity (ORAC) assays was done by Yamamoto (2009). The highest antioxidative activity strain screened by TBARS assay was *Lactobacillus sakei* PP6-S. The strain that demonstrated highest ORAC value was *Lactobacillus casei* JCM20024. Several preliminary analyses indicated that antioxidative activities detected by TBARS and ORAC assays were low molecular weight compound(s) and relatively heat stable. Incorporation of probiotics in food can provide a good strategy to supply dietary antioxidants, but more studies are needed to standardize methods and evaluate antioxidant properties of probiotics before they can be recommended for antioxidant potential.

9.5 Conclusion

Probiotic food products are a fast growing area of functional food, as found to be strongly accepted by the consumers. The application of probiotics in dairy products is already common. However, the food industry is seeking to

produce different varieties of probiotic food other than dairy products with potential health benefits. The success of new probiotic food depend on the ability of probiotics to provide sufficient numbers of viable cells that beneficially modify the gut microflora of the host along with its potential antioxidant activity. From the above-mentioned study, it can be inferred that probiotic bacteria posses a strong antioxidant activity, which have been assayed by different chemical methods. In order to improve the survival rates of probiotic microorganisms during food production, microencapsulation is considered to be a promising process. Encapsulation materials are recognized as safe ingredients and can be used in food applications. These extend the application of probiotics as a nutraceutical with antioxidant potential and may find therapeutic potential in management of degenerative diseases.

References

Abubakr, M. A. S., Z. Hassan, M. M. A. Imdakim, and N. R. S. A. Sharifah. 2012. Antioxidant activity of lactic acid bacteria (LAB) fermented skim milk as determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferrous chelating activity (FCA). *Afr J Microbiol Res* 6 (34):6358–6364.

Afify, A. M. R., R. M. Romeilah, S. I. M. Sultan, and M. M. Hussein. 2012. Antioxidant activity and biological evaluations of probiotic bacteria strains. *Int J Acad Res* 4 (6, A):131–139.

Andersson, H., N. G. Asp, A. Bruce, S. Roos, T. Wadstrom, and A. E. Wold. 2001. Health effects of probiotics and prebiotics: A literature review on human studies. *Scand J Nutr* 45:58–75.

Azcárate-Peril, M. A., M. Sikes, and J. M. Bruno-Bárcena. 2011. The intestinal microbiota, gastrointestinal environment and colorectal cancer: A putative role for probiotics in prevention of colorectal cancer? *Am J Physiol Gastrointest Liver Physiol* 301:G401–G424.

Chapman, C. M., G. R. Gibson, and I. Rowland. 2011. Health benefits of probiotics: Are mixtures more effective than single strains? *EurJ Nutr* 50:1–17.

Ejtahed, H. S., J. Mohtadi-Nia, A. Homayouni-Rad, M. Niafar, M. Asghari-Jafarabadi, and V. Mofid. 2012. Probiotic yogurt improves antioxidant status in type 2 diabetic patients. *Nutrition*. 28:539–543.

FAO/WHO. 2002. Guidelines for the Evaluation of Probiotics in Food. London, ON, April 30 and May 1, 2002. ftp://ftp.fao.org/es/esn/food/wgreport2.pdf.

Feed antioxidants, Market and markets.com. December 23, 2014. http://www.marketsandmarkets.com/Market-Reports/animal-feed-antioxidants market-138013648.html.

Ferrari, C. K. B. and E. A. F. S. Torres. 2003. Biochemical pharmacology of functional foods and preventing of chronic diseases of aging. *Biomed Pharmacother* 57:251–260.

Firuzi, O., R. Miri, M. Tavakkoli, and L. Saso. 2011. Antioxidant therapy: Current status and future prospects. *Curr Med Chem* 18:3871–3888.

Fuller, R. 1989. Probiotics in man and animal. *J Appl Bacteriol* 66:365–378.

Halliwell, B. and J. M. Gutteridge. 1990. The antioxidants of human extracellular fluids. *Arch Biochem Biophys* 280:1–8.

Holzapfel, W. H. and U. Schillinger. 2002. Introduction to pre- and probiotics. *Food Res Int* 35:109–116.

Hurrell, R. 2003. Influence of vegetable protein sources on trace element and mineral bioavailability. *J Nutr* 133(9):2973S–2977S.

Illupapalayam, V. V., S. C. Smith, and S. S. Gamlath. 2014. Consumer acceptability and antioxidant potential of probiotic-yogurt with spices. *LWT-Food Sci Technol* 55:255–262.

Kaizu, H., M. Sasaki, H. Nakajima, and Y. Suzuki. 1993. Effect of antioxidative lactic acid bacteria on rats fed a diet deficient in vitamin. *E J Dairy Sci* 76:2493–2499.
Kris-Etherton, P. M., K. D. Hecker, A. Bonanome, S. M. Coval, A. E. Binkoski, K. Hilpert, A. E. Griel, and T. D. Etherton. 2002. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. *Am J Med* 113 (Suppl 9B):71S–88S.
Kullisar, T., M. Zilmer, M. Mikelsaar, T. Vihalemm, H. Annuk, C. Kairanc, and A. Kilk. 2002. Two antioxidative lactobacilli strains as promising probiotics. *Int J Food Microb* 72:215–224.
Lin, M. Y. and C. L. Yen. 1999. Antioxidative ability of lactic acid bacteria. *J Agric Food Chem* 47:1460–1466.
Martarelli, D., M. C. Verdenelli, S. Scuri, M. Cocchioni, S. Silvi, C. Cecchini, and P. Pompei. 2011. Effect of a probiotic intake on oxidant and antioxidant parameters in plasma of athletes during intense exercise training. *Curr Microbiol* 62:1689–1696.
Mishra, V. and D. N. Prasad. 2005. Application of in vitro methods for selection of *Lactobacillus casei* strains as potential probiotics. *Int J Food Microbiol* 103:109–115.
Naruszewicz, M., M. L. Johansson, D. Zapolska-Downar, and H. Bukowska. 2002. Effect of *Lactobacillus plantarum* 299v on cardiovascular disease risk factors in smokers. *Am J Clin Nutr* 76:1249–1255.
Ouwehand, A. C., P. V. Kirjavainen, C. Shortt, and S. Salminen 1999. Probiotics: Mechanisms and established effects. *Int Dairy J* 9:43–52.
Pieniz, S., R. Andreazza, T. Anghinoni, F. Camargo, and A. Brandelli. 2014. Probiotic potential, antimicrobial and antioxidant activities of *Enterococcus durans* strain LAB18s. *Food Cont* 37:251–256.
Rossi, M. and A. Amaretti. 2010. Probiotic properties of bifidobacteria. In: Mayo, B., van Sinderen, D. (Eds.). *Bifidobacteria: Genomics and Molecular Aspects*. Rowan. House, UK: Horizon Scientific Press, pp. 97–123.
Sah, B. N. P., T. Vasiljevic, S. McKechnie, and O. N. Donkor. 2014. Effect of probiotics on antioxidant and antimutagenic activities of crude peptide extract from yogurt. *Food Chem* 156:264–270.
Saiga, A., S. Tanabe, and T. Nishimura. 2003. Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. *J Agric Food Chem* 12:3661–3667.
Serafini, M. and D. Del Rio. 2004. Understanding the association between dietary antioxidants, redox status and disease: Is the total antioxidant capacity the right tool?. *Redox Rep* 9:145–152.
Shklar, G. 1998. Mechanisms of cancer inhibition by anti-oxidant nutrients. *Oral Oncol* 34:24–29.
Sies, H. 1997. Oxidative stress: Oxidants and antioxidants. *Exp Physiol* 82 (2):291–295.
Songisepp, E., J. Kals, T. Kullisaar, R. Mändar, P. Hütt, M. Zilmer, and M. Mikelsaar. 2005. Evaluation of the functional efficacy of an antioxidative probiotic in healthy volunteers. *Nutr J* 4:22–32.
Spyropoulos, B. G., E. P. Misiakos, C. Fotiadis, and C. N. Stoidis. 2011. Antioxidant properties of probiotics and their protective effects in the pathogenesis of radiation-induced enteritis and colitis. *Dig Dis Sci* 56:285–294.
Stiles, M. E. and W. H. Holzapfel. 1997. Lactic acid bacteria of foods and their current taxonomy. *Int J Food Microbiol* 36:1–29.
Surh, Y. J. 1999. Molecular mechanisms of chemopreventive effects of selected dietary and medicinal phenolic substances. *Mutat Res* 428:305–327.
Talwalkar, A. and K. Kailasapathy. 2003. Metabolic and biochemical responses of probiotic bacteria to oxygen. *J Dairy Sci* 86:2537–2546.
Vasiljevic, T. and N. P. Shah. 2008. Probiotics—From Metchnikoff to bioactives. *Int Dairy J* 18:714–728.
Virtanen, T., A. Pihlanto, S. Akkanen, and H. Korhonen. 2007. Development of antioxidant activity in milk whey during fermentation with lactic acid bacteria. *J Appl Microbiol* 102:106–115.

Wang, Y. C., R. C. Yu, and C. C. Chou. 2006. Antioxidative activities of soymilk fermented with lactic acid bacteria and bifidobacteria. *Food Microbiol* 23:128–135.

World Health Organization Food and Agriculture Organization of the United Nations Rome. 2006. *FAO/WHO Guidance to Governments on the Application of HACCP in Small and/or Less-developed Food Businesses*. FAO Food and Nutrition paper, p. 86.

Wouters, D., N. Bernaert, N. Anno, B. Van Droogenbroeck, M. De Loose et al. 2013. Application' and validation of autochthonous lactic acid bacteria starter cultures for controlled leek fermentations and their influence on the antioxidant properties of leek. *Int J Food Microbiol* 165:121–133.

Yamamoto, Y. 2009. *Identification of Antioxidant(s) from Lactic Acid Bacteria and Screening of High Antioxidative Strain(s)*. NISR Research GRANT, Young Investigator Research Grant, Japan, pp. 57–58.

Zanoni, S., A. Pompei, L. Cordisco, A. Amaretti, M. Rossi, and D. Matteuzzi. 2008. Growth kinetics on oligo- and polysaccharides and promising features of three antioxidative potential probiotic strains. *J Appl Microbiol* 105:1266–1276.

Zubillaga, M., R. Weil, E. Postaire, C. Goldman, R. Caro, and J. Boccio. 2001. Effect of probiotics and functional foods and their use in different diseases. *Nutr Res* 21:569–579.

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

10

Antioxidant Nutraceuticals as Health Drinks for Prevention of Diseases

Kumar Rajendran, P. Latha, and Shampa Sen

Contents

10.1 Introduction

Research reports support the role of oxidative stress in the causation and progression of several chronic diseases, including cancer, Alzheimer's disease (AD) and cerebrovascular disease (Halliwell 1994; Ames et al. 1995). Free radicals, either as a by-product of normal metabolism or associated with inflammatory reactions, can contribute to several diseases such as cardiovascular disease and cancer. Numerous epidemiological studies suggest that the risks of many chronic diseases are diet-related and could be decreased significantly through the change of dietary habits. A nutraceutical is a food or part of food or nutrient that provides health benefits, including the prevention and treatment of a disease. Antioxidants have become the essential nutrients

of the nutraceutical and nutritional world over the past decade. Nowadays, the interest in the role of dietary antioxidants in the prevention of diseases has prompted research in the field of antioxidant-rich beverages. The market for nutraceutical beverages, that is, drinks with vitamin and mineral fortification, antioxidant or high polyphenol beverages containing ingredients such as green teas or berries, and drinks with certain selected herbs is growing continuously along with the health consciousness of the people. Antioxidants are perhaps some of the most added ingredients to beverages or available naturally in many juices. There are a number of commercial fruit juices and antioxidant-rich beverages, which base their marketing strategies on antioxidant potency.

Antioxidative systems include antioxidative enzymes, such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione-S-transferase (GST), and nonenzymatic substrates, such as vitamin C, vitamin A, vitamin E, glutathione, lipoic acid, uric acid, coenzyme Q, bilirubin, flavonoids, carotenoids (e.g., astaxanthin, zeaxanthin), lycopene, phosvitin, bioflavonoid resveratrol, ferritin, lactoferrin, transferrin, ceruloplasmin, hemopexin, haptoglobin, and so on. More than 3100 antioxidants have been identified in foods, such as fruit, vegetables, seeds, nuts, cereals, wholegrains, tea, garlic, herbs, and spices, which are regularly consumed by different cultures (Rajendran et al. 2014). Major antioxidants present in the health drinks are shown in **Table 10.1**. The most antioxidant-rich beverages are coffee (200–550 mg/cup), tea (150–400 mg/cup), and red wine (150–400 mg/glass) (Menshchikova et al. 2006). Morillas-Ruiz et al. (2010) suggested that the antioxidant drink can reduce the effects of inflammation and cardiovascular risk associated with AD and oxidative stress-related disease development. Bioavailability of antioxidants differs greatly from one phytochemical to another (Manach et al. 2004, 2005b; Williamson and Manach 2005), so the most antioxidant-rich foods do not necessarily provide highest concentrations of active metabolites in target tissues. The intestinal absorption of antioxidant compounds from fruit juices is even better than that of antioxidants from fruits (Bitsch et al. 2004). Sugar content has no effect on the antioxidant content and capacity of the drinks.

10.2 Antioxidant health drinks for prevention of diseases

10.2.1 Fruit and vegetable juices

Nutrition and health organizations recommend that an adult should consume at least two serves of fruit and five serves of vegetables everyday to decrease the incidence of several chronic diseases. Polyphenol-rich fruits may protect against oxidative stress (Heo et al. 2008). A number of vitamins such as C, A, E as well as carotene are excellent antioxidants that also contribute to good health as being cofactors for certain enzymes and involved in

Table 10.1 Antioxidants Present in Health Drinks and Their Roles in Prevention and Treatment of Diseases

Antioxidants	Health Benefits	Reference
Polyphenol	Prevent cardiovascular diseases	Manach et al. (2005a)
	Prevent neurodegenerative diseases	Halliwell (2001)
	Osteoporosis	Atmaca et al. (2008)
	Treatment of diabetes	Zunino et al. (2007)
	Inhibit non-heme iron absorption	Hurrell et al. (1999)
	Platelet aggregation	Russo et al. (2001)
	Lower risk of myocardial infarction	Schachinger et al. (2000)
	Anticarcinogenic	Yang et al. (2001)
	Parkinson's disease	Pan et al. (2003)
Vitamin C	Protects against cancers	Barry (2008)
	Protects from heart disease	Liu et al. (2002)
	Improvement of the health of bones and skin	Wang et al. (2007)
	Maintaining a healthy immune system	Wintergerst et al. (2006)
	Improvement in the antibody production	Woo et al. (2010)
	Increase in the absorption of nutrients	Thankachan et al. (2008)
	Increases protection against H_2O_2-induced DNA strand breaks	Riso et al. (2010)
	Cytoprotective effect on cirrhosis	Passoni and Coelho (2008)
Vitamin E	Prevents coronary heart disease	Pryor (2000)
	Decreases incidence of breast and prostate cancers	Weinstein et al. (2007)
	Reduces long-term risk of dementia	Devore et al. (2010)
	Decreases risk of Parkinson's disease	Miyake et al. (2010)
Carotenoids (lycopene)	Protection against oxidation of lipids, LDL, proteins, and DNA	Visioli et al. (2004)
	Abduction and free radical scavenging	Miller et al. (1996)

oxidation–reduction reactions in the body. **Table 10.2** shows the antioxidant content of juices and beverages.

Carrots are high in antioxidants such as phenolics (chlorogenic, p-coumaric, and caffeic acids), carotenoids, and vitamins C and E (Alasalvar et al. 2001). Oral intake of carrot juice showed increased levels of plasma antioxidants (Törrönen et al. 1996), reduced oxidative DNA damage (Pool-Zobel et al. 1998), reduced inflammation (Hu et al. 2004), and decreased plasma malondialdehyde production (Potter et al. 2011). Lycopene is the predominant carotenoid in tomatoes that exhibits highest antioxidant activity among all dietary carotenoids (Miller et al. 1996). Two hypotheses have been proposed to explain the cancer-preventing effects of carotenoids: (1) their ability to act as precursors

Table 10.2 Antioxidant Content of Juices and Beverages

Beverages/Juice	Antioxidant Content (mmol/100 g)
Amla syrup	29.7
Espresso coffee	12.64–15.83
Red wine	1.78–3.66
Pomegranate juice	1.59–2.57
Coffee	1.24–4.20
Green tea	0.57–2.62
Grape juice	0.69–1.74
Prune juice	0.83–1.13
Blackberry juice	1.27
Black tea	0.75–1.21
Cranberry juice	0.75–1.01
Orange juice	0.47–0.81
Raspberry juice	0.78
Strawberry juice	0.43
Cocoa with milk	0.26–0.45
Tomato juice	0.19–1.06
Apple juice	0.12–0.60
Apricots juice	0.14

Source: Carlsen, M.H. et al., *Nutr. J.* 9, 3, 2010.

of vitamin A and (2) their intrinsic antioxidant property. An inverse association between high intake of tomato products or lycopene and the risk of prostate cancer (Giovamrucci et al. 1995), digestive-tract cancers (Franceschi et al. 1994), and breast cancer (Potischman et al. 1990) was confirmed. Beetroot juice also delivers a high amount of bioaccessible antioxidants and may be a cost-effective method of increasing antioxidant status (Wootton-Beard and Ryan 2011). Flavonoids and carotenoids contribute to the antioxidant potential of beetroot juice. Beetroot juice lowers blood pressure and prevents heart attack, stroke, and other cardiovascular diseases.

Pomegranate juice contains antioxidants higher than most other fruit juice and beverages (Seeram et al. 2008). The most abundant compounds in pomegranate juice are hydrolysable tannins, anthocyanins, ellagic acid derivatives, and different flavanols, which have important antioxidant and atherosclerotic biological properties. Pomegranate juice has been endorsed as a preventive treatment for coronary heart disease (Basu and Penugonda 2009), chemoprevention and chemotherapeutic effects on human prostate cancer (Malik et al. 2005). Higher antioxidant activity was found in commercial juices (specially the organic juice) extracted from whole pomegranate fruits than in fresh

juices that were obtained only from the arils (Gil et al. 2000). This is due to the fact that the industrial processing extracted more hydrolysable tannins predominantly punicalagin from the fruit peels resulting in higher antioxidant activity in commercial juices.

Apple plays a major role in decreasing the risk of chronic diseases because of their fiber, flavonoids, polyphenols, and carotenoids (Basu et al. 2010). Apple juice can be used as a nonpharmacologic protective agent that improves the total antioxidant status and antioxidant enzymes, whereas it reduces oxidative stress. Further, the hypolipidemic and hypocholesterolemic effects of apple juice substantially protect against atherosclerosis. Orange juice and grape fruit juice are rich sources of antioxidants and polyphenols, and they cooperatively reduce blood lipid profiles and oxidative stress, making them a valuable choice for disease prevention especially among the elderly (Densupsoontorn et al. 2002; Kiefer et al. 2004). Total antioxidant activity of ready-to-drink orange juice and syrup ranges from 57.88 to 349.32 μmol TEAC/100 mL (Stella et al. 2011). Grape juice contains flavonoids, which effectively increase the levels of high-density lipoproteins. Resveratrol, an important antioxidant found in the juice made from dark purple Concord grapes, reduces the risk of heart disease. Blueberries, cranberries, and strawberries help in preventing a range of diseases, such as cancer, heart disease, urinary tract infection, slow the aging process and memory loss. Several studies reported the role of cranberries in the prevention and treatment of urinary tract infections (Côté et al. 2010). Lemon fruit (*Citrus limon*) is rich in citric acid, vitamin C, flavonoids, and minerals (Del Río et al. 2004). Lemon juice is widely used as a natural antioxidant substitute for the synthetic ascorbic or citric acids. Juices of watermelon, pink grapefruit, apricots, pink guava, papaya, and tomatoes are also rich sources of lycopene (Gross 1987). Lycopene or canthaxanthin also exhibits cancer-preventing effects.

10.2.2 Tea

Next to water, tea and coffee are the two most consumed beverages in the world. However, the consumption patterns vary among different cultures and countries. Approximately 76%–78% of the tea produced and consumed in the world is black tea, 20%–22% is green tea, and <2% is oolong tea. Black tea is consumed largely in Europe, North America, and North Africa, whereas in India, black tea is mixed with milk. Green tea is widely consumed in China, Japan, Korea, and Morocco. Oolong tea is prevalent in China and Taiwan. The importance of having tea can be best explained by an ancient proverb, "Better to be deprived of food for three days, than tea for one." The major antioxidants in tea and coffee are flavonoids and phenolic acids. Monomer catechins, (−)-epigallocatechin-3-gallate (EGCG), epigallocatechin, epicatechin gallate, and epicatechin are the major flavonoids present in green tea. Polymerized catechins theaflavin and thearubigins predominate in black tea in addition to quercetin and flavonols (McKay and Blumberg 2002, 2007). A cup of green tea may contain 90–200 mg of catechins and there is also a difference in

polyphenol content between black and green tea due to the manufacturing conditions. Green tea consumption has also been linked to the prevention of many types of cancer, including esophagus, mouth, stomach, small intestine, colon, pancreas, lung, kidney, and mammary glands (Koo and Cho 2004). A possible relationship between high consumption of green tea and the low incidence of breast and prostate cancers has been postulated by Chen et al. (1998). Green tea was reported to reduce the risk of esophageal cancer by 60% (Leventhal et al. 1999). Green tea and EGCG prevent cancer by numerous mechanisms that affect different aspects of the carcinogenic process, including the inhibition of key cellular enzymes, inhibition of growth factor signaling, inhibition of gene transcription, and induction of tumor suppressor genes (Chen et al. 2004; Khan et al. 2006; Yang et al. 2006; Tachibana 2009). The antioxidant activity and prooxidant effects of green tea polyphenols have also been suggested as potential mechanisms for cancer prevention (Hou et al. 2005; Shen et al. 2005; Butt and Sultan 2009). Green tea has the ability of lowering total cholesterol levels and improving the ratio of HDL and LDL cholesterol. Green tea may lower blood pressure, and thus may reduce the risk of stroke and coronary heart disease by reducing blood glucose levels and body weight (Tsuneki et al. 2004). The polyphenolic compounds present in tea are proved to be beneficial in reducing the risk of cardiovascular disease (Hodgson 2000). Further, green tea is thought to exert a beneficial impact on the body composition and weight management by decreasing body weight and visceral fat. Shim et al. (1995) found that green tea can block the cigarette-induced increase in sister chromatid exchange frequency among cigarette smokers. Green tea catechins inhibit *Helicobacter pylori* infection (Yee et al. 2002; Takabayashi et al. 2004), other bacterial infections (Toda et al. 1989; Yam et al. 1997), influenza virus, especially in its earliest stage, as well as against the Herpes simplex virus (Mukoyama et al. 1991) and adenovirus infection (Weber et al. 2003). Green tea polyphenols might protect against chronic diseases, including diabetes, AD, and Parkinson's diseases (PDs) and other neurodegenerative diseases (Pan et al. 2003; Weinreb et al. 2004). Green tea consumption offers protection against the risk of hip fractures by increasing the bone mineral density (Muraki et al. 2003). Green tea and its flavonoids have anti-diabetic effects (Wu et al. 2004; Iso et al. 2006; Wolfram et al. 2006) and were shown to have insulin-like activities (Waltner-Law et al. 2002) as well as insulin-enhancing activities (Anderson and Polansky 2002). Gomes et al. (1995) reported the antihyperglycemic effect of black tea. Black tea polyphenols are reported to prevent the degeneration of the matrix in bone and cartilage (Oka et al. 2012). EGCG inhibits intestinal glucose uptake by the sodium-dependent glucose transporter (SGLT1) (Kobayashi et al. 2000). Green tea and green tea extracts, especially EGCG consumption, may help in reducing body weight, mainly body fat by increasing postprandial thermogenesis, decrease diet-induced obesity by decreasing energy absorption, and increasing fat oxidation (Klaus et al. 2005). As a result of these beneficial effects, green tea is currently used in the preparation of a variety of foods, dentifrices, pharmaceutical preparations, and cosmetics (Arburjai and Natsheh 2003). However, bottled tea is not as

powerful as newly brewed tea regarding their antioxidant capacity. The antioxidant level in bottled tea drinks depends on the type of tea used and how it is brewed. Green tea consumption is recommended up to a maximum of six cups per day (four cups for pregnant women).

10.2.3 Coffee

Several different compounds, for example, caffeine, polyphenols, volatile aroma compounds (furans, pyrroles), and heterocyclic compounds contribute to coffee's antioxidant content (Fuster et al. 2000; Illy 2002; Yanagimoto et al. 2002; Nawrot et al. 2003). Green coffee beans contain large amounts of polyphenolic antioxidants, such as chlorogenic, caffeic, ferulic, and *n*-coumarinic acids. However, coffee roasting significantly alters the composition of polyphenols due to Maillard reaction (chemical reaction between amino acids and sugars). Phenylalanine (Farah and Donangelo 2006) and melanoidins (Steinhart et al. 2001), which are formed during the roasting process show high antioxidant activity. Many researchers believe that Maillard reaction products formed during the roasting process are also strong antioxidants (Eichner 1981; Nicoli et al. 1997; Del Castillo et al. 2002). Some epidemiological evidence shows that coffee drinkers are at lower risk for AD and PD (Maia and de Mendonca 2002). Ferulic acid has anti-inflammatory, antibacterial, antiviral, antiallergic, antiplatelet effects (Prior et al. 2003). Many of these antioxidants are efficiently absorbed, and hence increase the total plasma antioxidant content after coffee consumption (Olthof et al. 2001; Illy 2002). A cup of coffee may contain 70–350 mg of chlorogenic acid, and the content of caffeine and polyphenol varies greatly depending on how the coffee beans are roasted. Interestingly, chlorogenic acid and polyphenols in coffee are geographically related. Chlorogenic acid was found to be more in Arabica coffee fruit planted in Mexico and India compared to those grown in China (Mullen et al. 2013). Consumption of 2–3 cups of coffee made with roasted beans ensures the daily consumption rate of antioxidants.

10.2.4 Milk

Several antioxidants can be found in milk (Lindmark-Mansson and Akesson 2000). Antioxidant enzymes such as superoxide dismutase and catalase have been demonstrated in milk (Hoolbrook and Hicks 1978; Ito and Akuzawa 1983). The iron-binding protein, lactoferrin can act as an antioxidant. Vitamin E, carotenoids, and ubiquinol act as radical scavengers in the lipid phase, whereas vitamin C acts in the water phase. Others can react in both the lipid and the water phase. Some flavonoids operate both as radical scavengers and metal-ion binders (Lindmark-Mansson and Akesson 2000). Some carotenoids have provitamin A action as well as antioxidant functions. The bioavailability of polyphenols in milk is somewhat controversial (Leenen et al. 2000; Hoffman et al. 2001; Lorenz et al. 2007; Serafini et al. 2009; Gad and Abd El-salam 2010).

10.2.5 Red wine

Although red wine contains a variety of polyphenols (the quantity varies between each bottle), it is not a good source of antioxidants. Manufacturers of red wine recommend that the polyphenolic compound, resveratrol, has cardioprotective, antioxidant, and antiapoptotic effects (Penumathsa and Maulik 2009). Moderate consumption of red wine (containing grape-derived polyphenols) attenuates AD amyloid pathogenesis and cognitive deterioration and reduce the incidence of AD (Dai et al. 2006; Wang et al. 2006). The quantity of alcohol drunk is more important than the type of alcohol drunk.

10.2.6 Soybean milk drink

Soybean is rich in beneficial phytochemicals and is considered as a nutraceutical. Soybean milk drink has a high content of polyphenols such as isoflavones (Messina and Flickinger 2002; Omoni and Aluko 2005). Hydrolysis of soy protein improved antioxidant efficacy by releasing bound phenols from chelating agents such as phytic acid, and production of antioxidant peptide sequences (Wang et al. 2008). Consumers now prefer soy milk drink with various flavors because of its high protein and antioxidant content.

10.2.7 Cocoa drink

Incas Empire of South America considered cocoa drink as the drink of gods, an association that gave rise to the scientific name of the cocoa tree, *Theobroma cacao*, from the Greek words theo (god) and broma (drink). Aztec Emperor, Montezuma, considered it as a *divine drink*, which builds up resistance and fights fatigue (Hernán Cortés 1519). Cocoa flavanols and procyanidins exert strong antioxidant effects. Raw cocoa powder has high levels of polyphenols, which reduce risk factors for cardiovascular diseases, such as endothelial function and high blood pressure, and relief of angina pectoris, stimulation of the nervous system, facilitates digestion, and improves kidney and bowel function. Raw cocoa powder can be used for making a cocoa drink with the addition of milk. However, commercial cocoa powders sold for baking and drinking lose their antioxidants while processing, and thus are not good sources of antioxidants.

10.2.8 Herbal drinks

Water extract of different herbs (decoction) rich in antioxidant can be used to prepare ready-to-serve herbal drinks. People are using some herbs such as *Hibiscus sabdariffa*, *Aegle marmelos*, and *Chrysanthemum indicum* as panacea drinks for a long time. *Hibiscus sabdariffa* has potent antioxidant activity-related to the presence of anthocyanins (Ramirez-Rodrigues et al. 2012). Zobo drinks scavenge the ABTS, 2,2-diphenyl-1-picrylhydrazyl (DPPH),

and nitric oxide radicals (Oboh and Okhai 2012). It is prepared from vitamin C and flavonoid-rich roselle calyces of the *H. sabdariffa* (Wong et al. 2002). Zobo with ginger had a higher flavonoid and vitamin C content compared to the other *zobo* drinks. Some of the herbal juice/drinks rich in antioxidants include *Syzygium cumini* (Perera et al. 2014), Ginger (*Zingziber officinale*) (Shirin and Prakash 2010), *Crataegus pinnatifida* (Yang and Liu 2012). Handayani et al. (2015) suggested that the water attained from cooking pigmented rice have high potential to be developed into antioxidant drinks as the antioxidant compounds in the rice bran tend to seep out into the cooking water. The consumption of the hibiscus drink is widespread in Africa and Asia.

10.2.9 Ready-to-drink antioxidant beverages

L-arginine and vitamin C present in tender coconut water significantly reduce the free radical generation and lipid peroxidation (Loki and Rajamohan 2003). The antioxidant power of some ready-to-drink antioxidant beverages has been reported (Seeram et al. 2008). However, their recipes are confidential. Some of these beverages contain caffeine in coffee and tea and theobromine in chocolate drinks. Owing to their manufacture procedures, some soft drinks colored with caramel contain melanoidins that were reported to act as antioxidant (Borrelli et al. 2002; Wang et al. 2007). Caffeine is normally added in cola soft drinks (Chou and Bell 2007). Melanoidins are responsible for the strong antioxidant properties showed by coffee beverages (Borrelli et al. 2002; Anese and Nicoli 2003).

10.3 Impact of processing conditions on antioxidant drinks

Antioxidant content of any food component depends on the growing conditions, seasonal changes, and genetically different cultivars (Imeh and Khokhar 2002; Scalzo et al. 2005), storage conditions (Kalt et al. 1999; Mullen et al. 2002; Xianquan et al. 2005), and differences in manufacturing procedures and processing (Gil-Izquierdo et al. 2002; Ismail and Lee 2004; Hartmann et al. 2008). Since the antioxidants in fruits and vegetables are affected by extrinsic factors, processing and pasteurization conditions play an important role in the antioxidant capacity of juice (Hernández et al. 1999; Yildiz et al. 2009; Gastol et al. 2011). In particular, thermal treatments are the main cause of depletion of antioxidants in food (Liao and Seib 1988; Jonsson 1991). Hence, the antioxidant properties of foods should be maintained by processing under optimized conditions. On the other hand, heating can also induce the formation of further compounds with antioxidant properties during the development of the Maillard reaction (Eichner 1981; Ames 1988; O'Brien and Morrissey 1989; Rizzi 1994). For example, Nicoli et al. (1997) reported that the overall antioxidant properties were greatly increased with increasing degree of roasting.

Similarly, Anese et al. (1999) showed that heating increases the overall antioxidant potential of the tomato juice. However, short heat treatments supported an initial decrease in the original antioxidant potential of the product. Ultra high-pressure homogenization treated juices are capable of retaining the antioxidant capacity content than the thermally treated juices. Further, highest inlet and processing temperature could induce the degradation of antioxidant compounds.

10.4 Conclusion

The addition of antioxidants is an emerging trend for development of nutraceuticals and functional foods. Antioxidants are highly useful in the prevention of cancer, cardiovascular diseases, diabetes, and other chronic diseases related to oxidative stress. Whole foods such as fruit and vegetables and tea are a better choice than foods and drinks with added antioxidants as they may have additional health effects that are independent of their antioxidant component. The best antioxidant health drink contains 100% natural fruits or vegetables. Prevention is the definitive cure of several chronic diseases, including cancer, for which current therapy is expensive and difficult.

References

Alasalvar, C., J. M. Grigor, D. Zhang, P. C. Quantick, and F. Shahidi. 2001. Comparison of volatiles, phenolics, sugars, antioxidant vitamins, and sensory quality of different colored carrot varieties. *J Agr Food Chem* 49:1410–1416.

Ames, B. N., L. S. Gold, and W. C. Willet. 1995. Causes and prevention of cancer. *Proc Nat Acad Sci USA* 92:5258–5265.

Ames, J. M. 1988. The Maillard browning reaction—An update. *Chem Ind* 5:558–561.

Anderson, R. A. and M. M. Polansky. 2002. Tea enhances insulin activity. *J Agr Food Chem* 50:7182–7186.

Anese, M. and M. C. Nicoli. 2003. Antioxidant properties of ready-to-drink coffee brews. *J Agr Food Chem* 51:942–946.

Anese, M., L. Manzocco, M. C. Nicoli, and C. R. Lerici. 1999. Antioxidant properties of tomato juice as affected by heating. *J Sci Food Agr* 79:750–754.

Arburjai, T. and F. M. Natsheh. 2003. Plants used in cosmetics. *Phytother Res* 17:987–1000.

Atmaca, A., M. Kleerekoper, M. Bayraktar, and O. Kucuk. 2008. Soy isoflavones in the management of postmenopausal osteoporosis. *Menopause* 15:748–757.

Barry, I. 2008. Vitamin C: Friends or foe?. *Nat Rev Cancer* 8:830.

Basu, A. and K. Penugonda. 2009. Pomegranate juice: A heart-healthy fruit juice. *Nutr Rev* 67:49–56.

Basu, A., M. Rhone, and T. J. Lyons. 2010. Berries: Emerging impact on cardiovascular health. *Nutr Rev* 68 (3):168–177.

Bitsch, R., M. Netzel, T. Frank, G. Strass, and I. Bitsch. 2004. Bioavailability and biokinetics of anthocyanins from red grape juice and red wine. *J Biomed Biotechnol* 5:293–298.

Borrelli, R. C., A. Visconti, C. Mennella, M. Anese, and V. Fogliano. 2002. Chemical characterization and antioxidant properties of coffee melanoidins. *J Agr Food Chem* 50:6527–6533.

Butt, M. S. and M. T. Sultan. 2009. Green tea: Nature's defense against malignancies. *Crit Rev Food Sci Nutr* 49:463–473.

Carlsen, M. H., B. L. Halvorsen, K. Holte et al. 2010. The total antioxidant content of more than 3100 foods, beverages, spices, herbs, and supplements used worldwide. *Nutr J* 9:3.
Chen, D., K. G. Daniel, D. J. Kuhn, A. Kazi, M. Bhuiyan, L. Li, Z. Wang et al. 2004. Green tea and tea polyphenols in cancer prevention. *Front Biosci* 9:2618–2631.
Chen, Z. P., J. B. Schell, C. T. Ho, and K. Y. Chen. 1998. Green tea epigallocatechin gallate shows a pronounced growth inhibitory effect on cancerous cells but not on their normal counterparts. *Cancer Lett* 129:173–179.
Chou, K. H. and L. N. Bell. 2007. Caffeine content of prepackaged national-brand and private-label carbonated beverages. *J Food Sci* 72:C337–C342.
Corti, R., A. J. Flammer, N. K. Hollenberg, and T. F. Luscher. 2009. Cocoa and cardiovascular health. *Circulation* 119:1433–1441.
Côté, J., S. Caillet, G. Doyon, J. F. Sylvain, and M. Lacroix. 2010. Bioactive compounds in cranberries and their biological properties. *Crit Rev Food Sci Nutr* 50:666–679.
Dai, Q., A. R. Borenstein, Y. Wu, J. C. Jackson, and E. B. Larson. 2006. Fruit and vegetable juices and Alzheimer's disease: The Kame project. *Am J Med* 119 (9):751–759.
Del Castillo, M. D., J. M. Ames, and M. H. Gordon. 2002. Effect of roasting on the antioxidant activity of coffee brews. *J Agr Food Chem* 50:3698–3703.
Del Río, J. A., M. D. Fuster, P. Gómez, I. Porras, A. García-Lidón, and A. Ortuño. 2004. Citrus limon: A source of flavonoids of pharmaceutical interest. *Food Chem* 84 (3):457–461.
Densupsoontorn, N., P. Jirapinyo, N. Thamonsiri, R. Wongarn, P. Phosuya, A. Tritiprat et al. 2002. Comparison of the nutrient content of fresh fruit juices vs commercial fruit juices. *J Med Assoc Thai* 85(2):S732–S738.
Devore, E. E., F. Grodstein, F. J. van Rooij et al. 2010. Dietary antioxidant and long term risk of dementia. *Arch Neurol* 67:819–825.
Eichner, K. 1981. Antioxidative effect of Maillard reaction intermediates. *Progr Food Nutr Sci* 5:441–451.
Farah, A. and C. M. Donangelo. 2006. Phenolic compounds in coffee. *Braz J Plant Physiol* 18:23–36.
Franceschi, S., E. Bidoli, C. LaVeccia, R. Talamini, B. D'Avanzo, and E. Negri. 1994. Tomatoes and risk of digestive-tract cancers. *Int J Cancer* 59:181–184.
Fuster, M. D., A. E. Mitchell, H. Ochi, and T. Shibamoto. 2000. Antioxidant activities of the heterocyclic compounds formed in brewed coffee. *J Agr Food Chem* 48:5600–5603.
Gad, A. S. and M. H. Abd El-salam. 2010. The antioxidant properties of skim milk supplemented with rosemary and green tea extracts in response to pasteurisation, homogenisation and the addition of salts. *Int J Dairy Technol* 63:349–355.
Gastol, M., I. Domagala-Swiatkiewicz, and M. Krosniak. 2011. Organic verses conventional—A comparative study on quality and nutritional value of fruit and vegetable juices. *Biol Agr Hort* 27:310–319.
Gil, M. I., F. A. Tomas-Barbara, B. Hess-Pierce, D. M. Holcroft, and A. A. Kader. 2000. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J Agr Food Chem* 48:4581–4589.
Gil-Izquierdo, A., M. I. Gil, and F. Ferreres. 2002. Effect of processing techniques at industrial scale on orange juice antioxidant and beneficial health compounds. *J Agr Food Chem* 50:5107–5114.
Giovamrucci, E., A. Ascherio, E. B. Rimm, M. J. Stampfer, G. A. Colditz, and W. C. Willett. 1995. Intake of carotenoids and ratinol in relation to risk of prostate cancer. *J Nat Cancer Inst* 87:1767–1776.
Gomes, A., J. R. Vedasiromoni, M. Das, R. M. Sharma, and D. K. Ganguly. 1995. Antihyperglycemic effect of black tea (*Camellia sinensis*) in rat. *J Ethnopharmacol* 45:223–226.
Gross, J. 1987. *Pigments in Fruits*. London: Academic Press.
Halliwell, B. 1994. Free radicals, antioxidants and human disease: Curiosity, cause or consequence? *Lancet* 344:721–724.
Halliwell, B. 2001. Role of free radicals in the neurodegenerative diseases: Therapeutic implications for antioxidant treatment. *Drugs Aging* 18:685–716.

Handayani, A. P., R. Karim, and K. Muhammad. 2015. Optimization of processing conditions for aqueous pigmented rice xtracts as bases for antioxidant drinks. *J Rice Res* 3:135.

Hartmann, A., C. D. Patz, W. Andlauer, H. Dietrich, and M. Ludwig. 2008. Influence of processing on quality parameters of strawberries. *J Agr Food Chem* 56:9484–9489.

Heo, H. J., S. J. Choi, S. G. Choi, D. H. Shin, J. M. Lee, and C. Y. Lee. 2008. Effects of banana, orange, and apple on oxidative stress-induced neurotoxicity in PC12 cells. *J Food Sci* 73 (2):H28–H32.

Hernández, F., P. Melgarejo, F. A. Tomás-Barberán, and F. Artés. 1999. Evolution of juice anthocyanins during ripening of new selected pomegranate (*Punica granatum*) clones. *Europ Food Res Technol* 210:39–42.

Hodgson, J. M. 2000. Tea and cardiovascular disease: A review. *Proc Nutr Soc* 24:241–249.

Hoffman, P. C. H., K. H. VanHet Hof, L. B. M. Tijburg, and M. B. Atan. 2001. Addition of milk does not affect the absorption of flavonoids from tea in man. *Free Rad Res* 34:297–300.

Hoolbrook, J. J. and C. L. Hicks. 1978. Variation of superoxide dismutase in bovine milk. *J Dairy Sci* 61:1072–1077.

Hou, Z., S. Sang, H. You, M. J. Lee, J. Hong, K. V. Chin, and C. S. Yang. 2005. Mechanism of action of (−)-epigallocatechin-3-gallate: Auto-oxidation-dependent inactivation of epidermal growth factor receptor and direct effects on growth inhibition in human esophageal cancer KYSE 150 cells. *Cancer Res* 65:8049–8056.

Hu, P., D. B. Reuben, E. M. Crimmins, T. B. Harris, M. H. Huang, and T. E. Seeman. 2004. The effects of plasma beta-carotene concentration and burden of inflammation on all-cause mortality risk in high-functioning older persons: MacArthur studies of successful aging. *J Gerontol Ser A-Biol Sci Med Sci* 59:849–854.

Hurrell, R. F., M. Reddy, and J. D. Cook. 1999. Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages. *Br J Nutr* 81:289–295.

Illy, E. 2002. The complexity of coffee. *Sci Am* 286:86–91.

Imeh, U. and S. Khokhar. 2002. Distribution of conjugated and free phenols in fruits: Antioxidant activity and cultivar variations. *J Agr Food Chem* 50:6301–6306.

Ismail, A. and W. Y. Lee. 2004. Influence of cooking practice on antioxidant properties and phenolic content of selected vegetables. *Asia Pac J Clin Nutr* 13:S162.

Iso, H., C. Date, K. Wakai, M. Fukui, and A. Tamakoshi. 2006. The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes among Japanese adults. *Ann Int Med* 144:554–562.

Ito, O. and R. Akuzawa. 1983. Purification, crystallisation and properties of bovine milk catalase. *J Dairy Sci* 66:967–973.

Jonsson, I. 1991. Thermal degradation of carotenoids and influence of their physiological functions. In: Friedman, M. (Ed.). *Nutrition and Toxicological Consequences of Food Processing*. New York: Plenum Press, pp. 75–82.

Kalt, W., C. F. Forney, A. Martin, and R. L. Prior. 1999. Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. *J Agr Food Chem* 47:4638–4644.

Khan, N., F. Afaq, M. Saleem, N. Ahmad, and H. Mukhtar. 2006. Targeting multiple signaling pathways by green tea polyphenol (–)-Epigallocatechin-3-Gallate. *Cancer Res* 66: 2500–2505.

Kiefer, I., P. Prock, C. Lawrence, J. Wise, W. Bieger, P. Bayer et al. 2004. Supplementation with mixed fruit and vegetable juice concentrates increased serum antioxidants and folate in healthy adults. *J Am Coll Nutr* 23 (3):205–211.

Klaus, S., S. Pultz, C. Thone-Reineke, and S. Wolfram. 2005. Epigallocatechin gallate attenuates diet-induced obesity in mice by decreasing energy absorption and increasing fat oxidation. *Int J Obesity* 29 (6):615–623.

Kobayashi, Y., M. Suzuki, H. Satsu, S. Arai, Y. Hara, K. Suzuki, Y. Miyamoto, and M. Shimizu. 2000. Green tea polyphenols inhibit the sodium-dependent glucose transporter of intestinal epithelial cells by a competitive mechanism. *J Agr Food Chem* 48:5618–5623.

Koo, M. W. L. and C. H. Cho. 2004. Pharmacological effects of green tea on the gastrointestinal system. *Europ J Pharmacol* 500:177–185.

Leenen, R., A. J. Roodenburg, L. B. Tijburg, and S. A. Wiseman. 2000. A single dose of tea with or without milk increases plasma antioxidants in humans. *Europ J Clin Nutr* 54:87–92.

Leventhal, H., K. Kelly, and E. A. Leventhal. 1999. Population risk, actual risk, perceived risk and cancer control: A discussion. *J Nat Cancer Inst Monogr* 25:81–85.

Liao, M. L. and P. A. Seib. 1988. Chemistry of L-ascorbic acid related to foods. *Food Chem* 30:289–312.

Lindmark-Mansson, H. and B. Akesson. 2000. Antioxidative factors in milk. *Br J Nutr* 84:S103–S110.

Liu, L., S. P. Zhao, M. Gao, Q. C. Z. Zhou, Y. L. Li, and B. Xia. 2002. Vitamin C preserves endothelial function in patients with coronary heart disease after a high-fat meal. *Clin Cardiol* 25:219–224.

Loki, A. L. and T. Rajamohan. 2003. Hepatoprotective and antioxidant effect of tender coconut water on CCl4 induced liver injury in rats. *Indian J Biochem Biophys* 40:354–357.

Lorenz, M., N. Jochmann, A. Von Krosigk, P. Matris, G. Baumann, K. Stangl, and V. Stangl. 2007. Addition of milk prevents vascular protective effect of tea. *Europ Heart J* 28:219–233.

Maia, L. and A. de Mendonca. 2002. Does caffeine intake protect from Alzheimer's disease? *Europ J Neurol* 9:377–382.

Malik, A., F. Afaq, S. Sarfaraz, V. Adhami, D. Syed, and H. Mukhtar. 2005. Pomegranate fruit juice for chemoprevention and chemotherapy of prostate cancer. *Proc Nat Acad Sci USA* 102:14813–14818.

Manach, C., A. Mazur, and A. Scalbert. 2005a. Polyphenols and prevention of cardiovascular diseases. *Curr Opin Lipidol* 16:77–84.

Manach, C., A. Scalbert, C. Morand, C. Remesy, and L. Jimenez. 2004. Polyphenols: Food sources and bioavailability. *Am J Clin Nutr* 79:727–747.

Manach, C., G. Williamson, C. Morand, A. Scalbert, and C. Remesy. 2005b. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr* 81:230S–242S.

McKay, D. L. and J. B. Blumberg. 2002. The role of tea in human health: An update. *J Am Coll Nutr* 21:1–13.

McKay, D. L. and J. B. Blumberg. 2007. Roles of epigallocatechin gallate in cardiovascular disease and obesity: An introduction. *J Am Coll Nutr* 26:362s–365s.

Menshchikova, E. B., V. Z. Lankin, and N. K. Zenkov. 2006. *Oxidative Stress: Pro-Oxidants and Antioxidants*. Moscow, Russia: Slovo.

Messina, M. and B. Flickinger. 2002. Hypothesized anticancer effects of soy: Evidence points to isoflavones as the primary anticarcinogens. *Pharm Biol* 40:S6–S23.

Miller, N. J., J. Sampson, L. P. Candeias, P. M. Bramley, and C. A. Rice-Evans. 1996. Antioxidant activities of carotenes and xanthophylls. *FEBS Lett* 384:240–246.

Miyake, Y., W. Fukushima, K. Tanaka et al. 2010. Dietary intake of antioxidant vitamins and risk of Parkinson's disease: A case–control study in Japan. *Europ J Neurol* 18 (1):106–113.

Morillas-Ruiz, J. M., J. M. Rubio-Perez, M. D. Albaladejo, P. Zafrilla, S. Parra, and M. L. Vidal-Guevara. 2010. Effect of an antioxidant drink on homocysteine levels in Alzheimer's patients. *J Neurol Sci* 299:175–178.

Mukoyama, A., H. Ushijima, S. Nishimura, H. Koike, M. Toda, Y. Hara, and T. Shimamura. 1991. Inhibition of rotavirus and enterovirus infections by tea extracts. *Jpn J Med Sci & Biol* 44:181–186.

Mullen, W., A. J. Stewart, M. E. Lean, P. Gardner, G. G. Duthie, and A. Crozier. 2002. Effect of freezing and storage on the phenolics, ellagitannins, flavonoids, and antioxidant capacity of red raspberries. *J Agr Food Chem* 50:5197–5201.

Mullen, W., B. Nemzer, A. Stalmach, S. Ali, and E. Combet. 2013. Polyphenolic and hydroxycinnamate contents of whole coffee fruits from China, India, and Mexico. *J Agri Food Chem* 61: 5298–5309.

Muraki, S., S. Yamamoto, H. Ishibashi, T. Horiuchi, T. Hosoi, T. Suzuki, H. Orimo, and K. Nakamura. 2003. Green tea drinking is associated with increased bone mineral density. *J Bone Miner Res* 18:S241.

Nawrot, P., S. Jordan, J. Eastwood, J. Rotstein, A. Hugenholtz, and M. Feeley. 2003. Effects of caffeine on human health. *Food Addit Contam* 20:1–30.

Nicoli, M. C., M. Anese, L. Manzocco, and C. R. Lerici. 1997. Antioxidant properties of coffee brews in relation to the roasting degree. *Lebensmittel-Wissenschaft und -Technologie* 30:292–297.

O'Brien, G. P. and P. A. Morrissey. 1989. Nutritional and toxicological aspects of the Maillard reaction in food. *Crit Rev Food Sci Nutr* 28:211–248.

Oboh, H. A. and E. O. Okhai. 2012. Antioxidant and free radical scavenging abilities of some indigenous Nigerian drinks. *Nig J Basic Appl Sci* 20 (1):21–26.

Oka, Y., S. Iwai, H. Amano et al. 2012. Tea polyphenols inhibit rat osteoclast formation and differentiation. *J Pharmacol Sci* 118:55–64.

Olthof, M. R., P. C. Hollman, and M. B. Katan. 2001. Chlorogenic acid and caffeic acid are absorbed in humans. *J Nutr* 131:66–71.

Omoni, A. O. and R. E. Aluko. 2005. Soybean foods and their benefits: Potential mechanisms of action. *Nutr Rev* 63:272–283.

Pan, T., J. Jankovic, and W. Le. 2003. Potential therapeutic properties of green tea polyphenols in Parkinson's disease. *Drugs Aging* 20:711–721.

Passoni, C. R. and C. A. Coelho. 2008. Ascorbic acid supplementation has a cytoprotective effect on secondary biliary cirrhosis: Experimental study in young rats. *Jornal de Pediatria (Rio. J.)* 84:522–528.

Penumathsa, S. V. and N. Maulik. 2009. Resveratrol: A promising agent in promoting cardioprotection against coronary heart disease. *Can J Physiol Pharmacol* 87:275–86.

Perera, P. R. D., S. Ekanayake, and K. K. D. S. Ranaweera. 2014. Antiglycation and antioxidant activities of a ready to serve herbal drink of *Syzygium cumini* bark extract. *Med Aromatic Plants* 3:148.

Pool-Zobel, B. L. B. A., U. M. Liegibel, S. Treptow-van Lishaut, and G. Rechkemmer. 1998. Mechanisms by which vegetable consumption reduces genetic damage in humans. *Cancer Epidemiol, Biomarkers Prev* 7:891–899.

Potischman, N., C. E. McCulloch, T. Byers, T. Nemoto, N. Stubbe, R. Milch, R. Parker et al. 1990. Breast cancer and dietary and plasma concentrations of carotenoids and vitamin A. *Am J Clin Nutr* 52:909–915.

Potter, A. S., S. Foroudi, A. Stamatikos, B. S. Patil, and F. Deyhim. 2011. Drinking carrot juice increases total antioxidant status and decreases lipid peroxidation in adults. *Nutr J* 10:96.

Prior, R. L., H. Hoang, L. Gu, X. Wu, M. Bacchiocca, L. Howard, M. Hampsch-Woodill, D. Huang, and R. Jacob. 2003. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL)) of plasma and other biological and food samples. *J Agr Food Chem* 51:3273–3279

Pryor, W. L. 2000. Vitamin E and heart disease: Basic science to clinical intervention trials. *Free Radic Biol Med* 28:141–61.

Rajendran, P., N. Nandakumar, T. Rengarajan, R. Palaniswami, E. N. Gnanadhas, U. Lakshminarasaiah, J. Gopas, and I. Nishigaki. 2014. Antioxidants and human diseases. *Clinica Chimica Acta* 436:332–347.

Ramirez-Rodrigues, M. M., M. L. Plaza, A. Azeredo, M. O. Balaban, and M. R. Marshall. 2012. Phytochemical, sensory attributes and aroma stability of dense phase carbon dioxide processed *Hibiscus sabdariffa* beverage during storage. *Food Chem* 134:1425–1431.

Riso, P., D. Martini, P. Moller, S. Loft, G. Bonacina, M. Moro, and M. Porrini. 2010. DNA damage and repair activity after broccoli intake in young healthy smokers. *Mutagenesis* 25(6): 595–602.

Rizzi, G. P. 1994. The maillard reaction in foods. In: Labuza, T. P., Reineccius, G. A., Monnier, V. M., O'Brien, J., and Baynes, J. W. (Eds.). *Maillard Reaction in Chemistry*. Cambridge, UK: Food and Health, Royal Society of Chemistry Publisher, pp. 11–19.

Russo, P., I. Tedesco, M. Russo, G. L. Russo, A. Venezia, and C. Cicala. 2001. Effects of de-alcoholated red wine and its phenolic fractions on platelet aggregation. *Nutr, Metabol Cardiovasc Dis* 11:25–29.

Scalzo, J., A. Politi, N. Pellegrini, B. Mezzetti, and M. Battino. 2005. Plant genotype affects total antioxidant capacity and phenolic contents in fruit. *Nutrition* 21:207–213.

Schachinger, V., M. B. Britten, and A. M. Zeiher. 2000. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* 101:1899–1906.

Seeram, N. P., M. Aviram, Y. Zhang, S. M. Henning, L. Feng, M. Dreher et al. 2008. Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. *J Agr Food Chem* 56:1415–1422.

Serafini, M., M. F. Testa, D. Villano, M. Pecorari, K. V. Wieren, E. Azzini, A. Brambilla, and G. Maiani. 2009. Antioxidant activity of blueberry fruit is impaired by association with milk. *Free Radic Biol Med* 46:769–774.

Shen, G., C. Xu, R. Hu, M. R. Jain, S. Nair, W. Lin, C. S. Yang, J. Y. Chan, and A. N. Kong. 2005. Comparison of (-)-epigallocatechin-3-gallate elicited liver and small intestine gene expression profiles between C57BL/6J mice and C57BL/6J/Nrf2 (-/-) mice. *Pharm Res* 22:1805–1820.

Shim, J. S., M. H. Kang, Y. H. Kim, J. K. Roh, C. Roberts, and I. P. Lee. 1995. Chemopreventive effect of green tea (*Camellia sinensis*) among cigarette smokers. *Cancer Epidemiol, Biomarkers Prev* 4:387–391.

Shirin, A. P. R. and J. Prakash. 2010. Chemical composition and antioxidant properties of ginger root (*Zingiber officinale*). *J Med Plants Res* 4 (24):2674–2679.

Steinhart, H., A. Luger, and J. Piost. 2001. Antioxidative effect of coffee melanoidins. In: *Proceedings of the 19th International Scientific Collogue on Coffee*, Trieste, Italy, pp. 14–18.

Stella, S. P., A. C. Ferrarezi, K. O. dos Santos, and M. Monteiro 2011. Antioxidant activity of commercial ready-to-drink orange juice and nectar. *J Food Sci* 76 (3):C392–C397.

Tachibana, H. 2009. Molecular basis for cancer chemoprevention by green tea polyphenol EGCG. In: Yoshikawa, T. (Ed.). *Food Factors for Health Promotion. Forum Nutrition*. Basel, Switzerland: Karger, Vol. 61, pp. 156–169.

Takabayashi, F., N. Harada, M. Yamada, B. Murohisa, and I. Oguni. 2004. Inhibitory effect of green tea catechins in combination with sucralfate on *Helicobacter pylori* infection in Mongolian gerbils. *J Gastroenterol* 39:61–63.

Thankachan, P., T. Walczyk, S. Muthayya, A. V. Kurpad, and R. F. Hurrell. 2008. Iron absorption in young Indian women: The interaction of iron status with the influence of tea and ascorbic acid. *Am J Clin Nutr* 87:881–886.

Toda, M., S. Okubo, R. Ohnishi, and T. Shimamura. 1989. Antibacterial and bactericidal activities of zapanese green tea. *Nippon Saikingaku Zasshi* 44:669–672.

Törrönen, R. L. M., S. Häkkinen, O. Hänninen, and H. Mykkänen. 1996. Plasma β-carotene response to supplementation with raw carrots, carrot juice or purified β-carotene in healthy non-smoking women. *Nutr Res* 16:565–575.

Tsuneki, H., M. Ishizuka, M. Terasawa, J. B. Wu, T. Sasaoka, and I. Kimura. 2004. Effect of green tea on blood glucose levels and serum proteomic patterns in diabetic (db/db) mice and on glucose metabolism in healthy humans. *BMC Pharmacol* 4:18–21.

Visioli, F., S. Grande, P. Bogani, and C. Galli. 2004. The role of antioxidants in the mediterranean diets: Focus on cancer. *Europ J Cancer Prev* 13:337–343.

Waltner-Law, M. E., X. L. Wang, B. K. Law, R. K. Hall, M. Nawano, and D. K. Granner. 2002. Epigallocatecin gallate, a constituent of green tea, represses hepatic glucose production. *J Biol Chem* 277:34933–34940.

Wang, J., Z. Zhao, L. Ho, I. Seror, N. Humala, S. Percival et al. 2006. Moderate consumption of Cabernet Sauvignon attenuates β-amyloid neuropathology in a mouse model of Alzheimer's disease. *FASEB J* 20:2313–2320.

Wang, L., F. Zhu, J. Zhi, X. D. Chen, L. Zou, and M. Saito 2008. *In vitro* and *in vivo* studies on the antioxidant activities of the aqueous extracts of Douchi (a traditional Chinese salt fermented soybean. *Food Chem* 107 (4):1421–1428.

Wang, Y., A. M. Hodge, A. E. Wluka et al. 2007. Effect of antioxidante on knee cartilage and bone in healthy, middle-aged subjects: A cross-sectional study. *Arthritis Res Ther* 9:1–9.

Weber, J. M., A. Ruzindana-Umunyana, L. Imbeault, and S. Sircar. 2003. Inhibition of adenovirus infection and adenain by green tea catechins. *Antiviral Res* 58:167–173.

Weinreb, O., S. Mandel, T. Amit, and M. B. H. Youdim. 2004. Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases. *J Nutr Biochem* 15:506–516.

Weinstein, S. J., M. E. Wright, K. A. Lawson et al. 2007. Serum and dietary vitamin E in relation to prostate cancer risk. *Cancer Epidemiol, Biomarkers Prev* 16:1253–1258.

Williamson, G. and C. Manach. 2005. Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. *Am J Clin Nutr* 81:243S–255S.

Wintergerst, E. S., S. Maggini, and D. H. Homig. 2006. Immune-enhancing role of vitamin C and zinc and effect on clinical conditions. *Ann Nutr Metabol* 50:85–94.

Wolfram, S., D. Raederstorff, M. Preller, Y. Wang, S. R. Teixeira, C. Riegger, and P. Weber. 2006. Epigallocatechin gallate supplementation alleviates diabetes in rodents. *J Nutr* 136:3512–3518.

Wong, P., S. Yusof, H. M. Ghazah, and Y. E. Cheman. 2002. Physico-chemical characteristics of roselle (*Hibiscus sabdariffa* L.). *Nutr Food Sci* 32:68–73.

Woo, A., J. H. Kim, Y. J. Jeong et al. 2010. Vitamin C acts indirectly to modulate isotype switching in mouse B cells. *Anat Cell Biol* 43:25–35.

Wootton-Beard, P. C. and L. Ryan. 2011. A beetroot juice shot is a significant and convenient source of bioaccessible antioxidants. *J Funct Foods* 3 (4):329–334.

Wu, L. Y., C. C. Juan, L. T. Ho, Y. P. Hsu, and L. S. Hwang. 2004. Effect of green tea supplementation on insulin sensitivity in Sprague-Dawley rats. *J Agr Food Chem* 52:643–648.

Xianquan, S., J. Shi, Y. Kakuda, and J. Yueming. 2005. Stability of lycopene during food processing and storage. *J Med Food* 8:413–422.

Yam, T. S., S. Shah, and J. M. T. Hamilton-Miller. 1997. Microbiological activity of whole and fractionated crude extracts of tea (*Camellia sinensis*), and of tea components. *FEMS Microbiol Lett* 152:169–174.

Yanagimoto, K., K. G. Lee, H. Ochi, and T. Shibamoto. 2002. Antioxidative activity of heterocyclic compounds found in coffee volatiles produced by Maillard reaction. *J Agr Food Chem* 50:5480–5484.

Yang, B. and P. Liu. 2012. Composition and health effects of phenolic compounds in hawthorn (*Crataegus* spp.) of different origins. *J Sci Food Agr* 92 (8):1578–1590.

Yang, C. S., J. M. Landau, M. T. Huang, and H. L. Newmark. 2001. Inhibition of carcinogenesis by dietary polyphenolic compounds. *Ann Rev Nutr* 21:381–406.

Yang, C. S., S. Sang, J. D. Lambert, Z. Hou, J. Ju, and G. Lu. 2006. Possible mechanisms of the cancer-preventive activities of green tea. *Mol Nutr Food Res* 50:170–175.

Yee, Y. K., M. W. L. Koo, and M. L. Szeto. 2002. Chinese tea consumption and lower risk of *Helicobacter* infection. *J Gastroenterol Hepatol* 17:552–555.
Yildiz, H., H. Bozkurt, and F. Icier. 2009. Ohmic and conventional heating of pomegranate juice: Effects on rheology, color, and total phenolics. *Food Sci Technol Int* 15:503–512.
Zunino, S. J., D. H. Storms, and C. B. Stephensen. 2007. Diets rich in polyphenols and vitamin A inhibit the development of Type I autoimmune diabetes in nonobese diabetic mice. *J Nutr* 137:1216–1221.

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

11

The Prevention and Treatment of Coronary Heart Disease and Congestive Heart Failure with Antioxidants and Nutritional Supplements

Mark C. Houston

Contents

Summary

We have reached a limit in our ability to reduce the incidence of coronary heart disease (CHD), congestive heart failure (CHF), and cardiovascular disease (CVD) utilizing the traditional evaluation, prevention, and treatment strategies for the top five cardiovascular risk factors—hypertension, diabetes mellitus,

dyslipidemia, obesity, and smoking. Statistics show that approximately 50% of patients continue to have CHD or myocardial infarction (MI) despite *normal* levels of these five risk factors as traditionally defined. A more logical and in-depth understanding of these top five risk factors is necessary. Advanced testing should include 24-hour ambulatory blood pressure monitoring, advanced lipid profiles, dysglycemic parameters, visceral obesity with effects of adipokines, and evaluation of the three finite vascular endothelial responses of inflammation, oxidative stress, and immune vascular dysfunction. Congestive heart failure is most commonly due to CHD and presents with both systolic and diastolic heart failure. Understanding translational cardiovascular medicine allows appropriate correlation of the CHD risk factors to the presence or absence of vascular injury and disease utilizing noninvasive vascular testing. This provides for early identification, prevention, and treatment of CHD, CHF, and CVD.

11.1 Introduction

Cardiovascular medicine needs a complete functional and metabolic medicine reevaluation related to diagnosis, prevention, and integrative treatments. We have reached a limit in our ability to reduce CVD and CHD (Yusuf et al. 2004). The cardiovascular system is literarily *on fire*. Our present treatments are not always effective in reducing this vascular inflammation. CVD, CHD, and CHF remain the number 1 cause of morbidity and mortality in the United States (Houston 2012a). Statistics show that we spend approximately $80 billion a year treating CVD alone (Houston 2012a) and over 2200 U.S. citizens die from stroke or MI each day (Houston 2010, 2012a; O'Donnell and Nabel 2011; ACCORD Study Group, Gerstein et al. 2011). CHD includes angina, MI, ischemic heart disease, and ischemic cardiomyopathy with both systolic (low ejection fraction) and diastolic congestive heart failure (normal ejection fraction with stiff and noncompliant left ventricle). The most common cause of CHF in the United States is ischemic heart disease.

The traditional evaluation, prevention, and treatment strategies for the top five cardiovascular risk factors—hypertension, diabetes mellitus, dyslipidemia, obesity, and smoking—have resulted in what is now referred to as a *CHD gap* ACCORD Study Group, (Gerstein et al. 2011). Approximately 50% of patients continue to have CHD or MI despite having *normal* levels of these risk factors as currently defined in the medical literature (ACCORD Study Group, Gerstein 2011; Houston 2012a). We maintain a cholesterol-centric approach to the management of CHD but do not address the basic etiologies of CHD such as inflammation, oxidative stress, and immune vascular dysfunction. However, there are important details within each of these top five risk factors that are not being measured by physicians and are thus ignored in the prevention and treatment of CHD (Houston 2012a). In fact, there are at least 395 other risk factors that physicians either do not know about, ignore, or do not use appropriate techniques to identify and treat them. Thus, it is imperative that we utilize other methods to prevent and treat CVD (Houston 2012a).

11.2 Revolutionizing the treatment of cardiovascular disease

The blood vessel has three finite responses to an infinite number of insults (Houston 2012a). Those responses are inflammation, oxidative stress, and vascular immune dysfunction. Tracking backward from those three finite responses brings us to the genesis of CVD with the goal of starting effective treatments to resolve the downstream abnormalities.

Cell membrane physiology and cell membrane dysfunction are keys to this treatment strategy. This membrane barrier separates the outside and the inside of every cell. This includes the endothelium, enterocyte, the blood–brain barrier, or any other membrane. Membrane activation determines all of the signaling mechanisms that occur from the external to the internal milieu and the downstream internal cell signal pathways (Houston 2012a).

Any cell membrane insult such as high blood pressure, LDL-cholesterol, glucose, microbes, toxins, heavy metals, or homocysteine results in a reaction diffusion wave throughout the cell membrane that disrupts the signaling mechanisms and induces membrane damage and dysfunction (El Khatib et al. 2009; Youssef-Elabd et al. 2012). One small insult becomes a heightened response (metabolic memory) to create further cell damage (El Khatib et al. 2009; Youssef-Elabd et al. 2012). The blood vessel is really an innocent bystander in a correct but often a chronic and dysregulated vascular response to these infinite insults.

In the acute setting, any vascular insult results in a correct defensive response by the endothelium. The vascular immune dysfunction, oxidative stress, or inflammatory responses are usually short-lived, appropriate, and regulated (Houston 2012a). However, chronic insults result in a chronic exaggerated and dysregulated vascular dysfunction with preclinical then clinical CVD due to maladaptation of various systems such as the renin–angiotensin–aldosterone (RAAS) system, sympathetic nervous system (SNS), and others (Houston 2012a).

Most diseases are arbitrarily defined with a specific abnormal level of some test or measurement. Hypertension is defined as greater than 140/90 mm Hg, dyslipidemia as LDL-cholesterol over 100 mg/dL, and glucose intolerance as a fasting glucose over 99 mg/dL (Houston 2012a). However, it is very clear that there exists a continuum of risk starting at lower levels of BP, LDL-cholesterol, and glucose, as well as for most other CHD risk factors (Houston 2012a). For example, we know that the blood pressure risk for CVD actually starts at 110/70 mm Hg, and that LDL-cholesterol reduces nitric oxide in the endothelium at 60 mg/dL and fasting glucose at 75 mg/dL is the level at which CHD risk begins (Houston 2012a). There is a progressive continuum of risk with all of the CVD risk factors and mediators that effect the blood vessel, leading initially to functional abnormalities (endothelial dysfunction), then to structural abnormalities of the vascular and cardiac muscle (stiffness and hypertrophy), and to preclinical and clinical CVD.

Finally, it is important to understand the concept of *translational vascular medicine*. Do the risk factors that are measured actually translate into a vascular illness? Does the absence of those risk factors actually define vascular health? Functional and structural markers of vascular and endothelial dysfunction are not always used to predict the risk to identify the vascular effects of CHD risk factors or the presence of vascular disease. Risk factor scoring systems such as Framingham, American Heart Association, American College of Cardiology, or Consortium of Southeastern Hypertension Centers (COSEHC) are used to predict risk. We assume that if a patient has risk factors, he or she also has vascular disease; but if he or she doesn't, he or she may have vascular health. It is important to measure sensitive indicators of endothelial dysfunction and vascular structural disease that are induced by the insults. Early detection with aggressive treatment will reduce CVD.

11.2.1 The endothelium, endothelial function, and endothelial dysfunction

The endothelium is a very thin monolayer of vascular cells that forms an interface between the circulating luminal blood and the vascular smooth muscle (**Figure 11.1**) (Houston 2009, 2010, 2012a).

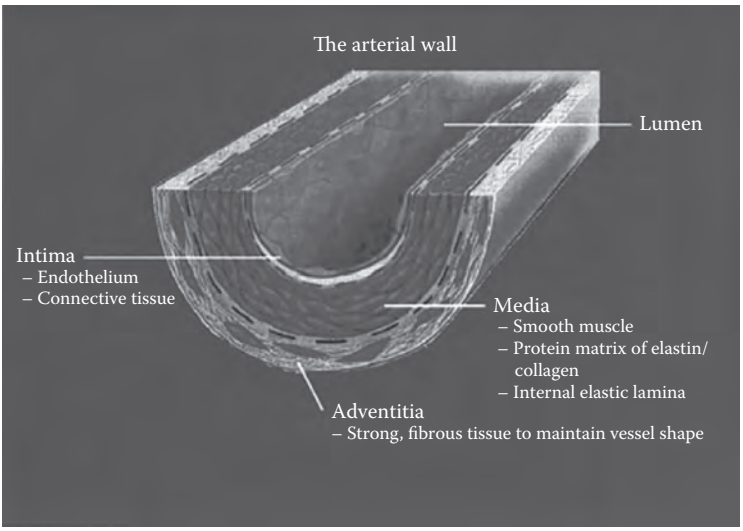


Figure 11.1 *The arterial wall includes the endothelium with connective tissue (intima), the media or vascular smooth muscle, and the adventitia with supporting fibrous tissue. (Modified from Ross, R.,* N. Engl. J. Med., *340, 115–126, 1999; Mulvany, M.J. et al.,* Physiol. Rev., *70, 921–961, 1990; Mark, C., Vascular Biology in Clinical Practice, Houston, TX, 2000.)*

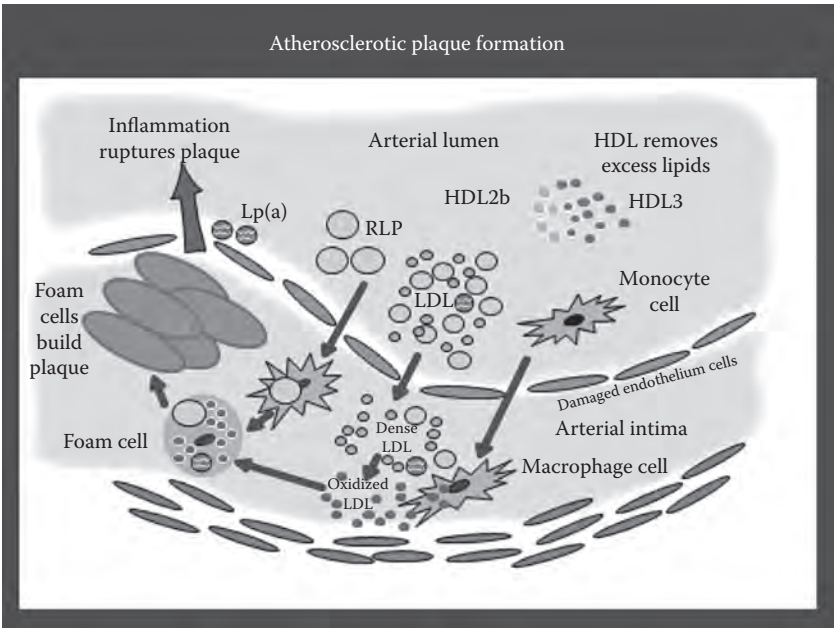


Figure 11.2 *A simplified illustration of atherosclerotic plaque formation.*

When the endothelium is working correctly (endothelial function), all the blood elements and the vascular smooth muscle have normal function and structure. However, when endothelial dysfunction occurs, the results are inflammation, oxidative stress, immune dysfunction, abnormal growth, vasoconstriction, increased permeability, thrombosis, and ultimately CVD (Houston 2009, 2010, 2012a, 2014a).

Figure 11.2 illustrates LDL-cholesterol's role in atherosclerotic plaque formation (Houston 2014b). Once inside the vessel wall in the subendothelium, LDL-cholesterol becomes modified and susceptible to oxidation with modification by free radicals, as well as by glycation and acetylation (Houston 2014b). Oxidized, glycated, and acetylated LDLs are toxic to the vessel wall. Higher LDL-particle number (LDL-P) and small dense LDL increase the risk for LDL modification, which leads to CHD. The modified LDL is consumed by scavenger receptors (SR-A and CD-36) on macrophages to form foam cells. Foam cells are not able to process any of the forms of modified LDL and continue to accumulate modified-LDL, forming fatty streaks and then a plaque, which may rupture to induce acute coronary thrombosis. This is the progression that must be interrupted. There are over 38 different steps in this process that can be treated to disrupt the dyslipidemia-induced vascular disease (Houston 2014b).

Vascular disease is a balance of vascular injury (angiotensin II and endothelin) and vascular protection with nitric oxide coupled with vascular repair

that includes endothelial progenitor cells (EPCs) produced in the bone marrow (Houston 2010, 2012a). The infinite insults result in preconditioned and heightened *metabolic memory* responses that trigger the three finite downstream responses that have a bidirectional communication involving endothelial dysfunction, vascular smooth muscle dysfunction, and cardiac dysfunction (Houston 2010; Youssef-Elabd et al. 2012). Once endothelial dysfunction has developed, a smaller insult occurring at a later time can result in a heightened response that induces more vascular damage (Houston 2010; Youssef-Elabd et al. 2012). This concept of metabolic memory was demonstrated by Youssef-Elabd et al. who found that short-term exposure of adipose cells to uncontrolled levels of saturated fatty acids and glucose leads to a long-term inflammatory insult within adipocytes (Youssef-Elabd et al. 2012).

11.2.2 The pathophysiology of vascular disease

- Oxidative stress with reactive oxygen species (ROS) and reactive nitrogen species (RNS) is increased in the arteries and kidneys and with a decreased oxidative defense.
- Inflammation is increased in the vasculature and kidneys with increased high-sensitivity C-reactive protein (HS-CRP), leukocytosis, increased neutrophils and decreased lymphocytes, and increased activity of the RAAS.
- Autoimmune dysfunction of the arteries and kidneys occurs with an increase in white blood cell count (WBC) and involvement of CD4+ (T-helper cells) and CD8+ (cytotoxic T-cells).

These insults result in abnormal vascular biology with endothelial dysfunction and cardiac and vascular smooth muscle hypertrophy and dysfunction. Of course, nutragenomics, genetics, and epigenetics also play a role in the pathophysiology of vascular disease O'Donnell and Nabel (2011).

Figure 11.3 offers an insight into the infinite insults that bombard the endothelium. The infinite insults are divided into two major categories: biomechanical (blood pressure, pulse pressure, shear stress, and oscillatory pressure within the arterial system) and biochemical (e.g., nutritional and biohumoral factors, microbes, sterile antigens, nonsterile antigens, and environmental toxins). Most plaques form at the bifurcation of arteries.

Endothelial cells express various receptors that determine the interaction between the insults and the downstream mediators. These include pattern recognition receptors (PRRs), toll-like receptors (TLRs), nod-like receptors (NLRs), and caveolae (Della Rocca and Pepine 2010; Lundberg and Yan 2011; Zhao et al. 2011; Houston 2012b). The TLRs and NLRs are membrane receptors that react to external insults with appropriate intracellular signaling that usually induces inflammation, oxidative stress, and immune dysfunction

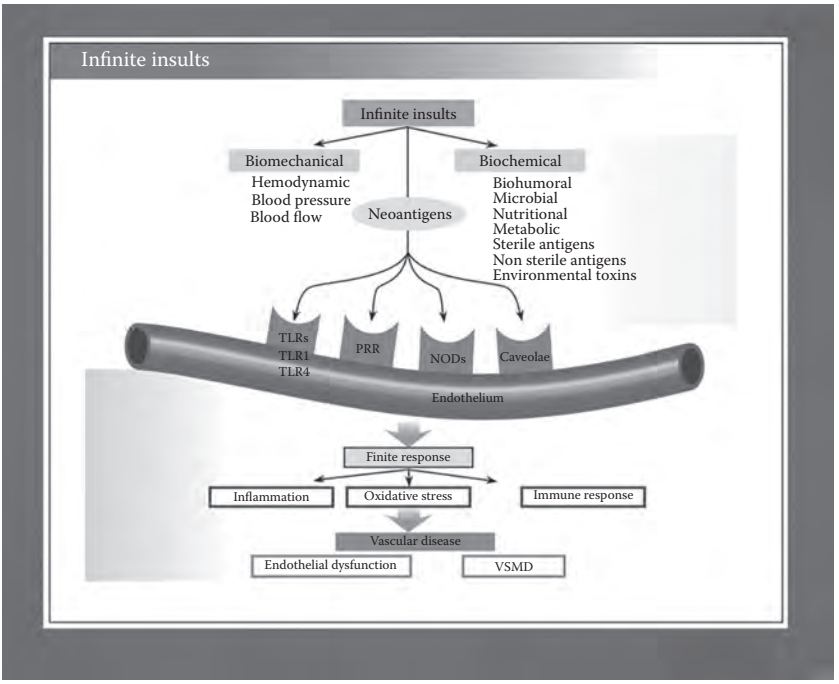


Figure 11.3 *The endothelium is subject to an infinite number of insults but can only elicit a finite number of responses to those insults.*

within the cell. The caveolae are membrane lipid microdomains that when interrupted or stimulated reduce endothelial nitric oxide synthase (eNOS) and nitric oxide levels with an increased BP, inflammation, dyslipidemia, oxidative stress, immune dysfunction, and atherosclerosis. The various risk factors and risk mediators attach to one of the receptors in the membrane and then set off a cascade of the three finite responses (inflammation, oxidative stress, and immune dysfunction), which leads to endothelial dysfunction and ultimately CVD (Della Rocca and Pepine 2010).

11.2.3 Interrupting the finite pathways

The key to the successful prevention and treatment of CVD is recognition of the risk factors, optimal aggressive and early treatment of the risk factors, and identification of treatments that will interrupt the pathways that connect the risk factors to these receptors. The TLR 1, 2, and 4 are the most common of the PRR-type TLRs related to the vascular membrane and endothelial dysfunction. The NLRs (NOD 1 and NOD 2) are also type of PRRs that involve the vascular membrane. There are many scientifically proven

nutraceuticals and dietary factors that reduce TLR and NLR activation (Zhao et al. 2011):

- *Curcurmin* (*tumeric*): TLR 4, NOD 1 (NLR), and NOD 2 (NLR).
- *Cinnamaldehyde* (*cinnamon*): TLR 4.
- *Sulforaphane* (*broccoli*): TLR 4.
- *Resveratrol* (*nutritional supplement, red wine, and grapes*): TLR 1.
- *Epigallocatechin gallate* (*EGCG*) (*green tea*): TLR 1.
- *Luteolin*: Celery, green pepper, rosemary, carrots, oregano, oranges, and olives: TLR 1.
- *Quercetin*: (*tea, apples, onion, tomatoes, and capers*): TLR 1.
- *Omega-3 fatty acids*: Interrupt caveolae lipid microdomains TLRs and NODs, decrease inflammation and HS CRP, lower BP, decrease LDL P, increase LDL and HDL size, improve glycation parameters and insulin sensitivity, decrease immune vascular dysfunction, decrease CHD plaque formation, and improve CHD and CHF symptoms and outcomes.

The goal is to use a dynamic systems biology, functional and metabolic medicine approach to establish cardiovascular ecology, balance, and allostasis (achieve stability through change), and minimize chronic internal and external cardiovascular stressors, mediators, and risk factors that insult the blood vessel. An attempt should be made to reduce the allostatic load and prevent, regulate, and treat the *abnormal* downstream finite responses.

The polygenetic codes for CVD identifies 30 separate loci that are associated with MI and CHD, but only a minority of those 30 loci have anything to do with the top five cardiovascular risk factors (O'Donnell and Nabel 2011). The majority of those loci deal directly with inflammatory pathways. Evaluation and treatment of only the top five risk factors and how they interact with our genome will never reduce CVD and the CHD gap will persist.

Atherosclerosis, endothelial dysfunction, and vascular disease are postprandial phenomena (Mah and Bruno 2012). Ingestion of sodium chloride, refined carbohydrates, and foods containing saturated fats and trans fats, trigger glucotoxicity, triglyceride toxicity, vascular endotoxemia, inflammation, oxidative stress, and immune dysfunction (Lundberg and Yan 2011; Mah and Bruno 2012; Youssef-Elabd et al. 2012). Furthermore, these responses may be perpetuated long after the original insult with a heightened continued inflammatory response (metabolic memory) (Youssef-Elabd et al. 2012). Fortunately, studies have shown that eating a diet rich in potassium and magnesium with low-glycemic foods (vegetables, fiber), monounsaturated and polyunsaturated omega-3 fats, polyphenols, and antioxidants can help to prevent postprandial endothelial dysfunction and reduce future CV events (Houston 2009, 2014a,b). Early evidence of CVD in the form of fatty streaks has been documented in children in the first and second decades of life (**Figure 11.4**) (Houston 2012a). The vascular disease is subclinical for 10–30 years or more prior to any cardiovascular event (Houston 2009, 2010, 2012a). Endothelial dysfunction is the earliest functional abnormality, followed

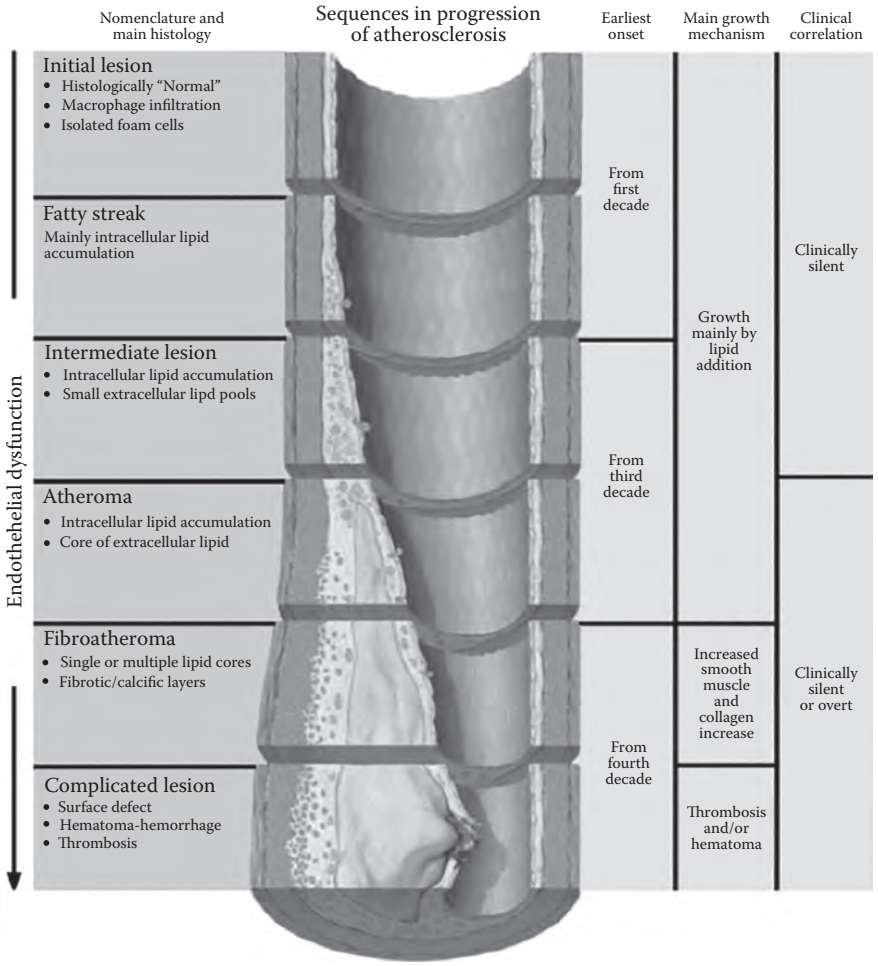


Figure 11.4 *Progression of atherosclerosis from the initial lesion to fatty streak, then to atheroma and complicated lesion with plaque formation, and subsequent plaque rupture with myocardial infarction.*

by changes in arterial compliance, stiffness, and elasticity. It is important to begin using technologies that allow earlier identification of cardiovascular dysfunction before any structural changes have occurred.

11.2.3.1 Coronary heart disease

Figure 11.5 illustrates the vessel changes that occur as CHD progresses. The image on the left of the figure shows a fairly normal artery. In the middle image, the CHD has progressed from minimal to moderate CHD with the subendothelium layer becoming thickened, but the lumen is still the same size.

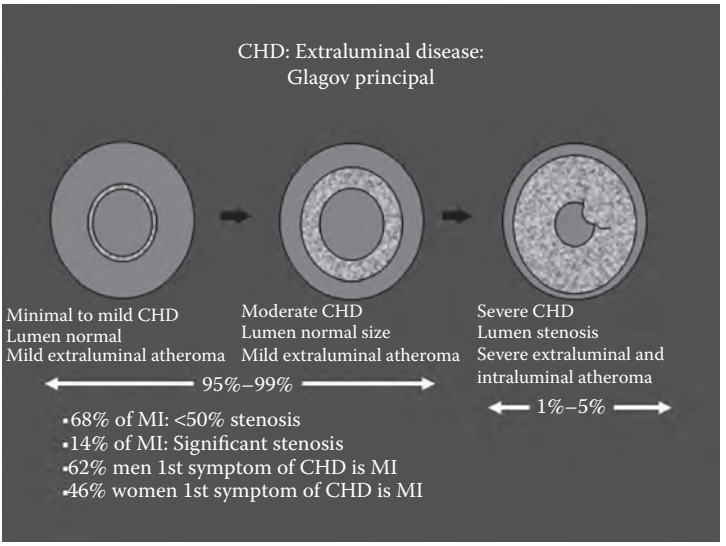


Figure 11.5 *Illustration of the vessel changes that occur as coronary heart disease progresses.*

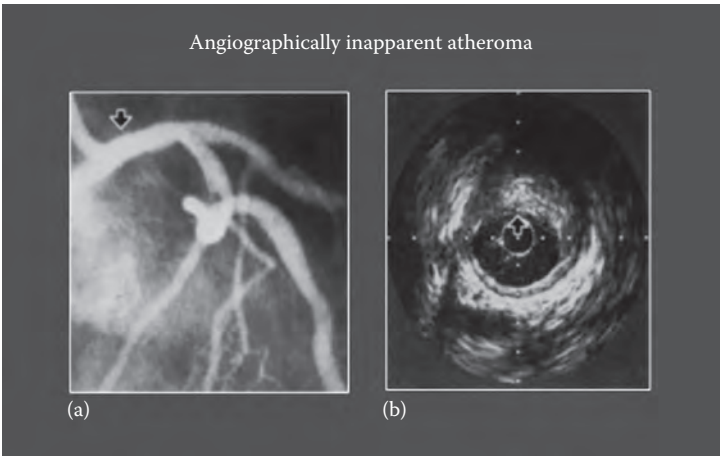


Figure 11.6 *Coronary heart disease that is not detectable by angiogram (a) is clearly evident using computed tomography (b). (From Nissen, S.E. et al., in Topol [Ed.], Interventional Cardiology Update, 14, 1995.)*

This extraluminal plaque and inflammation could be seen with computerized CT angiogram (CTA) or magnetic resonance angiogram (MRA) but missed by conventional coronary arteriogram (**Figure 11.6**). The image on the right in Figure 11.5 shows extensive extraluminal and intraluminal disease.

11.2.3.2 Coronary heart disease risk factors—hypertension

The lack of proper types of imaging, ignoring the majority of the 400 or more CHD risk factors, and not properly evaluating the top five risk factors are some of the reasons for the persistence of the CHD gap (Houston 2012a). For example, only a 24-hour ABM (ambulatory blood pressure monitor) can identify specific BP risks for CVD such as nocturnal BP, dipping, nondipping, BP surges, BP load, white coat and masked hypertension, and BP variability. Nondipping is defined as a less than 10% reduction in BP at night compared to daytime. Nocturnal BP is the primary determinate of CVD related to BP measurements. Nocturnal blood pressure is more clinically important than day blood pressure (27/15 mm Hg difference is optimal) (Houston 2009). The BP load is the number of BP readings over 140/90 mm Hg in 24 h. The normal BP load is less than 15% of the total BP readings over 140/90 mm Hg. BP surges that are high and rapid during the early hours between 3 a.m. and 9 a.m., as well as labile or variable BP, will increase CVD and plaque rupture (Houston 2009). Furthermore, morning blood pressure surges (level and rapidity) increase the risk of ischemic stroke, MI, and left ventricular hypertrophy (Houston 2009). Excessive dipping is associated with an increased risk of ischemic stroke and reverse dipping is associated with an increased risk of intracerebral hemorrhage. Hypertension is not a disease, it is really a marker for vascular dysfunction. Therefore, it is crucial that it is correctly identified. The following points should always be considered when evaluating blood pressure (Houston 2009):

- Normal blood pressure is 120/80 mm Hg, but there is a continuum of risk for CVD starting at 110/70 mm Hg.
- Each increase of 20/10 mm Hg doubles cardiovascular risk.
- Before age 50, the diastolic blood pressure predicts CVD risk best.
- After age 50, the systolic blood pressure predicts CVD risk best.
- 24-h ambulatory blood pressure monitoring is more accurate than office or home blood pressure measurements and should be the standard of care for defining blood pressure and CVD risk.
- *Mercury cuffs are best*: Electronic arm cuffs are good. Do not use wrist or finger monitors.
- *Blood pressure load*: The percent over 140/90 mm Hg should be less than 15 of total BP.
- White coat hypertension (office BP > home BP) and masked hypertension (home BP > office BP).

Nutritional and nutraceutical supplements and other life style changes that improve BP are shown in **Tables 11.1** and **11.2** (Houston 2014a).

Table 11.1 Natural Antihypertensive Compounds Categorized by Antihypertensive Class

Antihypertensive Therapeutic Class (Alphabetical Listing)	Foods and Ingredients Listed by Therapeutic Class	Nutrients and Other Supplements Listed by Therapeutic Class
Angiotensin-converting enzyme inhibitors	Egg yolk *Fish* (*specific*): Bonito, Dried salted fish, Fish sauce Sardine muscle/protein Tuna Garlic Gelatin Hawthorne berry Milk products (specific): Casein Sour milk Whey (hydrolyzed) Sake Sea vegetables (kelp) Sea weed (wakame) Wheat germ (hydrolyzed) Zein (corn protein)	Melatonin Omega-3 fatty acids Pomegranate Pycnogenol Zinc
Angiotensin receptor blockers	Celery Fiber Garlic MUFA	Coenzyme Q10 γ-Linolenic acid NAC Oleic acid Resveratrol Potassium Taurine Vitamin C Vitamin B6 (pyridoxine)
β-Blockers	Hawthorne berry	
Calcium channel blockers	Celery Garlic Hawthorn berry MUFA	α-Lipoic acid Calcium Magnesium *N*-acetyl cysteine Oleic acid Omega-3 fatty acids: Eicosapentaenoic acid Docosahexaenoic acid Taurine Vitamin B6 Vitamin C Vitamin E

(*Continued*)

Table 11.1 (*Continued*) Natural Antihypertensive Compounds Categorized by Antihypertensive Class

Antihypertensive Therapeutic Class (Alphabetical Listing)	Foods and Ingredients Listed by Therapeutic Class	Nutrients and Other Supplements Listed by Therapeutic Class
Central α agonists (reduce sympathetic nervous system activity)	Celery Fiber Garlic Protein	Coenzyme Q10 γ-Linolenic acid Potassium Restriction of sodium Taurine Vitamin C Vitamin B6 Zinc
Direct renin inhibitors		Vitamin D
Direct vasodilators	Celery Cooking oils with monounsaturated fats Fiber Garlic MUFA Soy	α-Linolenic acid Arginine Calcium Flavonoids Magnesium Omega-3 fatty acids Potassium Taurine Vitamin C Vitamin E
Diuretics	Celery Hawthorn berry Protein	Calcium Coenzyme Q10 Fiber γ-Linolenic acid L-carnitine Magnesium Potassium Taurine Vitamin B6 Vitamin C Vitamin E: High γ/δ-Tocopherols and tocotrienols

Table 11.2 An Integrative Approach to the Treatment of Hypertension

Intervention Category	Therapeutic Intervention	Daily Intake
Diet characteristics	DASH I, DASH II-Na^+ or PREMIER diet	Diet type
	Sodium restriction	1500 mg
	Potassium	5000 mg
	Potassium/sodium ratio	>3:1
	Magnesium	1000 mg
	Zinc	50 mg

(*Continued*)

Table 11.2 (*Continued*) An Integrative Approach to the Treatment of Hypertension

Intervention Category	Therapeutic Intervention	Daily Intake
Macronutrients	*Protein*: Total intake from nonanimal sources, organic lean or wild animal protein, or cold-water fish	30% of total calories, which 1.5–1.8 g/kg body weight
	Whey protein	30 g
	Soy protein (fermented sources are preferred)	30 g
	Sardine muscle concentrate extract	3 g
	Milk peptides (VPP and IPP)	30–60 mg
	Fat	30% of total calories
	Omega-3 fatty acids	2–3 g
	Omega-6 fatty acids	1 g
	Omega-9 fatty acids	2–4 tablespoons of olive or nut oil or 10–20 olives
	Saturated fatty acids from wild game, bison, or other lean meat	<10% total calories
	Polyunsaturated to saturated fat ratio	>2.0
	Omega-3-to-omega-6 ratio	1.1–1.2
	Synthetic *trans* fatty acids	None (completely remove from diet)
	Nuts in variety	*Ad libitum*
	Carbohydrates as primarily complex carbohydrates and fiber	40% of total calories
	Oatmeal or	60 g
	Oatbran or	40 g
	β-Glucan or	3 g
	Psyllium	7 g
Specific foods	Garlic as fresh cloves or aged Kyolic garlic	4 fresh cloves (4 g) or 600 mg aged garlic taken twice daily
	Sea vegetables, specifically dried wakame	3.0–3.5 g
	Lycopene as tomato products, guava, watermelon, apricots, pink grapefruit, papaya, or supplements	10–20 mg
	Dark chocolate	100 g
	Pomegranate juice or seeds	8 oz. or one cup
	Sesame	60 mg sesamin or 2.5 g sesame meal
Exercise	Aerobic	20 min daily at 4200 kJ/week
	Resistance	40 min per day
Weight reduction	Body mass index <25 Waist circumference: <35 in. for women <40 in. for men Total body fat: <22% for women <16% for men	Lose 1–2 lb. per week and increasing the proportion of lean muscle

(*Continued*)

Table 11.2 (*Continued*) An Integrative Approach to the Treatment of Hypertension

Intervention Category	Therapeutic Intervention	Daily Intake
Other lifestyle recommendations	Alcohol restriction: Among the choice of alcohol red wine is preferred due to its vasoactive phytonutrients	<20 g per day Wine < 10 oz. Beer < 24 oz. Liquor < 2 oz.
	Caffeine restriction or elimination depending on CYP 450 type	<100 mg per day
	Tobacco and smoking	Stop
Medical considerations	Medications which may increase blood pressure.	Minimize use when possible, such as by using disease-specific nutritional interventions
Supplemental foods and nutrients	α-Lipoic acid with biotin	100–200 mg twice daily
	Amino acids: Arginine	5 g twice daily
	Carnitine	1–2 g twice daily
	Taurine	1–3 g twice daily
	Chlorogenic acids	150–200 mg
	Coenzyme Q10	100 mg once to twice daily
	Grape seed extract	300 mg
	Hawthorne extract	500 mg twice a day
	Melatonin	2.5 mg
	N-acetyl cysteine (NAC)	500 mg twice a day
	Olive leaf extract (oleuropein)	500 mg twice a day
	Pycnogenol	200 mg
	Quercetin	500 mg twice a day
	Resveratrol (*trans*)	250 mg
	Vitamin B6	100 mg once to twice daily
	Vitamin C	250–500 mg twice daily
	Vitamin D3	Dose to raise 25-hydroxyvitamin D serum level to 60 ng/ml
	Vitamin E as mixed tocopherols	400 IU

11.2.3.3 Coronary heart disease risk factors—dyslipidemia

Dyslipidemia is another one of the top five cardiovascular risk factors. Proper measurement, risk assessment, and treatment using advanced lipid profiles is proven and recommended (van der Steeg et al. 2008; Della Rocca and Pepine 2010; Fazio and Linton 2010; Houston 2012b, 2014b). An advanced lipid profile will measure:

- LDL-C total
- *LDL-P*: LDL particle number (drives CHD risk)

- LDL size (dense type B versus large type A)
- Modified LDL (oxidized, glycated, glyco-oxidized, and acetylated)
- Antibodies to oxLDL and modified LDL
- Apolipoprotein (APO) B elevated
- APO B antibodies and immune complexes
- Lp(a)
- HDL-C total
- *HDL-P*: HDL particle number
- HDL size (large 2b versus small type 3)
- Dysfunctional HDL
- Pro-inflammatory and pro-atherogenic HDL
- Myeloperoxidase (MPO) and dysfunctional APO A
- Low APO A
- Low paraoxonase (PON)-1 and PON-2
- Increased APO-CIII
- Serum-free fatty acids
- VLDL and triglyceride (TG) total
- Large VLDL
- VLDL-P particle number
- Remnant particles

The primary driving cardiovascular risk related to LDL-cholesterol is the number of LDL-particle number (LDL-P) and apolipoprotein B particles (Houston 2012b, 2014b). HDL-P (particle number) is most protective, with larger HDL type 2b being a second important protective mechanism (Houston 2012b, 2014b). Larger number and size of HDL are more efficient at reverse cholesterol transport and cholesterol efflux and more protective to the vascular system in numerous other ways. It is also important to analyze dysfunctional HDL (van der Steeg et al. 2008; Fazio and Linton 2010; Houston 2012b, 2014b). The loss of HDL function reduces reverse cholesterol transport (RCT) and cholesterol efflux capacity (CEC), reduces oxidative defense, and increases oxidative stress and inflammation. Dysfunctional HDL my represent the most important protective factor of HDL compared to HDL-P or HDL size related to CHD. Patients who have a HDL of 85 mg/dL or more often have dysfunctional HDL that is not protective and may be pro-inflammatory or atherogenic (van der Steeg et al. 2008; Fazio and Linton 2010). VLDL, triglycerides, and remnant particles are very atherogenic and thrombogenic (Houston 2012b, 2014b).

Nutritional and nutraceutical supplements for the treatment of dyslipidemia are shown in **Table 11.3** (Houston 2012b, 2014b).

11.2.3.4 Coronary heart disease risk factors—dysglycemia

A fasting blood sugar (FBS) of over 75 mg increases CHD by 1% per increase of 1 mg/dL and induces endothelial dysfunction (Houston 2012a). If a patient has a FBS of 100 mg (often considered a normal level), the risk of

Table 11.3 Nutrition and Nutraceutical Supplements for the Treatment of Dyslipidemia

- Red yeast rice 2400–4800 mg at night with food
- Plant sterols 2.5 g per day
- Berberine 500 mg per day to twice per day
- Niacin (nicotinic acid B3) 500–3000 mg per day as tolerated pretreated with quercetin, apples, ASA. Take with food and avoid alcohol. Never interrupt therapy
- Omega-3 fatty acids with EPA/DHA at 3/2 ratio 4 grams/day with GLA at 50% of total EPA and GLA and γ/δ-tocopherol
- γ/δ-Tocotrienols 200 mg h
- Aged Kyolic garlic standardized 600 mg twice per day
- Sesame 40 g per day
- Pantethine 450 mg bid
- MUFA 20–40 g per day (EVOO 4 tablespoons per day)
- Lycopene 20 mg per day
- Luteolin 10 per day
- Trans resveratrol 250 mg per day
- NAC 500 mg twice per day
- Carnosine 500 mg twice per day
- Citrus bergamot 1000 mg per day
- Quercetin 500 mg twice per day
- Probiotics standardized 15–50 billion organisms bid
- Curcumin 500–1000 mg twice per day
- EGCG 500–1000 mg bid or 60–100 oz. of green tea per day
- Pomegranate one cup of seeds/day or 6 oz. of juice per day

CHD is increased by 25% (Houston 2012a). A 2-hour glucose tolerance test (GTT) over 110 mg increases CHD by 2% per 1 mg/dL increase in glucose (Houston 2012a). The current definition of an abnormal 2-hour GTT is value of more than 140 mg. If a patient's result is 140 mg, which again is currently classed as *normal*, CHD and MI are increased by 60%. Hyperinsulinemia is also an independent risk factor for CHD (Houston 2012a). Insulin resistance creates inflammation, reduces nitric oxide levels, and causes endothelial dysfunction and vascular disease through the mitogen-activated protein kinase (MAPK) pathway, which is inflammatory and atherogenic and induces hypertension, diabetes mellitur, and CVD, as opposed to the phosphatidylinositol 3-kinase (PI3K) pathway, which is anti-inflammatory, antihypertensive, and anti-atherogenic (Houston 2012a). It is important to measure all glycation parameters including fasting glucose, 2-hour GTT, insulin levels, C-peptide, and proinsulin, depending on the clinical setting (Fazio and Linton 2010).

Obesity with increased levels of inflammatory and oxidative stress–related adipokines contribute to CHD. Measurement of body weight, waist and hip circumference, waist-to-hip ratio, BMI, and body composition with total body fat and visceral fat with measurement of lean body mass and using body impedence analysis will help predict CHD risk (Houston 2003). Interval aerobic and resistance exercise should also be part of the comprehensive CHD prevention program.

11.2.3.5 Noninvasive vascular testing

Fortunately, there are a number of noninvasive tests to determine vascular pathology before it actually starts (Houston 2012a). A discussion of these techniques is beyond the scope of this book; however, the reader is encouraged to find out more about these technologies, particularly EndoPAT, a post-brachial artery study, which is very accurate at assessing endothelial function and diagnosing endothelial dysfunction, computerized arterial pulse wave analysis (CAPWA) for endothelial function and arterial compliance, carotid intimal medial thickness (IMT), heart rate variability and heart rate recovery time, ECHO, magnetic cardiography (MCG) and cardiac CT angiograms for obstructive CHD, and coronary calcium score (Houston 2003, 2009, 2012a; Bonetti et al. 2004; Kandori et al. 2010; Rozanski et al. 2011) (**Table 11.4**). EndoPAT is the most cost-effective and accurate noninvasive test to identify early endothelial dysfunction to predict future CVD and CHD. This test, along with 24-hour BP, advanced lipid testing, and glycation measurements, is the best initial way to evaluate the CV patient. Numerous other CHD risk factors with comprehensive CHD testing are listed in **Table 11.5**. Some of the most neglected and important CHD risk factors to evaluate include gender-specific hormones, thyroid function, toxins, homocyteine, and vitamin D. If proper coding is done, these and other tests reviewed are very cost-effective and covered by insurance.

Table 11.4 Noninvasive Vascular Testing for Cardiovascular Disease

Functional Tests	Structural Tests	Other
EndoPAT (endothelial dysfunction)	Carotid IMT duplex	Cardiac PET and SPECT
CAPWA: (computerized arterial pulse wave analysis)	EBT and CT angiogram (CTA) with CAC scoring	*IVUS*: intravascular ultrasound
DTM: (digital thermographic monitor)	Cardiac MRI (CMR)	Cardiac nuclear studies (MPI) with PET and SPECT
HRV: (heart rate variability)	*Echo*: rest and exercise	Coronary angiogram
EKG and TMT	*ABI*: rest and exercise	
MCG: (magnetocardiography)		

Table 11.5 Other Comprehensive Laboratory Testing for Cardiovascular Disease

Laboratory Testing	Laboratory Testing (Other)	Finite Response Testing: Blood Inflammation	Finite Response Testing: Blood Immune and Vascular Dysfunction	Finite Response Testing: Oxidative Stress
CBC w/diff	Vitamin D3	HS-CRP	Thyroid antibodies	Ox LDL and glycated LDL
UA	Fasting C-peptide, A1C, insulin, proinsulin, 2-h GTT	TNF-α	IL-1b, IL-4, IL-6, IL-10, IL-12, IL-17	MPO
CMP 12	PRA and aldosterone	Interleukins: IL-6 and IL-1b	TNF-α	GGT
Expanded advanced lipid profile	Free testosterone, SHBG, estradiol, estriol	Myeloperoxidase (MPO)	C3a, C4a, C3, and C4	GSH and GSJ/GSSG (reduced/oxidized ratio)
APO B, APO AI	Progesterone, DHEA, and DHEAS	Lp-PLA2	T-Lymphocyte subsets	MDA (malondialdehyde)
Free T3, T4, TSH, and RT3, thyroid antibodies	B12 and folate	Fibrinogen and ferritin	Total and epitope specific IgE and IgG-type 4	TBARS (Thiobarbituric acid reactive substances)
Magnesium	AM and PM cortisol, salivary cortisol	ESR	NKC percent	F2 isoprostane and *-OHdG (deoxyguanosine)
Iron, TIBC, and ferritin	ADMA	Omega-3 index	CBC with diff	Hexanoyl lysine (ELISA) and lipid hydroxyperoxide: Lipid OX
Fibrinogen	Adiponectine	Homocysteine	ELISA–ACT	Protein carbonyl
HSCRP	EKG, TMT, and CXR	Waist circumference, visceral obesity, body fat%, total body fat	ACTH/Cortisol ratio	SOD 1,2 and Catalase
Homocystine	Micro nutrient testing		MMP-9, MMP-2	Lp-PLA 2
Uric Acid	Omega-3 index		Infectious disease profile with IGM and IGG antibodies, such as *H. pylori*, CMV, EBV, Lyme, Mycoplasma, HSV, Chlamydia, Hep. A, B, C, and so on	WBC with diff

(*Continued*)

Table 11.5 (*Continued*) Other Comprehensive Laboratory Testing for Cardiovascular Disease

Laboratory Testing	Laboratory Testing (Other)	Finite Response Testing: Blood Inflammation	Finite Response Testing: Blood Immune and Vascular Dysfunction	Finite Response Testing: Oxidative Stress
Microalbuminuria	APO E			Uric acid
GGTP and hepatic profile	Blood viscosity			Haptoglobin
Myeloperoxidase (MPO)	Erythrocyte adhesion			Oxidative Defense TAS (total antioxidant status)
Cardiovascular SNP	Telomere test			Spectrox
Toxicology/heavy metal screen: Spot or UA	Gluten antibody test			
	MTHFR			
	Body impedance analysis, waist circumference, WHR			
	Rest and exercise BP and 24-h ambulatory blood pressure monitor			
	PFT, Dexa			

11.3 Treatment

The prevention and treatment of cardiovascular disease, CHD, and CHF require an early and aggressive program that includes optimal nutrition, antioxidants, nutritional supplements, weight management, resistance and aerobic interval exercise programs, tobacco cessation, and other life style changes that can be incorporated into a pharmaceutical regimen as necessary. The treatment of hypertension, dyslipidemia, dysglycemia, and obesity have been reviewed. In this chapter, prevention and treatment of endothelial dysfunction, CHD, angina, and CHF will be reviewed.

11.3.1 Endothelial dysfunction

Maintaining optimal endothelial function is most important in preventing CHD and future CV events. Maintaining optimal nitric oxide bioavailability is the key in maintaining endothelial function. This involves arginine, nitrates, and nitrites, eNOS function (endothelial nitric oxide synthase), and cofactors such as folate, tetrahydrobiopterin, glutathione, NADH, and FADH. Endothelial dysfunction is the first functional abnormality in CVD (Landberg et al. 2012; Larijani et al. 2013; Yamagata et al. 2015). The Mediterranean diet and DASH diets have been shown to improve endothelial function and reduce CV event rate by about 30% [20–22] (**Table 11.6**). Various supplements such as vitamin D

Table 11.6 Nutrition and Supplements to Improve Endothelial Function

DASH and Mediterranean diet
Vitamin D and C
Aged garlic
Quercetin
Coenzyme Q10
Lycopene
Omega-3 fatty acids
Polyphenols and flavonoids: Cacao, tea, catechis, berry anthocyanins, orange juice and hesperidein, wine polyphenols, beets, and beet root extract
Pomegranate
Curcumin
Arginine and citrullene
Nitrates and nitrites in dark green leafy vegetables and beets
Resveratrol
Glutathione
N-acetyl cysteine
Lipoic acid
B vitamins

to a blood level of about 60 ng/ml, vitamin C at 250 mg bid, aged garlic 600 mg bid, Quercetin 500–1000 mg bid, curcumin 500–1000 mg bid, Coenzyme Q10 to a blood level of 3 μg/ml, lycopene 20 mg per day and various polyphenols, flavonoids, beets, and beet root extract, and omega-3 fatty acids at 1000–5000 mg per day improve eNOS, nitric oxide, and endothelial function (Landberg et al. 2012; Larijani et al. 2013; Yamagata et al. 2015) (**Table 11.6**).

11.3.2 Coronary heart disease and congestive heart failure

CHD, systolic CHF with a low ejection fraction, and diastolic CHF can be effectively managed with various supplements as shown in **Table 11.7**. These supplements improve coronary artery endothelial function, reduce oxidative stress, improve oxidative defense, and decrease inflammation and cardiovascular immune dysfunction. They also improve cardiac contractility, ventricular compliance, metabolic and nutritional function of the cardiac myocyte, myocardial bioenergetics, ATP production, and oxygen delivery, reduce cardiac arrhythmias and plaque progression, enhance plaque regression, stabilize plaque, and reduce plaque rupture and MI, stent restenosis, and CABG restenosis (Xu et al. 2008; Wagner et al. 2009; Chiuve et al. 2011; Mingorance et al. 2011; DiNicolantonio et al. 2013; Nozue et al. 2013; Shea et al. 2013; Mortensen et al. 2014) (Table 11.7).

Table 11.7 Summary

***Summary Metabolic Cardiology*: Nondrug TX of Angina, CHD, and CHF**	***Summary Metabolic Cardiology:* Nondrug TX of Angina, CHD, and CHF**
Taurine 3 g bid	Vitamin K2 MK & 150 mg qd
D-Ribose 5 g tid or qid	Omega-3 FA 6 capsules bid, 5 g per day
Coenzyme Q10, 300 mg bid	*Glutathione precursors*: NAC 1000 mg bid, R-Lipoic acid extended release 300 mg bid, whey protein 40 g qd, selenium 200 mg qd
Magnesium chelates 500–1000 mg bid	Vitamin D3 to level of 60 ng/ml
High potassium diet 5000–10,000 mg qd	B Vitamins with 200 mg thiamine qd
Carnitine tartrate 3 g bid	Zinc 50 mg qd (test for copper levels)
R-Lipoic Acid 300–600 mg bid (pyruvate decarboxylase complex)	Selenium 200 mcg qd
Malic acid 240 mg bid	Proprietary vascular product 5 caps bid
Aged garlic 1200 qd	Trans-Resveratrol (Resvera-Sirt HP) 250 mg qd
Curcumin 500–1000 mg bid	Creatine 2000 mg qd
Vitamin C 500 mg bid	Quercetin 1000 mg bid
Sesame oil 200–300 ml qd	Sauna treatments
Carnosine 500–1000 mg qd	
Probiotics (*Saccharomyces boulardii* and others)	

11.4 Conclusion

The top five cardiovascular risk factors, as they are currently defined, are not an adequate explanation for CHD. In order to close the CHD gap, the top five risk factors must be better defined and treated while assessing the other 395 risk factors and mediators. Early detection and aggressive prevention and treatment of vascular disease are needed before any structural changes occur. New laboratory tests, such as the advanced lipid profiles, 24-hour BP monitoring, and specific tests to identify inflammation and oxidative stress such and immune vascular dysfunction are needed. In addition, vascular translational medicine will need to be evaluated with new imaging technologies, such as EndoPAT, CAPWA, carotid IMT, MCG, HRV, and CT Angiogram.

In order to truly revolutionize the treatment of CVD, new therapies will need to involve management of the pathophysiologic risk factors, mediators, and their downstream effects, as well as the finite vascular responses. This will be achievable by using a combination of targeted personalized treatments with genomics, proteomics, metabolomics, nutrition, nutraceutical supplements, vitamins, minerals, antioxidants, anti-inflammatory agents, anti-immunological agents, and pharmacologic agents. Future studies must begin to measure all the pertinent risk factors that have been reviewed here to correlate their direct relationship with CHD. Only by addressing all these factors will we be able to decrease or halt subsequent vascular aging, damage, and CVD.

References

ACCORD Study Group, H. C. Gerstein, M. E. Miller, S. Genuth, F. Ismail-Beigi, J. B. Buse, D. C. Goff Jr, J. L. Probstfield et al. 2011. Long-term effects of intensive glucose lowering on cardiovascular outcomes. *N Engl J Med* 364 (9):818–828.

Bonetti, P. O., G. M. Pumper, S. T. Higano, D. R. Holmes Jr, J. T. Kuvin, and A. J. Lerman. 2004. Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. *Am Coll Cardiol* 44 (11):2137–2141.

Chiuve, S. E., E. C. Korngold, J. L. Januzzi Jr, M. L. Gantzer, and C. M. Albert. 2011. Plasma and dietary magnesium and risk of sudden cardiac death in women. *Am J Clin Nutr* 93:253–260.

Della Rocca, D. G. and C. J. Pepine. 2010. Endothelium as a predictor of adverse outcomes. *Clin Cardiol* 33 (12):730–732.

DiNicolantonio, J. J., C. J. Lavie, H. Fares, A. R. Menezes, and J. H. O'Keefe. 2013. L-carnitine in the secondary prevention of cardiovascular disease: Systematic review and meta-analysis. *Mayo Clin Proc* 88:544–551.

El Khatib, N., S. Génieys, B. Kazmierczak, and V. Volpert. 2009. Mathematical modelling of atherosclerosis as an inflammatory disease. *Philos Transact A Math Phys Eng Sci* 367 (1908):4877–4886.

Fazio, S. and M. F. Linton. 2010. High-density lipoprotein therapeutics and cardiovascular prevention. *J Clin Lipidol* 4 (5):411–419.

Houston, M. 2003. *What Your Doctor May Not Tell You About Hypertension: The Revolutionary Nutrition and Lifestyle Program to Help Fight High Blood Pressure*. NY: Wellness Central. Hachette Book Group.

Houston, M. C. 2009. *Handbook of Hypertension*. Oxford UK: Wiley–Blackwell.

Houston, M. C. 2010. Nutrition and nutraceutical supplements in the treatment of hypertension. *Expert Rev Cardiovasc Ther* 8:821–833.
Houston, M. C. 2012a. *What Your Doctor May Not Tell You About Heart Disease. The Revolutionary Book that Reveals the Truth Behind Coronary Illnesses and How You Can Fight Them*. Grand Central Life and Style. Park Ave. NY: Hachette Book Group.
Houston, M. 2012b. The role of nutraceutical supplements in the treatment of dyslipidemia. *J Clin Hypertens* (*Greenwich*) 14 (2):121–132.
Houston, M. 2014a. The role of nutrition and nutraceutical supplements in the treatment of hypertension. *World J Cardiol* 6 (2):38–66.
Houston, M. 2014b. The role of nutrition and nutritional supplements in the treatment of dyslipidemia. *Clinical Lipidology* 9 (3):333–354.
Kandori, A., K. Ogata, T. Miyashita, H. Takaki, H. Kanzaki, S. Hashimoto, W. Shimizu, S. Kamakura, S. Watanabe, and K. Aonuma. 2010. Subtraction magnetocardiogram for detecting coronary heart disease. *Ann Noninvasive Electrocardiol* 5 (4):360–368.
Landberg, R., N. Naidoo, and R. M. van Dam. 2012. Diet and endothelial function: From individual components to dietary patterns. *Curr Opin Lipidol* 23:147–156.
Larijani, V. N., N. Ahmadi, I. Zeb, F. Khan, F. Flores, and M. Budoff. 2013. Beneficial effects of aged garlic extract and coenzyme Q10 on vascular elasticity and endothelial function: The FAITH randomized clinical trial. *Nutrition* 29:71–75.
Lundberg, A. M. and Z. Q. Yan. 2011. Innate immune recognition receptors and damage-associated molecular patterns in plaque inflammation. *Curr Opin Lipidol* 22 (5):343–349.
Mah, E. and R. S. Bruno. 2012. Postprandial hyperglycemia on vascular endothelial function: Mechanisms and consequences. *Nutr Res* 32 (10):7727–7740.
Mark, C. 2000. *Vascular Biology in Clinical Practice.* Houston, TX.
Mingorance, C., R. Rodriguez-Rodriguez, M. L. Justo, M. D. Herrera, and M. A. de Sotomayor. 2011. Pharmacological effects and clinical applications of propionyl-L-carnitine. *Nutr Rev* 69:279.
Mortensen, S. A., F. Rosenfeldt, A. Kumar, P. Dolliner, K. J. Filipiak, D. Pella, U. Alehagen, G. Steurer, and G. P. Littarru. 2014. Q-SYMBIO Study Investigators. The effect of coenzyme Q10 on morbidity and mortality in chronic heart failure: results from Q-SYMBIO: A randomized double-blind trial. *JACC Heart Fail* 2 (6):641–649.
Mulvany, M. J. et al. 1990. Structure and function of small arteries. *Physiol Rev* 70:921–961.
Nissen, S. E. et al. 1995. In: Topol (Ed.). Interventional Cardiology Update, Vol. 14, 1995.
Nozue, T., S. Yamamoto, S. Tohyama, K. Fukui, S. Umezawa, Y. Onishi, T. Kunishima et al. 2013. Effects of serum n-3 to n-6 polyunsaturated fatty acids ratios on coronary atherosclerosis in statin-treated patients with coronary artery disease. *Am J Cardiol* 111 (1):6–11.
O'Donnell, C. J. and E. G. Nabel. 2011. Genomics of cardiovascular disease. *N Engl J Med* 365 (22):2098–2109.
Ross, R. 1999. Atherosclerosis—An inflammatory disease. *N Engl J Med* 340:115–126.
Rozanski, A., H. Gransar, L. J. Shaw, J. Kim, L. Miranda-Peats, N. D. Wong, J. S. Rana et al. 2011. Impact of coronary artery calcium scanning on coronary risk factors and downstream testing the EISNER (Early Identification of Subclinical Atherosclerosis by Noninvasive Imaging Research) prospective randomized trial. *J Am Coll Cardiol* 57 (15):1622–1632.
Shea, M. K., S. L. Booth, M. E. Miller, G. L. Burke, H. Chen, M. Cushman, R. P. Tracy, and S. B. Kritchevsky. 2013. Association between circulating vitamin K1 and coronary calcium progression in community-dwelling adults: The multi-ethnic study of atherosclerosis. *Am J Clin Nutr* 98:197–208.
van der Steeg, W. A., I. Holme, S. M. Boekholdt, M. L. Larsen, C. Lindahl, E. S. Stroes, M. J. Tikkanen et al. 2008. High-density lipoprotein cholesterol, high-density lipoprotein particle size, and apolipoprotein A-I: Significance for cardiovascular risk: The IDEAL and EPIC-Norfolk studies. *J Am Coll Cardiol* 51 (6):634–642.

Wagner, S., J. Herrick, L. M. Shecterle, and J. A. 2009. St Cyr. D-ribose, a metabolic substrate for congestive heart failure. *Prog Cardiovasc Nurs* 24 (2):59–60.
Xu, Y. J., A. S. Arneja, P. S. Tappia, and N. S. Dhalla. 2008. The potential health benefits of taurine in cardiovascular disease. *Exp Clin Cardiol* 13 (2):57–65.
Yamagata, K., M. Tagami, and Y. Yamori. 2015. Dietary polyphenols regulate endothelial function and prevent cardiovascular disease. *Nutrition* 31:28–37.
Youssef-Elabd, E. M., K. C. McGee, G. Tripathi et al. 2012. Acute and chronic saturated fatty acid treatment as a key instigator of the TLR-mediated inflammatory response in human adipose tissue, in vitro. *J Nutr Biochem* 23:39–50.
Yusuf, S., S. Hawken, S. Ounpuu, T. Dans, A. Avezum, F. Lanas, M. McQueen et al. 2004. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): Case-control study. INTERHEART Study Investigators. *Lancet* 364 (9438):937–952.
Zhao, L., J. Y. Lee, and D. H. Hwang. 2011. Inhibition of pattern recognition receptor-mediated inflammation by bioactive phytochemicals. *Nutr Rev* 69 (6):310–320.

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

12

Nutraceuticals

A Preventative Measure against the Adverse Impact among Diabetic Patients

Suyansh Sharma and Sarvadaman Pathak

Contents

12.1 Introduction

Hesy-Ra, an ancient Egyptian in 1552 BC, was the first to notice what we now call a *diabetic* symptom. He noticed frequent urination among his patients, which somehow attracted ants. In the 1800s, the first chemical test was then developed to detect glucose level in urine. Diabetes is a metabolic disorder in which one's body is unable to absorb glucose. In order to absorb glucose, pancreas (β-cells) produce insulin, which maintains the blood glucose hemostasis in our body. Insulin secretion is usually triggered during post-prandial state, when glucose plasma level is higher. However, due to genetic

and environmental stimuli, insulin production can be reduced or nonfunctional in our system. The two main types of diabetes most frequently documented include type 1 diabetes (IDDM), a condition with an unknown cause that result in an absolute insulin deficiency due to a defective pancreatic β cells that do not produce insulin (Diabetes Fact Sheet 2013). Type 2 diabetes (NIDDM), on the other hand, is a progressive insulin secretory defect. In addition, the body also becomes *insensitive* to insulin. Certain genetic faults found in the pancreatic β-cell and even viral infection (Filippi and Von Herrath 2008) (Coxsackie infection) could lead toward developing diabetes. Since insulin production/function is repressed, this causes a progressive increase in blood glucose level, which can be life threatening in the long run. Increased blood glucose levels could harden the blood vessels (atherosclerosis), which would clog blood vessels, impeding oxygen and nutrients deliveries for tissues. Over time, increased blood glucose level increases the chance of getting heart disease, stroke, peripheral arterial disease, diabetic nephropathy, diabetic retinopathy, and even diabetic neuropathy. Poor blood circulation not only affects organs but also leads to skin ulcers, particularly on the foot. In absolute or relative insulin deficiency, the body relies solely on fat as an energy source. This process, however, produces ketones as the by-product, which is both toxic and lowers the pH of the blood. Prolonged low pH could lead to a fatal diabetic ketoacidosis. The International Diabetes Federation (IDF) stated that 387 million people are currently affected by diabetes worldwide (Key Findings 2014). The IDF also predicted that by 2035, the number of people with diabetes would increase to 592 million. Controlling glycemic level is not sufficient when treating diabetes; American Diabetes Association (ADA) described diabetes as a lifelong illness with multiple symptoms that go beyond just glucose level (American Diabetes Association 2015a).

Diabetic patients in the United States are faced with rising medical cost impacting patients with low socioeconomic status (American Diabetes Association 2015a). A recent study in Denmark found that healthcare resource usage for diabetic patients was almost twice that of their non-diabetic counterparts (Sortsø et al. 2015). Cost of treatment also spikes as diabetic patients develop major complications. In addition, these complications could also affect patients in their day-to-day life, jeopardizing their employment status. Diabetic patients have even self-reported greater *work impairment* in relation to their productivity (Rodbard et al. 2009). The Centers of Disease Control and Prevention (CDC) reported over 65,700 lower-limb amputations per year due to severe cases of diabetic ulcer (U.S. Centers for Disease Control and Prevention 2011). Patients with these complications may then be faced with limited employment opportunities with lower pay. The same study in Denmark also reported that unemployment is higher among diabetic patients compared with non-diabetic patients by 22% (Sortsø et al. 2015), also noting that diabetic patients earned less annually. This is where this chapter comes. Our mission is to educate about the cost-effective nutritional medical route that could be taken along with the prescribed allopathic medicine. These include herbal medicine and

even common culinary spices. This *alternative* medicine called *nutraceutical* is not anew, as studies have shown that 30% of diabetic patients use plants with medicinal value (Raman et al. 2012).

For decades, several drugs and treatments have been developed to help patients manage their diabetes. Although anti-diabetic agents can be used to manage hyperglycemia and reduce microvascular complications (i.e., retinopathy, neuropathy, and nephropathy), their use is associated with undesirable side effects (International Diabetes Federation 2005; American Diabetes Association 2015b). Dietary changes are effective in managing diabetes and should be continued for long term. However, dietary changes alone will not effectively control blood glucose. As diabetes develops, and especially if blood glucose remains uncontrolled, complications will significantly develop that result in increased cost and patient morbidity. In the following section, we will focus on the general treatment options for diabetic patients and look into alternatives mentioned earlier.

12.2 General treatment options for diabetes

12.2.1 Insulin

Type 1 diabetes is traditionally thought of as an absolute insulin deficiency and T2D a relative insulin deficiency. Both can be treated with exogenous administration of insulin. Our current knowledge of the cause of decreased insulin secretion is still limited, but one of the errors in the insulin pathway is the disturbance of phosphatidylinositol 3-kinase (PI3K) (Choi and Young-Bum 2010). Although T1D relies on insulin more than T2D, patients with T2D progressively lose the capability to produce insulin throughout disease progression. Since insulin is a peptide hormone, oral route is not an option, as the digestive enzymes found in the stomach could break them down before it reaches the bloodstream. Insulin is also used to treat diabetic ketoacidosis (Hamdy 2015). There are two main ways to administer insulin; the most common is by subcutaneous injection. The ADA recommends up to three to four injections per day to maintain glucose within goal ranges (American Diabetes Association 2015c). Note that daily dosages vary and it is advised to check with personal physician. There are several short-acting insulin therapies, which have shown decrease in blood glycemic level among patients (Rosenstock et al. 2004). Another option to receive insulin is by continuous subcutaneous insulin infusion, which is most commonly called *insulin pump*. Although this device is costly, it could be programmed to provide continuous and sufficient insulin through a plastic tube into the subcutaneous tissue. Studies have also shown that there is limited difference in hypoglycemia occurrences among the two-treatment options (Evans and Bahng 2000). Note that insulin delivery is usually a last resort when all other treatment options have been used. Recent study has also shown the danger of insulin administration, as it could trigger the development of T1D (Nishida et al. 2014).

12.2.2 Lifestyle changes

Patients with T2D are encouraged to make significant lifestyle changes such as reducing body weight, controlling glycaemia, and increasing physical activity. Depending on the frequency of insulin administration, patients may be required to monitor their blood glucose from one to four times daily or even more. The ADA recommends patients using multiple doses of insulin or an insulin pump to check their blood glucose prior to meals/snacks, at bedtime, prior to exercise, or critical tasks (e.g., driving), if low blood glucose is suspected, after treating low blood glucose, and occasionally postprandial (Amaryl® [package insert] 2009). Monitoring may also increase in the settings of stress, exercise, illness, or signs and symptoms of hypo/hyperglycemia. It is vital that patients are consistently educated regarding diet/nutrition, as elevated blood glucose is a major factor leading to diabetic complications. Patients with T1D often require extensive education to manage their diabetes, including insulin dosing, blood glucose management, and lifestyle adjustments. These points were detailed recently in a guideline targeted specifically at T1D (Chiang et al. 2014).

12.2.3 Non-insulin anti-diabetic agents

Both insulin and lifestyle changes are the common treatment options for both T1D and T2D. However, T2D has more medication options available, including numerous oral medications. Metformin (a biguanide) is typically the first-line agent used in the management of T2D (International Diabetes Federation 2005). Metformin (International Diabetes Federation 2005), being the mainstay of therapy for T2D, works by lowering glucose produced in the liver without increasing insulin production; therefore, there is low risk to develop hypoglycemia. Metformin, which may be beneficial in diabetic patients who are overweight/obese, is typically considered weight neutral. Metformin has been shown to be effective in combination with most other anti-diabetic agents including insulin (Yin et al. 2014). Although numerous claims have linked metformin with lactic acidosis, a 1-year longitudinal study in patients with T2D treated with metformin (compared diet therapy only) resulted in zero cases of lactic acidosis, with no significant difference in patient outcome (Cryer et al. 2005).

The most common non-insulin second- and third-line treatment options from the American Diabetes Association and European Association for the Study of Diabetes for T2D include sulfonylureas (SUs), thiazolidinediones (TZDs), dipeptidyl peptidase-4 (DPP-4) inhibitors, glucagon-like peptide-1 receptor agonists (GLP-1 RA), and sodium-glucose co-transporter 2 (SGLT2) inhibitors (International Diabetes Federation 2005). SUs (e.g., glimepiride, glipizide, and glyburide) bind to the sulfonylurea receptor in the pancreatic β-cell plasma membrane, leading to closure of the ATP-sensitive potassium channel, resulting in stimulation of insulin hormone release (Al-Romaiyan et al. 2013). Although SUs are known for their clinical effectiveness and cost-effectiveness,

they are associated with weight gain and hypoglycemia (Inzucchi et al. 2015). Additionally, as SU function is dependent on β-cells, SUs may progressively lose effect in T2D, as progressive disease results in progressive loss of β-cell function. TZDs (e.g., pioglitazone and rosiglitazone) are peroxisome proliferator-activated receptor-γ agonists and decrease insulin resistance in the periphery and liver Actos® (package insert). This results in increased insulin-dependent glucose disposal and decreased hepatic glucose output. Unlike sulfonylureas, TZDs do not stimulate insulin release and are therefore low risk for hypoglycemia. However, TZDs are contraindicated in New York Heart Association Class III and IV heart failure due to their effects on fluid retention. TZDs are also associated with weight gain. DPP-4 inhibitors (e.g., sitagliptin, saxagliptin, linagliptin, and alogliptin) slow inactivation of incretin hormones involved in glucose homeostasis (Green et al. 2015). DPP-4 inhibitors remain high-cost option in the United States, as they are only available as brand name medications. Recent studies have raised concern regarding DPP-4 inhibitors link with heart failure; however, results in multiple large-scale cardiovascular safety trials are conflicting between different agents (Scirica et al. 2013; White et al. 2013; Green et al. 2015). SGLT-2 inhibitors (e.g., canagliflozin, dapagliflozin, and empagliflozin) decrease renal glucose reabsorption, thereby stimulating urinary glucose excretion (Amaryl® [package insert] 2009). SGLT-2 inhibitors are associated with dehydration, as glucose functions as osmotic diuretic and increases risk of genitourinary infections. SGLT-2 inhibitors remain high-cost option in the United States, as they are only available as brand name medications. More recently, canagliflozin has been associated with an increase in bone fracture risk, and empagliflozin has been associated with a decrease in cardiovascular death and all-cause mortality (Watts et al. 2015; Zinman et al. 2015). Finally, GLP-1 RAs (i.e., exenatide, liraglutide, albiglutide, and dulaglutide) help maintain glucose homeostasis through increased glucose-dependent insulin release, decreased glucose-dependent glucagon secretion, and decreased gastric emptying rate (Victoza package insert). As insulin release is glucose dependent and glucagon secretion is not impaired at low glucose concentration, GLP-1 RAs are low risk for hypoglycemia. GLP-1 RAs also consistently result in weight loss for patients (InterAct Consortium 2012). However, they are high-cost option, injectable only, and can result in significant gastrointestinal disturbance (Inzucchi et al. 2015).

12.3 Nutraceutical approach

Nutraceutical have shown promises in the management of diabetes. Nutraceutical are either taken as oral supplements or as a *functional food*. Stephen Defelice, MD, first coined the term nutraceutical in 1989 as a combination of *nutrition* and *pharmaceutical* (Brower 1998). He stated that these are functional foods that convey many health benefits against numerous diseases. Since these are naturally occurring ingredients, there are many advantages including low cost and reduced/no side effects (Chauhan et al. 2013). As society becomes more

health-focused, nutraceutical may offer preventative benefit from developing complications of chronic diseases. Nutraceuticals contain essential ingredients such as amino acids, vitamins, and minerals that combat and prevent chronic diseases. Nutraceuticals are also known for their specific regional availability. One nutraceutical example is the extract found from the *Morinda lucida* plant. *M. lucida* is commonly found in Africa and not in the United States. The use of nutraceuticals as it relates to maintaining health should be considered in conjunction with lifestyle adjustments (Shori 2015). Lifestyle adjustments include dietary changes and, as it relates to diabetes, a focus on consuming more functional foods with anti-diabetic properties. It is highly recommended that each diabetic individual consults his or her nutritionist as well as primary care physician to identify quality nutritional recommendations (Hamdy 2015). As discussed in the following section, nutraceuticals may be beneficial in different aspects/targets in the prevention of T2D, and therapeutic lifestyle changes are required following the diagnosis of T2D.

12.3.1 Therapeutic benefits

There are many targets for nutraceutical intervention to improve outcomes in patients with diabetes. Potential targets for therapy include enzymes responsible for hyperglycemia, lowering toxic glucose output, blood pressure, and inflammation. Nutraceuticals may also directly influence insulin secretion to aid in glucose hemostasis. There are numerous enzymes found throughout our digestive system that break down food for absorption. One of the enzymes responsible for producing glucose is α-amylase. This specific enzyme breaks down glycogen and starch, converting them into glucose and maltose (Pugh 2000). Throughout the brush border of the small intestine, α-glucosidase converts disaccharides to monosaccarides (e.g., glucose) to facilitate absorption (MeSH Descriptor Data 1992; Shori 2015). Both of these enzymes lead to an increased postprandial glucose level. Many nutraceuticals and functional foods have been studied to inhibit these two enzymes to slow glucose absorption. Functional foods that contain phenolic compounds have been found to inhibit α-amylase and α-glucosidase, as well as angiotensin-converting enzymes (ACE) (Manteiga et al. 1997). Note that controlled blood pressure could delay the progression of diabetic complications such as nephropathy.

Aside from targeting enzymes, nutraceuticals have other strategies including antioxidants, weight management, and many others to prevent further diabetic complications. World Health Organization (WHO) has advised the use of medicinal plants as they have less or no side effects compared with allopathic medicine (Day 1998). The nutraceuticals discussed will vary from medicinal plants, tea, and even spices.

12.3.1.1 Medicinal plants

Morinda lucida is a plant from West Africa and commonly called Brimstone tree. It is known for its role in diabetes, as it reduces blood glucose level

(Olajide 1999; Odutuga 2010). The extract itself contains active compounds such as flavonoids, saponins, and tannins. These compounds contribute toward the inhibition of α-amylase and α-glucosidase (Kazeem et al. 2013). Aside from *M. Lucida*, other plants such as *Irvingia gabonensis* (*O'Rorke*) *Bail*, *Eucalyptus torreliana*, and *Securidata longependuculata* Fers. also inhibit α-glucosidase activity (Ogunwande et al. 2007). Phenolic compounds have also been shown to exhibit antioxidant effects. Several medicinal plants such as Molle (*Schinus molle*), Zarzaparrilla (*Smilax officinalis*), Cat's claw (*Uncaria tomentosa*), and Chancapiedra (*Phyllantus niruri* L.) carry anti-α-glucosidase activities. Several medicinal plants such as *Maca* and *Caigua* also inhibit ACE.

Pterocarpus marsupium is a well-known plant drug with its Indian and Ayurvedic roots. *P. marsupium* belongs to a group called *rasayana*, which is common for its antioxidant properties (Govindarajan et al. 2005). Since oxidative stress had been linked to hyperglycemic state, this medicinal plant could modulate the immunological signal associated with inflammation (Manning et al. 2004; Srinivasan et al. 2010). The extract of *P. marsupium* contains flavonoid fraction, which is found to regenerate pancreatic β-cells (Chakravarty et al. 1980).

Sweet potato (*Ipomoea batatas*) commonly found in Central America and also South America provides glucose control in diabetes. In human study, patients who were given high-dose *I. batatas* displayed lowered glucose concentrations compared with the patients who received placebo (Meinert and Susan 1986). In addition, treated patients displayed higher insulin sensitivity along with lowered LDL cholesterol. *I. batatas*'s leaf and stem extract contains polyphenol caffeoylquinic acid (CQA), which helps regulate blood glucose. CQA directly triggers the production of glucagon-like peptide-1, influencing insulin production (Nagamine et al. 2014).

Fenugreek seed (*Trigonella foenum-graecum*) is a common ingredient found in Indian dishes, also called *methi*. Studies found that when these seeds were supplemented with pharmaceutical anti-diabetic medications, glycated hemoglobin (Hba1) was significantly lowered by 0.9% ($p < 0.001$) when compared with those who were only taking anti-diabetic medications (Ansari and Saiqaa 2011). A 3-year study examined the likelihood of patients with prediabetes to develop diabetes (Gaddam et al. 2015). In the fenugreek treatment group ($n = 74$), 5 g of fenugreek was given twice a day, while the control group ($n = 66$) received none. After 3 years, patients taking fenugreek had a lowered risk for developing diabetes (23%) compared with those assigned to control (55%). Fenugreek seeds not only help normalize blood glucose level but also increase insulin secretion.

Increasing functional pancreatic β-cells is essential in managing diabetes, as it leads to increase in insulin production; a nutraceutical called *Gurmar* (*Gymnema sylvestre*) does this (Yeh et al. 2003; Saxena and Vikram 2004). Gurmar, most commonly found in India and across southeast Asia, has been

used to treat diabetes for almost 2000 years. The extract, generated from the Gurmar leaf, contains gymnemic acid, which has anti-inflammatory and anti-diabetic properties. Gymnemic acid contains several saponins and also suppresses *sweet* taste, decreasing glucose intake (Sahu et al. 1996) Gurmar's anti-diabetic properties also help regulate blood glucose by enhancing enzymes responsible for glucose breakdown (Shanmugasundaram et al. 1983). What makes Gurmar different than all the other nutraceuticals discussed is its ability to influence insulin release. *G. sylvestre*'s extract called OSA® was found to directly influence β-cell lines to produce insulin (Liu et al. 2009). This action has also been replicated in an obese mouse model with hyperglycemia. It was concluded that glucose homeostasis was restored due to increased insulin secretion and decreased glucose absorption (Al-Romaiyan et al. 2013).

Known for its vitamin and nutrient-rich (including protein!) (Padmavathi and Rao 1990) leaf, *Sauropus androgynous* is widely used in southeast Asia for its anti-diabetic properties. Also known as Sweet Leaf Bush, *S. androgynous* has been widely used in southeast Asia to treat wound, urinary infection, and fever and even improving lactation for women (Benjapak et al. 2008; Bhaskare et al. 2009; Soka et al. 2010). A recent study has shown a marked reduction in blood glucose in diabetes-induced induced mice compared with the placebo group (Kumar and George 2015). In addition to controlling glycemic level, *S. androgynous* is a potent antioxidant due to the high level of flavonoids (Andarwulan et al. 2010).

12.3.1.2 Fruit

Momordica charantia goes by many names, such as bitter melon, kerela, balsam pear, and so on. It had been used to treat diabetes in regions throughout Asia, South America, East Africa, and the Caribbean (Cousens 2008). *M. charantia* is rich in vitamins, antioxidant, and minerals and a reliable source of fiber (Bakare et al. 2010). AMP protein kinase has many roles in metabolic regulations and one of them includes glucose uptake. *M. charantia*'s stem contains cucurbitane triterpenoid, which could increase AMP protein kinase activity (Chang et al. 2008; Tan et al. 2008). This in turn would enhance glucose uptake by increasing GLUT4 translocation (Xu et al. 2015). In a study with diabetic mice, a significant body weight loss was also seen (Tan et al. 2008).

Garcinia cambogia is a tropical fruit from Indonesia and South India. The fruit itself contains an hydroxycitric acid that aids in weight loss by suppressing appetite and reducing the body's ability to make adipocytes (Sethi 2011). Studies have shown that weight loss decreases the risk of diabetes (Herman 2015).

12.3.1.3 Herbal tea

Herbal teas have remained popular in the United States for decades, and research has revealed potential health benefits (Manteiga et al. 1997). A study in the

United Kingdom found that those who drink herbal tea, at least four cups per day compared with non-tea drinkers, had a 16% lower risk for developing T2D (InterAct Consortium 2012). *Chamomile tea* (*Matricaria chamomilla*) is one of the most popular herbal teas with documented health benefits. Chamomile tea carries numerous bioactive compounds, such as umbelliferone, *herniarin*, *esculetin*, *isoscopoletin*, *apigenin*, and so on, which may alleviate diabetic symptoms due to its anti-inflammatory nature (McKay and Jeffrey 2006; Peña et al. 2006). Sorbitol along with hepatic glycogen levels decreased among diabetes-induced rats when exposed to these compounds (Kato et al. 2008). These effects may be beneficial, as diabetes is associated with an inflammatory state, and sorbitol accumulation throughout the body may lead to complications such as nephropathy, neuropathy, and retinopathy. Chamomile tea therefore may be protective against some long-term complications of diabetes. Other teas such as *Linden tea tilo* (*Tilia platyphyllos*) and *Boldo* (*Peumus boldus*) also inhibit α-glucosidase (Ogunwande et al. 2007).

12.3.1.4 Spices

Another polyphenol-rich spice with flavorful aroma, which gained popularity, is *Cinnamon* (*Cinnamomum cassia*). Cinnamon supplements are highly in demand due to their role as antioxidants with anti-inflammatory properties (Brahmachari et al. 2009; Aggarwal 2010). This makes cinnamon suitable for adjunctive therapy in diabetes, with potential benefits regarding long-term complications. One study in patients with T2D compared groups given different doses of cinnamon or placebo (Khan et al. 2003). It was found that patients who were given 1–6 g of cinnamon per day had reduced fasting serum glucose, triglycerides, LDL-cholesterol, and total cholesterol. However, skepticism exists regarding these findings, as all patients were taking a sulfonylurea and the results were not compared to the placebo group (Rafehi et al. 2012). Cinnamon is thought to function through extracts that increase insulin receptor activation by inhibiting protein tyrosine phosphatase-1 (Imparl-Radosevich et al. 1998).

Garlic (*Allium satvium*) is not only known for its culinary role but its abundant health benefits. Garlic had been used for almost 5000 years by the Egyptians for strength (Moyers 1996). Studies have shown that *A. satvium* may be beneficial in cardiovascular disease by decreasing blood pressure (both systolic and diastolic) along with reducing the rate of carotid artery thickening (Ashraf et al. 2013; Mahdavi-Roshan et al. 2015). This may have benefit in diabetic patients, as complications of diabetes include atherosclerosis and hypertension. *A. satvium* contains abundant molecules including sulfur-based compounds, oil, and vitamins (Kumar et al. 2013). One study ($n = 60$) compared combination of *A. satvium* with metformin to metformin alone. Both groups showed significantly reduced fasting blood glucose ($p < 0.01$); however, patients receiving both metformin and garlic had a greater reduction ($p < 0.05$) (Kumar et al. 2013).

Ginger (*Zingiber officinale*), with its origin in southeast Asia, has been widely used for culinary purposes mostly in India and China (Wang and Wang 2005). In addition, it also served medicinal purposes of relieving pain, vomiting, cold, and indigestions (Wang and Wang 2005). Numerous studies have been done to evaluate the ability of ginger in treating diabetes. The pharmacokinetics of ginger is not limited to only controlling glycemic level but also decreasing cholesterol and increasing insulin level. This shows that ginger focuses on the overall being of the body (Akhani et al. 2004; Al-Amin et al. 2006). A dose of ginger juice was shown to prevent serotonin-induced hyperglycemia. The ethyl acetate extract of the ginger was able to inhibit α-glucosidase (Priya Rani et al. 2011). Diabetes-induced rats given ethanolic ginger extracts for 30 days showed decreased carbohydrate level (Shanmugam et al. 2009).

Asia *ginseng* (*Panax ginseng*) and American *ginseng* (*Panax quinquefolis*) are the two most common types of ginseng that are researched for their medicinal purposes. These two distinct ginseng species have different effects. The Asian ginseng is found to improve blood flow, decrease fatigue, and serves as antioxidant (Zhao et al. 2004). The American ginseng provides anti-aging effects and improves digestions (Vuksan et al. 2000). These two ginsengs were found to differ in terms of the active compound called glycosides. Ginseng were found to reduce and maintain blood glucose level for both diabetic and nondiabetic patients (Vuksan et al. 2008; Luo and Luo 2009; Shishtar et al. 2014). This could also be explained by its ability to improve and protect pancreatic β-cells from IL-4-induced apoptosis along with reduced insulin resistance (Luo and Luo 2006, 2009). Uncoupling protein 2 (UCP-2), found in the inner of mitochondria, inhibits insulin production (Zhang et al. 2001). Ginseng is able to reduce UCP-2, thereby increasing insulin production (Luo and Luo 2006).

The list of all those spices discussed that have received recognition for their antidiabetic properties would not be complete without turmeric (*Curcuma longa*). A study was done to assess the benefits of turmeric in diabetes. Over the course of 9 months, daily dosage of turmeric had prevented prediabetic patients to develop diabetes (Chuengsamarn et al. 2012). Turmeric could also regenerate damaged pancreatic β-cells in mice induced with type 1 diabetes (Aziz et al. 2013). A recent study has shown that turmeric itself could aid obese patients with type 2 diabetes. *C. longa* inhibits sterol regulatory element binding proteins (SERPs). SERP is responsible for producing cholesterol, triglycerides, and fatty acids, and this inhibition was found to prevent obesity and insulin resistivity.

12.3.1.5 Others

Similar to cardiovascular diseases, diabetes is a disorder in which reactive oxidative species (ROS) plays a major role in inflammation, which also increases C-reactive protein. *Honey* was shown to have a phenolic compound that reduces oxidative species (Frankel et al. 1998). While all honey is expected to contain antioxidants, darker honeys contain greater amounts of antioxidants (Frankel et al. 1998).

Abound® formula contains β-*hydroxy*-β-*methylbutyrate, arginine*, and *glutamine*. These three amino acids have important roles in accelerating wound healing (Alon et al. 2001; Wu et al. 2009) and may play a role in managing foot ulcers, a complication of diabetes associated with significant morbidity and economic impact. Typically, diabetic foot ulcers heal slowly due to poor blood circulation and inflammation. One retrospective study of 11 dialysis-dependent diabetic patients with foot ulcers examined the effect of 4-week treatment with β-hydroxy-β-methylbutyrate, arginine, and glutamine on wound healing. Following treatment, seven patients had improved wound depth and eight patients had improved wound appearance. No deterioration was observed in any patient (Sipahi et al. 2013).

12.4 Conclusion

The management of diabetes requires a multifaceted approach targeted at achieving evidence-based glycemic goals. Metformin remains the first-line pharmacologic agent to treat T2D and insulin being the last resort. The purpose of this chapter is to inform the efficacy of nutraceuticals in managing diabetes. In addition to its ability to maintain baseline glucose level, nutraceuticals have anti-inflammatory properties, control cholesterol level, protect pancreatic β-cells, and even aid in weight loss. Several nutraceuticals such as *G. sylvestre*'s gymnemic acid along with sweet potato's (*I. batatas*) CQA may aid glucose control, secondary to their effects on influencing insulin release (Kurihara 1992; Sahu et al. 1996; Liu et al. 2009). Herbal teas have shown promise due to their significant anti-inflammatory properties (McKay and Jeffrey 2006; Peña et al. 2006).

Nutraceuticals have less risk of developing side effects compared with allopathic medicine. In addition, nutraceuticals can be used along with the prescribed medication (e.g., metformin, SU), which displays significantly better result (Khan et al. 2003; Ansari and Saiqaa 2011; Kumar et al. 2013). Another benefit of nutraceutical is that unlike prescribed medications, they don't cause hypoglycemia. Recall that diabetic patients treated with ginseng had significantly lower blood glucose compared with healthy individuals (Shishtar et al. 2014). More studies are needed to assess the specific roll of nutraceuticals in the management of diabetes.

References

Actos® (package insert). Takeda Pharmaceuticals America, Deerfield, IL. Highlights of prescribing information. Available from http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/021073s043s044lbl.pdf. Accessed: January 12, 2015.

Aggarwal, B. B. 2010. Targeting inflammation-induced obesity and metabolic diseases by curcumin and other nutraceuticals. *Ann Rev Nutr* 30:173.

Akhani, S. P., S. L. Vishwakarma, and R. K. Goyal. 2004. Antidiabetic activity of Zingiber officinale in streptozotocininduced type I diabetic rats. *J Pharm Pharmacol* 56 (1):101–105.

Al-Amin, Z. M., M. Thomson, K. K. Al-Qattan, and M. Ali. 2006. Anti-diabetic and hypolipidaemic properties of ginger (*Zingiber officinale*) in streptozotocin-induced diabetic rats. *Br J Nutr* 96 (4):660–666.

Alon, T., B. Debasis, and G. P. Harry. 2001. Supplementing with beta-hydroxy-beta-methylbutyrate (HMB) to build and maintain muscle mass: A review. *Res Commun Mol Pathol Pharmacol* 111 (1–4):139–151.

Al-Romaiyan, A. et al. 2013. A novel extract of *Gymnema sylvestre* improves glucose tolerance *in vivo* and stimulates insulin secretion and synthesis *in vitro*. *Phytother Res* 27 (7):1006–1011.

Amaryl® [package insert]. 2009. Sanofi-aventis US. LLC., Bridgewater, NJ. Available from https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/020496s021lbl.pdf. Accessed: January 12, 2015.

American Diabetes Association. 2015a. Introduction—2015. *Diabetes Care* 38: S1–S93.

American Diabetes Association. 2015b. 6. Glycemic targets. *Diabetes Care* 38 (1):S33–S40.

American Diabetes Association. 2015c. 7. Approaches to glycemic treatment. *Diabetes Care* 38 (1):S41–S48.

Andarwulan, N., R. Batari, D. A. Sandrasari, B. Bolling, and H. Wijaya. 2010. Flavonoid content and antioxidant activity of vegetables from Indonesia. *Food Chem* 121 (4):1231–1235.

Ansari, R. and A. Saiqaa. 2011. *Effectiveness of Fenugreek for Lowering Hemoglobin* (*HbA1c*) *in Patients with Self-Management of Type 2 Diabetes: A Randomized Controlled Trial*. INTECH Open Access Publisher.

Ashraf, R. et al. 2013. Effects of Allium sativum (garlic) on systolic and diastolic blood pressure in patients with essential hypertension. *Pak J Pharm Sci* 26 (5):859–863.

Aziz, M. T. A., M. F. El-Asmar, A. M. Rezq, S. M. Mahfouz, M. A. Wassef, H. H. Fouad, H. H. Ahmed, and F. M. Taha. 2013. The effect of a novel curcumin derivative on pancreatic islet regeneration in experimental type-1 diabetes in rats (long term study). *Diabetol Metab Syndr* 5 (1):75.

Bakare, R. I., O. A. Magbagbeola, A. I. Akinwande, and O. W. Okunowo. 2010. Nutritional and chemical evaluation of Momordica charantia. *J Med Plants Res* 4 (21):2189–2193.

Benjapak, N., P. Swatsitang, and S. Tanpanich. 2008. Determination of antioxidant capacity and nutritive values of Pak-Wanban (*Sauropus androgynus* L. Merr.). *Khon-Kean University Sci J* 36:279–289.

Bhaskar, A., K. V. Ramesh, and S. Rajeshwari. 2009. Wound healing profile of sauropus androgynus in wistar rats. *J Nat Remed* 9 (2):159–164.

Brahmachari, S., J. Arundhati, and P. Kalipada. 2009. Sodium benzoate, a metabolite of cinnamon and a food additive, reduces microglial and astroglial inflammatory responses. *J Immunol* 183 (9):5917–5927.

Brower, V. 1998. Nutraceuticals: poised for a healthy slice of the healthcare market?. *Nat Biotechnol* 16:728–732.

Chakravarty, B. K., S. Gupta, S. S. Gambhir, and K. D. Gode. 1980. Pancreatic beta cell regeneration. A novel antidiabetic mechanism of *Pterocarpus marsupium Roxb*. *Indian J Pharmacol* 12:123–127.

Chang, C.-I., C.-R. Chen, Y.-W. Liao, H.-L. Cheng, Y.-C. Chen, and C.-H. Chou. 2008. Cucurbitane-type triterpenoids from the stems of momordica charantia. *J Nat Prod* 71 (8):1327–1330.

Chauhan, B. et al. 2013. Current concepts and prospects of herbal nutraceutical: A review. *J Adv Pharm Technol Res* 4 (1):4.

Chiang, J. L. et al. 2014. Type 1 diabetes through the life span: A position statement of the American diabetes association. *Diabetes Care* 37 (7):2034–2054.

Choi, K. and K. Young-Bum. 2010. Molecular mechanism of insulin resistance in obesity and type 2 diabetes. *Korean J Int Med* 25 (2):119–129.

Chuengsamarn, S., S. Rattanamongkolgul, R. Luechapudiporn, C. Phisalaphong, and S. Jirawatnotai. 2012. Curcumin extract for prevention of type 2 diabetes. *Diabetes Care* 35 (11):2121–2127.

Cousens, G. 2008. *There is a Cure for Diabetes: The Tree of Life 21 Day Program*. Berkeley, CA: North Atlantic Books, pp. 191–192.
Cryer, D. R., S. P. Nicholas, D. H. Henry, D. J. Mills, and B. V. Stadel. 2005. Comparative outcomes study of metformin intervention versus conventional approach the COSMIC approach study. *Diabetes Care* 28 (3):539–543.
Day, C. 1998. Traditional plant treatments for diabetes mellitus: Pharmaceutical foods. *Br J Nutr* 80 (1):5.
Diabetes Fact Sheet. WHO. n.p., October 2013, July 15, 2015.
Evans, J. L. and M. Bahng. 2000. Non-pharmaceutical intervention options for type 2 diabetes: Diets and dietary supplements (Botanicals, Antioxidants, and Minerals).
Filippi, C. M. and M. G. Von Herrath. 2008. Viral trigger for type 1 diabetes: Pros and cons. *Diabetes* 57 (11):2863–2871.
Frankel, S., G. E. Robinson, and M. R. Berenbaum. 1998. Antioxidant capacity and correlated characteristics of 14 unifloral honeys. *J Apicul Res* 37 (1):27–31.
Gaddam, A. et al. 2015. Role of fenugreek in the prevention of type 2 diabetes mellitus in prediabetes. *J Diabetes Metab Disord* 14 (1):1–10.
Ginger. 2016. *Encyclopædia Britannica. Encyclopædia Britannica Online*, May 12.
Govindarajan, R., M. Vijayakumar, and P. Pushpangadan. 2005. Antioxidant approach to disease management and the role of "Rasayana" herbs of ayurveda. *J Ethnopharmacol* 99 (2):165–178.
Green, J. B. et al. 2015. Effect of sitagliptin on cardiovascular outcomes in type 2 diabetes. *N Engl J Med* 373 (3):232–242.
Hamdy, O. 2015. Diabetic ketoacidosis treatment and management. *Medscape*. WebMD, 13 Aug. 2015. Web. 14 Oct. 2015.
Herman, W. H. 2015. The cost-effectiveness of diabetes prevention: Results from the diabetes prevention program and the diabetes prevention program outcomes study. *Clin Diabetes Endocrinol* 1 (1):9.
Imparl-Radosevich, J. et al. 1998. Regulation of PTP-1 and insulin receptor kinase by fractions from cinnamon: Implications for cinnamon regulation of insulin signalling. *Horm Res Paediatr* 50 (3):177–182.
InterAct Consortium. 2012. Tea consumption and incidence of type 2 diabetes in Europe: The EPIC-InterAct case-cohort study. *PLoS One* 7 (5):e36910.
International Diabetes Federation. 2005. Clinical guidelines task force. Chapter 9: Glucose control: Oral therapy. *Global Guideline for Type 2 Diabetes*. Belgium, Germany: International Diabetes Federation, 2005. n.p. Print.
Inzucchi, S. E. et al. 2015. Management of hyperglycemia in type 2 diabetes, 2015: A patient-centered approach: Update to a position statement of the American diabetes association and the European association for the study of diabetes. *Diabetes Care* 38 (1):140–149.
Kato, A. et al. 2008. Protective effects of dietary chamomile tea on diabetic complications. *J Agric Food Chem* 56 (17):8206–8211.
Kazeem, M. I., J. O. Adamson, and I. A. Ogunwande. 2013. Modes of inhibition of α-amylase and α-glucosidase by aqueous extract of *Morinda lucida* Benth leaf. *BioMed Res Int*.
Key Findings. 2014. International Diabetes Federation. n.p., n.d. July 15, 2015.
Khan, A. et al. 2003. Cinnamon improves glucose and lipids of people with type 2 diabetes. *Diabetes Care* 26 (12):3215–3218.
Kim, E. S. and L. P. Greg. 2015. AFREZZA® (insulin Human) inhalation powder: A review in diabetes mellitus. *Drugs* 75 (14):1679–686.
Kumar, P. R. and P. George. 2015. Antidiabetic effect of *Sauropus androgynus* L. leaves in alloxan induced diabetic mice. *J Pure Appl Microbiol* 9 (3):2565–2570.
Kumar, R. et al. 2013. Antihyperglycemic, antihyperlipidemic, anti-inflammatory and adenosine deaminase–lowering effects of garlic in patients with type 2 diabetes mellitus with obesity. *Diabetes, Metab Syndr Obes: Tar Ther* 6:49.
Kurihara, Y. 1992. Characteristics of antisweet substances, sweet proteins, and sweetness-inducing proteins. *Crit Rev Food Sci Nutr* 32 (3):231–252.

Liu, B. et al. 2009. Characterisation of the insulinotropic activity of an aqueous extract of *Gymnema sylvestre* in mouse β-cells and human islets of Langerhans. *Cell Physiol Biochem* 23 (1–3):125–132.
Luo, J. Z. and L. Luo. 2006. American ginseng stimulates insulin production and prevents apoptosis through regulation of uncoupling protein-2 in cultured β cells. *Evid-Based Compl Altern Med* 3 (3):365–372.
Luo, J. Z. and L. Luo. 2009. Ginseng on hyperglycemia: Effects and mechanisms. *Evid Based Compl Altern Med* 6:423–427.
Mahdavi-Roshan, M. et al. 2015. Effect of garlic powder tablet on carotid intima-media thickness in patients with coronary artery disease a preliminary randomized controlled trial. *Nutr Health*: 0260106014563446.
Manning, P. J., S. A. De Jong, W. H. Sultherland, M. McGrath, and S. M. Williams. 2004. Changes in circulating postprandial proinflammatory cytokine concentration in diet-controlled type 2 diabetes and the effect of ingested fat. *Diabetes Care* 27:2509–2511.
Manteiga, R., L. P. Douglas, and S. A. Syed. 1997. Risks associated with consumption of herbal teas. *Reviews of Environmental Contamination and Toxicology*. NY: Springer New York, pp. 1–30.
Maureen Barlow Pugh, Ed. 2000. *Stedman's Medical Dictionary* (27th Ed.). Baltimore, MA: Lippincott Williams & Wilkins, p. 65.
McKay, D. L. and B. B. Jeffrey. 2006. A review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita* L.). *Phytother Res* 20 (8):619–633.
McKendry, J. B., K. Kuwayti, and P. P. Rado. 1959. Clinical experience with DBI (Phenformin) in the management of diabetes. *Can Med Assoc* 10th ser. 80:773–778.
Meinert, C. L. and T. Susan. 1986. *Clinical Trials: Design, Conduct, and Analysis*. NY: Oxford UP, pp. 53–54.
MeSH Descriptor Data. 1992. *National Library of Medicine—Medical Subject Headings*, February 26, 1992. *XP002553615*.
Moyers, S. 1996. History of garlic. *Garlic in Health, History and World Cuisine*. Petersburg, FL: Suncoast Press St., pp. 1–36.
Nagamine, R. et al. 2014. Dietary sweet potato (*Ipomoea batatas* L.) leaf extract attenuates hyperglycaemia by enhancing the secretion of glucagon-like peptide-1 (GLP-1). *Food and Funct* 5 (9):2309–2316.
Nishida, W., M. Nagata, A. Imagawa, T. Hanafusa, J. Ohashi, K. Takahashi, T. Suehiro. et al. 2014. Insulin administration may trigger type 1 diabetes in Japanese type 2 diabetes patients with type 1 diabetes high-risk HLA class II and the insulin gene VNTR genotype. *J Clin Endocrinol Metabol* 99 (9):E1793–E1797.
Odutuga, A. A., J. O. Dairo, J. B. Minari, and F. A. Bamisaye. 2010. Anti-diabetic effect of *Morinda lucida* stem bark extracts on alloxan-induced diabetic rats. *Res J Pharmacol* 4 (3):78–82.
Ogunwande, I. A. et al. 2007. α-Glucosidase inhibitory profile of Nigerian medicinal plants in immobilized assay system. *Food Sci Technol Res* 13 (2):169–172.
Olajide, O. A. et al. 1999. Evaluation of the anti-diabetic property of morinda lucida leaves in streptozotocin-diabetic rats. *J Pharm Pharmacol* 51 (11):1321–1324.
Padmavathi, P. and M. P. Rao. 1990. Nutritive value of sauropus androgynus leaves. *Plant Foods Hum Nutr* 40 (2):107–113.
Peña, D. et al. 2006. Anti-inflammatory and anti-diarrheic activity of *Isocarpha cubana* Blake. *Pharmacologyonline* 3:744–749.
Priya Rani, M., K. P. Padmakumari, B. Sankarikutty, O. Lijo Cherian, V. M. Nisha, and K. G. Raghu. 2011. Inhibitory potential of ginger extracts against enzymes linked to type 2 diabetes, inflammation and induced oxidative stress. *Int J Food Sci Nutr* 62 (2):106–110.
Rafehi, H., K. Ververis, and T. C. Karagiannis. 2012. Controversies surrounding the clinical potential of cinnamon for the management of diabetes. *Diabetes, Obes Metab* 14 (6):493–499.

Raman, B. V., N. V. Krishna, N. B. Rao, P. M. Saradhi, and B. M. V. Rao. 2012. Plants with antidiabetic activities and their medicinal values. *Int Res J Pharm* 3 (3):11–15.

Rodbard, H. W., M. F. Kathleen, and G. Susan. 2009. Impact of obesity on work productivity and role disability in individuals with and at risk for diabetes mellitus. *Am J Heal Prom* 23 (5):353–360.

Rosenstock, J., D. R. Hassman, R. D. Madder, S. A. Brazinsky, J. Farrell, N. Khutoryansky, and P. M. Hale. 2004. Repaglinide versus nateglinide monotherapy: A randomized, multicenter study. *Diabetes Care* 27 (6):1265–1270.

Sahu, N. P. et al. 1996. Triterpenoid saponins from *Gymnema sylvestre*. *Phytochemistry* 41 (4):1181–1185.

Saxena, A. and N. K. Vikram. 2004. Role of selected Indian plants in management of type 2 diabetes: A review. *J Alter Compl Med* 10 (2):369–378.

Scirica, B. M. et al. 2013. Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. *NEng J Med* 369 (14):1317–1326.

Sethi, A. 2011. A review on garciniacambogia—A weight controll in gagent. *Int Periodontics Restorative Den* 3 (10):13–24.

Shanmugam, K. R., C. H. Ramakrishna, K. Mallikarjuna, and K. S. Reddy. 2009. The impact of ginger on kidney carbohydrate metabolic profiles in STZ induced diabetic rats. *Asian J Exp Sci* 23 (1):127–134.

Shanmugasundaram, K. R. et al. 1983. Enzyme changes and glucose utilisation in diabetic rabbits: The effect of *Gymnema sylvestre*, R. Br. *J Ethnopharmacol* 7 (2):205–234.

Shishtar, E., J. L. Sievenpiper, V. Djedovic, A. I. Cozma, V. Ha, V. H. Jayalath, D. J. A. Jenkins et al. 2014. The effect of ginseng (the genus *Panax*) on glycemic control: A systematic review and meta-analysis of randomized controlled clinical trials. *PLoS One* 9 (9):e107391.

Shori, A. B. 2015. Screening of antidiabetic and antioxidant activities of medicinal plants. *J Integr Med* 13 (5):297–305.

Sipahi, S. et al. 2013. The effect of oral supplementation with a combination of beta-hydroxy-beta-methylbutyrate, arginine and glutamine on wound healing: A retrospective analysis of diabetic haemodialysis patients. *BMC Nephrol* 14 (1):8.

Soka, S., H. Alam, N. Boenjamin, T. W. Agustina, and M. T. Suhartono. 2010. Effect of sauropus androgynus leaf extracts on the expression of prolactin and oxytocin genes in lactating BALB/C mice. *J Nutrigenet Nutrigenomics* 3 (1):31–36.

Sortsø, C., A. Green, P. B. Jensen, and M. Emneus. 2015. Societal costs of diabetes mellitus in Denmark. *Diabetic Med* 6 (10):1150–1166.

Srinivasan, B. P., K. Halagappa, and H. N. Girish. 2010. The study of aqueous extract of Pterocarpus marsupium Roxb. on cytokine TNF-α in type 2 diabetic rats. *Indian J Pharmacol* 42 (6):392.

Tan, M.-J., J.-M. Ye, N. Turner, C. Hohnen-Behrens, C. Q. Ke, C. P. Tang, T. Chen et al. 2008. Antidiabetic activities of triterpenoids isolated from bitter melon associated with activation of the AMPK pathway. *Chem Biol* 15 (5):520.

U.S. Centers for Disease Control and Prevention. 2011 Diabetes public health resource: 2011 national diabetes fact sheet (Internet). Available from http://www.cdc.gov/diabetes/pubs/pdf/ndfs_2011.pdf. Accessed: October 6, 2015.

Vuksan, V., M. P. Stavro, J. L. Sievenpiper, V. Y. Koo, E. Wong, U. Beljan-Zdravkovic, and T. Francis. 2000. American ginseng improves glycemia in individuals with normal glucose tolerance: Effect of dose and time escalation. *J Am Coll Nutr* 19 (6):738–744.

Vuksan, V., M. K. Sung, J. L. Sievenpiper, P. M. Stavro, A. L. Jenkins, M. Di Buono, K. S. Lee et al. 2008. Korean red ginseng (*Panax ginseng*) improves glucose and insulin regulation in well-controlled, type 2 diabetes: Results of a randomized, double-blind, placebo-controlled study of efficacy and safety. *Nutr, Metab Cardiovas Dis* 18 (1):46–56.

Wang, W. H. and Z. M. Wang. 2005. Studies of commonly used traditional medicine-ginger. *Zhongguo Zhongyao Zazhi* 30 (20):1569–1573.

Watts, N. B. et al. 2015. Effects of canagliflozin on fracture risk in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 10 (1):157–166.
White, W. B. et al. 2013. Alogliptin after acute coronary syndrome in patients with type 2 diabetes. *NEng J Med* 369 (14):1327–1335.
Wu, G. et al. 2009. Arginine metabolism and nutrition in growth, health and disease. *Amino Acids* 37 (1):153–168.
Xu, X., B. Shan, C. H. Liao, J. H. Xie, P. W. Wen, and J. Y. Shi. 2015. Anti-diabetic properties of *Momordica charantia* L. polysaccharide in alloxan-induced diabetic mice. *Inte J Biol Macromol* 81:538–543.
Yeh, G. Y. et al. 2003. Systematic review of herbs and dietary supplements for glycemic control in diabetes. *Diabetes Care* 26 (4):1277–1294.
Yin, J., H. Deng, S. Qin, W. Tang, L. Zeng, and B. Zhou. 2014. Comparison of repaglinide and metformin versus metformin alone for type 2 diabetes: A meta-analysis of randomized controlled trials. *Diabetes Res Clin Prac* 105 (3):e10–e15.
Zhang, C. Y., G. Baffy, P. Perret, S. Krauss, O. Peroni, D. Grujic et al. 2001. Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, beta cell dysfunction, and type 2 diabetes. *Cell* 105:745–755.
Zhao, S. J., C. Y. Li, Y. C. Qian, X. P. Luo, X. Zhang, X. S. Wang et al. 2004. Induction of hairy roots of *Panax ginseng* and studies on suitable culture condition of ginseng hairy roots. *Sheng Wu Gong Cheng Xue Bao* 20:215–220.
Zinman, B. et al. 2015. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *NEng J Med* 373 (22):2117–2128.

13

Antioxidant Nutraceuticals

Novel Strategies for Combating Chronic Inflammatory Diseases

Adeleke Badejo and Ashim Malhotra

Contents

13.1 The emergence of nutraceuticals

Recent advancements in our understanding of the causation of human and veterinary disease, and the design and implementation of novel technologies and strategies for battling them, have vastly improved the medical arsenal against global human and animal morbidity and mortality. This has been made possible by an incredible interprofessional endeavor that has included researchers, pharmaceutical companies, small business, and social networking in the creation and popularization of novel approaches to disease state management, coupled with an ever-expanding knowledge base and medical

awareness among laypersons. Interestingly, modern medical practice is no longer limited to cutting-edge technological advancement in the hands of few highly trained and skilled, and therefore somewhat relegated, technical specialists. The merger of many fields of human knowledge has allowed modern researchers and clinicians to blend different ideologies and try novel strategies to overcome disease-related morbidity and mortality.

Examples of this multimodal interprofessional approach abound in modern medical practice. This approach can be seen in the treatment of aggressive and recalcitrant cancers, such as pancreatic adenocarcinoma, which has a very poor 5-year prognosis after detection and a high mortality rate. Until the mid-to-late 1990s, the antimetabolite drug gemcitabine was the corner stone of pancreatic adenocarcinoma therapy, but it was not extremely effective *per se*. In an attempt to bolster the anti-cancer effect of this drug, many research groups across the world tried different combinations of drugs and natural products in consort with gemcitabine. Some have proven effective, and further research is being carried out. This strategy of adopting a combinatorial therapeutic approach to achieve maximal drug benefits is now gaining rapid traction in the treatment of other complex and recalcitrant diseases. The amalgamation of drug regimens with the incorporation of natural products and modified dietary components recognizes the importance of social factors and behavior such as diet and lifestyle contribution to human health and disease. This holistic approach of pharmaceuticals and natural cures has given birth to the nutraceutical movement.

The idea underlying the development and therapeutic usage of nutraceuticals is a simple one—many nutraceuticals are dietary components, regularly and easily available from various food products, which have been *enhanced* in their physiological activity or effects either by pharmaceutical formulation-based modification or by other means. Thus, the term nutraceutical covers vast ground—including both commonly occurring and ubiquitous phytochemicals and animal vitamins and products, and some more exotic and less-common phytochemicals, with the important difference that compared with their normal quantities in every day meals, they are provided in higher concentrations, or in more purified form, or with extensive pharmaceutical manipulation to increase their bioavailability.

13.1.1 Nutraceuticals: Definition and first use

The consumption of particular foods or their constituent components either through specific diets, or by the addition of supplements, with the specific goal to provide medical or health benefits, including the prevention and treatments of particular diseases, is the overall premise underlying the nutraceutical field. The therapeutic effectiveness of nutraceuticals has been investigated for organ system disorders as disparate as cardiovascular, diabetic, metabolic, and inflammatory disease, suggesting the universality of its implementation and usage (DiGiacomo et al. 1989).

Historically, one of the first nutraceuticals studied for its potential benefit in amelioration of disease progression was a fatty acid called eicosapentaenoic acid found in fish oil. Preliminary studies conducted to elucidate the biological activity of eicosapentaenoic acid hinted at its interference with the cellular arachidonic acid metabolism pathway, suggesting that this dietary component possessed anti-inflammatory properties (Prescott 1984).

13.1.2 Eicosapentaenoic acid: An anti-inflammatory fish oil fatty acid

Eicosapentaenoic acid is a member of the 20-carbon family of oxygenated fatty acids referred to as eicosanoids. Eicosanoids are derived from arachidonic acid, a major component of phospholipids in the cell membrane, where they are released from the phospholipids through phospholipase A_2 and other phospholipases. Arachidonic acid is then metabolized through three separate pathways resulting in different families of biological cell mediators. Depending on the end product of these pathways, metabolites of arachidonic acid can contribute to the inflammation through mediators such as thromboxane A2 (TXA2) and leukotriene B4 (LTB4), or attenuate the inflammatory response as with prostacyclin (PGI2) (Agarwal et al. 2009). Eicosapentaenoic acid has been shown to interfere with the conversion of arachidonic acid within the cyclooxygenase and lipoxygenase pathways, which resulted in diminished prostaglandin and leukotriene production, thereby producing an anti-inflammatory effect (Needleman et al. 1979; Lee et al. 1984; Prescott 1984). These findings led to a rapid focus on the use of fish oil as a therapeutic adjuvant for the treatment of diseases that have an inflammatory component. One such trial was conducted by Kremer and colleagues to determine if fish oil supplements could relieve the symptoms associated with rheumatoid arthritis (RA). Thirty-three patients, all with active disease, were given either fish oil supplements or placebo in addition to their prescribed treatment regimen. Out of the 33 subjects, there were 25 women and 8 males, with the average age of participants being 56.8 years, ranging from 23 to 74 years old. The supplements were administered for 4 weeks, followed by a washout period to prepare patients to crossover from fish oil supplements to placebo and vice versa. All patients were treated with either aspirin or a nonsteroidal anti-inflammatory drug (NSAID) and instructed to maintain any additional medications they were prescribed. Patients who received fish oil supplements not only showed significant improvement in the number of inflamed joints but also improved assessment scores of the overall severity of the disease. In addition to symptom relief in response to the fish oil, the clinical improvements were shown to remain statistically significant as compared to the patients' own baseline values following the 4-week washout period. These findings indicated that not only did fish oil supplements provide relief to patients with RA, but their benefits were sustained weeks after use was discontinued (Kremer et al. 1987).

Similar studies have evaluated the benefits of fish oil supplementation in patients with other inflammatory disorders such as psoriasis, where again an overall lasting therapeutic benefit of nutraceutical supplementation were in evidence (Belch et al. 1988; Gupta et al. 1989; Kragballe and Fogh 1989). The belief that nutraceuticals could potentially provide better outcomes, either as an adjuvant or as an alternative to current therapies, has led to extensive research in this field in recent years. Further, since nutraceuticals are derived from natural sources, they are believed to possess low toxicity profiles and cause minimal adverse effects as opposed to conventional synthetic pharmaceuticals.

13.1.3 The biological impact of reactive oxidative species and antioxidants

13.1.3.1 Reactive oxygen species

Definition: Free radicals are elements or compounds that have an unpaired electron in their atomic or molecular orbitals. These unpaired electrons make free radicals highly reactive with other compounds and molecules. Reactive oxygen species (ROS) are an important cellular example of biological free radicals found in almost all eukaryotic cells. ROS are formed when oxygen acting as an electron acceptor forms oxygen free radicals. However, ROS are not the only chemically unstable species in the cellular milieu. Separate from ROS are reactive nitrogen species (RNS) formed from the nitric oxide free radical. Nitric oxide is a highly permeable gas that is an important second messenger in humans, especially in the cardiovascular system, where it regulates vascular relaxation.

ROS and RNS are naturally occurring by-products of cellular metabolism. At physiological levels, ROS are known to regulate a variety of cellular pathways, such as the mitogen-activated protein kinase (MAPK) and the phosphoinositide 3-kinase (PI3K) pathways (Ray et al. 2012). However, if left unchecked, at higher concentrations, these species can cause cellular and tissue damage and disease through direct DNA damage, in addition to the unintended oxidation of proteins and lipids. To prevent the elevation of ROS concentration to pathological levels, the body has a natural defense system composed of antioxidants that protects against the damaging oxidation by ROS.

13.1.3.2 Antioxidants

Antioxidants may be defined as substances that prevent the oxidation of a substrate. Interestingly, this definition is broad enough to encompass starkly different molecular species; thus, both cellular enzymes and complex bioorganic chemicals can act as antioxidants. Examples of enzymes that produce antioxidant physiological effects include mitochondrial enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx). Examples of complex molecules evincing antioxidant

properties include various vitamins such as vitamins C and E, phytochemicals such as carotenoids, flavonoids, and others (Valko et al. 2007), and the cellular oxidative buffer system of glutathione. Antioxidants can further be subdivided by the source, either being produced by the cells or provided through diet.

13.1.3.2.1 Endogenous antioxidants The endogenous antioxidant defense system is made up of a mixture of enzymatic, nonenzymatic systems, and low-molecular-weight compounds, such as uric and lipoic acids. There are multiple ways in which antioxidants counteract the destructive potential of ROS and free radicals. Examples include (1) reduction of local molecular oxygen concentration, (2) the enzymatic transformation of ROS into more inert compounds, or (3) through the action of a scavenger, trapping ROS, radicals, and metal ions. The prominent enzymatic antioxidants found in cells are superoxide dismutase (SOD), catalase, and glutathione peroxidase. These enzymes work in combination in stepwise reactions, which result in the conversion of the superoxide free radical to the final products of water and oxygen in the following manner. First, superoxide dismutase converts superoxide into hydrogen peroxide and oxygen. This is followed by the enzyme catalase, which converts hydrogen peroxide to water. Similar to catalase, glutathione peroxidase catalyzes the reduction of hydrogen peroxide into water. In addition, glutathione peroxidase also reduces organic hydroperoxides to water (Pisoschi and Pop 2015). In this process, glutathione peroxidase with glutathione acts as an electron donor and also oxidizes the nonenzymatic antioxidant glutathione. This is critical because glutathione and the ratio of the reduced (GSH) to oxidized (GSSG) formed is critical in cellular redox homeostasis. Major antioxidant effects of glutathione are mediated through the direct scavenging action of GSH on hydroxyl radical and singlet oxygen and the regeneration of the antioxidants, and vitamins C and E (Valko et al. 2007). The low-molecular-weight scavengers of the endogenous antioxidant system, as the name suggests, work through the trapping of reactive oxygenated species.

13.1.3.2.2 Exogenous antioxidants In addition to the body's natural pool, foods rich in antioxidants such as fruits and vegetables or dietary supplements provide an alternative source of these compounds. Common dietary examples of exogenous antioxidants include vitamin C, which scavenges hydroxyl, alkosyl, and superoxide radicals, and vitamin E, which also acts as a radical scavenger, forming a low-reactivity by-product preventing cell membrane–lipid peroxidation (Descamps-Latscha et al. 2001). Other important antioxidants are potent phytochemicals such as the carotenoids, which are plant pigments found in vegetables such as carrots. Lycopene, a carotenoid found in tomatoes, is known for its potent antioxidant activity. Its potent scavenger capability is based on its chemical structure of multiple double bonds, which are readily attacked by electrophilic reagents, thereby providing enhanced reactivity with oxygen and free radicals (Kelkel et al. 2011).

A number of acute and chronic inflammatory diseases involve the formation of excess ROS as a major etiological factor. Therefore, the critical role of antioxidants in the prevention of damage to cellular biomolecules by ROS cannot be overstated.

13.1.3.3 Oxidative stress

It is commonly accepted in the context of disease that when the production of oxygen free radicals is elevated and overwhelms the body's antioxidant defenses, and results in an imbalance within the cells and tissues, the organism is said to be under *oxidative stress* (nitrosative stress when the imbalance is in terms of RNS). Such oxidative stress has been documented in multiple different pathologies, as diverse as ranging from reperfusion injury following cardiac surgery for myocardial infarction to the oxidative stress experienced during the growth stage of most tumor cells. Interestingly, oxidative stress with respect to neoplasms makes the cells susceptible to further damage by chemotoxic agents. This desirable role of oxidative stress as a therapeutic enabler that can potentially synergize the cytotoxic effect of chemotherapy emphasizes that the nature of oxidative stress, and the consequent use of antioxidant therapy must be examined on a case-by-case basis before designing and implementing an antioxidant-based intervention. With the increasing knowledge of the role of ROS and oxidative stress in the etiology of certain diseases, there has been increased interest in the use of antioxidants and nutraceuticals in the prevention of disease. The capacity of oxidative stress to induce cellular and tissue damage has led to the examination of its contribution in the modulation of the immune response in recent years. This has also lead many researchers to attempt to elucidate the possible links between ROS and its contribution to the pathogenesis of chronic inflammatory diseases.

13.1.3.4 ROS, oxidative stress, and human disease

Multiple attempts have been made to link the production of ROS to disease severity in order to provide a potential diagnostic tool to evaluate disease progression and to allow physicians to stage the disease. These studies have used specific ROS species as *biomarkers* to gauge disease progression, emphasizing the role of ROS in the development and progression of chronic inflammatory diseases. For instance, in evaluating the relationship between ROS and psoriasis, Kadam et al. measured malondialdehyde (MDA), a by-product of lipid peroxidation commonly used as a biomarker for oxidative stress. The patients' levels of the antioxidants SOD and catalase were also recorded. The serum levels of MDA of psoriatic patients was compared with those of healthy controls. These results stated that MDA was not only significantly elevated compared to controls, but also the level of oxidative stress was statistically elevated relative to disease severity. An inverse relationship was observed when evaluating the antioxidants in the study population and disease severity.

The levels of the antioxidants, SOD and catalase, measured were significantly diminished with levels decreasing with disease severity (Kadam et al. 2010). Next, we will explore the role of oxidative stress in known inflammatory diseases.

13.1.4 The use of antioxidant nutraceuticals in treating chronic inflammatory diseases

Chronic inflammatory disease is a general term that encompasses disorders in which the immune system responds to an initial stimulus, evoking long-term inflammation, which ultimately results in undesirable histological changes and tissue damage. Such diseases include RA, Crohn's disease (CD), sarcoidosis, and psoriasis. Common to these disorders is an elevated level of the proinflammatory cytokines (tumor necrosis factor alpha [TNF-α], interleukin-1 [IL-1], and interleukin-6 [IL-6]), increased production of Reactive Oxygen Species (ROS), and a diminished antioxidant capacity. The benefits of nutraceutical antioxidant agents in the treatment of chronic inflammatory diseases were first demonstrated in experimental cellular and animal models for RA and inflammatory bowel disease (IBD). These first studies laid the ground work for the ongoing research into the potential benefits of nutraceuticals in the treatment of chronic inflammatory diseases. We shall briefly examine select instance of these pioneering studies.

As previously mentioned, a pivotal clinical trial that evaluated the possible therapeutic potential of the addition of food extracts or dietary supplements to the treatment of the chronic inflammatory RA was performed by Kremer and colleagues. Interestingly, this important study was published 2 years prior to the coining of the term *nutraceuticals* by DeFelice. The goal of the trial was to determine the effectiveness of fish oil fatty acid supplements in relieving the severity and symptoms of RA in patients with active disease. The results of this study showed that patients who received fish oil supplements showed significant improvement of not only the number of inflamed joints but also improved assessment scores of the overall severity of the disease. Interestingly enough, following a 4-week washout period to prepare patients to crossover from supplements to placebo and vice versa, clinical improvements quantified as *duration to fatigue* were shown to still be statistically significant as compared to the patients' own baseline values (Kremer et al. 1987), suggesting an overall lasting impact of dietary supplementation in the selected patient population (Kremer et al. 1987; Belch et al. 1988; DiGiacomo et al. 1989; Gupta et al. 1989; Kragballe and Fogh 1989).

Another example of the utility of antioxidant nutraceuticals is their use in the treatment of IBD. It is important to underline that for many inflammatory disorders there are limited therapeutic options and, in general, a scarcity of targeted therapy. Current treatment goals include the management and alleviation

of symptoms, slowing the progression of the disease, and the improvement of patient's quality of life. Treatment modalities typically follow a step-up approach, where drug potency and toxicity is taken into account to address the current state of disease severity. Physicians must then in response to increasing severity of disease or lack of relief from therapy decide to either increase medication dosage or revert to more potent and/or medications with a narrow therapeutic index. Furthermore, the adverse effects of these agents must be taken into account in the context of the demographics of the patient population. For example, a large percentage of patients with the gastrointestinal chronic inflammatory CD are under the age of 18, and therefore the combination of weight loss and poor nutrition due to the altered gastrointestinal tissue morphology may contribute to overall growth restriction. This is of additional concern with the fact that TNF-α inhibitors may contribute to growth restriction. Thus, the use of complementary alternative or supplemented therapies, such as the use of antioxidant nutraceuticals, is of interest in this patient population.

13.1.4.1 Specific examples of the use of antioxidant nutraceuticals in therapy of chronic inflammatory disease

13.1.4.1.1 Rheumatoid arthritis

13.1.4.1.1.1 The need for antioxidant nutraceuticals in rheumatoid arthritis therapy RA is an autoimmune inflammatory disease affecting synovial joints. Chronic inflammation in these joints results in severe structural changes and pathology such as subcutaneous rheumatoid nodules, ulnar deviation, and subluxation. As is common in the treatment of other chronic inflammatory diseases, in RA too, drug regimens are directed to suppressing inflammation and reducing pain. Typical drug prescriptions include NSAIDs, glucocorticoids, and Disease-modifying antirheumatic drug (DMARDs). Thus, the intended targets of current remedies are alleviation of the heightened tissue inflammatory response through the suppression of the immune system or through immunomodulation or through the interference of cytokine signaling pathways. Although there have been improvements in the current medications available to treat such disorders, especially with the discovery and advancement of cytokine inhibitors and biologics, these agents do present undesirable adverse effects. A recent meta-analysis of controlled trials that evaluated the benefit and safety of biologics in the treatment of RA illustrates this point. Although this study showed that these agents provide benefit in the treatment of RA, it also reported that patients who took these agents experienced statistically significant more adverse effects compared to placebo, indicated by the withdrawal rate from these clinical trials (Singh et al. 2009). Drug toxicity is even more of a pressing issue when in light of advance disease; physicians' only recourse is often to increase dosage, thereby increasing likelihood of adverse reactions.

With the emergence of nutraceuticals, more effort recently has been directed toward the evaluation of the therapeutic potential of these agents in the

treatment of chronic inflammatory diseases. A series of recent studies have been performed to evaluate the effects of nutraceuticals on experimental models of chronic inflammatory diseases; specifically, if nutraceuticals may have therapeutic benefits by addressing the inflammatory process and or the oxidative imbalance in these disorders. The potential benefits of these agents have been shown in both cellular and animal models for this category of diseases.

13.1.4.1.1.2 Fish oil and rheumatoid arthritis As stated earlier, NSAID and DMARD provide some relief for RA patients, but they also produce undesired adverse effects. The anti-inflammatory properties of nutraceuticals have potential benefits in the treatment of RA and a possible alternative treatment for joint pain management. Such therapeutic potential was shown in a 9-week study wherein patients were administrated with the combination of the supplements of *Lemon Verbena* element and fish oil omega-3 fatty acid. The trial reported significantly improvement in the pain, stiffness, and functional assessment of patients with joint pain (Caturla et al. 2011). Future studies are needed to assess the potential of such nutraceuticals as a sole or supplement therapeutic option compared to current RA treatment modalities.

13.1.4.1.1.3 Antioxidant vitamins and rheumatoid arthritis As with other chronic diseases, RA patients have been shown to be deficient in multiple nutrients such as calcium, vitamin D, and vitamin B. Therefore, possible benefits of supplementing current therapy with these nutrients have been investigated. One such study evaluated the anti-inflammatory effect of vitamin B6 in RA patients. Although prior to this report, previous studies indicated vitamin B6 supplementation did not suppress inflammation in RA patients, Huang et al. suggested these previous findings may be due to low supplement dosage and or inadequate treatment duration. By addressing these concerns, it was shown that patients, after 12 weeks of treatment with these nutraceutical vitamin supplements, had significantly lower levels of the proinflammatory cytokines IL-6 and TNF-α when compared with baseline values and controls.

To understand the relationship between tissue-specific inflammation and vitamin B6 status, Chiang et al. compared the plasma pyridoxal 5′-phosphate concentrations of RA patients with those of healthy controls. It was shown that RA patients had significantly lower levels of pyridoxal 5′-phosphate than controls. This group then induced arthritis in rats and compared their vitamin B6 status with that of controls. As expected, the tissue-specific inflammation resulted in significantly lower levels of vitamin B6 than of normal animals (Chiang et al. 2005). Collectively, these results imply that the diminished vitamin B6 in inflammatory diseases is not an underlying causation but a consequence of the disorder.

13.1.4.1.1.4 The antioxidant "Compound K" and rheumatoid arthritis In a recent study, Chen et al. evaluated the possible benefits of compound K in RA. A metabolic product of ginsenoside, compound K is the active ingredient

found in dietary supplement called ginseng. For this study, Chen and his group used an induced RA experimental rat model wherein, following the onset of arthritis, rats were subdivided into treatment groups that received either differing doses of compound K or the DMARD, methotrexate. Compound K was shown to produce possible therapeutic benefit as indicated by a decrease in arthritis index and animals' paw swelling. Untreated rats were shown to have elevated inflammatory infiltration of the joints, which was diminished in the treated groups, suggesting that relief from compound K was due to its anti-inflammatory effect (Chen et al. 2015). This research group further corroborated this notion of an anti-inflammatory benefit by showing that compound K also suppressed the activation of T cells and their production of cytokine IL-2 (Chen et al. 2015). These findings are in agreement with those of a previous in vitro study that also demonstrated the potential benefit of compound K in RA. Cells similar to the ones found in the joints of diseased patients were shown to produce metalloproteinases (MMPs) when induced by TNF-α. MMPs are a family of proteolytic enzymes that digest proteins of the extracellular matrix (ECM), the scaffold-like structure composed of different proteins and material that provides critical cellular functions such as organization and cell-to-cell adhesion and communication. Through the degradation of ECM proteins, MMPs have been shown to play a role in both the pathological processes such as tumor and metastatic progression and the normal physiological function such as the migration of immune cells from the bloodstream into tissue during the immune response. Compound K was shown to reduce the production of MMP in dose-dependent manner (Choi et al. 2013). Further, it should be noted in the animal model of RA that compound K when administered at its highest dose (160 mg/kg) was shown to be as effective as methotrexate (Chen et al. 2014). This is of great significance due to the role of FLS and the MMP-secreted enzyme in the pathogenesis of RA.

13.1.4.1.2 Inflammatory bowel diseases The benefits of vitamin B6 with respect to IBDs were studied. In two separate reports, two different animal models were utilized: dextran sodium sulfate-induced colitis and colonic inflammation of the IL-10 knockout mice. In both studies, animals were feed either a vitamin-poor, a nutritional sufficient, or a high-vitamin diet. However, both studies showed that pyridoxal-5-phosphate supplementation had significant attenuation of molecular and histological markers of inflammation; these studies also noted that animals fed a vitamin B6–poor diet also showed improvements (Benight et al. 2011; Selhub et al. 2013). More studies are needed to clarify these opposing findings. The potent anti-inflammatory and antioxidant properties of these compounds may serve as effective alternative remedies, either as monotherapies or in combination with current drug regiments. In the following chapters, with respect to nutraceuticals and various inflammatory diseases, recent clinical trials and their findings will be discussed.

13.1.4.1.3 Psoriasis Psoriasis is a common chronic inflammatory dermatosis with a prevalence of 2.9% in the United States, which translates to

approximately 7.4 million American adults who are inflicted with this disorder (Rachakonda et al. 2014). The importance of discovering more effective therapies that treats psoriasis is critical. This is best illustrated from a recent multinational survey of patients with psoriasis or psoriasis arthritis. The study results indicated that 47% of the patients surveyed have not seen their healthcare provider (HCP) about their psoriasis. Out of this population, 19% stated the reason they did not seek out assistance is because they felt their HCP could not help. However, the proportion with patients with psoriatic arthritis who have not seen a HCP in the past year was less than that of patients with psoriasis alone (18.5%); 30% indicated that it was due to belief the provider cannot provide relief. Of the patients surveyed, 24% of all patients said they had been prescribed a traditional oral medication such as cyclosporine or methotrexate, whereas 11% were prescribed biologic therapy. Of these patient populations, 57% and 45% had discontinued oral and biologic therapies, respectively. In both groups, tolerability was the most common reason (43% and 25%), followed by lack/loss of effectiveness (30% and 22%). Evidently, there is a significant need to discover new and more effective therapies for psoriasis that are better tolerated and will allow for greater patient compliance. The use of nutraceuticals as monotherapy or in addition to current therapies may serve as a possible solution to address this problem (Lebwohl et al. 2014).

Multiple studies have shown that patients with psoriasis were found to be deficient in vitamin D (Gisondi et al. 2012; Orgaz-Molina et al. 2012; Al-Mutairi et al. 2013; Ricceri et al. 2013). It was suggested that the degree of vitamin D deficiency was correlated with disease severity (Ricceri et al. 2013). Thus, supplementation with vitamin D could improve therapeutic outcome. Initial clinical trials investigated the effects of dietary supplementation on psoriasis treatment. Unfortunately, these preliminary studies did not show any significant improvements on either the disease severity assessment score or patient quality of life when compared to placebo. It was suggested that the lack of observed benefit was due to the small sample size of the studies and relatively lower dose of vitamin D.

13.1.4.1.4 Psoriatic co-morbidities and nutraceutical therapy In addition to the need for more efficacious therapeutic options that are better tolerated by the patients, a growing concern for psoriasis therapy is the prevalence of multiple comorbidities. A recent cross-sectional study of psoriasis patients in the United Kingdom showed that the mean Charlson comorbidity index was higher at all severities. As expected, the odds of having one or more major comorbidities compared with controls were elevated. Diseases associated with psoriasis included diabetes, mild liver disease, myocardial infarction, renal disease, and rheumatologic disease (Yeung et al. 2013).

Metabolic syndrome is a comorbidity of concern with chronic inflammatory diseases. The term metabolic syndrome denotes a collection of health risks such as obesity, decreased insulin sensitivity, hypertension, and hyperlipidemia that coalesce to result in increased risk of developing diseases such

as Type II diabetes, myocardial infarction, stroke, and other cardiovascular disorders. An alarming proportion of the American population lives a sedentary lifestyle. Combined with a poor diet and decreased physical activity, this has led to an epidemic of individuals falling under the criteria of metabolic syndrome and the emergence of a major public health crisis. A correlation exists between metabolic syndrome prevalence and the severity of psoriasis (Langan et al. 2012; Armstrong et al. 2013). It has been proposed that the pro-inflammatory state that is associated with the metabolic syndrome acts as the link between psoriasis and the syndrome. It is reasonable to assume that treating metabolic syndrome while treating psoriasis can improve therapeutic outcomes. The potential of enhancing current psoriasis treatment with the addition of nutrient supplements to address the metabolic parameters of the metabolic syndrome was evaluated in a recent open-label clinical trial. This study compared the effectiveness of the standard treatment of etanercept when supplemented by a combination of nutraceuticals. The results showed that psoriatic patients whose standard therapy of etanercept with the addition of a combinatory supplement containing Coenzyme Q10, Krill oil, lipoic acid, resveratrol, *Vitis vinifera* seed oil, vitamin E, and selenium showed significantly improved (HDL) and triglycerides levels compared with their baseline values. The addition of these nutraceuticals also resulted in significant improvement of these metabolic syndrome parameters compared to patients who received etanercept alone (Skroza et al. 2013). Further studies are needed to determine if improved therapeutic outcomes can be achieved by the addition of nutraceuticals to current psoriatic therapies, especially if these supplements are directed at the metabolic syndrome that is prevalent in this patient population.

Another complication of psoriasis is the development of psoriatic arthritis and erythrodermic psoriasis. Treatment requires the use of potent drugs, potentially resulting in more adverse effects. Possible nutraceutical intervention for the management of psoriasis in the context of these complications was investigated and the effect of antioxidant supplementation was evaluated. It was observed that patients in both the arthritic and the erythrodermic groups who received supplemental therapy experienced an accelerated rate of recovery and significant decrease in the severity of symptoms when compared to patients who received conventional therapy alone. As with management of chronic inflammatory diseases, a specific goal is to prevent relapse (Kharaeva et al. 2009). This study suggested that in the face of worsening disease, supplementing current therapy with nutraceuticals may provide a viable alternative remedy without the negative consequence of additional adverse reactions.

An elevated oxidative state is a major accompanying complication of psoriasis. Therefore, the effect of nutraceutical supplementation on the status of oxidative stress has been investigated. For example, granulocytes from patients with psoriatic arthritis and erythrodermic psoriasis when activated *in vitro* produced elevated levels of the superoxide free radical when compared to cells from healthy controls. The addition of antioxidants in both

disease groups accelerated the desensitization of granulocytes to synthesize the ROS superoxide. The supplementation caused SOD and catalase production to return to normal levels at an accelerated rate in both diseased groups, indicating that the antioxidant properties of the supplements may have alleviated the toll on endogenous antioxidants, allowing the latter to return to normal (Kharaeva et al. 2009).

13.1.4.1.5 CD, IBD, and antioxidant nutraceuticals CD and IBD are both chronic inflammatory gastrointestinal diseases, the etiologies of which are incompletely understood. However, both are associated with structural changes at the tissue and cellular level in the gastrointestinal tract during disease progression, leading to poor absorption of vitamins and nutrients. Thus, a primary concern may be deficient nutritional status of the patient.

13.1.4.1.5.1 CD, IBD, and vitamin D Farraye et al. evaluated the ability of CD patients to absorb vitamin D. In the study, only CD patients whose disease was in remission at the time were included. The results indicated that this population absorbed the vitamin at a 30% deficiency compared to control. This diminished absorbance was independent of the location of the disease along the gastrointestinal tract (Farraye et al. 2011). A similar study performed in Denmark also compared vitamin D supplementation in CD patients who were in remission; however, the endpoint in this particular study was clinical relapse. Comparing the treatment group with controls, patients who received supplements showed fewer relapses compared with placebo; however, this did not meet statistical significance ($p = 0.06$) (Jørgensen et al. 2010). The authors noted that the lack of statistical significance could be attributed to the small sample size.

Another study examined the effect of vitamin D level on CD patients' quality of life by generating and using a short inflammatory bowel disease questionnaire (SIBDQ). SIBDQ is a self-administered questionnaire wherein scores are totaled and placed on a graded scale, where 10 indicates poor and 70 is the best state of quality of life (Lam et al. 2009). Unlike other vitamin D and IBDs studies, this research group accounted for the seasonal effect on serum levels and patients' quality of life. As expected, vitamin D level was significantly higher in the summer than in the winter due perhaps to ample sunshine. It was noted that the SIBDQ scores in the winter was significantly higher in patients who had normal vitamin D serum concentrations than patients who had low levels. It is interesting to note that in the summer season, the SIBDQ scores were similar when comparing patients with low and normal vitamin D levels. It was also noted that the questionnaire scores were similar when comparing patients who had low summer vitamin levels with patients who had normal winter levels. This finding suggested that sunlight alone had positive effects on Crohn patients' quality of life regardless of the serum vitamin D levels. In addition, when comparing patients who received vitamin D supplements to controls, there was no significant difference of

either the decline of serum levels from summer to winter or the SIBDQ score (Hlavaty et al. 2014). Although more studies are necessary to definitively determine the benefits of vitamin D supplements in CD patients, preliminary clinical trials indicate that vitamin D supplements may not provide any significant benefit to the treatment of this disease or improvement to the quality of life of these individuals.

13.1.4.1.5.2 Endogenous Vitamin D and Cancer-related Complication of IBD It has been shown that reduced vitamin D levels puts individuals at a higher risk for developing cancer, in particular colorectal, hepatocellular, pancreatic, and bladder (Jenab et al. 2010; Mondul et al. 2010; Wolpin et al. 2012; Fedirko et al. 2014; Chandler et al. 2015). Difference in vitamin D concentrations has also been suggested as a possible factor that could explain difference in cancer survival rates (Grant 2006). Taking into account vitamin deficiencies are common in the IBD patient population, one would expect a higher incidence of cancer associated with this disease. To find an answer to this question, one cohort study was set to determine if patients who have low vitamin D levels in addition to IBD are at increased risk for developing cancer. The results of this report indicated that IBD patients with reduced vitamin D levels indeed had an elevated risk of developing cancer. Out of the 2809 individuals who entered the study, 196 patients (7% ratio) developed cancer. The cohort included 881 patients who initially had deficient levels of vitamin D, of which 88 (or 10%) developed cancer compared to the 4% of patients with normal levels. This yielded an odds ratio of 2.38. By increasing the vitamin D level by 1 ng/mL, the odds ratio was adjusted to be just below 1 (0.97–0.98). Colon cancer was shown to have the strongest correlation between diminished vitamin D and incidence (Ananthakrishnan et al. 2014). Although there is little evidence that supplementing current CD remedies with vitamin D showed any therapeutic benefits, these findings raise the question, should patients with CD have vitamin D supplements added to their therapy regiment in order to prevent the development of cancer.

13.1.4.1.5.3 Vitamin D and Osteoporosis-related Complications of IBD and CD Osteoporosis results in demineralization of bones and the thinning and weakening of the skeletal system, leading to increased fractures. Interestingly, osteoporosis has a high prevalence within the IBD and CD patient populations (Ardizzone et al. 2000; Miznerova et al. 2013). The etiology of osteoporosis is not yet elucidated, and many factors have been suggested as contributors, such as body mass index (BMI), height, and gender. Though it is often assumed that osteoporosis in the IBD and CD patient population arises due to chronic steroid drug use as a consequence of therapy, a recent study reported a prevalence of low bone mass density among patients with IBD who were never treated with corticosteroids (22.36%) (Miznerova et al. 2013). Recent reports have suggested that the inflammatory state associated with CD may contribute to this pathology. This high prevalence of osteoporosis is related to an increased occurrence of vertebral fractures in patients with CD. A study of

293 CD patients who underwent bone density scans showed that 156 (53.2%) of these individuals were osteopenic (n = 111) or osteoporotic (n = 45). Of these 156 patients, 21.8% had one or more vertebral fractures (Klaus et al. 2002). In fact, a recent report has shown an inverse correlation between vitamin D deficiency and bone mass density (Nakajima et al. 2011). Further studies are needed to confirm the role of vitamin deficiency and of the utility of subsequent vitamin supplementation.

References

Agarwal, S., G. V. Reddy, and P. Reddanna. 2009. Eicosanoids in inflammation and cancer: The role of COX-2. *Expert Rev Clin Immunol* 5 (2):145–165.

Al-Mutairi, N., B. El Eassa, and V. Nair. 2013. Measurement of vitamin D and cathelicidin (LL-37) levels in patients of psoriasis with co-morbidities. *Indian J Dermatol Venereol Leprol* 79 (4):492–496.

Ananthakrishnan, A. N., S. C. Cheng, T. Cai, A. Cagan, V. S. Gainer, P. Szolovits, S. Y. Shaw et al. 2014. Association between reduced plasma 25-hydroxy vitamin D and increased risk of cancer in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol* 12 (5):821–827.

Ardizzone, S., S. Bollani, P. Bettica, M. Bevilacqua, P. Molteni, and G. Bianchi Porro. 2000. Altered bone metabolism in inflammatory bowel disease: There is a difference between Crohn's disease and ulcerative colitis. *J Intern Med* 247 (1):63–70.

Armstrong, A. W., C. T. Harskamp, and E. J. Armstrong. 2013. Psoriasis and metabolic syndrome: A systematic review and meta-analysis of observational studies. *J Am Acad Dermatol* 68 (4):654–662.

Belch, J. J., D. Ansell, R. Madhok, A. O'Dowd, and R. D. Sturrock. 1988. Effects of altering dietary essential fatty acids on requirements for non-steroidal anti-inflammatory drugs in patients with rheumatoid arthritis: A double blind placebo controlled study. *Ann Rheum Dis* 47 (2):96–104.

Benight, N. M., B. Stoll, S. Chacko, V. R. da Silva, J. C. Marini, J. F. Gregory 3rd, S. P. Stabler, and D. G. Burrin. 2011. B-vitamin deficiency is protective against DSS-induced colitis in mice. *Am J Physiol Gastrointest Liver Physiol* 301 (2):G249–G259.

Busbee, P. B., M. Rouse, M. Nagarkatti, and P. S. Nagarkatti. 2013. Use of natural AhR ligands as potential therapeutic modalities against inflammatory disorders. *Nutr Rev* 71 (6):353–369.

Caturla, N., L. Funes, L. Pérez-Fons, and V. Micol. 2011. A randomized, double-blinded, placebo-controlled study of the effect of a combination of lemon verbena extract and fish oil omega-3 fatty acid on joint management. *J Altern Complement Med* 17 (11):1051–1063.

Chandler, P. D., J. E. Buring, J. E. Manson, E. L. Giovannucci, M. V. Moorthy, S. Zhang, I. M. Lee, and J. H. Lin. 2015. Circulating vitamin D levels and risk of colorectal cancer in women. *Cancer Prev Res (Phila)* 8 (8):675–682.

Chen, J., H. Wu, Q. Wang, Y. Chang, K. Liu, S. Song, P. Yuan et al. 2014. Ginsenoside metabolite compound K alleviates adjuvant-induced arthritis by suppressing T cell activation. *Inflammation* 37 (5):1608–1615.

Chen, J., H. Wu, Q. Wang, Y. Chang, K. Liu, and W. Wei. 2015. Ginsenoside metabolite compound K suppresses T-cell priming via modulation of dendritic cell trafficking and costimulatory signals, resulting in alleviation of collagen-induced arthritis. *J Pharmacol Exp Ther* 353 (1):71–79.

Chiang, E. P., J. Selhub, P. J. Bagley, G. Dallal, and R. Roubenoff. 2005. Pyridoxine supplementation corrects vitamin B6 deficiency but does not improve inflammation in patients with rheumatoid arthritis. *Arthritis Res Ther* 7 (6):R1404–R1411.

Choi, Y. S., E. H. Kang, E. Y. Lee, H. S. Gong, H. S. Kang, K. Shin, E. B. Lee, Y. W. Song, and Y. J. Lee. 2013. Joint-protective effects of compound K, a major ginsenoside metabolite, in rheumatoid arthritis: In vitro evidence. *Rheumatol Int* 33 (8):1981–1990.

Datta, S., S. Kundu, P. Ghosh, S. De, A. Ghosh, and M. Chatterjee. 2014. Correlation of oxidant status with oxidative tissue damage in patients with rheumatoid arthritis. *Clin Rheumatol* 33 (11):1557–1564.

Descamps-Latscha, B., T. Drüeke, and V. Witko-Sarsat. 2001. Dialysis-induced oxidative stress: Biological aspects, clinical consequences, and therapy. *Semin Dial* 14 (3): 193–199.

DiGiacomo, R. A., J. M. Kremer, and D. M. Shah. 1989. Fish-oil dietary supplementation in patients with Raynaud's phenomenon: A double-blind, controlled, prospective study. *Am J Med* 86 (2):158–164.

Farraye, F. A., H. Nimitphong, A. Stucchi, K. Dendrinos, A. B. Boulanger, A. Vijjeswarapu, A. Tanennbaum, R. Biancuzzo, T. C. Chen, and M. F. Holick. 2011. Use of a novel vitamin D bioavailability test demonstrates that vitamin D absorption is decreased in patients with quiescent Crohn's disease. *Inflamm Bowel Dis* 17 (10):2116–2121.

Fedirko, V., T. Duarte-Salles, C. Bamia, A. Trichopoulou, K. Aleksandrova, D. Trichopoulos, E. Trepo et al. 2014. Prediagnostic circulating vitamin D levels and risk of hepatocellular carcinoma in European populations: A nested case-control study. *Hepatology* 60 (4):1222–1230.

Gisondi, P., M. Rossini, A. Di Cesare, L. Idolazzi, S. Farina, G. Beltrami, K. Peris, and G. Girolomoni. Vitamin D status in patients with chronic plaque psoriasis. *Br J Dermatol* 166 (3):505–510.

Grant, W. B. 2006. Lower vitamin-D production from solar ultraviolet-B irradiance may explain some differences in cancer survival rates. *J Natl Med Assoc* 98 (3):357–364.

Hlavaty, T., A. Krajcovicova, T. Koller, J. Toth, M. Nevidanska, M. Huorka, and J. Payer. 2014. Higher vitamin D serum concentration increases health related quality of life in patients with inflammatory bowel diseases. *World J Gastroenterol* 20 (42): 15787–15796.

Huang, S. C., J. C. Wei, D. J. Wu, and Y. C. Huang. 2010. Vitamin B6 supplementation improves pro-inflammatory responses in patients with rheumatoid arthritis. *Eur J Clin Nutr* 64 (9):1007–1013.

Jenab, M., H. B. Bueno-de-Mesquita, P. Ferrari, F. J. van Duijnhoven, T. Norat, T. Pischon, E. H. Jansen, N. Slimani, G. Byrnes, S. Rinaldi et al. 2010. Association between pre-diagnostic circulating vitamin D concentration and risk of colorectal cancer in European populations: A nested case-control study. *BMJ* 340:b5500.

Jørgensen, S. P., J. Agnholt, H. Glerup, S. Lyhne, G. E. Villadsen, C. L. Hvas, L. E. Bartels, J. Kelsen, L. A. Christensen, and J. F. Dahlerup. 2010. Clinical trial: Vitamin D3 treatment in Crohn's disease—A randomized double-blind placebo-controlled study. *Aliment Pharmacol Ther* 32 (3):377–383.

Kadam, D. P., A. N. Suryakar, R. D. Ankush, C. Y. Kadam, and K. H. Deshpande. 2010. Role of oxidative stress in various stages of psoriasis. *Indian J Clin Biochem* 25 (4): 388–392.

Kelkel, M., M. Schumacher, M. Dicato, and M. Diederich. 2011. Antioxidant and antiproliferative properties of lycopene. *Free Radic Res* 45 (8):925–940.

Kharaeva, Z., E. Gostova, C. De Luca, D. Raskovic, and L. Korkina. 2009. Clinical and biochemical effects of coenzyme Q(10), vitamin E, and selenium supplementation to psoriasis patients. *Nutrition* 25 (3):295–302.

Klaus, J., G. Armbrecht, M. Steinkamp, J. Brückel, A. Rieber, G. Adler, M. Reinshagen, D. Felsenberg, and C. von Tirpitz. 2002. High prevalence of osteoporotic vertebral fractures in patients with Crohn's disease. *Gut* 51 (5):654–658.

Kragballe, K. and K. Fogh. 1989. A low-fat diet supplemented with dietary fish oil (MaxEPA) results in improvement of psoriasis and in formation of leukotriene B5. *Acta Derm Venereol* 69 (1):23–28.

Kremer, J. M., W. Jubiz, A. Michalek, R. I. Rynes, L. E. Bartholomew, J. Bigaouette, M. Timchalk, D. Beeler, and L. Lininger. 1987. Fish-oil fatty acid supplementation in active rheumatoid arthritis. *Ann Intern Med* 106 (4):497–503.

Kundu, S., P. Ghosh, S. Datta, A. Ghosh, S. Chattopadhyay, and M. Chatterjee. 2012. Oxidative stress as a potential biomarker for determining disease activity in patients with rheumatoid arthritis. *Free Radic Res* 46 (12):1482–1489.

Lam, M. Y., H. Lee, R. Bright, J. R. Korzenik, and B. E. Sands. 2009. Validation of interactive voice response system administration of the short inflammatory bowel disease questionnaire. *Inflamm Bowel Dis* 15 (4):599–607.

Langan, S. M., N. M. Seminara, D. B. Shin, A. B. Troxel, S. E. Kimmel, N. N. Mehta, D. J. Margolis, and J. M. Gelfand. 2012. Prevalence of metabolic syndrome in patients with psoriasis: A population-based study in the United Kingdom. *J Invest Dermatol* 132 (3 Pt 1):556–562.

Lebwohl, M. G., H. Bachelez, J. Barker, G. Girolomoni, A. Kavanaugh, R. G. Langley, C. F. Paul, L. Puig, K. Reich, and P. C. van de Kerkhof. 2014. Patient perspectives in the management of psoriasis: Results from the population-based multinational assessment of psoriasis and psoriatic arthritis survey. *J Am Acad Dermatol* 70 (5): 871–881.

Maor, I., T. Rainis, A. Lanir, and A. Lavy. 2008. Oxidative stress, inflammation and neutrophil superoxide release in patients with Crohn's disease: Distinction between active and non-active disease. *Dig Dis Sci* 53 (8):2208–2214.

Miznerova, E., T. Hlavaty, T. Koller, J. Toth, K. Holociova, M. Huorka, Z. Killinger, and J. Payer. 2013. The prevalence and risk factors for osteoporosis in patients with inflammatory bowel disease. *Bratisl Lek Listy* 114 (8):439–445.

Mondul, A. M., S. J. Weinstein, S. Männistö, K. Snyder, R. L. Horst, J. Virtamo, and D. Albanes. 2010. Serum vitamin D and risk of bladder cancer. *Cancer Res* 70 (22):9218–9223.

Nakajima, S., H. Iijima, S. Egawa, S. Shinzaki, J. Kondo, T. Inoue, Y. Hayashi et al. 2011. Association of vitamin K deficiency with bone metabolism and clinical disease activity in inflammatory bowel disease. *Nutrition* 27 (10):1023–1028.

Needleman, H., E. Gunnoe, A. Leviton, R. Reed, H. Peresie, C. Maker, and P. Barrett. 1979. Deficits in psychologic and classroom performance of children with elevated dentine lead levels. *N Engl J Med* 300:689–695.

Orgaz-Molina, J., A. Buendía-Eisman, M. A. Arrabal-Polo, J. C. Ruiz, and S. Arias-Santiago. 2012. Deficiency of serum concentration of 25-hydroxyvitamin D in psoriatic patients: A case-control study. *J Am Acad Dermatol* 67 (5):931–938.

Pisoschi, A. M. and A. Pop. 2015. The role of antioxidants in the chemistry of oxidative stress: A review. *Eur J Med Chem* 97:55–74.

Prescott, S. M. 1984. The effect of eicosapentaenoic acid on leukotriene B production by human neutrophils. *J Biol Chem* 259 (12):7615–7621.

Rachakonda, T. D., C. W. Schupp, and A. W. Armstrong. 2014. Psoriasis prevalence among adults in the United States. *J Am Acad Dermatol* 70 (3):512–516.

Ray, P. D., B. W. Huang, and Y. Tsuji. 2012. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cell Signal* 24 (5):981–990.

Ricceri, F., L. Pescitelli, L. Tripo, and F. Prignano. 2013. Deficiency of serum concentration of 25-hydroxyvitamin D correlates with severity of disease in chronic plaque psoriasis. *J Am Acad Dermatol* 68 (3):511–512.

Selhub, J., A. Byun, Z. Liu, J. B. Mason, R. T. Bronson, and J. W. Crott. 2013. Dietary vitamin B6 intake modulates colonic inflammation in the IL10-/- model of inflammatory bowel disease. *J Nutr Biochem* 24 (12):2138–2143.

Singh, J. A., R. Christensen, G. A. Wells, M. E. Suarez-Almazor, R. Buchbinder, M. A. Lopez-Olivo, E. T. Ghogomu, and P. Tugwell. 2009. A network meta-analysis of randomized controlled trials of biologics for rheumatoid arthritis: A cochrane overview. *CMAJ* 181 (11):787–796.

Skroza, N., I. Proietti, N. Bernardini, G. La Viola, F. Nicolucci, R. Pampena, E. Tolino et al. 2013. Efficacy of food supplement to improve metabolic syndrome parameters in patients affected by moderate to severe psoriasis during anti-TNFα treatment. *G Ital Dermatol Venereol* 148 (6):661–665.

Valko, M., D. Leibfritz, J. Moncol, M. T. Cronin, M. Mazur, and J. Telser. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 39 (1):44–84.

Wolpin, B. M., K. Ng, Y. Bao, P. Kraft, M. J. Stampfer, D. S. Michaud, J. Ma, J. E. Buring, H. D. Sesso, I. M. Lee et al. 2012. Plasma 25-hydroxyvitamin D and risk of pancreatic cancer. *Cancer Epidemiol Biomarkers Prev* 21 (1):82–91.

Yeung, H., J. Takeshita, N. N. Mehta, S. E. Kimmel, A. Ogdie, D. J. Margolis, D. B. Shin, R. Attor, A. B. Troxel, and J. M. Gelfand. 2013. Psoriasis severity and the prevalence of major medical comorbidities: A population-based study. *JAMA Dermatol* 149 (10):1173–1179.

14

Immunomodulation and Antioxidant Nutraceuticals

Gregory L. Smith

Contents

14.1 Immunomodulation

Immunomodulation refers to changing the various immune and inflammatory processes in the body through therapeutic means. It refers to the balancing of this immune response through external means, in this case, nutrients, to reduce or prevent adverse health effects.

The body and brain have separate but similar immune systems that protect healthy cells from infection, foreign materials, or foreign cells. The immune system also assists with removing injured or unhealthy cells and with wound healing. The immune system is constantly surveying for problems and ready to respond with innate immune cells, such as natural killer (NK) cells and acquired cellular and humoral immunity. If the immune system is overactivated or out of balance, then it can actually do more harm than those things from which it is protecting the cells. In addition, some immunomodulators, if used in excess, can cause immunosuppression. Immunomodulation can affect innate immunity, acquired humoral (B-cell) or cellular (T-cell) immune responses, or all three.

14.2 Antioxidants

Antioxidant, refers to a substance that stabilizes harmful free radicals into stable harmless compounds using redox-based mechanisms. There are millions of antioxidants in nature. The body generates free radicals as an inevitable consequence of turning food into energy. Sugar in the diet, in particular, produces a large number of free radicals. There are also free radicals already in some of the foods we eat and in the air we breathe. In addition, UV and other radiation can generate free radicals that especially impact the skin and eyes. However, free radicals are normal physiological components of the signaling generated by cytokines, growth factors, and neurotropic peptides only when produced in low amounts and in a controlled manner.

Our bodies make a wide variety of innate antioxidants to handle the constant production of free radicals. The body's natural production of antioxidants can decline with age (Fusco et al. 2007). The body's innate antioxidants are not enough to handle the load of free radicals. Our bodies must get antioxidants from our food nutrients in order to help keep the entire process in balance.

These compounds can act as direct antioxidants through free radical scavenging mechanisms and/or as indirect antioxidants by enhancing the antioxidant status.

When there are too many free radicals for the available antioxidants, then this is called oxidative stress. The excess free radicals then cause damage to lipid and proteins in cell and mitochondrial membranes, to enzymes, and to nucleic acids in our DNA and RNA. Antioxidants therefore protect both the structure and function of cells. Repeated or prolonged oxidative stress has also been implicated in the onset of senescence in cells, especially immune cells (De la Fuente 2002).

There are three primary types of antioxidants in foods. These are phytochemicals, vitamins, and microminerals. Antioxidants occur mostly in plants to protect the effects of the large number of free radicals caused by exposure to UV radiation. Antioxidants are absorbed from the food and have their effects both within intestinal tract microbiome and systemically after absorption.

14.3 Nutraceutical

Nutraceutical is a word made up of the words *nutrition* and *pharmaceutical*. It is a broad umbrella term that refers to naturally occurring nutrients or foods that have beneficial health effects over and above the nutritional value found in the food. Antioxidant nutraceuticals is one subset of nutraceuticals. Many antioxidants can be obtained directly from the diet (e.g., ascorbic acid, α-tocopherol, carotenoids, and polyphenols) or require micronutrients as integral components, for example, selenium in the metalloenzyme glutathione peroxidase.

According to data from the U.S. National Health and Nutrition Examination Survey (NHANES), 93% of the U.S. population do not meet the estimated average requirement for antioxidant vitamin E, 56% for magnesium, 44% for vitamin A, 31% for vitamin C, 14% for vitamin B6, and 12% for zinc (Moshfegh et al. 2005). Because micronutrients play crucial roles in the development and expression of immune responses, selected micronutrient deficiencies can cause immunosuppression and thus increased susceptibility to infection and disease.

14.4 Antioxidant nutraceuticals

The discussion will be limited to antioxidant nutraceuticals. There is a wide variety of antioxidant nutraceuticals, and new categories continue to be discovered. The amount of research on each of these various antioxidant nutraceuticals varies widely. There is a large body of research on most of these antioxidants. Currently, close to 2000 clinical trials using antioxidants are ongoing (www.clinicaltrials.com).

In addition to the type of antioxidant, the location of antioxidant effects also varies widely. Even very similar nutrients can have a huge difference in their potential as antioxidants. For example, vitamin E has many isoforms: α-, β-, and δ-tocopherol and α-, β-, and δ-tocotrienol. Only α-tocopherol functions as an antioxidant in the body (Davis et al. 2015). However, ascorbic acid (vitamin C) has only one form, and most of its antioxidant properties are provided by regenerating the antioxidant capacity of α-tocopherol (vitamin E) (Davis et al. 2015).

So, this chapter will discuss the current state of the art of nutraceutical substances that have an antioxidant effect at a cellular level that results in beneficial effects on innate, humoral, and/or cellular immune responses.

14.5 Oxidation

Oxidation refers to the loss of electrons by an atom or molecule. There is a concomitant reduction reaction with the gain of an electron by a different atom or molecule. Collectively, these are known as reduction/oxygenation (redox) reactions. These redox reactions take place in cells as part of aerobic metabolic production of energy, namely adenosine triphosphate (ATP).

Endogenous free radicals are generated as a natural by-product of these metabolic redox reactions. These free radicals are generated in cell membranes, mitochondria, peroxisomes, and endoplasmic reticulum (Muller 2000). Exogenous free radicals can also be produced by ingesting xenobiotics (environmental toxins, cigarette smoke, and drugs). Other exogenous free radicals are generated from ionizing radiation interacting with water in the body's tissues.

Free radicals are highly reactive molecules containing one of more unpaired electrons. They play a role in cell signaling and homeostasis. A broader, more inclusive term is reactive oxygen species (ROS), which includes the oxygen-centered radicals (superoxide O_2- and hydroxyl OH) and some non-radical species of oxygen such as hydrogen peroxide (H_2O_2), singlet oxygen, and hypochlorous acid (HOCl).

14.6 Oxidative stress

An excess ROS is known as oxidative stress. The ROS cause damage to various parts of the cell. They are particularly damaging to long-chain polyunsaturated fatty acids (PUFAs) found in cell membranes. The process of lipid peroxidation results in a self-perpetuating chain reaction creating extensive damage along the cell membrane.

ROS also cause protein carbonylation. This refers to oxidation of side chains of lysine, arginine, proline, and other amino acids. This leads to loss of protein

function. Heavily carbonylated proteins tend to form high-molecular-weight aggregates that are resistant to degradation and accumulate as damaged or unfolded proteins (Dalle-Donne et al. 2006). In addition, ROS damage nucleic acids in DNA and RNA at several locations, resulting in transcription errors. Excessive or prolonged oxidative stress can influence T-cell function, contributing to a T-cell phenotype, which is hyporesponsive to growth and death signals and persists at the site perpetuating the immune response. Acute excess oxidative stress can lead to apoptosis or other means of cell death.

14.7 Oxidation and the immune system

The immune system is particularly susceptible to oxidative stress. Excess ROS induce the production of COX-2, inflammatory cytokines (TNF-α, IL-1, and IL-6), and chemokines (IL-8).

The immune system is impacted in several other ways. A very important aspect of keeping the immune system in balance is accurate cell–cell signaling. This signaling is done in particular via membrane-bound receptors. Damage to the signaling systems occurs via damage to the PUFA cell membrane integrity from ROS peroxidation chain reactions. This results in a reduction of cell membrane receptor expression. This significantly impairs the immune responsiveness (Stark 2005).

In order for the immune system to initiate an antibody response as part of humoral immunity, naive B cells must first be activated by an antigen. B-cell receptors recognize an antigen and then enlist CD4+ T cells to stimulate clonal expansion. B-cell production of ROS is a natural consequence of this response. In fact, generation of ROS following antigen receptor ligation is critical in promoting B-cell proliferation and antibody production and CD4+ T-cell responses. This suggests that while antioxidants may play a therapeutic role in the prevention of chronic diseases, there may be negative effects on the humoral immune response to acute infectious illness or immunization response (Crump et al. 2013, Lanzavecchia 1985).

Immune cells are unusual, as compared with other somatic cells, in that they contain high levels of antioxidant vitamins, presumably providing protection against lipid peroxidation and immunosuppression, both of which are well-known risks posed by high PUFA content of immune cells (Hughes 1999a).

14.8 Oxidative burst

Second, phagocytic cells produce ROS as a main part of its defense system. The phagocyte engulfs the microbe, foreign, or damaged cell. Lysosomes then fuse with the phagocytic vesicle and the microbe or cell is destroyed by the oxidative burst. When phagocytes are activated to produce this burst,

a multicomponent flavoprotein, NADPH oxidase, is assembled and this catalyzes the production of large amounts of superoxide anion radical (O_2^-) and also hydrogen peroxide (H_2O_2). Adequate amounts of neutralizing antioxidants are required to prevent damage to the lysosome membranes. Damage can lead to a release of hydrolytic enzymes in the phagocyte's cytoplasm (Puertollano et al. 2011). In addition, activated neutrophils and monocytes release the hemoprotein myeloperoxidase into the extracellular space, where it catalyzes the oxidation of Cl^- by H_2O_2 to yield hypochlorous acid (HOCl), which is a nonspecific oxidizing and chlorinating agent that reacts rapidly with a variety of biological compounds such as sulfhydryls, PUFAs, DNA, pyridine nucleotides, aliphatic and aromatic amino acids, and nitrogen-containing compounds (Brambilla et al. 2008).

14.9 Innate antioxidants

The body has innate enzyme- and nonenzyme-based systems to decompose ROS. Catalase and glutathione peroxidase are enzymes used by phagocytes to decompose of H_2O_2 and other ROS produced during the killing of phagocytized cells or microbes. Superoxide dismutase (SOD) scavenges other free radicals. Glutathione, is the most important innate antioxidant. However, there is usually not enough innate antioxidant to protect again excess ROS and oxidative stress. So, dietary antioxidants are necessary to keep the ROS in balance.

14.10 Antioxidant nutraceuticals

Antioxidant nutraceuticals include vitamins, phytochemicals, microminerals, and coenzymes.

14.10.1 Vitamins

The four major antioxidant vitamins are ascorbic acid, α-tocopherol, β-carotene (a precursor of vitamin A), and folic acid.

14.10.1.1 Vitamin E

α-Tocopherol is the most important lipid-soluble antioxidant. Strong evidence supports a significant impact of α-tocopherol on the modulation or immune functions. Deficiency of α-tocopherol has been shown to impair both humoral and cell-mediated immune functions. α-Tocopherol deficiency states are associated with depressed B-cell antibody production. α-Tocopherol supplementation, especially in the aged, has been shown to enhance immune response (Serafini 2000).

α-Tocopherol is the first line of defense terminating lipid peroxidation chain reactions. It therefore plays a major role in maintaining cell membrane integrity.

Immune system cells are particularly high in α-tocopherol because of the high PUFA content.

α-Tocopherol supplementation significantly enhances lymphocyte proliferation, IL-2 production, and delayed type hypersensitivity skin response and decreases prostaglandin E2 (PGE2) production by affecting COX activity. This immunoenhancing effect is thought to be due to reduced PGE2 production by macrophages and increased IL-2 production by T cells (Adolfsson et al. 2001).

Studies have shown, for example, that administration of α-tocopherol supplement (800 mg for 30 days) to healthy elderly patients produced an increased antibody titer to both hepatitis B and tetanus vaccine, thus enhancing T-cell-mediated functions (Meydani et al. 1997, Park et al. 2003).

α-Tocopherol has been shown in mice to protect the CD8+ and CB4+ T-cell membranes from oxidative damage (Matsushita et al. 2015). T cells have a repair enzyme, glutathione peroxidase. This enzyme repairs oxidative damage to the cell membrane. If this enzyme is defective or insufficient, the T cells die off as they divide instead of forming clones of themselves to fight allergen or infection. α-Tocopherol acts and the next line of antioxidant defense.

The evidence suggests that the current recommended daily allowance (RDA) for men and women of 22.4 IU tocopherol equivalents is inappropriately low, especially for elderly persons. However, too high a dose is associated with adverse consequences. The tolerable upper intake levels (Uls) for α-tocopherol at 1500 IU for adults (Institute of Medicine and Food and Nutrition Board 2000).

14.10.1.2 Vitamin C

Vitamin C, also known as ascorbic acid, is a water-soluble vitamin. It is not synthesized in the body and must be obtained exogenously. It has antioxidant properties on proteins, lipids, carbohydrates, and nucleic acids. It is a potent reducing agent and, therefore, readily donates electrons, preventing damage from ROS (Carr and Frei 1999, Wintergerst et al. 2006). Ascorbic acid concentrations in plasma and leukocytes rapidly decline during infections and other causes of oxidative stress.

Ascorbic acid is the main water-soluble free radical scavenger. It is important in neutralizing phagocyte-derived ROS that have been released extracellularly. Separately, ascorbic acid regenerates α-tocopherol from its oxidized state (Bruno et al. 2006). Ascorbic acid is found in high concentrations in phagocytes and T cells. It is actively transported into leukocytes, with intracellular concentrations 80 times that found in plasma. It has been shown to be effective in the treatment of some phagocytic cell dysfunction syndromes.

The belief that ascorbic acid can prevent or modify the *common cold* has not been substantiated scientifically. Modest effects on the duration of illness

have been seen. Studies have shown that glucose has an inhibitory effect on ascorbic acid's antioxidant effects (Wintergerst et al. 2006).

Unlike α-tocopherol, all forms of ascorbic acid supplements are equally effective as antioxidants. No toxic effects up to 10 grams/day in adults. Kidney stone formers should avoid a dose of more than 1000 mg/day. A large number of clinical trials with intake of 1000 mg of ascorbic acid, with or without 30 mg of zinc, are available. The tolerable Uls for ascorbic acid are 2000 mg for adults.

14.10.1.3 Vitamin A

Vitamin A is the name of a group of fat-soluble retinoids, including retinol, retinal, and retinyl esters. β-Carotene is a phytochemical. It is the main dietary source of vitamin A. The beauty of β-carotene, a vitamin A precursor, as a nutraceutical is that the body will only convert as much of it to vitamin A as the body needs. However, excess vitamin A intake can have several toxic sequelae. Vitamin A and β-carotene each independently has beneficial effects on the immune system.

Unrelated to vitamin A levels, β-carotene is an antioxidant and several human trials using dietary β-carotene supplementation have shown that it enhances humoral and cell-mediated immune responses, particularly in the elderly (Hughes 1999b).

14.10.1.4 β-Carotene

β-Carotene is one of a group of red, orange, and yellow pigments found in nutrients called carotenoids. Other carotenoids include lycopene, astaxanthin, and canthaxanthin. These, however, are not provitamins of vitamin A. All of these carotenoids have antioxidant effects and are beneficial for humoral and cell-mediated immunity. These benefit the immune system as antioxidants in ways similar to α-tocopherol (Chew and Park 2004). The tolerable Uls for vitamin A are 10,000 IU for adults.

14.10.1.5 Folic acid

Folic acid is a water-soluble vitamin B. Severe folate deficiency has been shown to impair cell-mediated immunity, resulting in decreased humoral response. Folic acid plays a crucial role in DNA and protein synthesis. Any mechanism in which cell proliferation plays a role can be affected by folate deficiency (Dhur et al. 1990).

Folic acid works as an antioxidant. It mostly functions as a scavenger of ROS. In addition, even though water soluble, it has also been shown to inhibit lipid peroxidation (Atteia et al. 2009). The RDA is 400 mcg per day, and the tolerable Uls are 1000 mcg for adults.

14.10.2 Phytochemicals

Phytochemicals are substances found in plants. They are often responsible for the color or odor of the plant. There are many thousands of different phytochemicals. Many of them are antioxidants. Refined or processed foods often contain little of the active phytochemicals (Halvorsen et al. 2006).

14.10.2.1 Carotenoids

β-Carotene, discussed earlier, is a carotenoid. It is the only carotenoid provitamin. There are 600 other carotenoids, many of which have antioxidant properties. There is a wide variety of basic science and human studies on various carotenoids. In general, a diet with plenty of colorful fresh vegetables and fruits will have copious amounts of carotenoids and other antioxidant phytochemicals. Unlike vitamins, the emphasis with phytochemical nutraceuticals should not be on a specific dose but on getting a wide array of many types of antioxidant phytochemicals for the beneficial effects on the immune system.

14.10.2.2 Polyphenols

Polyphenols are phytochemicals involved with defense against UV radiation in plants. There are 8000 different polyphenols in groups such as phenolic acids, flavonoids, and lignans. These are found in fruits, vegetables, tea, coffee, and red wines. A typical serving of tea, coffee, or red wine contains 100 mg of polyphenols. Flavonoids are the most studied group of polyphenols.

Bioavailability is highly variable. Many of the polyphenols are strong antioxidants having effects in plasma antioxidation and protecting against lipid peroxidation of PUFA membranes and nucleic acids. They have also been shown to inhibit the inflammatory mediator COX-1 (Seeram et al. 2003).

The grape polyphenol, resveratrol, has many purported *anti-aging* effects and is a potent antioxidant. It scavenges plasma ROS and lipid peroxidation ROS (Pandey and Rizvi 2009).

In general, the best way to obtain these nutrients is through a diet plentiful with fresh fruits, vegetables, and nuts. In addition, another variety of polyphenols is best obtained through regular consumption of green and black tea, coffee, and red wine.

14.10.2.3 Flavonoids

Flavonoids are polyphenolic substances found in a wide range of plants. Many of them are antioxidants and scavenge for ROS. Like the polyphenols in general, there is not a great deal of research on the flavonoids, especially in humans. Very little is known on the absorption, distribution, metabolism, and excretion of these compounds. Both the absorbed flavonoids and their

metabolites have antioxidant properties, impacting plasma antioxidant status, and preventing damage to PUFA membranes. Studies suggest that they also have a positive modulating effect on cells that respond to stimuli, such as the immune system cells (Middleton 1998). Several hundred milligrams of flavonoids are consumed daily in a typical Western diet. There is no RDA.

14.10.2.4 Allyl sulfides

These are phytochemicals, also known as organosulfides. They are most commonly found in garlic; up to 94% of garlic oil is comprised of these. They are considered to be antioxidants. Several studies suggest that high doses of these organosulfides can increase the NK immune cell activity. On the other hand, several cancer studies suggest that they promote the generation of ROS, leading to oxidative stress and apoptosis. More studies are needed to make any firm recommendations.

14.10.2.5 Cannabis

Cannabis can be considered to contain phytochemicals that are nutraceuticals. Cannabis in the form of oil and seeds have been used food/nutrients for 10,000 years. There are approximately 85 cannabinoids found in cannabis; of these, tetrahydrocannabinol (THC) and cannabidiol (CBD) are the most studied and well known. THC is the chemical in cannabis that has psychoactive effects, as well as temporary side effects of psychosis and anxiety, and is associated with dependency syndromes. On the other hand, CBD is non-psychoactive and very safe, with essentially no side effects unless it is given is very high doses, in which case, it can cause immunosuppression (Machado Bergamaschi et al. 2011).

In addition to cannabinoids, there are hundreds of terpenes in cannabis that can be considered nutraceutical antioxidants. THC, CBD, and several other cannabinoids are potent immunomodulators and exert their effects through the endocannabinoid system (ECS). Immune system cells in the body and brain have most of the cannabinoid 2 receptors (CB2). The body makes several natural endocannabinoids chemicals that bind to these receptors and regulate the immune system. CBD and THC interact with the CB2 receptors in different ways to suppress overactive immune responses.

Cannabinoids use the ECS to induce apoptosis and inhibit proliferation of macrophages and T cells. They also suppress cytokine production and induce T-regulatory cells. Separately, THC and CBD are also potent antioxidants via mechanisms unrelated to cannabinoid receptors and the ECS. In fact, CBD has been shown to be 30%–50% more potent antioxidant than α-tocopherol and ascorbic acid (Hampson et al. 2000).

14.10.2.5.1 THC THC has been shown to trigger marked apoptosis of T cells and dendritic cells in the brain, resulting in immunosuppression. THC also upregulates T-regulatory cells to suppress inflammatory responses (Nagarkatti et al. 2009).

14.10.2.5.2 CBD CBD is a very safe, non-psychoactive cannabinoid. When it is extracted from hemp versions of *cannabis sativa*, it is actually considered a food additive and sold over the counter in several nutraceutical supplements. CBD does not bind well with CB1 or CB2 receptors. It indirectly affects mostly CB2 receptors by decreasing the enzymatic breakdown of endocannabinoids. Some of its actions occur independently of the ECS or via a putative third cannabinoid receptor (Kaplan et al. 2008). Research is still ongoing. It has been demonstrated to be a potent suppressor of T-cell function and production of IL-2 and interferon (Zaric et al. 2010).

CBD is more potent for immunomodulation than THC. Long-term use of too high a dose of CBD can actually result in immunosuppression, aggravation of HIV, liver fibrosis, and other conditions (Nagarkatti et al. 2009).

14.10.2.5.3 Terpenes Cannabis has a wide array of terpenes. These are volatile aromatic oils. They are all fat soluble. Several of them are potent antioxidants. The most common is β-caryophylline, abbreviated BCP. It can make up as much as 35% of the essential oils of cannabis. This is considered a food additive, and is also present in significant quantities in black pepper, hops, cinnamon, oregano, basil, and many other nutrients. The estimated daily intake of BCP from the typical diet is 15–200 mg.

BCP acts as a cannabinoid, and binds to CB2 receptors, found mostly on immune system cells, and is a functional CB2 agonist (Gertsch et al. 2008). Separately, it is a potent antioxidant, impacting the immune system in this way as well.

14.10.3 Microminerals

Several microminerals are involved in immunomodulation via enzyme-related antioxidant effects.

14.10.3.1 Zinc

Zinc is a dietary micronutrient and potent antioxidant. It is second only to iron in its concentration in the body. Zinc is also known to play a central role in the immune system. It affects multiple mechanisms of the immune system. Zinc deficiency is associated with increased sensitivity to oxidative stress. It is essential to the development and function of cells mediating nonspecific immunity such as neutrophils and NK cells. It also affects development of acquired immunity, including T-cell activation, cytokine production, antibody production, and phagocytosis. Supplemental amounts of zinc (25 mg/day) have been associated with increased levels of CD4 T cells and NK cells in older adults (Shankar and Prasad 1998). Studies in the elderly have shown that zinc supplementation results in two-third decrease in incidence of infections. There was also a decrease in oxidative stress biomarkers and in cytokines (Prasad 2014).

Supplemental zinc ingestion results in antagonism of the redox catalytic metals iron and copper. This results in protection of protein sulfhydryl groups and reduction of some free radical formation. Chronic exposure to zinc induces other antioxidants, such as the metallothioneins. Excess ingestion of zinc can result in temporary diarrhea, vomiting, and abdominal cramping. Long-term use of excess zinc can result in copper or iron deficiency. The RDA is 8–11 mg a day.

14.10.3.2 Magnesium

Magnesium is a dietary micronutrient. It is an antioxidant and a cofactor in over 325 enzyme reactions. It is found in highest concentrations in the kidneys and liver and related to removal of toxins from the body. More studies have been conducted on magnesium deficiency as opposed to magnesium supplementation.

Low-grade magnesium deficiency is common and underdiagnosed. Chronic low-grade magnesium deficiency is strongly correlated with low-grade system inflammation. Magnesium deficiency is associated with high levels of ROS, low levels of intracellular glutathione, an increase in cytokines, and elevated leukocyte and phagocyte activation (Rayssiguier et al. 2006). Magnesium plays a key role as a cofactor for antibody synthesis and for macrophage response to cytokines. Adults who consume less than 75% of the RDA for magnesium are almost twice as likely to have elevated levels of CRP (Song et al. 2005).

Magnesium is involved with several aspects of the innate and acquired immune response. It is essential for the proper functioning of the enzyme gamma glutamyl transpeptidase (GGT). GGT speeds up glutathione peroxidase and the resulting reaction between glutathione and ROS (Zhang et al. 2005). GGT is involved in cysteine delivery to tissue demanding it in response to acute oxidative stress (Tam et al. 2003). Magnesium supplementation studies have yielded inconsistent results (Bo et al. 2006). The RDA for magnesium is 320–400 mg a day. However, higher daily intake of 600–1000 mg is safe and felt to achieve maximum benefits for the immune system. As many of 60% of adults don't consume the RDA (Laires and Monteiro 2008).

14.10.3.3 Selenium

Selenium is a dietary micronutrient and a potent antioxidant. It carries out biological effects through incorporation in selenoproteins, which play a role in regulating ROS in nearly all tissues. Several of the innate antioxidant enzymes contain selenium, including glutathione peroxidase and methionine sulfoxide reductase.

Selenium is an integral component of glutathione peroxidase, which, as discussed earlier, is intimately involved with the immune response. It is found concentrated in the liver, spleen, lymph nodes, and immune system cells.

Supplemental selenium has been shown to boost cell-mediated immune responses. It may also have a secondary effect of upregulating the expression of IL-2 receptors on T cells.

Selenoproteins are important for initiating immunity and regulating excessive immune response. Likewise, selenium deficiency is associated with decreased immune cell activation, differentiation, and proliferation. Supplementation in experimental animals has been associated with increases in NK cells and T-cell proliferation. Excess selenium can impair immune cell function as well and is associated with liver cirrhosis and neurological damage (Rayman 1997). Most adults in the United States get the RDA of 55–70 mcg per day of selenium (Hoffmann and Berry 2008). The tolerable Ul is 400 mcg per day for adults.

14.10.4 Enzymes

There are antioxidant enzymes synthesized from the proteins and minerals that we eat. They include SOD, glutathione peroxidase, glutathione reductase, and catalases. In order for antioxidant enzymes to provide optimum antioxidant activity, they require cofactors such as iron, copper, selenium, magnesium, and zinc. The quality of the protein source has an impact on the quality of the antioxidant enzymes. However, these are not true nutraceuticals.

14.10.4.1 Coenzyme Q10

Coenzyme Q10 (CoQ10) is an antioxidant nutrient found in high levels in organ meats such as liver and kidney. It is found is almost every cell in the body. It is fat soluble and found in the membranes of mitochondria and in plasma lipoproteins. It is an essential part of the cellular machinery that produces ATP. It is a primary scavenger of ROS and found in close proximity to PUFA chains in membranes (Littarru and Tiano 2007). The recommended dose for CoQ10 supplementation is 30–200 mg daily. Soft gels tend to be better absorbed than capsules or other preparations. Higher doses may be recommended for specific conditions.

14.11 Additional immunity-modulating antioxidants

14.11.1 Omega-3 fatty acids

Omega-3-polyunstaurated fatty acids are found in fish oil. The specific active compounds are docosahexaenoic acid and eicosapentaenoic acid. They have a wide array of powerful effects on immunomodulatory activities and inflammation (Ergas et al. 2002). Human supplementation studies have not been conclusive.

Several short-term studies using fish oil high in omega-3 fatty acids show a short-term anti-inflammatory effect. It is especially effective on chronic inflammatory conditions, probably through downregulation of cytokines.

Chronic daily intake probably reduces the risk of autoimmunity through promotion of apoptosis of autoreactive T cells (Fritsche 2006). Although they are antioxidants, they should not be considered super-antioxidants. There is no RDA for these fatty acids, although two to three servings of fish are recommended per week.

14.11.2 α-Lipoic acid

This is a dietary chemical, which was once thought to be a vitamin. It is primarily found in some meats. It is unusual in that it is both water and fat soluble. Unlike most antioxidants, it crosses the blood–brain barrier readily. It helps turn glucose into energy. It plays a vital role in mitochondrial dehydrogenase reactions. It is a potent antioxidant by increasing antioxidant enzymes. It interacts with ascorbic acid and glutathione to help recycle α-tocopherol. It also stimulates the production of the innate antioxidant, glutathione. α-Lipoic acid has been shown to inhibit expression of cytokines IL-6 and TNF-α, modulating the immune response to oxidative stress (Packer et al. 1995).

14.11.3 Mushrooms

Of the thousands of mushroom species, only about 20 are used for dietary purposes. Mushrooms contain many antioxidants. Mushrooms have some of the highest antioxidant activity of all foods. They are also high in selenium. Shiitake mushrooms have been associated with better functioning of T cells and decreased release of IL-8 and TNF-α (Dai et al. 2015). Taking 3–6 g a day of Turkey Tail mushrooms was associated with increased populations of NK cells.

Many mushrooms species contain a unique antioxidant called ergothioneine (ET). This is a derivative of the amino acid histidine and is not synthesized in the body. Its exact function is not known, but the presence of high levels of a specific transporter protein in mitochondria and cell nuclei implies an important physiologic role. ET is able to get into the mitochondria much more efficiently than other dietary antioxidants and acts as a potent intramitochondrial antioxidant. ET is as potent as the innate antioxidant glutathione. It is considered to be an antioxidant cytoprotectant (Paul and Snyder 2010). Deficiency is associated with cytotoxicity. It may represent a new vitamin.

14.12 Nutraceuticals through dietary choices

Many antioxidants such as ascorbic acid, ergothioneine, lutein, and the zeaxanthins are not synthesized by the human body and must be obtained from foods. Ideally, we should try to obtain all of the necessary antioxidants in sufficient amounts through dietary means rather than taking artificial

supplements. The concept of a nutraceutical supplement implies that there is an artificially manufactured tablet, powder, or liquid that contains higher or more concentrated amounts of the nutraceutical than that which is found in nature. In addition, there is the implication that dietary food choices alone would not provide the therapeutic effects of these supplements. However, for a large portion of society, it is not reasonable or feasible to obtain optimal daily amounts of fresh fruits, vegetables, and unprocessed meats. The use of antioxidant nutraceuticals represents an alternative and convenient, and perhaps economical, way to obtain health benefits from these substances. Awareness and education should be provided to make certain that excessive amounts of these substances are not ingested.

14.13 Summary

There is a wide array of antioxidants found in our diet, including vitamins, microminerals, phytochemicals, and other dietary substances. The body's innate antioxidant systems are not sufficient to deal with the ROS and dietary antioxidants are mandatory to prevent oxidative stress. Antioxidants protect both the structure and function of cells. Immune cells are particularly susceptible to the damaging effects of oxidative stress. This is due to the importance of cell signaling via cell membrane receptors and the formation of excessive ROS during the oxidative burst of phagocytosis.

The immune system is usually affected in a positive way by antioxidant nutraceuticals. Antioxidants work in four different areas. Water-soluble antioxidants reduce ROS in plasma. Fat-soluble antioxidants usually protect PUFA cell and mitochondrial membranes from peroxidation damage. Other antioxidant effects include protecting proteins and their enzymatic effects and protecting nucleic acids from causing transcription errors.

It is challenging to obtain optimal levels of antioxidant nutrients from our diet on a daily basis. So, in addition to a diet with a wide variety of fresh fruits, vegetables, and unprocessed meats, appropriate doses of supplemental antioxidants may be indicated.

References

Adolfsson, O., B. T. Huber, and S. N. Meydani. 2001. Vitamin E-enhanced IL-2 production in old mice: Naive but not memory T cells show increased cell division cycling and IL-2-producing capacity. *J Immunol* 167 (7):3809–3817.

Atteia, B. M. R., A. E. A. A. El-Kak, P. A. Lucchesi, and P. Delafontane. 2009. Antioxidant activity of folic acid: From mechanism of action to clinical application. *FASEB J* 23 (1 Supplement):103.7–103.7.

Bo, S., M. Durazzo, S. Guidi, M. Carello, C. Sacerdote, B. Silli, R. Rosato, M. Cassader, L. Gentile, and G. Pagano. 2006. Dietary magnesium and fiber intakes and inflammatory and metabolic indicators in middle-aged subjects from a population-based cohort. *Am J Clin Nutr* 84 (5):1062–1069.

Brambilla, D., C. Mancuso, M. R. Scuderi, P. Bosco, G. Cantarella, L. Lempereur, G. Di Benedetto, S. Pezzino, and R. Bernardini. 2008. The role of antioxidant supplement in immune system, neoplastic, and neurodegenerative disorders: A point of view for an assessment of the risk/benefit profile. *Nutr J* 7 (1):1–9. doi: 10.1186/1475-2891-7-29.

Bruno, R. S., S. W. Leonard, J. Atkinson, T. J. Montine, R. Ramakrishnan, T. M. Bray, and M. G. Traber. 2006. Faster plasma vitamin E disappearance in smokers is normalized by vitamin C supplementation. *Free Rad Bio Med* 40 (4):689–697. doi: 10.1016/j.freeradbiomed.2005.10.051.

Carr, A. C. and B. Frei. 1999. Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. *Am J Clin Nutr* 69 (6):1086–1107.

Chew, B. P. and J. S. Park. 2004. Carotenoid action on the immune response. *J Nutr* 134 (1):257S–261S.

Crump, K. E., P. K. Langston, S. Rajkarnikar, and J. M. Grayson. 2013. Antioxidant treatment regulates the humoral immune response during acute viral infection. *J Virol* 87 (5):2577–2586.

Dai, X., J. M. Stanilka, C. A. Rowe, E. A. Esteves, C. Nieves Jr, S. J. Spaiser, M. C. Christman, B. Langkamp-Henken, and S. S. Percival. 2015. Consuming *Lentinula edodes* (Shiitake) mushrooms daily improves human immunity: A randomized dietary intervention in healthy young adults. *J Am Coll Nutr* 34 (6):478–487.

Dalle-Donne, I., G. Aldini, M. Carini, R. Colombo, R. Rossi, and A. Milzani. 2006. Protein carbonylation, cellular dysfunction, and disease progression. *J Cell Mol Med* 10 (2): 389–406. doi: 10.1111/j.1582-4934.2006.tb00407.x.

Davis, S., B. M. Davis, J. L. Richens, K.-A. Vere, P. G. Petrov, C. P. Winlove, and P. O'Shea. 2015. α-Tocopherols modify the membrane dipole potential leading to modulation of ligand binding by P-glycoprotein. *J Lipid Res* 56 (8):1543–1550.

De la Fuente, M. 2002. Effects of antioxidants on immune system ageing. *Europ J Clin Nutr* 56 (S3):S5.

Dhur, A., P. Galan, and S. Hercberg. 1990. Folate status and the immune system. *Progr Food Nutr Sci* 15 (1–2):43–60.

Ergas, D., E. Eilat, S. Mendlovic, and Z. M. Sthoeger. 2002. n-3 fatty acids and the immune system in autoimmunity. *Isr Med Ass J* 4 (1):34–38.

Fritsche, K. 2006. Fatty acids as modulators of the immune response. *Ann Rev Nutr* 26 (1): 45–73. doi: 10.1146/annurev.nutr.25.050304.092610.

Fusco, D., G. Colloca, M. R. L. Monaco, and M. Cesari. 2007. Effects of antioxidant supplementation on the aging process. *Clin Interv Aging* 2 (3):377–387.

Gertsch, J., M. Leonti, S. Raduner, I. Racz, J.-Z. Chen, X.-Q. Xie, K.-H. Altmann, M. Karsak, and A. Zimmer. 2008. Beta-caryophyllene is a dietary cannabinoid. *Proc Nat Acad Sci USA* 105 (26):9099–9104. doi: 10.1073/pnas.0803601105.

Halvorsen, B. L., M. H. Carlsen, K. M. Phillips, S. K. Bøhn, K. Holte, D. R. Jacobs, and R. Blomhoff. 2006. Content of redox-active compounds (ie, antioxidants) in foods consumed in the United States. *Am J Clin Nutr* 84 (1):95–135.

Hampson, A. J., M. Grimaldi, M. Lolic, D. Wink, R. Rosenthal, and J. Axelrod. 2000. Neuroprotective antioxidants from marijuana. *Ann NYAcad Sci* 899:274–82.

Hoffmann, P. R. and M. J. Berry. 2008. The influence of selenium on immune responses. *Mol Nutr Food Res* 52 (11):1273–1280. doi: 10.1002/mnfr.200700330.

Hughes, D. 1999a. Effects of dietary antioxidants on the immune function of middle-aged adults. *Proc Nutr Soc* 58 (1):79–84.

Hughes, D. A. 1999b. Effects of carotenoids on human immune function. *Proc Nutri Soc* 58 (3):713–718.

Institute of Medicine and Food and Nutrition Board. 2000. *Dietary Reference Intakes: Vitamin C, Vitamin E, Selenium, and Carotenoids*. Washington, DC: National Academies Press.

Kaplan, B. L. F., A. E. B. Springs, and N. E. Kaminski. 2008. The profile of immune modulation by cannabidiol (CBD) involves deregulation of nuclear factor of activated T cells (NFAT). *Biochem Pharmacol* 76 (6):726–737. doi: 10.1016/j.bcp.2008.06.022.

Laires, M. J. and C. Monteiro. 2008. Exercise, magnesium and immune function. *Mag Res* 21 (2):92–96.

Lanzavecchia, A. 1985. Antigen-specific interaction between T and B cells. *Nature* 314 (6011):537–539.

Littarru, G. P. and L. Tiano. 2007. Bioenergetic and antioxidant properties of coenzyme Q10: Recent developments. *Mol Biotechnol* 37 (1):31–37.

Machado Bergamaschi, M., Q. R. Helena Costa, S. Crippa, A. Waldo Zuardi, and J. Alexandre. 2011. Safety and side effects of cannabidiol, a cannabis sativa constituent. *Curr Drug Saf* 6 (4):237–249. doi: 10.2174/157488611798280924.

Matsushita, M., S. Freigang, C. Schneider, M. Conrad, G. W. Bornkamm, and M. Kopf. 2015. T cell lipid peroxidation induces ferroptosis and prevents immunity to infection. *J Exp Med* 212 (4):555–568.

Meydani, S., M. Meydani, J. B. Blumberg et al. 1997. Vitamin e supplementation and in vivo immune response in healthy elderly subjects: A randomized controlled trial. *Jama* 277 (17):1380–1386.

Middleton, E. 1998. Effect of plant flavonoids on immune and inflammatory cell function. In: *Flavonoids in the Living System*. Boston, MA: Springer US, pp. 175–182.

Moshfegh, A., J. Godlman, and L. Cleveland. 2005. What we eat in America NHANES 2001–2002: Usual nutrient intakes from food compared to dietary reference intakes. *US Depart Agri, Agri Res Ser*.

Muller, F. 2000. The nature and mechanism of superoxide production by the electron transport chain: Its relevance to aging. *J Am Aging Ass* 23 (4):227–253. doi: 10.1007/s11357-000-0022-9.

Nagarkatti, P., R. Pandey, S. A. Rieder, V. L. Hegde, and M. Nagarkatti. 2009. Cannabinoids as novel anti-inflammatory drugs. *Fut Med Chem* 1 (7):1333–1349. doi: 10.4155/fmc.09.93.

Packer, L., E. H. Witt, and H. J. Tritschler. 1995. Alpha-lipoic acid as a biological antioxidant. *Free Rad Bio Med* 19 (2):227–250. doi: 10.1016/0891-5849(95)00017-R.

Pandey, K. B. and S. I. Rizvi. 2009. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid Med Cell Longev* 2 (5):270–278.

Park, O. J., H. Y. Kim, W. K. Kim, Y. J. Kim, and S. H. Kim. 2003. Effect of vitamin E supplementation on antioxidant defense systems and humoral immune responses in young, middle-aged and elderly Korean women. *J Nutr Sc Vitaminol* 49 (2):94–99.

Paul, B. D. and S. H. Snyder. 2010. The unusual amino acid L-ergothioneine is a physiologic cytoprotectant. *Cell Death Differ* 17 (7):1134–1140. doi: 10.1038/cdd.2009.163.

Prasad, A. S. 2014. Zinc is an antioxidant and anti-inflammatory agent: Its role in human health. *Front Nutr* 1:14. doi: 10.3389/fnut.2014.00014.

Puertollano, M. A., E. Puertollano, G. Alvarez de Cienfuegos, and M. A. de PabloM. 2011. Dietary antioxidants: Immunity and host defense. *Curr. Top. Med Chem* 11 (14):1752–1766.

Rayman, M. P. 1997. Dietary selenium: Time to act. *Br Med J* 314 (7078):387–388.

Rayssiguier, Y., E. Gueux, W. Nowacki, E. Rock, and A. Mazur. 2006. High fructose consumption combined with low dietary magnesium intake may increase the incidence of the metabolic syndrome by inducing inflammation. *Mag Res* 19 (4):237–243.

Seeram, N. P., R. H. Cichewicz, A. Chandra, and M. G. Nair. 2003. Cyclooxygenase Inhibitory and antioxidant compounds from crabapple fruits. *J Agri Food Chem* 51 (7):1948–1951. doi: 10.1021/jf025993u.

Serafini, M. 2000. Dietary vitamin E and T cell-mediated function in the elderly: Effectiveness and mechanism of action. *Int J Dev Neurosci* 18 (4–5):401–410. doi: 10.1016/S0736-5748(00)00016-2.

Shankar, A. H. and A. S. Prasad. 1998. Zinc and immune function: The biological basis of altered resistance to infection. *Am J Clin Nutr* 68 (2):447S–463S.
Song, Y., P. M. Ridker, J. E. Manson, N. R. Cook, J. E. Buring, and S. Liu. 2005. Magnesium intake, C-reactive protein, and the prevalence of metabolic syndrome in middle-aged and older U.S. Women. *Diabetes Care* 28 (6):1438–1444.
Stark, G. 2005. Functional consequences of oxidative membrane damage. *J Membr Biol* 205 (1):1–16. doi: 10.1007/s00232-005-0753-8.
Tam, M., S. Gomez, M. Gonzalez-Gross, and A. Marcos. 2003. Possible roles of magnesium on the immune system. *Europ J Clin Nutr* 57 (10):1193–1197.
Wintergerst, E. S., S. Maggini, and D. H. Hornig. 2006. Immune-enhancing role of vitamin C and zinc and effect on clinical conditions. *Ann Nutr Metabol* 50 (2):85–94.
Zaric, V., B. Busbee, P. Nagarkatti, and M. Nagarkatti. 2010. Potential of cannabinoids to modulate the inflammatory response in colitis. *J Immunol* 184 (Meeting Abstracts 1): 87–91.
Zhang, H., H. J. Forman, and J. Choi. 2005. γ-Glutamyl Transpeptidase in Glutathione Biosynthesis. *Meth Enzymol* 401:468–483.

15

Free Radicals and Antioxidants in Better Healthcare

Shannon Kelly and Rohini Nimbalkar

Contents

15.1 Introduction

A healthy balance between free radicals and antioxidants is necessary for proper functioning of the biological systems within the human body. Antioxidants are molecules that inhibit oxidation of free radicals, which would otherwise react and cause damage to cells. The conversion of free radicals into harmless water and oxygen can occur via various mechanisms, including catalysis, the Weiss reaction, chain-breaking antioxidants, and chemical quenching. Antioxidants can be categorized into two different groups: primary antioxidants, which are naturally occurring, and secondary antioxidants, which are synthetic. Neither group can be produced by the body, and must therefore be obtained through proper diet, vitamins, and/or supplements. Free radicals, though dangerous, are simply unpaired electrons in the outer shell of an atom. These electrons alone have the ability to pierce cell membranes and destroy DNA. However, free radicals can also be beneficial to the human body. Their uses range from regulation of intracellular signaling to the induction of cell division and apoptosis. The two main categories of free radicals are reactive oxygen species (ROS) and reactive nitrogen species (RNS); both can be produced by enzymatic and nonenzymatic reactions. Health problems ensue when there is an accumulation of free radicals, also known as oxidative stress. If left untreated, oxidative stress can then lead to cancer, cardiovascular diseases, neurological diseases, pulmonary diseases, rheumatoid arthritis, ocular diseases, preeclampsia, renal disorder, and even aging. With such a broad spectrum for the beneficial and dangerous effects of free radicals, methods have been created that measure free radical activity in order to aid in the development of commercial products and diagnosis. The concentrations of antioxidants, oxidized macromolecules, and free radicals are measured using GC–MS, high-performance liquid chromatography (HPLC), and electron spin resonance methods. Continued research toward the sensitive balance of antioxidants and free radicals within the human body will help to improve healthcare overall.

15.2 Antioxidants

In recent years, antioxidants have been exploited for their therapeutic potential in treating diseases caused by oxidative stress (Percival 1998; Gupta and Sharma 2006; Rathore et al. 2011). Antioxidants are molecules that inhibit the oxidation of free radicals. Free radicals such as ROS and RNS produced as a result of oxidation are harmful to the cell. Antioxidants such as ascorbic acid in vitamin C and retinol in β-carotene help to prevent and terminate these free radicals from reacting and causing damage. Free radicals are discussed in detail later in Section 15.3. The destructive effects of free radicals can be prevented with the addition of antioxidants or antioxidant supplements in the diet. A good antioxidant complex supplement actually has advantages over

natural sources in that the complex has several different types of antioxidants which seek out and destroy free radicals at various cellular sites. A single antioxidant, for example, vitamin E, protects the outer fatty layers of the cell but will not stabilize DNA. Rather, this is one of the main effects of the antioxidant vitamin C. The process by which different antioxidants disperse through the bloodstream to protect cells at different sites is referred to as *antioxidant synergy*. When a specific antioxidant meets a free radical in the bloodstream at its appropriate activity site, it naturally interacts with it and converts the free radical to harmless water and oxygen. As a result, as antioxidant levels increase due to the supplementation of higher amounts of a greater variety of antioxidants, cellular damage lessens, and performance and health improve (Hamid et al. 2010).

Characteristics of antioxidants: The major antioxidants currently used in foods are monohydroxy or polyhydroxy phenol compounds with various ring substitutions. These compounds have low activation energies to donate hydrogen. Hence, the resulting antioxidant radical does not initiate another free radical due to the stabilization of the delocalized radical electron. Propagation and initiation of free radicals chain reaction can be delayed or minimized by the donation of hydrogen from the antioxidants and metal-chelating agent. The resulting antioxidant free radical is not subject to rapid oxidation due to its stability. Antioxidant free radicals can also react with lipid free radicals to form a stable complex compound, thereby preventing some of their damages (Hamid et al. 2010).

Antioxidants fight against free radicals and oxidative stress by the following mechanisms (Sen and Chakraborty 2011):

1. *The catalytic systems to neutralize or divert ROS*: Defenses in plants are more sophisticated and specific as need increases. Oxygen is a waste product for plants. This causes the catalytic systems to divert superoxide to the less reactive hydrogen peroxide and transform the ROS into water (Benzie 2003).
2. *Binding or inactivation of metal ions prevents generation of ROS by Haber–Weiss reaction*: Metal chelators decrease oxidation by preventing metal redox cycling, forming insoluble metal complexes, or providing steric hindrance between metals and food components or their oxidation intermediates (Graf and Eaton 1990). Ethylenediaminetetraacetic acid (EDTA) and citric acid are the most common metal chelators found in foods. Most chelators are water soluble, but citric acid can be dissolved in oils with some limitation to chelate metals in the oil system. Phospholipids also act as metal chelators (Koidis and Boskou 2006). Flavonoids can also bind to metal ions (Rice-Evans et al. 1996) and the activity is closely related to the structural features: 3′,4′-dihydroxy group in the B ring, the 4-carbonyl and 3-hydroxy group in the C ring, or the 4-carbonyl

$$^1O_2 + Q \rightarrow [O_2^- \text{ --- } Q^+]^1 \rightarrow [O_2^- \text{ --- } Q^+]^3 \rightarrow {}^3O_2 + Q$$

Figure 15.1 *Quenching of ROS. (From Choe, E. and Min, D.B. Compr. Rev. Food Sci. Food Saf., 8, 345–358, 2009.)*

group in the C ring together with the 5-hydroxy group in the A ring (Hudson and Lewis 1983; Feralli 1997). Lignans, polyphenols, ascorbic acid, and amino acids, such as carnosine and histidine, can also chelate metals (Choe and Min 2009).

3. *Suicidal and chain-breaking antioxidants scavenge and destroy ROS*: When a radical is released or gains an electron, a secondary radical formation takes place. The last radical formed exerts the same action on another molecule and continues until either the free radical formed is stabilized by a chain-breaking antioxidant (vitamin C, E, carotenoids, etc.), or it simply disintegrates to form an inoffensive product. The classic example of such a chain reaction is lipid peroxidation (Pham-Huy et al. 2008).
4. *Absorb energy, electron, and quenching of ROS*: Any reaction of a free radical that does not yield free radicals as products is referred to as free radical quenching (Hess 1994). Chemical quenching of singlet oxygen is a reaction involving the oxidation of a quencher, thus producing breakdown or oxidation products of a quencher. β-Carotene, tocopherols, ascorbic acid, amino acids (such as histidine, tryptophan, cysteine, and methionine), peptides, and phenolics are oxidized by singlet oxygen, and they are all chemical quenchers of singlet oxygen. When a quencher has high reduction potential and low triplet energy, it quenches singlet oxygen by a charge transfer mechanism as shown in **Figure 15.1**. These types of quenchers are amines, phenols (including tocopherols), sulfides, iodide, and azides, all of which have many electrons (Min et al. 1989). The quencher donates electron to singlet oxygen to form a singlet state charge transfer complex and then changes the complex to the triplet state by intersystem crossing. Finally, the triplet state charge transfer complex is dissociated into triplet oxygen and a quencher.

15.2.1 Classification

Broadly, antioxidants are classified into the following categories:

1. *Primary or natural antioxidants*: Primary antioxidants are mainly phenolic in nature and convert lipid radicals into more stable products by breaking chain. Examples include
   a. *Minerals*: Minerals such as selenium, copper, zinc, manganese, and iron act as cofactors of antioxidants enzyme. They play a role in metabolism of macromolecules such as carbohydrates.

b. *Vitamins*: Vitamins such as B, C, and E act as antioxidants in the body's metabolic functions.
c. *Phytochemicals*: Phytochemicals are usually from plant sources. These include flavonoids, catechins, and carotenoids (Hamid et al. 2010).

2. *Secondary or synthetic antioxidants*: These are preventive antioxidants. Secondary antioxidants do not stabilize free radicals like primary antioxidants. Instead, they act as chelators or catalysts. They enhance the antioxidant activity of primary antioxidants. Examples include acids such as citric, malic, succinic, tartaric, EDTA, ascorbic, and erythorbic acids. Other examples are phosphates, sulfites, and ascorbyl palmitate (Wanasundara 2005).

15.2.2 Sources

- Since antioxidants cannot be produced by the body, they must come from a person's diet or another external source. The main sources of antioxidants are food, vitamins, and supplements (**Table 15.1**).

15.2.2.1 Food

Fruits and vegetables, which are high in nutrients, are often great sources of antioxidants because they contain high levels of vitamins and phytochemicals. The foods rich in antioxidants include, turmeric, soybean, wine, tea, garlic, and olive oil (Smythies 1998).

15.2.2.2 Vitamins

Vitamins like A, C, and E possess antioxidant activity. β-Carotene (pro-vitamin A) is a carotenoid and possesses antioxidant activity. Retinol also has an antioxidant effect. These are essential in maintaining healthy vision (Dasgupta and Klein 2014). Vitamin C (ascorbic acid) is the only water-soluble antioxidant and plays an important role in many intracellular functions. It plays a role in protein and lipid metabolism, biosynthesis of collagen and carnitine, and absorption of iron. Vitamin C can be obtained from foods such as citrus fruits, melons, and cherries, and vegetables such as tomatoes, leafy greens, broccoli, and cauliflower. Vitamin E exists in eight different forms namely, α-,

Table 15.1 Sources of Antioxidants

Source	Examples
Food	Tumeric, soybean, wine, tea, garlic, and olive oil
Vitamins	Vitamins A, C, E, and retinol
Supplements	Selenium supplements

β-, γ-, δ-tocopherol and α-, β-, γ-, δ-tocotrienol (Dasgupta and Klein 2014). Major sources of vitamin E in the diet include vegetable oils, wheat germ, and nuts. Vitamin E maintains lipid structure. Vitamin E requires the presence of vitamin C for proper antioxidant effect. Together, they prevent oxidation of lipoproteins.

15.2.2.3 Supplements

Selenium is an important antioxidant supplement. It is important in metabolic pathways, thyroid hormone metabolism, and immunity. The main sources for selenium are whole grains and shellfish.

15.3 Free radicals

At the atomic level, free radicals are unpaired electrons in the outer shell of an atom. Although they may seem trivial, they can be very reactive and cause much damage to the cell by destroying cell membranes or DNA. The two main types of radical species are ROS and RNS (**Table 15.2**). The oxygen species radicals include hydroxyl, peroxyl, superoxide, alkoxyl, and lipid peroxyl. The nitrogen radicals include nitrogen dioxide and nitric oxide (Sen and Chakraborty 2011). Hydrogen peroxide (H_2O_2), ozone (O_2), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl), nitrous acid (HNO_2), peroxynitrite ($ONOO^-$), dinitrogen trioxide (N_2O_3), and lipid peroxide (LOOH) are not free radicals. They are generally called oxidants, but can easily lead to free radical reactions in living organisms (Genestra 2007).

15.3.1 Production

Enzymatic and nonenzymatic reactions produce free radicals. Examples of these reactions are shown in **Figure 15.2**. Oxygen is required during these reactions for the production of ROS and RNS. They are formed during the breakage of a chemical bond, usually during a redox reaction. They are

Table 15.2 Oxidants by Classification

ROS	RNS	Not Free Radicals
$\bullet OH$	$\bullet NO_2$	H_2O_2
HO_2^-	$\bullet NO$	O_3
O_2^-		$^1O_2$
CH_3O^-		HOCl
		HNO_2
		$ONOO^-$
		N_2O_3
		LOOH

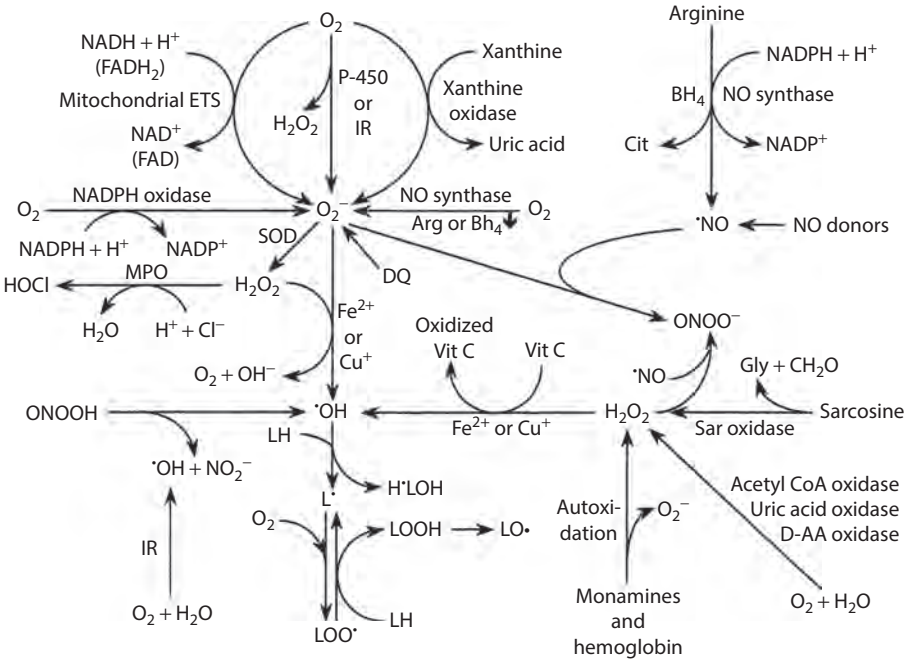


Figure 15.2 *Free radical production. (From Fang, Y. et al., Nutrition, 18, 872–879, 2002.)*

created as a by-product of ATP production in the mitochondria. Enzymatic reactions that generate free radicals include those taking place in the respiratory chain, phagocytosis, prostaglandin synthesis, and the cytochrome P450 system. Free radicals can be produced from nonenzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing radiations. It is also possible that the nonenzymatic process occurs during oxidative phosphorylation.

15.3.2 Benefits

When discussing free radicals, it is common to focus on the negative consequences. At low and even moderate concentrations, free radicals can be beneficial to the body. For example, free radicals play a key role in the regulation of intracellular signaling and the induction of cell division. Free radicals also defend against infectious agents by phagocytosis. They detoxify xenobiotics by cytochrome P450, which generates ATP in the mitochondria. Free radicals are also involved in cell growth and the induction of mitogenic responses (Sen and Chakraborty 2011). They can induce apoptosis of effete or defective cells, which explains their role in the eradication of cancerous cells (Devasagayam et al. 2004).

15.4 Oxidant-antioxidant balance

15.4.1 Oxidative stress

Oxidative stress can be defined as the accumulation of free radicals. This process can lead to deleterious effects on health causing damage to cells and the proteins within. Oxidative stress takes place when excess free radicals are not destroyed causing imbalance between formation and neutralization of reactive species. Some of the major chronic diseases and disorders caused by oxidative stress include the following:

1. *Cancer*: DNA damage by oxidation is a leading cause of cancer. The free radicals of ROS deactivate the detoxifying enzymes that are responsible for the neutralizing potent carcinogens. This interferes in cell growth causing mutations and carcinogenesis.
2. *Cardiovascular diseases*: Arteriosclerosis is the primary cause for major cardiovascular diseases. There is much evidence supporting that oxidative stress caused by oxidation of low-density lipoprotein (LDL) is a risk factor and plays a significant role in the pathogenic pathway (Berliner and Heinecke 1996). Free radicals initiate lipid peroxidation resulting in the oxidation of LDL. The underlying biochemical process includes a layer of foam cells formed upon the intake of oxidized LDL and scavenger receptor.
3. *Neurological diseases*: Oxidative stress might lead to neuronal degeneration related to diseases like Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease (Kondo 1996). Free radicals generated due to oxidation can cause both necrosis and apoptosis. Lipid peroxidation involved in this process then causes the cell membrane to rupture. This might result in necrotic cell death of neurons (Diplock et al. 1998).
4. *Pulmonary diseases*: Oxidative stress can cause muscular dysfunction of the peripheral skeletal muscles and eventually lead to chronic obstructive pulmonary disease (COPD). The leading cause of COPD is smoking cigarettes. The mechanism involves activation of necrosis factor kb in monocytes or macrophages. This causes the release of proinflammatory mediators in lung epithelial fluid and amplifies inflammatory response (Rahman 2005).
5. *Rheumatoid arthritis*: Rheumatoid arthritis is an autoimmune disease that is characterized by inflammation of the tissue surrounding the joints. This is associated with the infiltration of macrophages and activated T cells. The underlying pathophysiology for this disease is the generation of ROS and RNS at the site of inflammation. Increased levels of isoprostanes and prostaglandins in serum and synovial liquid causes oxidative damage, leading to rheumatoid disease (Mahajan and Tandon 2004).
6. *Ocular diseases*: Proteins in the lens of the eye possess an extremely long duration of life. They are often prone to oxidative damage, since

they have chronic exposure to light and oxygen. This causes formation of ROS, which may react with the proteins in the lens. Subsequently, the damaged proteins aggregate, precipitate, and lose their regular function.

7. *Fetal growth*: Oxidative stress can cause vascular dysfunction of the placenta and preeclampsia (Myatt et al. 2000). Preeclampsia is a disease of pregnancy. It is a life-threatening and hypertensive condition characterized by the presence of autoantibodies responsible for activating the major angiotensin receptors (Xia and Kellems 2009).

15.4.2 Role in disease

Oxidative stress plays a critical role in the causation of many diseases including cancer, cardiovascular disease, rheumatoid arthritis, neurological disease, renal disorders, pulmonary diseases, ocular disorders, and aging.

15.4.2.1 Cancer

Free radicals often react with the DNA of the cells, causing mutations in DNA sequences. DNA mutation is a key step in the development of carcinogens and cancer in the body. In cancer, there is a mutant cell that reproduces uncontrollably, causing formation of tumors. This mutant cell could be made due to oxidative stress in the body. Antioxidants may act by making the free radical a *normal* cell again and restoring the *normal* cell cycle to prevent the growth of these tumors. Vitamins like A, C, and E exert antioxidant effects. Vitamin C lowers the risk of breast and cervical cancer. It provides the greatest protection against stomach cancer. This can be attributed to its role in reducing nitrosamines (Borek 1997). β-Carotene reduces the risk of breast, cervix, esophagus, lungs, and stomach cancers (Borek 1997). The photoprotective property of β-carotene helps in protecting against ultraviolet light-induced cancers (Sen and Chakraborty 2011). Vitamin E has inverse relation in preventing and inducing cancer in humans. At low levels, there is five times increase in breast cancer (Borek 1997). Vitamin E supplements reduce the risk of cancer in the oral cavity, esophagus, and larynx.

15.4.2.2 Cardiovascular disease

Oxidative stress can play a key role in pathogenesis of cardiovascular diseases such as ischemic heart disease and atherosclerosis. Antioxidant vitamins can potentiate endothelial nitric oxide levels and inhibit vascular inflammation, lipid peroxidation, platelet aggregation, and oxidation of LDL. This is beneficial in preventing endothelial dysfunction. Cardiovascular disease induced by oxidative stress can be prevented by consuming fruits, vegetables, or legumes. This activity is due to presence of unique dietary antioxidant components. These components reduce oxidative stress by influencing a variety of cellular activities. They inhibit lipid oxidation, cause an increase in plasma antioxidant

capacity, aid the reduction of platelet aggregation and plasma lipid levels reduction, and scavenge free radicals. Antioxidants reduce intracellular ROS generation, the induction of glutathione (GSH), antiapoptotic effect, increase in bioavailability of nitric oxide, reduction in matrix metalloproteinase production, anti-inflammatory responses related to cardiovascular health, and decreases the chance of mortality (Maxwell and Lip 1997; Hercberg et al. 1998; Blomhoff 2005; Tandon et al. 2005; Wang et al. 2011).

15.4.2.3 Rheumatoid arthritis

Rheumatoid arthritis is an inflammatory disorder that affects not only the joints but also the skin, eyes, lungs, and heart. It is an autoimmune disorder that occurs when the immune system attacks the body's own tissue as though it were a pathogen. This disease is linked to the formation of free radicals at the inflammation site. Oxidative stress caused by the production of too many free radicals can contribute to inflammation. Using antioxidants such as vitamin C has proven to alleviate the effects of rheumatoid arthritis (Jaswal et al. 2003).

15.4.2.4 Neurological disease

Neurological disorders are usually characterized by damage to the nerve cells in the brain and spinal cord leading to alterations in sensory perceptions. Examples of such diseases include Parkinson's disease and Alzheimer's disease. Free radicals play a role in the development of neurological diseases because the brain itself consumes a significant portion of the oxygen in the body. Also, the brain has relatively less antioxidants to combat free radicals than other parts of the body, so there is more scope for oxidative stress and alterations that could lead to various neurological diseases.

15.4.2.5 Renal disorders

The renal system, also known as the urinary system, consists of the kidneys, ureters, bladder, and urethra. This organ system can also be affected by the presence of free radicals because the free radicals can react with proteins in the cell membranes of nephrons, which are the functional units of the kidneys. The membranes of the nephrons play an extremely important role in their functions because the function of the kidney is to filter out substances using its selectively semipermeable membrane. Damage to the membrane would cause damage to the whole kidney and therefore to the entire renal system. The antioxidant vitamin E has proven to be beneficial in situations of declined kidney function and renal failure.

15.4.2.6 Pulmonary disease

The pulmonary system is also known as the respiratory system and is responsible for maintaining oxygen and carbon dioxide levels in the body. It can be

caused by both inflammation of the airway and oxidative stress. One of the main sources of free radical production is smoke and air pollution. Therefore, smoking could increase the production of free radicals as well as increase the risk of pulmonary diseases such as asthma. In other cases, oxidative stress causes inflammation similarly to rheumatoid arthritis. Inflammation of the airway can make breathing difficult, causing other pulmonary issues.

15.4.2.7 Ocular disorders

The eyes are one of the most sensitive organs of the body and can be damaged easily. Oxidative stress in the eyes has been recognized as a cause of eye disorders such as cataracts. The proteins in the lens of the eye can be damaged when forced to interact and react with a free radical. In the case of such ocular disorders, the antioxidant vitamin E has proven to be the most beneficial treatment.

15.4.2.8 Aging

The process of aging can be traced to free radical production in the body. Free radicals tend to react with biological molecules such as proteins and damage them, contributing to aging. The theory that free radicals cause aging is known as the *free radical theory*.

15.5 Other antioxidant effects

Antioxidants play a key role in reducing the accumulation of free radicals, thereby protecting the body from oxidative stress. However, when an antioxidant interacts with a free radical, the antioxidant is oxidized and may become ineffective or toxic. These oxidized antioxidants can produce ROS and RNS in some cases, thereby acting as prooxidants (Pham-Huy et al. 2008). Some antioxidants have been rumored by popular media to have other benefits. For instance, β-carotene is widely believed to improve vision and other faculties. However, the Physicians' Health Study only showed improvements in cognitive function in men after 18 years of routine β-carotene consumption (Grodstein et al. 2007). It has also been hypothesized that vitamin E can reduce exercise-induced free radical damage (Sarma et al. 2010). More study is required to substantiate these claims.

15.5.1 Risks

Antioxidants are essential to the body's immune defense system. Without antioxidants, the body would fall victim to a host of diseases. However, the consumption of antioxidants is not without risks. For instance, β-carotene supplements may increase the risk of stroke and lung cancer in smokers (Sen and Chakraborty 2011). Many antioxidants produce antagonistic effects

when consumed in large quantities. An intake of more than 3000 mg of vitamin C daily can lead to the formation of kidney stones (Sen and Chakraborty 2011). Vitamin A can cause fatigue, breast soreness, gastrointestinal stress, renal dysfunction, vascular inflammation, or thyroid problems when consumed in excess of 1600–3200 mg/kg (Sen and Chakraborty 2011). In high doses, Coenzyme Q10 can cause severe hemorrhaging and may even act as a prooxidant (Sen and Chakraborty 2011).

Though natural antioxidants are generally believed to be safe, antioxidant supplements have been reported to have antagonistic effects (Sen and Chakraborty 2011). This may be because the antioxidant was already present in the food consumed, resulting in a large dose of the antioxidant. It may also be due to an interaction with other antioxidants that may have been consumed. The effects of combining antioxidants from food and from supplements are yet to be identified (Seifried et al. 2003). Antioxidants have been known to have interactions with other drugs, however. For instance, the combination of vitamin E supplements with anticoagulants can increase the risk of bleeding (NCCAM 2010). Furthermore, long-term use of synthetic antioxidants may lead to chronic health conditions (Sen and Chakraborty 2011). The effect of antioxidants supplements and their interactions requires further study.

15.6 Assessing free radical activity

Assessing free radical activity is important to the development of commercial products and health standards as well as diagnosis. Depending on the circumstances, different methods may be used to assess free radical activity. One such method is to find the levels of endogenous antioxidants. This is usually completed by determining the concentration of antioxidants present in blood plasma and cells (Fang et al. 2002). Common antioxidants tested include α-tocopherol, γ-tocopherol, β-carotene, lycopene, carotenoids, and ascorbic acid (Diplock et al. 1998). Diplock et al. outlined methods to complete this using HPLC (1998).

An alternative method to assess free radical activity is by quantifying the products of oxidized macromolecules, including lipids and proteins (Fang et al. 2002). Lipid peroxidation can be measured by determining the peroxides and isoprostanes present in blood and urine. This can be determined via GC–MS (Aruoma 1998). However, this method cannot distinguish between products due to oxidative stress and those that were ingested (Diplock et al. 1998). Oxidative damage to DNA is continuous *in vivo*. A *steady state* is presumed to exist between the molecule's damage and repair stages (Aruoma 1998). Cells and tissues can be analyzed for the products of oxidative damage using HPLC or GC–MS (Diplock et al. 1998). In this steady state, an increase in damage products may be due to an increase in oxidative damage, a decrease in repair, or a combination of both (Aruoma 1998). Analyzing urine may show DNA base products as well. However, these results cannot distinguish what is

produced from DNA from what has been produced from other molecules in the body (Diplock et al. 1998).

Lastly, free radical activity can be assessed by measuring the free radicals within the body directly. Electron spin resonance can be used to accomplish this. However, this method is limited due to the water content of body tissues. Spin trapping techniques operate by making active free radicals nearly inert. Electron spin resonance can then be used to analyze the samples (Fang et al. 2002).

15.7 Future trends

Studies indicate that oxidative stress plays a key role in many diseases, including cancer. This in turn suggests that antioxidants may be a valid treatment. However, more research into this correlation and the effect of antioxidant supplements is needed to determine their viability as a treatment. For example, Rodgers et al. (2016) studied the effect of antioxidants such as GSH on leukemia-induced fatigue and oxidative stress in children. Specifically, the ratio of GSH to glutathione disulfide (GSSG) present in the cerebrospinal fluid of pediatric leukemia patients was analyzed. It was determined that patients with low GSH/GSSG ratios experienced oxidative stress. Furthermore, patients with low GSH/GSSG ratios also indicated high fatigue during treatment (Rodgers et al. 2016). This suggests a relationship between GSH/GSSG ratios, oxidative stress, and fatigue. More study is needed to determine a treatment.

Jiang et al. (2016) studied the antioxidative effects of the polysaccharide GCPB-1b. The study reported that GCPB-1b had a higher scavenging effect on DPPH• radicals than vitamin C at low concentrations (Jiang et al. 2016). Khajehnasiri et al. (2016) studied the effect of omega-3 fatty acids and vitamin C on depression. It was determined that omega-3 fatty acids had a better impact on depression than vitamin C (Khajehnasiri et al. 2016). Balogun et al. (2016) studied the effect of various vitamin supplements on miscarriage. They concluded that while no vitamin can prevent a miscarriage, the consumption of multivitamins, iron, and folic acid showed a decreased risk for stillbirth (Balogun et al. 2016). It is clear that more research is needed to determine the viability of antioxidants as effective treatments for many conditions.

15.8 Conclusion

Understanding antioxidants and free radicals is essential to better healthcare. Oxidative stress caused by the accumulation of free radicals in the body has been linked to the development of several conditions, including cancer and aging. For instance, Rodgers et al. (2016) found a relationship between low GSH/GSSG ratios, oxidative stress, and fatigue in pediatric leukemia patients. The body combats the accumulation of free radicals naturally with antioxidants. For example, α-tocopherol, a vitamin E isomer, protects cell lines from

lipid peroxidation (Pham-Huy et al. 2008). In addition, studies have implicated that the consumption of antioxidant supplements has an impact on disease. For example, Khajehnasiri et al. (2016) found that omega-3 fatty acids have an impact on depression. However, antioxidants can be harmful to the body as well. When an antioxidant interacts with a free radical, the antioxidant becomes oxidized and may even act as a prooxidant (Pham-Huy et al. 2008). Therefore, more research is needed to determine the viability of antioxidant supplements as treatment for various diseases.

References

Aruoma, O. I. 1998. Free radicals, oxidative stress, and antioxidants in human health and disease. *Journal of the American Oil Chemists' Society* 75:199–212.

Balogun, O. O., K. da Silva Lopes, E. Ota, Y. Takemoto, A. Rumbold, M. Takegata, R. Mori. 2016. Vitamin supplementation for preventing miscarriage. *Cochrane Database Systematic Reviews* 5:CD004073.

Benzie, I. F. F. 2003. Evolution of dietary antioxidants. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology* 136:113–126.

Berliner, J. A., J. W. Heinecke. 1996. The role of oxidized lipoproteins in atherogenesis. *Free Radical Biology and Medicine* 20:707–727.

Blomhoff, R. 2005. Dietary antioxidants and cardiovascular disease. *Current Opinion in Lipidology* 16:47–54.

Borek, C. 1997. Antioxidants and cancer. *Science and Medicine* 4:52–61.

Choe, E., D. B. Min. 2009. Mechanisms of antioxidants in the oxidation of foods. *Comprehensive Reviews in Food Science and Food Safety* 8:345–358.

Dasgupta, A., K. Klein. 2014. *Antioxidants in Food, Vitamins and Supplements: Prevention and Treatment of Disease*. San Diego, CA: Elsevier.

Devasagayam, T. P. A., J. C. Tilak, K. K. Boloor, K. S. Sane, S. S. Ghaskadbi, R. D. Lele. 2004. Free radicals and antioxidants in human health: Current status and future prospects. *Journal of the Association of Physicians of India* 52:794–804.

Diplock, A. T., J.-L. Charleux, G. Crozier-Willi, F. J. Kok, C. Rice-Evans, M. Roberfroid, W. Stahl, J. Viña-Ribes. 1998. Functional food science and defence against reactive oxidative species. *British Journal of Nutrition* 80:S77–S112.

Fang, Y., S. Yang, G. Wu. 2002. Free radicals, antioxidants, and nutrition. *Nutrition* 18:872–879.

Genestra, M. 2007. Oxyl radicals, redox-sensitive signalling cascades and antioxidants. *Cell Signalling* 19:1807–1819.

Graf, E., J. W. Eaton. 1990. Antioxidant functions of phytic acid. *Free Radical Biology and Medicine* 8:61–9.

Grodstein, F., J. H. Kang, R. J. Glynn, N. R. Cook, J. M. Gaziano. 2007. A randomized trial of beta carotene supplementation and cognitive function in men: The physicians' health study II. *Archives of Internal Medicine* 167:2184–2190.

Gupta, V. K., S. K. Sharma. 2006. Plants as natural antioxidants. *Natural Product Radiance* 5:326–334.

Hamid, A. A., O. O. Aiyelaagbe, L. A. Usman, O. M. Ameen, A. Lawal. 2010. Antioxidants: Its medicinal and pharmacological applications. *African Journal of Pure and Applied Chemistry* 4:142–151.

Hercberg, S., P. Galan, P. Preziosi, M. Alfarez, C. Vazquez. 1998. The potential role of antioxidant vitamins in preventing cardiovascular diseases and cancers. *Nutrition* 14:513–520.

Hess, J. L. 1994. Free radical scavenging. In: *Plant Responses to the Gaseous Environment.* Alscher, R. G. and A. R. Wellburn (Eds.), pp. 99–120. Dordrecht, the Netherlands: Springer Science.

Hudson, B. J. F., J. I. Lewis. 1983. Polyhydroxy flavonoid anti-oxidant for edible oils-structural criteria for activity. *Food Chemistry* 10:147–155.

Jaswal, S., H. C. Mehta, A. K. Sood, J. Kaur. 2003. Antioxidant status in rheumatoid arthritis and role of antioxidant therapy. *Clinica Chimica Acta* 338:123–129.

Jiang, J., F. Kong, N. Li, D. Zhang, C. Yan, H. Lv. 2016. Purification, structural characterization and in vitro antioxidant activity of a novel polysaccharide from Boshuzhi. *Carbohydrate Polymers* 147:365–371.

Khajehnasiri, F., S. Akhondzadeh, S. B. Mortazavi, A. Allameh, G. Sotoudeh, A. Khavanin, Z. Zamanian. 2016. Are supplementation of omega-3 and ascorbic acid effective in reducing oxidative stress and depression among depressed shift workers? *International Journal of Vitamin and Nutrition Research* 10:1–12.

Koidis, A., D. Boskou. 2006. The contents of proteins and phospsholipids in cloudy (veiled) virgin olive oils. *European Journal of Lipid Science and Technology* 108:323–328.

Kondo, T. 1996. Parkinson's disease and free radicals. *Annals of the New York Academy of Sciences* 786:206–216.

Mahajan, A., V. R. Tandon. 2004. Antioxidants and rheumatoid arthritis. *Journal of Indian Rheumatology Association* 12:139–142.

Maxwell, S. R., G. Y. Lip. 1997. Free radicals and antioxidants in cardiovascular disease. *British Journal of Clinical Pharmacology* 44:307–317.

Min, D. B., S. H. Lee, E. C. Lee. 1989. Singlet oxygen oxidation of vegetable oil. In: *Flavor Chemistry of Lipid Foods.* Min, D. B. and T. H. Smouse (Eds.), pp. 57–97. Champaign, IL: AOCS Press.

Myatt, L., W. Kossenjans, R. Sahay, A. Eis, D. Brockman. 2000. Oxidative stress causes vascular dysfunction in the placenta. *Journal of Maternal-Fetal Medicine* 9:79–82.

NCCAM. 2010. Antioxidants and Health: An Introduction. National Center for Complementary and Alternative Medicine "get the facts" pamphlet. Retrieved from https://nccih.nih.gov/health/antioxidants/introduction.htm.

Percival, M. 1998. Antioxidants. *Clinical Nutrition Insights* 31:1–4.

Pham-Huy, L. A., H. He, C. Pham-Huy. 2008. Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science* 4:89–96.

Rahman, I. 2005. Oxidative stress in pathogenesis of chronic obstructive pulmonary disease: Cellular and molecular mechanisms. *Cell Biochemistry and Physics* 43:167–188.

Rathore, G. S., M. Suthar, A. Pareek, R. N. Gupta. 2011. Nutritional antioxidants: A battle for better health. *Journal of Natural Pharmaceuticals* 2:2–14.

Rice-Evans C. A., N. J. Miller, G. Paganga. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine* 20:933–956.

Rodgers, C., C. Sanborn, O. Taylor, P. Gundy, A. Pasvogel, I. M. Moore, M. J. Hockenberry. 2016. Fatigue and oxidative stress in children undergoing leukemia treatment. *Biological Research for Nursing.* doi:10.1177/1099800416647794.

Sarma, A. D., A. R. Mallick, A. K. Ghosh. 2010. Free radicals and their role in different clinical conditions: An overview. *International Journal of Pharma Science and Research* 1:185–192.

Seifried, H. E., S. S. McDonald, D. E. Anderson, P. Greenwald, and J. A. Milner. 2003. The antioxidant conundrum in cancer. *Cancer Research* 63:4295–4298.

Sen, S. and R. Chakraborty. 2011. The role of antioxidants in human health. In: *Oxidative Stress: Diagnostics, Prevention, and Therapy.* Andreescu, S. and M. Hepel (Eds.), pp. 1–37. Washington, DC: American Chemical Society.

Smythies, J. R. 1998. *Every Person's Guide to Antioxidants.* New Brunswick, NJ: Rutgers University Press.

Tandon, V. R., S. Verma, J. B. Singh, A. Mahajan. 2005. Antioxidants and cardiovascular health. *Drug Reviews* 7:61–64.
Wanasundara, P. K. J. P. D., F. Shahidi. 2005. Antioxidants: Science, technology, and applications. In: *Bailey's Industrial Oil and Fat Products*. Bailey, A. E. and F. Shahidi (Eds.), pp. 1–11. Hoboken, NJ: John Wiley & Sons.
Wang, S., J. P. Melnyk, R. Tsao, M. F. Marcone. 2011. How natural dietary antioxidants in fruits, vegetables and legumes promote vascular health. *Food Research International* 44:14–22.
Xia, Y., R. E. Kellems. 2009. Is preeclampsia an autoimmune disease? *Clinical Immunology* 133:1–12.

16

Pharmacology and Pharmacokinetics of Natural Antioxidants in the Human Body

Aishwarya Potdar, Danielle Dantuma,
Charles Preuss, and Yashwant Pathak

Contents

16.1 Endogenous antioxidants

16.1.1 Glutathione

Glutathione is a tripeptide produced naturally by the liver and is found in most aerobic life forms (Lobo et al. 2010). It is recognized as one of the most important intracellular antioxidants due to its crucial role in maintaining redox balance and in protecting the cell against electrophiles, hydrogen peroxide, and free radicals (Gul et al. 2000, Lobo et al. 2010). Glutathione has remarkable free radical scavaging activity and is the most abundant cytosolic thiol.

Glutathione has many therapeutic applications, including treatment of chronic liver diseases and cataract. Various studies have shown elevated blood and tissue levels of oxidative stress markers in diabetic patients and demonstrated the ability of antioxidant supplementation to attenuate complications in diabetic animals (Augustyniak et al. 2010). Depletion of glutathione in the mitochondria has been associated with tissue damage that developed during subsequent reperfusion in rats with ischemia and other brain disorders (Anderson et al. 2004).

Glutathione demonstrates antioxidant activity by directly reducing oxidants and supporting the recycling of other antioxidants, vitamin C and E (Gul et al. 2000). The cysteine moiety of glutathione is responsible for its antioxidant action (Lobo et al. 2010). Cysteine contains a thiol group with strong electron-donating character, which is reversibly oxidized and reduced (Lobo et al. 2010). Glutathione becomes oxidized when electrons

are lost and two oxidized glutathione molecules link by a disulfide bridge, forming glutathione disulfide. Reduced glutathione is constantly regenerated by a redox cycle with glutathione reductase and NADPH (Ndhlala et al. 2010). Decreased levels of reduced glutathione increase cellular exposure to reactive oxygen species (ROS), causing oxidative damage (Augustyniak et al. 2010).

Glutathione is a cofactor for various enzymes responsible for protecting the cell against damage: glutathione *S*-transferases, peroxidases, and transhydrogenases. Glutathione *S*-transferases catalyze the conjugation of glutathione to exogenous electrophiles, preventing serious damage to biomolecules (Gul et al. 2000). Peroxidases use glutathione to detoxify the peroxides made from free radical attack (Gul et al. 2000). Transhydrogenases reverse oxidative damage by using glutathione to reduce oxidized portions of biomolecules.

Administering glutathione as a therapeutic agent is complicated due to its low bioavailability when administered orally or intraperitoneally (Gul et al. 2000). The synthetic antioxidant, *N*-acetylcysteine, solves this issue by acting as a proglutathione drug that increases glutathione levels within tissues *in vivo* (Gul et al. 2000).

Glutathione clearance follows first-order, concentration-independent, kinetics. The synthesis and degradation of glutathione are catalyzed by the γ-glutamyl cycle consisting of six enzymes (Gul et al. 2000). Γ-glutamyl transpeptidase is responsible for the breakdown of glutathione by catalyzing the transfer of the γ-glutamyl moiety of glutathione to other molecules such as cysteine, glutamine, methionine, dipeptides, water, or glutathione itself (Gul et al. 2000). This route of metabolism is mainly extracellular due to the fact that γ-glutamyl transpeptidase is typically found on the external surface of the cell membrane (Gul et al. 2000). Once a glutathione molecule is transported outside the cell, it interacts with γ-glutamyl transpeptidase. The γ-glutamyl amino acids formed by this interaction are then transported into the cytosol where they enter the γ-glutamyl cycle and are used to regenerate glutathione (Gul et al. 2000).

16.1.2 α-Lipoic acid

α-Lipoic acid, also known as thioctic acid, is synthesized endogenously in many life forms, including humans (Brown et al. 2006). In addition to its antioxidant properties, α-lipoic acid is essential to mitochondrial bioenergetic reactions, where it acts as a cofactor in the pyruvate dehydrogenase complex (Mayr et al. 2014).

α-Lipoic acid plays an important role in many health processes. The R-enantiomer of α-lipoic acid, the more natural and active form, has been shown to amplify recovery of the conduction velocity of sensory and motor nerves that have been affected by diabetic neuropathy (Brufani and Figliola 2014). α-Lipoic acid also has antioxidant and neuroprotective effects in other various nervous system diseases (Kamchatnov et al. 2014). Patients with

type-2 diabetes show increased insulin sensitivity when administered with oral α-lipoic acid (Thirunavukkarasu et al. 2004, Brown et al. 2006). In addition, a recent study showed that α-lipoic acid plays a protective role against cardiovascular disease by reducing oxidative stress, uric acid levels, and improving vascular responses (Thirunavukkarasu et al. 2004, Saygin et al. 2015). This compound has also been shown to reverse schizophrenia-like symptoms induced by ketamine in mice (Vasconcelos et al. 2015).

α-Lipoic acid and its reduced form, dihydrolipoic acid, act as antioxidants through three distinct mechanisms: (1) metal chelation to prevent the potent oxidizing ability of heavy metals, (2) scavenging of ROS, and (3) regeneration of other endogenous antioxidants (Brown et al. 2006, Keith et al. 2012).

Both the R and S enantiomers of α-lipoic acid are absorbed in the gastrointestinal tract, with approximately 20%–30% of the dose reaching circulation (Keith et al. 2012, Brufani and Figliola 2014). α-lipoic acid has rapid absorption when administered orally and the measured median time to maximum concentration (t_{max}) ranges from 0.5 to 2 hours (Keith et al. 2012). The two enantiomers of α-lipoic acid have different absorption, distribution, degradation, and elimination phases (Shay et al. 2009, Chng et al. 2009). As stated previously, the R-enantiomer is more natural, making it less toxic and biologically more efficient and available than the S-enantiomer (Chng et al. 2009, Brufani and Figliola 2014).

α-Lipoic acid is transported into most tissues; however, it mainly accumulates in the skeletal muscles, heart, and liver (Keith et al. 2012). Following absorption, α-lipoic acid is rapidly cleared from the body by transport into tissues, glomerular filtration, and renal excretion (Keith et al. 2012). Within 3 hours, it is almost completely cleared from the plasma (Keith et al. 2012).

16.1.3 Ubiquinone

Ubiquinone, also known as coenzyme Q10, is an essential electron carrier and lipid component that is present in all eukaryotic cell membranes (Cordero et al. 2009, Rodriguez-Bies et al. 2015). In mammals, this antioxidant has a 2,3-dimethoxy-5-methylbenzoquinone core with a hydrophobic 45- to 50-carbon chain at the 6 position (Kelso et al. 2001).

Ubiquinone is involved in many essential activities including antioxidant activities, metabolism, and cell survival pathways (Rodriguez-Bies et al. 2015). It acts as the electron carrier between complexes I, II and complex III of the mitochondrial respiratory chain, and regulates uncoupling proteins, the transition core, and β oxidation of fatty acids (Kelso et al. 2001, Cordero et al. 2009). A recent study has shown that ubiquinone cooperates with α-tocopherol, an antioxidant factor in plasma, to prevent oxidative damage (Cordero et al. 2009).

Ubiquinone has been shown to have many clinical uses including a therapeutic efficacy for migraine prophylaxis (Sandor et al. 2005), protection against

Adriamycin-induced cardiotoxicity (Conklin 2005), enhancing outcomes for coronary artery bypass graft surgery (Littarru and Tiano 2005), treating hypertrophic cardiomyopathy (Adarsh et al. 2008), and improving oxidative balance in Down syndrome patients (Tiano et al. 2008).

Several intracellular and extracelluar processes are regulated in part by ubiquinone. A one- or two-electron process reduces quinones. One-electron reduction results in free radical semiquinones that may be oxidized by oxygen to form superoxide, a source of oxidative stress and damage to cells. By contrast, two-electron reduction produces hydroquinones, relatively stable compounds that can be further stabilized by conjugation (Sedlacek et al. 2015).

Ubiquinone plays a central part in the redox system of quinone oxidoreductase 1 (NQO1), a cytosolic flavoprotein that contains two flavin adenine dinucleotide (FAD) molecules per homodimer (Sedlacek et al. 2015, Rodriguez-Bies et al. 2015). NQO1 functions as an antioxidant enzyme by generating and maintaining ubihydroquinone, which is an effective lipid-soluble antioxidant that protects against lipid peroxidation (Sedlacek et al. 2015).

Ubiquinone and ubiquinol work together to protect the cell from oxidative stress. Ubiquinol acts as an antioxidant by donating a hydrogen atom from one of its hydroxyl groups to a lipid peroxyl radical, decreasing lipid peroxidation within the inner mitochondrial membrane. The ubisemiquinone radicals then either undergo a disproportionation reaction to form ubiquinone and ubiquinol or react with oxygen to form superoxide. Ubiquinone transfers the radical to the aqueous phase for detoxification by superoxide dismutase and peroxidases. Ubiquinone is then recycled back to ubiquinol via the respiratory chain (Kelso et al. 2001).

Ubiquinone is practically insoluble in aqueous solutions because of its lipophilic 10-carbon chain. Because of that characteristic, the first step of ubiquinone uptake is incorporation into chylomicrons in the small intestine for transport into the lymph and to the peripheral blood. The ubiquinone is then taken up by liver cells and secreted along with bile via the biliary tract. During this time, the small intestine reabsorbs part of it. It is thought that a nonlinear or zero-order absorption is associated with this process in the gastrointestinal tract (Miles 2007).

16.1.4 Uric acid

Uric acid is an important water-soluble antioxidant and powerful free radical scavenger that is derived from the catabolism of purines (Duarte et al. 2005, Bowman et al. 2010, Barros et al. 2012). It provides around 60% of free radical scavenging capacity in plasma (Campos et al. 2010, Fabbrini et al. 2014). One of its scavenging functions is to maintain ascorbic acid (vitamin C) in its reduced form in biological fluids (Duarte et al. 2005, Bowman et al. 2010). Uric acid may have a physiological role as an active component of the neuronal antioxidant pool (Duarte et al. 2005).

Uric acid is a molecule with both beneficial and harmful effects on human health (Duplancic et al. 2011). High levels of uric acid have been observed in patients with Down syndrome and other pathologies where oxidative stress is involved, including kidney disease and metabolic syndrome (Campos et al. 2010). Uric acid also has roles in Alzheimer's disease, cognitive decline, autism, and sleep apnea (Campos et al. 2010). It is a major circulating antioxidant in obese people and might provide a protective mechanism to prevent increased systemic oxidative damage by free radicals (Fabbrini et al. 2014).

Uric acid has been shown to have both antioxidant and prooxidant effects. They can be seen in its role within the ultrafiltrate for low-density lipoproteins (LDLs). Uric acid rapidly reduces Cu^{2+} to Cu^{+}. The decreased concentration of Cu^{2+} inhibits Tocopherol-mediated peroxidation, and the generation of it promotes rapid breakdown of lipid hydroperoxides into lipid radicals in mildly oxidized LDL (Patterson et al. 2003).

A recent study also showed that uric acid stimulated an increase in NADPH oxidase activity and ROS production in mature adipocytes. This stimulation of NADPH oxidase-dependent ROS resulted in activation of mitogen-activated protein (MAP) kinases p38 and extracellular-signal-regulated kinase (ERK)1/2, a decrease in nitric oxide (NO) bioavailability, and an increase in protein nitrosylation and lipid oxidation (Sautin et al. 2007).

Uric acid accounts for over half of the scavenging activity in plasma by quenching superoxide and singlet oxygen (Bowman et al. 2010). It has also been proven to be an efficient antioxidant and chelating agent for iron ions (Bowman et al. 2010). It alters the redox potential of chelated Fe^{2+}/Fe^{3+} and prevents Fenton-like reactions in many biological systems (Barros et al. 2012). Uric acid shows high scavenging rate constants against O^{2-} and RO and low rate constants against CH3 and *t*-BuOO (Kamogawa and Sueishi 2014). In addition, uric acid is converted to 5-hydroxy isourate and H_2O_2 by urate oxidase, which leads to the formation of allantoin. Urate inhibits lipid peroxidation by the products of the reaction of H_2O_2 with hemoglobin (Hershfield et al. 2010).

16.1.5 Melatonin

Melatonin (*N*-acetyl-5-methoxytryptamine) is a small, highly conserved pineal hormone involved in a variety of physiological functions including circadian rhythm, reproduction, mood, metabolism, and immune function (Bonnefont-Rousselot and Collin 2010, Zhang and Zhang 2014, Harpsoe et al. 2015). It is well known for its ability to neutralize free radicals, reduce inflammation, and defer age-related dysfunction of several organs (Favero et al. 2015). Melatonin has been shown as a specific antioxidant due to its amphiphilic feature that allows it to cross physiological barriers, reducing oxidative damage in both lipid and aqueous cell environments (Bonnefont-Rousselot and Collin 2010).

Melatonin is a small, highly conserved antioxidant molecule with numerous receptor-mediated and receptor-independent actions (Favero et al. 2015). It acts through G-protein-coupled melatonin receptors type 1 and 2 (MT1 and MT2) and retinoid Z receptor (RZR)/retinoid orphan receptor (ROR). Both receptors are present in the central and peripheral nervous systems and have been associated with cell differentiation. In addition, melatonin receptors are expressed on the membranes of CD4 T cells, CD8 T cells, and B cells (Adamczyk-Sowa et al. 2014).

Melatonin has been shown to be a major therapeutic tool for treating neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's disease (Ganie et al. 2015). It may also be useful for sickle cell anemia management (da Silva et al. 2015). The anti-aging properties of melatonin can be seen through its protection of skeletal muscle not only from toxin or drug exposure but also from degenerative changes associated with aging (Bonnefont-Rousselot and Collin 2010, Favero et al. 2015).

The mitochondrial membrane selectively absorbs melatonin, allowing it to be involved with mitochondrial bioenergetics functions (Ganie et al. 2015). Melatonin protects proteins of the electron transport chain (ETC) and mitochondrial DNA (mtDNA) from ROS and reactive nitrogen species (RNS)-induced oxidative damage. This protection limits the loss of intramitochondrial glutathione, improves ETC activity, and reduces mtDNA damage. Expression of complex IV and activity of complexes I and IV of the ETC are increased due to melatonin as well (Korkmaz et al. 2009).

Melatonin acts as an antioxidant through various direct and indirect mechanisms. These mechanisms include reducing activation of prooxidant enzymes, maintaining mitochondrial homeostasis, and scavenging of free radicals (Bonnefont-Rousselot and Collin 2010, Zhang and Zhang 2014, Favero et al. 2015). This indoleamine directly scavenges a variety of ROS such as H_2O_2 and the highly deleterious hydroxyl radical (Favero et al. 2015). It indirectly stimulates the expression and activity of several antioxidant enzymes, including glutathione peroxidase, superoxide dismutase (SOD), glutathione reductase, and catalase (Favero et al. 2015). Melatonin also induces the activity of γ-glutamylcysteine synthetase, stimulating the production of intracellular glutathione, another antioxidant (Korkmaz et al. 2009).

Unlike most other antioxidants, melatonin has inducible nitric oxide synthase (iNOS)-inhibitory and $ONOO^-$-scavenging properties. $ONOO^-$, formed by the coupling of NO and O_2^-, determines the fate of cells in many inflammatory processes. iNOS causes high NO production and is expressed in inflammatory cells. High levels of NO with excess O_2^- and high levels of $ONOO^-$ are referred to as a *devil's triangle*. Melatonin is currently the only antioxidant that is able to combat all aspects this *devil's triangle* (Korkmaz et al. 2009).

Although melatonin is mostly viewed as an antioxidant, a few recent studies have classified melatonin as a conditional prooxidant agent. They found that

melatonin promoted the generation of ROS at pharmacological concentrations in several tumor and non-tumor cells. It may stimulate ROS production through its interaction with calmodulin. Melatonin may also interact with mitochondrial complex III or the mitochondrial transition pore to promote ROS production (Zhang and Zhang 2014).

16.1.6 L-Carnitine

L-Carnitine (β-hydroxy-γ-trimethyl-amino-butyric acid) is a non-protein amino acid that is synthesized from the essential amino acids, lysine and methionine. L-Carnitine is involved in many processes in the human body including β-oxidation of long-chain fatty acids, metabolism of branched chain amino acids, stabilization of cellular membranes, and protection against oxidative damage by scavenging free radicals (Evans and Fornasini 2003, Lee et al. 2014).

Many studies have reported a variety of clinical applications of L-Carnitine. It has been shown to improve abstract and concrete thinking and memory function in discirculatory encephalopathy patients (Suslina et al. 2003). L-Carnitine has protective effects against coronary artery disease, chronic illnesses involving excessive oxidative stress, diabetic vascular complications, and ulcerative colitis (Irat et al. 2003, Cetinkaya et al. 2006, Cao et al. 2011, Lee et al. 2014). It may also prevent selenite-induced cataractogenesis by preventing abnormal expression of lenticular genes that govern apoptosis (Elanchezhian et al. 2010). A recent study found that L-carnitine has been linked to an increase in sperm quality and output in patients with infertility due to infection (Gual-Frau et al. 2015).

L-Carnitine administration was shown to prevent thiobarbituric acid-reactive substance formation in the cerebral cortex, cerebellum, hypothalamus, hippocampus, and striatum of 24-month-old rats. Age-associated changes were reversed and results of a recent study suggest that the neuroprotective effect on the brains of old rats was achieved by the elevation of antioxidants with L-carnitine (Rani and Panneerselvam 2002).

L-Carnitine has a direct stimulatory effect on gene and protein expression of the oxidative stress markers heme oxygenase 1 (HO-1) and endothelial nitric oxide synthase (ecNOS). Both of the markers are known to have antioxidant, antiproliferative, and anti-inflammatory properties. Their increased expression, due to L-carnitine, would lead to protection from oxidative stress-related risk factors (Calo et al. 2006). L-Carnitine is also able to scavenge superoxide anion and hydrogen peroxide free radicals, as well as to participate in metal chelating on ferrous ions (Gulcin 2006).

Endogenous L-carnitine comprises of free L-carnitine and short, medium, and long-chain esters. It is maintained in the body by dietary absorption, biosynthesis, and renal tubular reabsorption from glomerular filtrate. Although the bioavailability of dietary L-carnitine may be as high as 75%, supplemental

doses are absorbed much less efficiently. L-Carnitine and its short-chain esters do not bind to plasma proteins and the rate of distribution between erythrocytes and plasma is extremely slow in whole blood. In tissues that depend on fatty acid oxidation, carrier-mediated transport keeps the tissue-to-plasma concentrations high. L-Carnitine is eliminated from the body mainly by urinary excretion. The renal clearance of L-carnitine is much less than the glomerular filtration rate, which indicates tubular reabsorption (Evans and Fornasini 2003).

16.1.7 Amino acids

16.1.7.1 Aspartate

Many studies have demonstrated that the *N*-methyl-D-aspartate (NMDA) receptor has important cardiovascular effects, such as regulation of mean arterial pressure and splanchnic sympathetic nerve activity (Gao et al. 2007). NMDA-R activation caused the shift to oxidized condition of the redox state, which subsequently leads to neuron death in the hippocampus in the model of glutamate-associated neuronal disease (Ueda et al. 2007).

16.1.7.2 Glutamate

Administration of monosodium glutamate at a dose level of 4 mg/g body weight was shown to induce oxidative stress by lowering the activities of antioxidant enzymes like superoxide dismutases, catalase, and glutathione metabolizing enzymes, such as glutathione reductase and glutathione peroxidase in the arterial tissue (Singh and Ahluwalia 2003). A study also found that stress produced by glutamate in vulnerable brain regions persisted for extended periods of time (Singh et al. 2003). The antioxidant response element (ARE)-driven gene expression of glutamate–cysteine ligase via a mitogen-activated protein kinase kinase (MEK)/nuclear factor E2-related factor 2 (Nrf2) pathway may help protect macrophages from oxidative stress due to hyperhomocysteinemia (Bea et al. 2009).

16.1.7.3 Lysine

Carbocysteine lysine salt monohydrate (CLS) could act by interfering with the conversion of xanthine dehydrogenase into superoxide-producing xanthine oxidase. The antioxidant activity of CLS could contribute to its therapeutic activity by reducing radical damage to different lung structures (Pinamonti et al. 2001). Lysine has also been shown to reduce lipid peroxidation.

16.1.7.4 Arginine

L-Arginine supplementation can provide some benefits to the antioxidant system of skeletal muscle cells in culture (Da Silva and Lambertucci 2015).

16.2 Antioxidant vitamins

16.2.1 Ascorbic acid

Ascorbic acid, one form of Vitamin C, is a natural occurring essential micronutrient that has various biological functions (Ullah et al. 2011, Naksuriya and Okonogi 2015). It is a cofactor with a reactivity toward peroxyl and other radical species (Riabchenko et al. 2010). In addition, ascorbic acid plays a role in several enzymatic steps in the synthesis of collagen, monoamines, amino acids, peptide hormones, and carnitines and plays an important role in antioxidant defense (Santos et al. 2009).

Ascorbic acid is involved in cancer and other chronic diseases. Subchronic treatment with aspirin and ascorbic acid in aged rats was shown to enhance cognitive performance and increase the expressions of several receptors related to learning and memory process (Kara et al. 2014). Ascorbic acid is cytotoxic to a variety of cancer cells (Ullah et al. 2011). It has a radioprotective effect against UVA-dependent melanogenesis possibly through the improvement of antioxidant defense capacity and inhibition of NO production (Mathew et al. 2007, Panich et al. 2011). Ascorbic acid could also have a protective effect during seizures (Santos et al. 2009).

Ascorbic acid is a water-soluble antioxidant that protects the cell from oxidative damage, inhibits lipid peroxidation in the central nervous system, and can enter mitochondria by a facilitative glucose transporter and protect it against oxidative injury. It directly metabolizes ROS, scavenges oxygen radicals, acts to maintain α-tocopherol (Vitamin E) in its reduced form, and mediates electron transfer to ascorbate-dependent peroxidases (Santos et al. 2009, Uluata et al. 2015).

Ascorbic acid exhibits potent free radical scavenging activity toward the superoxide anion free radicals and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals (Wagner et al. 2008). Ascorbic acid has been shown to inhibit glutathione depletion, oxidant formation, and NO production through suppression of endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) mRNA (Panich et al. 2011).

Several studies have demonstrated that ascorbic acid has both antioxidant and prooxidant properties (Riabchenko et al. 2010). When administered at low doses in the brain, ascorbic acid acts as an antioxidant but at high doses, it was shown to act as a prooxidant (Mendes-da-Silva et al. 2014). It was also shown to act as a prooxidant during a cold ischemia/reperfusion study (Park and Lee 2008).

16.2.2 α-Tocopherol

α-Tocopherol is the most common and biologically active form of vitamin E. It is a lipid-soluble antioxidant that has heat, light, and oxygen lability (Engin 2009,

Noronha et al. 2014). α-tocopherol is involved in gene regulation and inhibits cytotoxic response. It contains multiple transfer proteins and specific membrane receptors (Engin 2009).

α-Tocopherol can be synergized with lipoic acid to reduce oxidative damage in the brain, cardiac ischemia, and oxidation of LDLs (Thomas et al. 2014). A recent study showed that it may play a role in treatment of erectile dysfunction in hypertensive patients by improving neuronal or endothelial function related to NO and carbon monoxide (Ushiyama et al. 2008). It has been shown to prevent heat-induced alterations in some enzyme activities within aged groups of cells (Stojkovski et al. 2013). α-Tocopherol is also used as a food additive due to the encapsulated tocopherol that allows a controlled release and better solubility in hydrophilic matrices of the lipophilic compound (Noronha et al. 2014).

α-Tocopherol has a major role in terminating free radical chain reactions that result from the oxidation of polyunsaturated fatty acids (Fernandes et al. 2013). These molecules can interrupt free radical chain reactions by capturing the free radical. The free hydroxyl group on the aromatic ring of α-tocopherol is responsible for the antioxidant properties. The hydrogen from this group is donated to the free radical, resulting in a relatively stable free radical (Engin 2009). Enrichment of mitochondria with protective α-tocopherol weakens production of ROS and antioxidant enzyme activities (Stojkovski et al. 2013).

Protein kinase C (PKC) is one of the pathways that α-tocopherol uses. α-Tocopherol inhibits free radical formation and tyrosine kinase activity in tissue plasminogen activator (TPA)-induced primary human fibroblasts or human promyelocytic leukemia cell line (HL-60) cells. In smooth muscle, it is shown to have an antiproliferative effect (Engin 2009).

Once digested, α-tocopherol is easily absorbed from the intestinal lumen and dispersed between lipids and proteins in cell membranes (Engin 2009). It is prone to be retained within the stratum corneum due to its high lipophilicity (Thomas et al. 2014).

16.2.3 Carotenoids (β-carotene, lycopene, lutein)

Carotenoids are a class of orange-, yellow-, and red-colored pigments that are involved in many processes in the human body and represent a provitamin A source (Carranco Jauregui et al. 2011). Because of their conjugated double bond system, carotenoids are incorporated into the biological membranes in various ways (Young and Lowe 2001). They take part in cellular signaling pathways, influence expression of certain genes, and act as inhibitors of regulator enzymes (Stahl et al. 2002). Carotenoids stimulate gap junctional communications and impact regulation of cell growth and induction of detoxifying enzymes, such as cytochrome P450-dependent monooxygenases (Stahl et al. 2002). Oxidized derivatives of carotenoids interact with a network of transcription systems that lead to synergistic inhibition of cell

growth. Mixtures of carotenoids have been shown to be more effective than single compounds (Stahl and Sies 2003).

Carotenoids may reduce the incidence of certain diseases including cancer, heart disease, and age-related macular degeneration, in addition to other uses (Carranco Jauregui et al. 2011). They protect the skin against photooxidative damage by filtering blue light and protecting the macula lutea (Stahl and Sies 2003). Low levels of carotenoids may increase the risk of persistent human papillomavirus (HPV) infection, which is the primary etiological factor for cervical cancer (Peterson et al. 2010). High plasma levels of lutein are associated with lower risk of acute myocardial infarction (Ciccone et al. 2013). Carotenoids may also have potential erythroprotective properties because they prevent peroxyl radicals (ROO)-induced toxicity in human erythrocytes (Chiste et al. 2014).

Carotenoids act as antioxidants through several mechanisms. Several studies have reported their free radical scavenging properties (Stahl and Sies 2003, Skibsted 2012, Ciccone et al. 2013). Carotenoids scavenge singlet molecular oxygen and peroxyl radicals at low partial pressure of oxygen and quench electronically excited states (Stahl and Sies 2003). These networks seem to be thermodynamically controlled and depend on the balance between ionization energy and electron affinity of the individual carotenoids (Skibsted 2012). Carotenoids help LDL cholesterol to resist oxidation inductors. They also take part in protecting cellular membranes against oxidative damage due to their lipophilic nature. Carotenoids have several cooperative effects with other antioxidants, increasing their efficiency (Ciccone et al. 2013).

Several studies have reported that carotenoids do not necessarily display prooxidant properties. However, they may lose their effectiveness as antioxidants at high concentrations or high partial pressures of oxygen (Young and Lowe 2001).

16.3 Antioxidant minerals

16.3.1 Selenium

Selenium is a nonmetal trace element linked to many health benefits in the human body (Nogueira and Rocha 2011, Asci et al. 2015). In addition to being a part of antioxidant defense, it is also involved with thyroid function, boosting immune function, and improving tissue respiration (Kolesnikova et al. 2015, Pawlas et al. 2015). Selenium has been shown to protect the heart from cardiovascular diseases and hyperglycemia or ischemia/reperfusion-induced injury (Deletioglu et al. 2015).

A recent finding concluded that high-dose parenteral selenium, due to its antioxidant properties, might be associated with a reduction in mortality among critically ill patients with systematic inflammatory response syndrome (Manzanares and Hardy 2009). Another study has shown that stabilized

selenium nanoparticles at a certain concentration can be a potential anti-arthritis drug supplement, restoring antioxidant levels in the kidney, liver, and spleen (Malhotra et al. 2015).

Although selenium has many antioxidant properties, a novel study recently found selenium to have apparent prooxidant properties when in high concentrations (Deletioglu et al. 2015).

Selenium works as an antioxidant in several ways. Selenoproteins help to eliminate ROS induced by metals (Pawlas et al. 2015).

One way consists of being a part of an antioxidant enzyme called glutathione peroxidase (GPx) (Mugesh et al. 2001, Pawlas et al. 2015). The GPx family contains four enzymes, all of which require selenium in their active site (Mugesh et al. 2001). With selenium as a cofactor, GPx utilizes hydrogen peroxide to convert reduced glutathione to its oxidized form to protect cells from free radical oxidation (Kolesnikova et al. 2015, Pawlas et al. 2015).

GPx and other selenoproteins, such as selenomethionine, have been reported to reduce peroxynitrite (PN), a strong oxidant that causes DNA damage, inactivates a variety of biologically important enzymes, and initiates lipid peroxidation (Mugesh et al. 2001). Selenomethionine is reduced by glutathione and forms an Se:N transient species, which eventually reduces PN (Mugesh et al. 2001).

16.3.2 Zinc

Zinc is an important signaling trace element necessary for many physiological functions including involvement in the immune system, redox states, enzyme activity, gene transcription, energetic metabolism, cell migration, apoptosis, and proliferation (Ruttkay-Nedecky et al. 2013, Prasad 2014). According to the Protein Data Bank, it is also a component for more than 2700 enzymes (Ganger et al. 2015).

Zinc supplementation has been successfully used as a therapeutic and preventative for many conditions, including chronic hepatitis C, leprosy, tuberculosis, pneumonia, and acute lower respiratory tract infection (Prasad 2009). It has also been shown to have produced decreased incidents of infections, oxidative stress, and generation of inflammatory cytokines in the elderly (Prasad 2014).

In addition, zinc has improved oxidative stress in patients with type 2 diabetes by reducing chronic hyperglycemia (Cruz et al. 2015). A recent study has concluded that Zn^{2+} is a dual functioning ion. It has antioxidant properties at low concentrations and prooxidant properties at high concentrations (Deletioglu et al. 2015).

Although Zn (II) itself does not have redox capability, it is still considered a potent antioxidant agent and acts in direct and indirect manners through various mechanisms (Powell 2000). A decrease in cellular zinc has been associated

with an increase in oxidant properties in several studies (Kojima-Yuasa et al. 2005, Aimo et al. 2010).

Zinc is a well-known inducer of metallothionein (MT), a low molecular weight metal-binding protein that contains high cysteine content, and has high heat stability (Powell 2000, Ruttkay-Nedecky et al. 2013). MT participates in protecting the human body against oxidative stress from reactive oxygen and nitrogen species, as well as maintaining essential metal ion homeostasis. It can bind 5–7 g of zinc (mol/protein) and under physiological conditions, it exists as Zn–MT (Powell 2000). Zinc induces MT in different organs including the liver, kidney, and intestine by directly binding to its Zn finger domains (Ruttkay-Nedecky et al. 2013). Metal regulatory transcription factor 1 (MTF-1), a 753 amino acid transcription factor, directly responds to increased levels of zinc (II). It binds the metal responsive element of the MT gene and initiates MT transcription (Ruttkay-Nedecky et al. 2013).

Zinc is also involved in the protection of certain enzyme sulfhydryls from oxidation (Powell 2000, Zago and Oteiza 2001). Several mechanisms have been proposed to account for this stabilization: direct binding of zinc to the sulfhydryl group, binding to another protein site in close proximity to the sulfhydryl group resulting in steric hindrance, and binding to another site on the protein that would cause a conformational change (Powell 2000).

In addition, Zinc plays an important role in regulating glutathione (GSH) synthesis. Many studies have revealed that a zinc deficiency is accompanied by a glutathione deficiency (Kojima-Yuasa et al. 2005). Glutamate–cysteine ligase (GCL), a key regulatory enzyme in GSH synthesis, was identified as a link in these conditions. GCL acts to catalyze a reaction of glutamate and cysteine to gamma-L-glutamyl-L-cysteine (GCLC). Zinc supplementation protects endothelial cells from peroxide-induced death by increasing the production of GCLC and GSH (Cortese et al. 2008, Ruttkay-Nedecky et al. 2013).

16.4 Phenolic antioxidants

16.4.1 Phenolic acids

Phenolic acids are aromatic secondary metabolites that contain a carboxylic acid function group and a phenolic ring (Nakamura et al. 2000, Robbins 2003). Derivatives of phenolic acids have a number of hydroxyl groups and other substituents in different positions leading to differences in structure and function (Nakamura et al. 2000).

Phenolic compounds have antioxidative properties such as ROS scavenging, electrophile scavenging, metal chelation, and inhibition of ROS generation systems (Nakamura et al. 2000). In addition, phenolic acids have been found to be involved in antimutagenic, anticarcinogenic, anti-inflammatory, antiallergic, antimicrobial, and antiviral activities (Nakamura et al. 2000, Chen et al. 2015).

The antioxidant capacity of phenolic acids correlates well with their reducing activity, both of which reflect their tendency to donate electrons (Ma and Qian 2010). Phenolic compounds generally exert their antioxidant activities by three different mechanisms: hydrogen atom transfer (HAT), single electron transfer–proton transfer (SETPT), and sequential proton loss-electron transfer (SPLET) (Chen et al. 2015). All of the mechanisms are believed to play a role in determining radical scavenging activities (M, N, and M, 2011, Chen et al. 2015). The radical scavenging ability of phenolic acid compounds is determined by the ability of the compound to satisfy the color of stable radicals such as the DPPH radical (Lu et al. 2006, Ma and Qian 2010, Chen et al. 2015).

The phenolic O–H bond dissociation energy (BDE), ionization potential (IP), proton dissociation enthalpy (PDE), proton affinity (PA), and electron transfer enthalpy (ETE) can be used to describe the three mechanisms (Chen et al. 2015). It has been found that HAT is the preferred method in the gas phase, and benzene and the SPLET method is more favorable in water and ethanol (Chen et al. 2015).

Some common phenolic acids are described in the following.

16.4.1.1 Gallic acid

Gallic acid is a naturally occurring polyphenol antioxidant and anti-carcinogen that has recently been shown to have potential health benefits (Sohi et al. 2003, Lu et al. 2006). It exhibits different hydrophobicity and can cross through the liposome membrane to react with the DPPH free radical, as well as scavenge for other free radicals (Lu et al. 2006).

A recent study concluded that gallic acid inhibits human rhinovirus because of its role as an antioxidant and the mode of action derived from the inhibition of virus absorption (Choi et al. 2010). Another finding showed that gallic acid may be a double-edged sword that exhibits antioxidant behavior in the presence of H_2O_2, while exhibiting prooxidant behavior in the presence of H_2O_2. Its antioxidant behavior inhibits oxidative species in the peroxidase cycle of peroxidases and its prooxidant behavior may promote cell injury (Serrano et al. 2010).

16.4.1.2 Protocatechuic acid

Protocatechuic acid is a major benzoic acid derivative that shows a strong antioxidative effect (Nakamura et al. 2000, Guan et al. 2006). A research study has found that this compound increases mitochondrial membrane potential, increases glutathione production, and decreases the formation of ROS (Guan et al. 2006). Protocatechuic acid has also been shown to have chemopreventive effects on colon and oral carcinogens in rats, as well as the ability to attenuate diabetic complications because of its triglyceride-lowering effects (Nakamura et al. 2000, Lin et al. 2009).

16.4.1.3 Caffeic acid

Caffeic acid is the hydroxycinnamic acid in the human diet (Genaro-Mattos et al. 2015). It shows many biological and pharmacological effects including roles in antioxidation, antivirality, immunostimulation, and tumor cell cytotoxicity (Chen et al. 2001, Russo et al. 2002). Caffeic acid has been shown to inhibit human LDL lipoperoxidation induced by cupric ions by reducing lipoperoxyl radicals by donating a hydrogen atom to its corresponding hydroperoxide, leading to lipid peroxidation inhibition (Genaro-Mattos et al. 2015).

16.4.2 Phenolic diterpenes

Phenolic diterpenes have important antioxidant, anti-tumor, and anti-HIV properties and are also involved in protection against neurodegenerative diseases (Fischedick et al. 2013). When isolated from *Hyptis incana*, they have multiple anti-tumor effects on neuroblastoma cells in a dose-dependent manner (Tabata et al. 2012).

Phenolic diterpenes act as antioxidants by directly scavenging several free radicals including the lipid free radical and the superoxide anion. They inhibit low-density lipid peroxidation by synergistic effects such as blocking direct modification and inhibiting adduct formations between aldehydes and lysine residues (Zeng et al. 2001). Several phenolic diterpenes and their effects are described in the following.

16.4.2.1 Carnosol

Carnosol inhibits mitochondria and microsomal lipid peroxidation induced by NADH or NADPH (Zeng et al. 2001). It has also been reported to induce apoptosis of leukemia cells and to induce G2/M-phase cell-cycle arrest in colon and prostate cancer cells via the mitochondrial pathway (Tabata et al. 2012). Carnosol protects against hydrogen peroxide-induced premature senescence (Carvalho et al. 2015). It also has a cytoprotective effect against oxidant challenges with tert-butyl hydroperoxide (Carvalho et al. 2015).

16.4.2.2 Carnosic acid

Carnosic acid possesses antimicrobial, anti-cancer, anti-inflammatory, and lipid-lowering properties (Fischedick et al. 2013). It protects against glutamate toxicity in primary rat cortical cultures and also against cerebral ischemia by activating the nuclear factor E2-related factor 2 (Nrf2) and the Keap1 pathway (Fischedick et al. 2013).

16.4.2.3 Rosmanol

Rosmanol, in addition to carnosol, has also been shown to inhibit mitochondria and microsomal lipid peroxidation induced by NADH or NADPH (Zeng et al. 2001). It is stronger than carnosol as a scavenger of lipid radicals. Rosmanol has an ortho position diphenolic group that easily gives off H· to form ortho-position diquinion H· that can react with other free radicals (Zeng et al. 2001).

16.4.3 Flavonoids

Flavonoids have been shown to have antioxidant, antiviral, and protective effects. They protect the cell from nickel toxicity and have antiproliferative ability against chemically induced colon tumorigenesis (Amudha and Pari 2011, Aranganathan and Nalini 2013). Flavonoids may also be useful in the treatment of hypoxia in hepatopulmonary syndrome and as an antiviral agent for dengue (Muhamad et al. 2010, Atalay et al. 2013). Flavonoids have a NO-inhibiting effect (Atalay et al. 2013). They play a synergistic role with ethanol, allowing it to scavenge DPPH and hydroxyl radicals (Habbu et al. 2010). Flavonoids are also able to reduce ROS-forming enzymes, such as NADPH oxidase and myeloperoxidase (Nishimura Fde et al. 2013). Following are some examples of flavonoids.

16.4.3.1 Quercetin

Quercetin, 3,3′,4′,5,7-pentahydroxyflavone, is one of the most representative compounds in the flavonoid group and has many health benefits including antioxidant, cancer prevention, and cardioprotective activity (Liu et al. 2012). It has recently started becoming a promising agent in the treatment of diabetes mellitus (Maciel et al. 2013). Quercetin plays a role in protecting the spinal cord by inhibiting activation of the p38MAPK/iNOS signaling pathway, regulating secondary oxidative stress (Song et al. 2013). Its antioxidant effects also contribute to its antidepressive properties (Holzmann et al. 2015).

Quercetin exerts antioxidant activity by a variety of mechanisms, including scavenging free radicals, chelating transition metal ions, and inducing the antioxidant defense system regulated by nuclear factor erythroid 2-related factor 2 (Nrf2). It also increases glutathione levels and reduces the generation of ROS (Liu et al. 2012, Zerin et al. 2013, Papiez and Krzysciak 2014). Quercetin can interrupt the redox cycle of etoposide by reducing the amount of phenoxyl radicals in cells (Papiez and Krzysciak 2014).

16.4.3.2 Naringenin

Naringenin (4,5,7-trihydroxy-flavonone) is a flavonoid found mainly in citrus fruit, tomatoes, and cocoa. Pharmacological activities of naringenin include

anti-tumor, anti-inflammatory, antibacterial, antiviral, anti-diabetic, cardioprotective, neuroprotective, and nephroprotective properties (Hermenean et al. 2014). It shows hepatoprotective effects against injuries induced by CCl4 and antihyperglycemic effects in experimental diabetic rats, acting as a cholinesterase inhibitor (Annadurai et al. 2012, Rahigude et al. 2012). Naringenin has also been shown to have therapeutic potential in the abatement of ethanol-induced hepatotoxicity (Jayaraman et al. 2009).

Naringenin acts as an antioxidant through scavenging ROS, inhibiting the activity of ROS-forming enzymes, and directly inhibiting myeloperoxidase chlorinating activity (Nishimura Fde et al. 2013). It acts against increased lipid peroxidation when exposed to cadmium, arsenic, lead, dimethylnitrosamine, or oxytetracycline. The biological effects of narigenin (NGN) are limited by its lower solubility and minimal oral bioavailability due to its largely hydrophobic ring structure (Hermenean et al. 2014).

16.4.3.3 Rutin

Rutin, a quercetin glycoside, demonstrates several pharmacological activities including anti-inflammatory, anti-allergic, and vasoactive properties (Li et al. 2014b). It has been used in ROS-related diseases such as gastric lesions, diabetes, hypoxic pulmonary vasoconstriction, and cisplatin-induced nephrotoxicity (Li et al. 2014b, Kamel et al. 2014). Rutin has a hepatoprotective effect by enhancing its antioxidant effect through amelioration of oxidative stress genes (Al-Rejaie et al. 2013). It protects against ischemia/reperfusion injury by affecting dynamic cardiac factors and by enhancing SOD and DPPH activity (Bhandary et al. 2012). Rutin inhibits abnormal cell proliferation induced by hypoxia by reducing the production of ROS, mainly NAPDH oxidase and mitochondria respiratory chain activity (Li et al. 2014b). It has also been shown to reduce lipid peroxidation.

16.4.3.4 Kaempferol

Kaempferol (3,4′,5,7-tetrahydroxyflavone) is one of the most commonly found dietary flavonoids (Leung et al. 2007). It can be safely used as a chemoprotective agent in colorectal cancer and may reduce or prevent osteoblast degeneration in osteoporosis (Choi 2011, Nirmala and Ramanathan 2011). Kaempferol has been shown to improve the recovery of cardiac function, reduce intracellular oxidation status, and decrease the myocardial apoptosis induced by ischemia.

16.4.4 Volatile oils

Volatile oils have antioxidant, antifungal, and antibacterial properties (Bozin et al. 2006). They may also play a role in cancer chemoprevention and in the preservation of pharmacological products (Trevisan et al. 2006).

The bioactivity of these oils results from complex interactions between their constituents, producing both synergistic and antagonistic responses (Sarrou et al. 2013).

Volatile oils act as antioxidants through several mechanisms. They are able to reduce ferric ions with an average capacity of 3.5–220 mmol/kg oil (Lado et al. 2004). They also have very strong free radical scavenging capacities and scavenge the DPPH radical (Bozin et al. 2006, Sarrou et al. 2013). In addition, volatile oils can inhibit lipid peroxidation induced by (2+)/ascorbate or by Fe(2+)/H(2)O(2) (Bozin et al. 2006). Some common volatile oils are described in the following.

16.4.4.1 Eugenol

Eugenol (4-allyl-2-methoxyphenol) is a naturally occurring phenolic compound (Nagababu et al. 2010). It may offer potential benefits in the management of cardiac hypertrophy and liver toxicity (Choudhary et al. 2006, Yogalakshmi et al. 2010). Its protective effects are due to interception of secondary radicals derived from endoplasmic reticulum lipids (Nagababu et al. 2010). Eugenol also plays a role in prolonging the lag phase and suppressing propagation rates, adding to its protective effects (Ito et al. 2005).

Eugenol's antioxidant properties are documented by multiple studies. Several of these properties can be explained because eugenol forms complexes with reduced metals (Ito et al. 2005). It has been shown to completely inhibit both iron and Fenton reagent-mediated lipid peroxidation (Nagababu et al. 2010). Eugenol can inhibit LDL oxidation by reducing copper ions and suppressing the free radical 1,1′-diphenyl-2-picrylhydrazyl (DPPH) cascade of lipid peroxidation (Ito et al. 2005). A few studies have reported that eugenol has some prooxidant properties that can lead to adverse effects such as allergic and inflammatory reaction (Fujisawa et al. 2002, Atsumi et al. 2005). Cytotoxicity of eugenol is mediated by ROS-independent mechanisms, possibly involving phenoxyl radicals and/or eugenol quinone methide (Fujisawa et al. 2002, Atsumi et al. 2005).

16.4.4.2 Carvacrol

Carvacrol is a hydrophobic phenolic compound found primarily in oils of oregano, thyme, and marjoram. It has antispasmodic, anti-inflammatory, angiogenic, antiparasitic, antiplatelet, inhibition of acetylcholinesterase, anti-elastase, insecticidal, antihepatotoxic, and hepatoprotective activities (Aristatile et al. 2009, Guimaraes et al. 2010). Carvacrol is active against *Escherichia coli* and inhibits the growth of *Penicillium citrinum* (De Martino et al. 2009). It has protective effects against oxidative damage to membrane polyunsaturated fatty acids.

Carvacrol has the ability to prevent lipid peroxidation. It also acts as a free radical scavenger and NO and the superoxide ion, in addition to

ROS (Guimaraes et al. 2010). It may preclude the formation of PN because of its ability to separately scavenge both ROS and NO. Carvacrol has a weak acid character, and when it reacts with a free radical, it donates hydrogen atoms with an unpaired electron that produces another radical that is stabilized by a molecular resonance structure (Guimaraes et al. 2010). Some findings have suggested that carvacrol can exhibit damaging effects depending on cell resistance, concentration, and time (Ozkan and Erdogan 2012).

16.4.4.3 Thymol

Thymol is a phenolic compound that has redox properties that play an important role in absorbing and neutralizing free radicals including PN. It displays antioxidant and anti-inflammatory properties in human cells (Braga et al. 2006).

16.4.4.4 Menthol

Methanol is a compound that mainly affects mucus secretion and prostaglandin E2 (PGE2) production. It shows potent antioxidant and anti-inflammatory effects. Methanol causes increases in glutathione and the enzymes GSH-Px and glutathione reductase (GR), as well as decreases in the level of superoxide dismutase enzyme. It diminishes the levels of the proinflammatory cytokines TNF-α and IL-6 while increasing the level of anti-inflammatory factor IL-10 (Rozza et al. 2014).

16.4.5 Stilbenoids

Stilbenoids are bioactive compounds that show beneficial effects on human health. Their broad structure due to oligomerization and modifications gives rise to anti-tumor properties and helps increase rate of survival (Chen et al. 2012, Yu et al. 2013). Stilbenoids have a polyphenol-mediated anti-HSV response, which links antihepatic activity to their innate immunity (Chen et al. 2012). They have been shown to have DPPH and superoxide anion radical scavenging abilities, ferric reducing power, and lipid peroxidation inhibition properties (Chai et al. 2012).

16.4.5.1 Resveratrol

Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) is a representative stilbenoid that consists of two aromatic rings that are attached by a methylene bridge (Yu et al. 2013). It is a natural phenol and phytoalexin with anti-aging, anti-inflammatory, anti-cancer, antioxidative, and chemoprotective properties (Yu et al. 2013, Li et al. 2014a). It is a free radical scavenger of hydroxyl, superoxide, and metal-induced radicals and effectively antagonizes oxidation induced by irradiation (Li et al. 2014a). A recent study found that resveratrol may interact with hemoglobin to protect against oxidative damage (Tellone et al. 2014).

Resveratrol is involved in various processes. It may prevent progression of ligature-induced periodontitis and may improve systemic nitrosative stress (Tamaki et al. 2014). It improves levels of 8-hydroxydeoxyguanosine, dityrosine, NO metabolism, nitrotyrosine, and proinflammatory cytokines (Leopoldini et al. 2011). Resveratrol is a neuroprotective agent against diabetes-induced oxidative damage (Sadi and Konat 2015). It can also inhibit the activation of proinflammatory mediators and cytokines at the early gene expression stage (Rocha et al. 2015).

16.4.6 Phenylethanoids

Phenylethanoids are inhibitors of LDL oxidation and can break peroxidative chain reactions (Tuck et al. 2001). They have inhibitory effects on free radical-induced hemolysis of red blood cells and can participate in free radical scavenging activities (He et al. 2000). Some radicals that are scavenged by phenylethanoids are the superoxide radical anion and 1-diphenyl-2-picrylhydrazyl (Yu et al. 2007, Qu et al. 2012).

16.4.6.1 Hydroxytyrosol/Tyrosol

Tyrosol, the major compound in extra virgin olive oil, is also a naturally occurring phenol with many biological properties including anti-cancer, antidepressant, stress-protective, and anti-osteoporosis effects (Wang et al. 2011). It is effective in preserving cellular antioxidant defenses by intracellular accumulation (Di Benedetto et al. 2007). Tyrosol protects DNA against dioxin toxicity and prevents alterations in levels of other antioxidant enzymes (Kalaiselvan et al. 2014). Tyrosol is sometimes oxidized to hydroxytyrosol, a naturally occurring orthodiphenolic antioxidant (Di Benedetto et al. 2007).

A recent group studied bioavailability of tyrosol and hydroxytyrosol. When administered in an olive oil solution, hydroxytyrosol had 99% bioavailability, and when dosed as an aqueous solution, it had 75%. Tyrosol had 98% bioavailability in olive oil and 71% when dosed as an aqueous solution (Tuck et al. 2001).

References

Adamczyk-Sowa, M., K. Pierzchala, P. Sowa, S. Mucha, I. Sadowska-Bartosz, J. Adamczyk, and M. Hartel. 2014. Melatonin acts as antioxidant and improves sleep in MS patients. *Neurochem Res* 39 (8):1585–1593. doi: 10.1007/s11064-014-1347-6.

Adarsh, K., H. Kaur, and V. Mohan. 2008. Coenzyme Q10 (CoQ10) in isolated diastolic heart failure in hypertrophic cardiomyopathy (HCM). *Biofactors* 32 (1–4):145–149.

Aimo, L., G. N. Cherr, and P. I. Oteiza. 2010. Low extracellular zinc increases neuronal oxidant production through nadph oxidase and nitric oxide synthase activation. *Free Radic Biol Med* 48 (12):1577–1587. doi: 10.1016/j.freeradbiomed.2010.02.040.

Al-Rejaie, S. S., A. M. Aleisa, M. M. Sayed-Ahmed, O. A. Al-Shabanah, H. M. Abuohashish, M. M. Ahmed, K. A. Al-Hosaini, and M. M. Hafez. 2013. Protective effect of rutin on the antioxidant genes expression in hypercholestrolemic male Westar rat. *BMC Complement Altern Med* 13:136. doi: 10.1186/1472-6882-13-136.

Amudha, K. and L. Pari. 2011. Beneficial role of naringin, a flavanoid on nickel induced nephrotoxicity in rats. *Chem Biol Interact* 193 (1):57–64. doi: 10.1016/j.cbi.2011.05.003.

Anderson, M. F., M. Nilsson, and N. R. Sims. 2004. Glutathione monoethylester prevents mitochondrial glutathione depletion during focal cerebral ischemia. *Neurochem Int* 44 (3):153–159.

Annadurai, T., A. R. Muralidharan, T. Joseph, M. J. Hsu, P. A. Thomas, and P. Geraldine. 2012. Antihyperglycemic and antioxidant effects of a flavanone, naringenin, in streptozotocin-nicotinamide-induced experimental diabetic rats. *J Physiol Biochem* 68 (3): 307–318. doi: 10.1007/s13105-011-0142-y.

Aranganathan, S. and N. Nalini. 2013. Antiproliferative efficacy of hesperetin (citrus flavanoid) in 1,2-dimethylhydrazine-induced colon cancer. *Phytother Res* 27 (7):999–1005. doi: 10.1002/ptr.4826.

Aristatile, B., K. S. Al-Numair, C. Veeramani, and K. V. Pugalendi. 2009. Effect of carvacrol on hepatic marker enzymes and antioxidant status in D-galactosamine-induced hepatotoxicity in rats. *Fundam Clin Pharmacol* 23 (6):757–765. doi: 10.1111/j.1472-8206.2009.00721.x.

Asci, A., O. Surmeli-Onay, P. Erkekoglu, S. Yigit, M. Yurdakok, and B. Kocer-Gumusel. 2015. Oxidant and antioxidant status in neonatal proven and clinical sepsis in relation to selenium status. *Pediatr Int* 57 (6):1131–1137. doi: 10.1111/ped.12698.

Atalay, T., M. Cakir, A. Tekin, T. Kucukkartallar, S. Kargin, A. Kartal, and A. Kaynak. 2013. The effects of flavanoid on the treatment of hepatopulmonary syndrome. *J Korean Surg Soc* 85 (5):219–224. doi: 10.4174/jkss.2013.85.5.219.

Atsumi, T., S. Fujisawa, and K. Tonosaki. 2005. A comparative study of the antioxidant/prooxidant activities of eugenol and isoeugenol with various concentrations and oxidation conditions. *Toxicol In Vitro* 19 (8):1025-33. doi: 10.1016/j.tiv.2005.04.012.

Augustyniak, A., G. Bartosz, A. Cipak, G. Duburs, L. Horakova, W. Luczaj, M. Majekova et al. 2010. Natural and synthetic antioxidants: An updated overview. *Free Radic Res* 44 (10):1216–62. doi: 10.3109/10715762.2010.508495.

Barros, M. P., D. Ganini, L. Lorenco-Lima, C. O. Soares, B. Pereira, E. J. Bechara, L. R. Silveira, R. Curi, and T. P. Souza-Junior. 2012. Effects of acute creatine supplementation on iron homeostasis and uric acid-based antioxidant capacity of plasma after wingate test. *J Int Soc Sports Nutr* 9 (1):25. doi: 10.1186/1550-2783-9-25.

Bea, F., F. N. Hudson, H. Neff-Laford, C. C. White, T. J. Kavanagh, J. Kreuzer, M. R. Preusch, E. Blessing, H. A. Katus, and M. E. Rosenfeld. 2009. Homocysteine stimulates antioxidant response element-mediated expression of glutamate-cysteine ligase in mouse macrophages. *Atherosclerosis* 203 (1):105–111. doi: 10.1016/j.atherosclerosis.2008.06.024.

Bhandary, B., C. S. Piao, D. S. Kim, G. H. Lee, S. W. Chae, H. R. Kim, and H. J. Chae. 2012. The protective effect of rutin against ischemia/reperfusion-associated hemodynamic alteration through antioxidant activity. *Arch Pharm Res* 35 (6):1091–1097. doi: 10.1007/s12272-012-0617-6.

Bonnefont-Rousselot, D. and F. Collin. 2010. Melatonin: Action as antioxidant and potential applications in human disease and aging. *Toxicology* 278 (1):55–67. doi: 10.1016/j.tox.2010.04.008.

Bowman, G. L., J. Shannon, B. Frei, J. A. Kaye, and J. F. Quinn. 2010. Uric acid as a CNS antioxidant. *J Alzheimers Dis* 19 (4):1331–1336. doi: 10.3233/JAD-2010-1330.

Bozin, B., N. Mimica-Dukic, N. Simin, and G. Anackov. 2006. Characterization of the volatile composition of essential oils of some lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. *J Agric Food Chem* 54 (5):1822–1828. doi: 10.1021/jf051922u.

Braga, P. C., M. Dal Sasso, M. Culici, L. Galastri, M. T. Marceca, and E. E. Guffanti. 2006. Antioxidant potential of thymol determined by chemiluminescence inhibition in human neutrophils and cell-free systems. *Pharmacology* 76 (2):61–68. doi: 10.1159/000089719.

Brown, M. K., J. L. Evans, and Y. Luo. 2006. Beneficial effects of natural antioxidants EGCG and alpha-lipoic acid on life span and age-dependent behavioral declines in Caenorhabditis elegans. *Pharmacol Biochem Behav* 85 (3):620–628. doi: 10.1016/j.pbb.2006.10.017.

Brufani, M. and R. Figliola. 2014. (R)-alpha-lipoic acid oral liquid formulation: pharmacokinetic parameters and therapeutic efficacy. *Acta Biomed* 85 (2):108–115.

Calo, L. A., E. Pagnin, P. A. Davis, A. Semplicini, R. Nicolai, M. Calvani, and A. C. Pessina. 2006. Antioxidant effect of L-carnitine and its short chain esters: relevance for the protection from oxidative stress related cardiovascular damage. *Int J Cardiol* 107 (1):54–60. doi: 10.1016/j.ijcard.2005.02.053.

Campos, C., R. Guzman, E. Lopez-Fernandez, and A. Casado. 2010. Urinary uric acid and antioxidant capacity in children and adults with down syndrome. *Clin Biochem* 43 (3):228–233. doi: 10.1016/j.clinbiochem.2009.09.017.

Cao, Y., H. J. Qu, P. Li, C. B. Wang, L. X. Wang, and Z. W. Han. 2011. Single dose administration of L-carnitine improves antioxidant activities in healthy subjects. *Tohoku J Exp Med* 224 (3):209–213. doi: 10.1620/tjem.224.209.

Carranco J. M. E., CML Carrillo, and F. P. Romo. 2011. Carotenoids and their antioxidant function: a review. *Arch Latinoam Nutr* 61 (3):233–241.

Carvalho, A. C., A. C. Gomes, C. Pereira-Wilson, and C. F. Lima. 2015. Redox-dependent induction of antioxidant defenses by phenolic diterpenes confers stress tolerance in normal human skin fibroblasts: Insights on replicative senescence. *Free Radic Biol Med* 83:262–272. doi: 10.1016/j.freeradbiomed.2015.02.022.

Cetinkaya, A., E. Bulbuloglu, B. Kantarceken, H. Ciralik, E. B. Kurutas, M. A. Buyukbese, and Y. Gumusalan. 2006. Effects of L-carnitine on oxidant/antioxidant status in acetic acid-induced colitis. *Dig Dis Sci* 51 (3):488–494. doi: 10.1007/s10620-006-3160-9.

Chai, Y. Y., F. Wang, Y. L. Li, K. Liu, and H. Xu. 2012. Antioxidant activities of stilbenoids from rheum emodi wall. *Evid Based Complement Alternat Med* 2012:603678. doi: 10.1155/2012/603678.

Chen, X., H. Qiao, T. Liu, Z. Yang, L. Xu, Y. Xu, H. M. Ge, R. X. Tan, and E. Li. 2012. Inhibition of herpes simplex virus infection by oligomeric stilbenoids through ROS generation. *Antiviral Res* 95 (1):30–36. doi: 10.1016/j.antiviral.2012.05.001.

Chen, Y. J., M. S. Shiao, and S. Y. Wang. 2001. The antioxidant caffeic acid phenethyl ester induces apoptosis associated with selective scavenging of hydrogen peroxide in human leukemic HL-60 cells. *Anticancer Drugs* 12 (2):143–149.

Chen, Y., H. Xiao, J. Zheng, and G. Liang. 2015. Structure-thermodynamics-antioxidant activity relationships of selected natural phenolic acids and derivatives: an experimental and theoretical evaluation. *PLoS One* 10 (3):e0121276. doi: 10.1371/journal.pone.0121276.

Chiste, R. C., M. Freitas, A. Z. Mercadante, and E. Fernandes. 2014. Carotenoids are effective inhibitors of in vitro hemolysis of human erythrocytes, as determined by a practical and optimized cellular antioxidant assay. *J Food Sci* 79 (9):H1841–H1847. doi: 10.1111/1750-3841.12580.

Chng, H. T., L. S. New, A. H. Neo, C. W. Goh, E. R. Browne, and E. C. Chan. 2009. Distribution study of orally administered lipoic acid in rat brain tissues. *Brain Res* 1251:80–86. doi: 10.1016/j.brainres.2008.11.025.

Choi, E. M. 2011. Kaempferol protects MC3T3-E1 cells through antioxidant effect and regulation of mitochondrial function. *Food Chem Toxicol* 49 (8):1800–1805. doi: 10.1016/j.fct.2011.04.031.

Choi, H. J., J. H. Song, L. R. Bhatt, and S. H. Baek. 2010. Anti-human rhinovirus activity of gallic acid possessing antioxidant capacity. *Phytother Res* 24 (9):1292–1296. doi: 10.1002/ptr.3101.

Choudhary, R., K. P. Mishra, and C. Subramanyam. 2006. Prevention of isoproterenol-induced cardiac hypertrophy by eugenol, an antioxidant. *Indian J Clin Biochem* 21 (2):107–113. doi: 10.1007/BF02912923.

Ciccone, M. M., F. Cortese, M. Gesualdo, S. Carbonara, A. Zito, G. Ricci, F. De Pascalis, P. Scicchitano, and G. Riccioni. 2013. Dietary intake of carotenoids and their antioxidant and anti-inflammatory effects in cardiovascular care. *Mediators Inflamm* 2013:782137. doi: 10.1155/2013/782137.

Conklin, K. A. 2005. Coenzyme Q10 for prevention of anthracycline-induced cardiotoxicity. *Integr Cancer Ther* 4 (2):110–130. doi: 10.1177/1534735405276191.

Cordero, M. D., A. M. Moreno-Fernandez, M. deMiguel, P. Bonal, F. Campa, L. M. Jimenez-Jimenez, A. Ruiz-Losada et al. 2009. Coenzyme Q10 distribution in blood is altered in patients with fibromyalgia. *Clin Biochem* 42 (7–8):732–735. doi: 10.1016/j.clinbiochem.2008.12.010.

Cortese, M. M., C. V. Suschek, W. Wetzel, K. D. Kroncke, and V. Kolb-Bachofen. 2008. Zinc protects endothelial cells from hydrogen peroxide via Nrf2-dependent stimulation of glutathione biosynthesis. *Free Radic Biol Med* 44 (12):2002–2012. doi: 10.1016/j.freeradbiomed.2008.02.013.

Cruz, K. J., A. R. de Oliveira, and N. Marreiro Ddo. 2015. Antioxidant role of zinc in diabetes mellitus. *World J Diabetes* 6 (2):333–337. doi: 10.4239/wjd.v6.i2.333.

da Silva, D. G., O. Ricci, Jr., E. A. de Almeida, and C. R. Bonini-Domingos. 2015. Potential utility of melatonin as an antioxidant therapy in the management of sickle cell anemia. *J Pineal Res* 58 (2):178–188. doi: 10.1111/jpi.12204.

Da Silva, E. P., Jr., and R. H. Lambertucci. 2015. Effects of N-acetylcysteine and L-arginine in the antioxidant system of C2C12 cells. *J Sports Med Phys Fitness* 55 (6):691–699.

De Martino, L., V. De Feo, F. Fratianni, and F. Nazzaro. 2009. Chemistry, antioxidant, antibacterial and antifungal activities of volatile oils and their components. *Nat Prod Commun* 4 (12):1741–1750.

Deletioglu, V., E. Tuncay, A. Toy, M. Atalay, and B. Turan. 2015. Immuno-spin trapping detection of antioxidant/pro-oxidant properties of zinc or selenium on DNA and protein radical formation via hydrogen peroxide. *Mol Cell Biochem* doi: 10.1007/s11010-015-2508-x.

Di Benedetto, R., R. Vari, B. Scazzocchio, C. Filesi, C. Santangelo, C. Giovannini, P. Matarrese, M. D'Archivio, and R. Masella. 2007. Tyrosol, the major extra virgin olive oil compound, restored intracellular antioxidant defences in spite of its weak antioxidative effectiveness. *Nutr Metab Cardiovasc Dis* 17 (7):535–545. doi: 10.1016/j.numecd.2006.03.005.

Duarte, A. I., M. S. Santos, C. R. Oliveira, and A. C. Rego. 2005. Insulin neuroprotection against oxidative stress in cortical neurons—Involvement of uric acid and glutathione antioxidant defenses. *Free Radic Biol Med* 39 (7):876–889. doi: 10.1016/j.freeradbiomed.2005.05.002.

Duplancic, D., L. Kukoc-Modun, D. Modun, and N. Radic. 2011. Simple and rapid method for the determination of uric acid-independent antioxidant capacity. *Molecules* 16 (8):7058-68. doi: 10.3390/molecules16087058.

Elanchezhian, R., M. Sakthivel, P. Geraldine, and P. A. Thomas. 2010. Regulatory effect of acetyl-l-carnitine on expression of lenticular antioxidant and apoptotic genes in selenite-induced cataract. *Chem Biol Interact* 184 (3):346–351. doi: 10.1016/j.cbi.2010.01.006.

Engin, K. N. 2009. Alpha-tocopherol: Looking beyond an antioxidant. *Mol Vis* 15:855–860.

Evans, A. M. and G. Fornasini. 2003. Pharmacokinetics of L-carnitine. *Clin Pharmacokinet* 42 (11):941–967. doi: 10.2165/00003088-200342110-00002.

Fabbrini, E., M. Serafini, I. C. Baric, S. L. Hazen, and S. Klein. 2014. Effect of plasma uric acid on antioxidant capacity, oxidative stress, and insulin sensitivity in obese subjects. *Diabetes* 63 (3):976–981. doi: 10.2337/db13-1396.

Favero, G., L. F. Rodella, L. Nardo, L. Giugno, M. A. Cocchi, E. Borsani, R. J. Reiter, and R. Rezzani. 2015. A comparison of melatonin and alpha-lipoic acid in the induction of antioxidant defences in L6 rat skeletal muscle cells. *Age (Dordr)* 37 (4):9824. doi: 10.1007/s11357-015-9824-7.

Fernandes, K. S., A. H. Silva, S. A. Mendanha, K. R. Rezende, and A. Alonso. 2013. Antioxidant effect of 4-nerolidylcatechol and alpha-tocopherol in erythrocyte ghost membranes and phospholipid bilayers. *Braz J Med Biol Res* 46 (9):780–788. doi: 10.1590/1414-431×20132940.

Fischedick, J. T., M. Standiford, D. A. Johnson, and J. A. Johnson. 2013. Structure activity relationship of phenolic diterpenes from *Salvia officinalis* as activators of the nuclear factor E2-related factor 2 pathway. *Bioorg Med Chem* 21 (9):2618–2622. doi: 10.1016/j.bmc.2013.02.019.

Fujisawa, S., T. Atsumi, Y. Kadoma, and H. Sakagami. 2002. Antioxidant and prooxidant action of eugenol-related compounds and their cytotoxicity. *Toxicology* 177 (1):39–54.

Ganger, R., R. Garla, B. P. Mohanty, M. P. Bansal, and M. L. Garg. 2015. Protective effects of zinc against acute arsenic toxicity by regulating antioxidant defense system and cumulative metallothione in expression. *Biol Trace Elem Res* 169 (2):218-229. doi: 10.1007/s12011-015-0400-x.

Ganie, S. A., T. Dar, A. Bhat, K. Dar, S. Anees, A. Masood, and M. A. Zargar. 2015. Melatonin—A potential antioxidant therapeutic agent for mitochondrial dysfunctions and related disorders. *Rejuvenation Res* 19(1): 21-40. doi: 10.1089/rej.2015.1704.

Gao, M., F. Kondo, T. Murakami, J. W. Xu, N. Ma, X. Zhu, K. Mori, and T. Ishida. 2007. 1-Aminocyclopropanecarboxylic acid, an antagonist of N-methyl-D-aspartate receptors, causes hypotensive and antioxidant effects with upregulation of heme oxygenase-1 in stroke-prone spontaneously hypertensive rats. *Hypertens Res* 30 (3):249–257. doi: 10.1291/hypres.30.249.

Genaro-Mattos, T. C., A. Q. Mauricio, D. Rettori, A. Alonso, and M. Hermes-Lima. 2015. Antioxidant activity of caffeic acid against iron-induced free radical generation-A chemical approach. *PLoS One* 10 (6):e0129963. doi: 10.1371/journal.pone.0129963.

Gual-Frau, J., C. Abad, M. J. Amengual, N. Hannaoui, M. A. Checa, J. Ribas-Maynou et al. 2015. Oral antioxidant treatment partly improves integrity of human sperm DNA in infertile grade I varicocele patients. *Hum Fertil (Camb)*:1–5. doi: 10.3109/14647273.2015.1050462.

Guan, S., B. Jiang, Y. M. Bao, and L. J. An. 2006. Protocatechuic acid suppresses MPP^+-induced mitochondrial dysfunction and apoptotic cell death in PC12 cells. *Food Chem Toxicol* 44 (10):1659–1666. doi: 10.1016/j.fct.2006.05.004.

Guimaraes, A. G., G. F. Oliveira, M. S. Melo, S. C. Cavalcanti, A. R. Antoniolli, L. R. Bonjardim, F. A. Silva et al. 2010. Bioassay-guided evaluation of antioxidant and antinociceptive activities of carvacrol. *Basic Clin Pharmacol Toxicol* 107 (6):949–957. doi: 10.1111/j.1742-7843.2010.00609.x.

Gul, M., F. Z. Kutay, S. Temocin, and O. Hanninen. 2000. Cellular and clinical implications of glutathione. *Indian J Exp Biol* 38 (7):625–634.

Gulcin, I. 2006. Antioxidant and antiradical activities of L-carnitine. *Life Sci* 78 (8):803–811. doi: 10.1016/j.lfs.2005.05.103.

Habbu, P. V., K. M. Mahadevan, P. V. Kulkarni, C. Daulatsingh, V. P. Veerapur, and R. A. Shastry. 2010. Adaptogenic and *in vitro* antioxidant activity of flavanoids and other fractions of *Argyreia speciosa* (Burm.f) Boj. in acute and chronic stress paradigms in rodents. *Indian J Exp Biol* 48 (1):53–60.

Harpsoe, N. G., L. P. Andersen, I. Gogenur, and J. Rosenberg. 2015. Clinical pharmacokinetics of melatonin: A systematic review. *Eur J Clin Pharmacol* 71 (8):901–909. doi: 10.1007/s00228-015-1873-4.

He, Z. D., K. M. Lau, H. X. Xu, P. C. Li, and P. P. H. But. 2000. Antioxidant activity of phenylethanoid glycosides from *Brandisia hancei*. *J Ethnopharmacol* 71 (3):483–486.

Hermenean, A., A. Ardelean, M. Stan, N. Hadaruga, C. V. Mihali, M. Costache, and A. Dinischiotu. 2014. Antioxidant and hepatoprotective effects of naringenin and its beta-cyclodextrin formulation in mice intoxicated with carbon tetrachloride: A comparative study. *J Med Food* 17 (6):670–677. doi: 10.1089/jmf.2013.0007.

Hershfield, M. S., L. J. Roberts, 2nd, N. J. Ganson, S. J. Kelly, I. Santisteban, E. Scarlett, D. Jaggers, and J. S. Sundy. 2010. Treating gout with pegloticase, a PEGylated urate oxidase, provides insight into the importance of uric acid as an antioxidant in vivo. *Proc Natl Acad Sci USA* 107 (32):14351–14356. doi: 10.1073/pnas.1001072107.

Holzmann, I., L. M. da Silva, J. A. Correa da Silva, V. M. Steimbach, and M. M. de Souza. 2015. Antidepressant-like effect of quercetin in bulbectomized mice and involvement of the antioxidant defenses, and the glutamatergic and oxidonitrergic pathways. *Pharmacol Biochem Behav* 136:55–63. doi: 10.1016/j.pbb.2015.07.003.

Irat, A. M., F. Aktan, and G. Ozansoy. 2003. Effects of L-carnitine treatment on oxidant/antioxidant state and vascular reactivity of streptozotocin-diabetic rat aorta. *J Pharm Pharmacol* 55 (10):1389–1395. doi: 10.1211/0022357021909.

Ito, M., K. Murakami, and M. Yoshino. 2005. Antioxidant action of eugenol compounds: Role of metal ion in the inhibition of lipid peroxidation. *Food Chem Toxicol* 43 (3):461–466. doi: 10.1016/j.fct.2004.11.019.

Jayaraman, J., M. Veerappan, and N. Namasivayam. 2009. Potential beneficial effect of naringenin on lipid peroxidation and antioxidant status in rats with ethanol-induced hepatotoxicity. *J Pharm Pharmacol* 61 (10):1383–1390. doi: 10.1211/jpp/61.10.0016.

Kalaiselvan, I., S. M. Dicson, and P. D. Kasi. 2014. Olive oil and its phenolic constituent tyrosol attenuates dioxin-induced toxicity in peripheral blood mononuclear cells via an antioxidant-dependent mechanism. *Nat Prod Res*:1–4. doi: 10.1080/14786419.2014.989393.

Kamchatnov, P. R., B. A. Abusueva, and A. Y. Kazakov. 2014. Alpha-lipoic acid in treatment of nervous system diseases. *Zh Nevrol Psikhiatr Im S S Korsakova* 114 (10):131–135.

Kamel, K. M., O. M. Abd El-Raouf, S. A. Metwally, H. A. Abd El-Latif, and M. E. El-sayed. 2014. Hesperidin and rutin, antioxidant citrus flavonoids, attenuate cisplatin-induced nephrotoxicity in rats. *J Biochem Mol Toxicol* 28 (7):312–319. doi: 10.1002/jbt.21567.

Kamogawa, E. and Y. Sueishi. 2014. A multiple free-radical scavenging (MULTIS) study on the antioxidant capacity of a neuroprotective drug, edaravone as compared with uric acid, glutathione, and trolox. *Bioorg Med Chem Lett* 24 (5):1376–1379. doi: 10.1016/j.bmcl.2014.01.045.

Kara, Y., D. K. Doguc, E. Kulac, and F. Gultekin. 2014. Acetylsalicylic acid and ascorbic acid combination improves cognition; via antioxidant effect or increased expression of NMDARs and nAChRs? *Environ Toxicol Pharmacol* 37 (3):916–927. doi: 10.1016/j.etap.2014.02.019.

Keith, D. J., J. A. Butler, B. Bemer, B. Dixon, S. Johnson, M. Garrard, D. L. Sudakin, J. M. Christensen, C. Pereira, and T. M. Hagen. 2012. Age and gender dependent bioavailability of R- and R, S-alpha-lipoic acid: A pilot study. *Pharmacol Res* 66 (3):199–206. doi: 10.1016/j.phrs.2012.05.002.

Kelso, G. F., C. M. Porteous, C. V. Coulter, G. Hughes, W. K. Porteous, E. C. Ledgerwood, R. A. Smith, and M. P. Murphy. 2001. Selective targeting of a redox-active ubiquinone to mitochondria within cells: Antioxidant and antiapoptotic properties. *J Biol Chem* 276 (7):4588–96. doi: 10.1074/jbc.M009093200.

Kojima-Yuasa, A., K. Umeda, T. Ohkita, D. O. Kennedy, S. Nishiguchi, and I. Matsui-Yuasa. 2005. Role of reactive oxygen species in zinc deficiency-induced hepatic stellate cell activation. *Free Radic Biol Med* 39 (5):631–640. doi: 10.1016/j.freeradbiomed.2005.04.015.

Kolesnikova, L. I., E. A. Karpova, B. Y. Vlasov, B. G. Sukhov, and B. A. Mov. 2015. Lipid peroxidation-antioxidant defense system during toxic liver damage and its correction with a composite substance containing selenium and arabinogalactan. *Bull Exp Biol Med* 159 (2):225-8. doi: 10.1007/s10517-015-2928-3.

Korkmaz, A., R. J. Reiter, T. Topal, L. C. Manchester, S. Oter, and D. X. Tan. 2009. Melatonin: Aan established antioxidant worthy of use in clinical trials. *Mol Med* 15 (1–2):43–50. doi: 10.2119/molmed.2008.00117.

Lado, C., M. Then, I. Varga, E. Szoke, and K. Szentmihalyi. 2004. Antioxidant property of volatile oils determined by the ferric reducing ability. *Z Naturforsch C* 59 (5–6):354–358.
Lee, B. J., J. S. Lin, Y. C. Lin, and P. T. Lin. 2014. Effects of L-carnitine supplementation on oxidative stress and antioxidant enzymes activities in patients with coronary artery disease: A randomized, placebo-controlled trial. *Nutr J* 13:79. doi: 10.1186/1475-2891-13-79.
Leopoldini, M., N. Russo, and M. Toscano. 2011. The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food Chem* (125):288–306.
Leung, H. W., C. J. Lin, M. J. Hour, W. H. Yang, M. Y. Wang, and H. Z. Lee. 2007. Kaempferol induces apoptosis in human lung non-small carcinoma cells accompanied by an induction of antioxidant enzymes. *Food Chem Toxicol* 45 (10):2005–2013. doi: 10.1016/j.fct.2007.04.023.
Li, J., L. Feng, Y. Xing, Y. Wang, L. Du, C. Xu, J. Cao, Q. Wang, S. Fan, Q. Liu, and F. Fan. 2014a. Radioprotective and antioxidant effect of resveratrol in hippocampus by activating Sirt1. *Int J Mol Sci* 15 (4):5928–5939. doi: 10.3390/ijms15045928.
Li, Q., Y. Qiu, M. Mao, J. Lv, L. Zhang, S. Li, X. Li, and X. Zheng. 2014b. Antioxidant mechanism of Rutin on hypoxia-induced pulmonary arterial cell proliferation. *Molecules* 19 (11):19036–19049. doi: 10.3390/molecules191119036.
Lin, C. Y., C. S. Huang, C. Y. Huang, and M. C. Yin. 2009. Anticoagulatory, antiinflammatory, and antioxidative effects of protocatechuic acid in diabetic mice. *J Agric Food Chem* 57 (15):6661–6667. doi: 10.1021/jf9015202.
Littarru, G. P. and L. Tiano. 2005. Clinical aspects of coenzyme Q10: An update. *Curr Opin Clin Nutr Metab Care* 8 (6):641–646.
Liu, H., L. Zhang, and S. Lu. 2012. Evaluation of antioxidant and immunity activities of quercetin in isoproterenol-treated rats. *Molecules* 17 (4):4281–4291. doi: 10.3390/molecules17044281.
Lobo, V., A. Patil, A. Phatak, and N. Chandra. 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev* 4 (8):118–126. doi: 10.4103/0973-7847.70902.
Lu, Z., G. Nie, P. S. Belton, H. Tang, and B. Zhao. 2006. Structure-activity relationship analysis of antioxidant ability and neuroprotective effect of gallic acid derivatives. *Neurochem Int* 48 (4):263–274. doi: 10.1016/j.neuint.2005.10.010.
Ma, X. and W. Qian. 2010. Phenolic acid induced growth of gold nanoshells precursor composites and their application in antioxidant capacity assay. *Biosens Bioelectron* 26 (3):1049–1055. doi: 10.1016/j.bios.2010.08.055.
Maciel, R. M., M. M. Costa, D. B. Martins, R. T. Franca, R. Schmatz, D. L. Graca, M. M. Duarte et al. 2013. Antioxidant and anti-inflammatory effects of quercetin in functional and morphological alterations in streptozotocin-induced diabetic rats. *Res Vet Sci* 95 (2):389–397. doi: 10.1016/j.rvsc.2013.04.028.
Malhotra, S., M. N. Welling, S. B. Mantri, and K. Desai. 2015. In vitro and in vivo antioxidant, cytotoxic, and anti-chronic inflammatory arthritic effect of selenium nanoparticles. *J Biomed Mater Res B Appl Biomater*. doi: 10.1002/jbm.b.33448.
Manzanares, W. and G. Hardy. 2009. Selenium supplementation in the critically ill: Posology and pharmacokinetics. *Curr Opin Clin Nutr Metab Care* 12 (3):273–280. doi: 10.1097/MCO.0b013e32832a0cc2.
Mathew, D., C. K. Nair, J. A. Jacob, N. Biswas, T. Mukherjee, S. Kapoor, and T. V. Kagiya. 2007. Ascorbic acid monoglucoside as antioxidant and radioprotector. *J Radiat Res* 48 (5):369–76.
Mayr, J. A., R. G. Feichtinger, F. Tort, A. Ribes, and W. Sperl. 2014. Lipoic acid biosynthesis defects. *J Inherit Metab Dis* 37 (4):553–563. doi: 10.1007/s10545-014-9705-8.
Mendes-da-Silva, R. F., A. A. C. Lopes-de-Morais, M. E. Bandim-da-Silva, G. de Araujo Cavalcanti, A. R. O. Rodrigues, B. L. da Silveira Andrade-da-Costa, and R. C. A. Guedes. 2014. Prooxidant versus antioxidant brain action of ascorbic acid in well-nourished and malnourished rats as a function of dose: A cortical spreading depression and malondialdehyde analysis. *Neuropharmacology* 86:155–160. doi: 10.1016/j.neuropharm.2014.06.027.

Miles, M. V. 2007. The uptake and distribution of coenzyme Q10. *Mitochondrion* 7 Suppl:S72-7. doi: 10.1016/j.mito.2007.02.012.
Mugesh, G., W. W. du Mont, and H. Sies. 2001. Chemistry of biologically important synthetic organoselenium compounds. *Chem Rev* 101 (7):2125–2179.
Muhamad, M., L. Y. Kee, N. A. Rahman, and R. Yusof. 2010. Antiviral actions of flavanoid-derived compounds on dengue virus type-2. *Int J Biol Sci* 6 (3):294–302.
Nagababu, E., J. M. Rifkind, S. Boindala, and L. Nakka. 2010. Assessment of antioxidant activity of eugenol in vitro and in vivo. *Methods Mol Biol* 610:165–180. doi: 10.1007/978-1-60327-029-8_10.
Nakamura, Y., K. Torikai, Y. Ohto, A. Murakami, T. Tanaka, and H. Ohigashi. 2000. A simple phenolic antioxidant protocatechuic acid enhances tumor promotion and oxidative stress in female ICR mouse skin: Dose-and timing-dependent enhancement and involvement of bioactivation by tyrosinase. *Carcinogenesis* 21 (10):1899–907.
Naksuriya, O. and S. Okonogi. 2015. Comparison and combination effects on antioxidant power of curcumin with gallic acid, ascorbic acid, and xanthone. *Drug Discov Ther* 9 (2):136–41. doi: 10.5582/ddt.2015.01013.
Ndhlala, A. R., M. Moyo, and J. Van Staden. 2010. Natural antioxidants: Fascinating or mythical biomolecules? *Molecules* 15 (10):6905–6930. doi: 10.3390/molecules 15106905.
Nirmala, P. and M. Ramanathan. 2011. Effect of kaempferol on lipid peroxidation and antioxidant status in 1,2-dimethyl hydrazine induced colorectal carcinoma in rats. *Eur J Pharmacol* 654 (1):75–79. doi: 10.1016/j.ejphar.2010.11.034.
Nishimura F. D. C., A. C. de Almeida, B. A. Ratti, T. Ueda-Nakamura, C. V. Nakamura, V. F. Ximenes, and S. D. O. Silva. 2013. Antioxidant effects of quercetin and naringenin are associated with impaired neutrophil microbicidal activity. *Evid Based Complement Alternat Med* 2013:795916. doi: 10.1155/2013/795916.
Nogueira, C. W. and J. B. Rocha. 2011. Toxicology and pharmacology of selenium: Emphasis on synthetic organoselenium compounds. *Arch Toxicol* 85 (11):1313–1359. doi: 10.1007/s00204-011-0720-3.
Noronha, C. M., S. M. de Carvalho, R. C. Lino, and P. L. Barreto. 2014. Characterization of antioxidant methylcellulose film incorporated with alpha-tocopherol nanocapsules. *Food Chem* 159:529–535. doi: 10.1016/j.foodchem.2014.02.159.
Ozkan, A. and A. Erdogan. 2012. A comparative study of the antioxidant/prooxidant effects of carvacrol and thymol at various concentrations on membrane and DNA of parental and drug resistant H1299 cells. *Nat Prod Commun* 7 (12):1557–1560.
Panich, U., V. Tangsupa-a-nan, T. Onkoksoong, K. Kongtaphan, K. Kasetsinsombat, P. Akarasereenont, and A. Wongkajornsilp. 2011. Inhibition of UVA-mediated melanogenesis by ascorbic acid through modulation of antioxidant defense and nitric oxide system. *Arch Pharm Res* 34 (5):811–820. doi: 10.1007/s12272-011-0515-3.
Papiez, M. A. and W. Krzysciak. 2014. The antioxidant quercetin protects HL-60 cells with high myeloperoxidase activity against pro-oxidative and apoptotic effects of etoposide. *Acta Biochim Pol* 61 (4):795–799.
Park, S. W. and S. M. Lee. 2008. Antioxidant and prooxidant properties of ascorbic acid on hepatic dysfunction induced by cold ischemia/reperfusion. *Eur J Pharmacol* 580 (3):401–406. doi: 10.1016/j.ejphar.2007.11.023.
Patterson, R. A., E. T. Horsley, and D. S. Leake. 2003. Prooxidant and antioxidant properties of human serum ultrafiltrates toward LDL: important role of uric acid. *J Lipid Res* 44 (3):512–21. doi: 10.1194/jlr.M200407-JLR200.
Pawlas, N., M. Dobrakowski, A. Kasperczyk, A. Kozlowska, A. Mikolajczyk, and S. Kasperczyk. 2015. The level of selenium and oxidative stress in workers chronically exposed to lead. *Biol Trace Elem Res* doi: 10.1007/s12011-015-0435-z.
Peterson, C. E., R. L. Sedjo, F. G. Davis, C. A. Beam, and A. R. Giuliano. 2010. Combined antioxidant carotenoids and the risk of persistent human papillomavirus infection. *Nutr Cancer* 62 (6):728–733. doi: 10.1080/01635581003693074.

Pinamonti, S., L. Venturoli, M. Leis, M. Chicca, A. Barbieri, S. Sostero, F. Ravenna, L. Daffonchio, R. Novellini, and A. Ciaccia. 2001. Antioxidant activity of carbocysteine lysine salt monohydrate. *Panminerva Med* 43 (3):215–220.

Powell, S. R. 2000. The antioxidant properties of zinc. *J Nutr* 130 (5S Suppl):1447S–1454S.

Prasad, A. S. 2009. Zinc: Role in immunity, oxidative stress and chronic inflammation. *Curr Opin Clin Nutr Metab Care* 12 (6):646–652. doi: 10.1097/MCO.0b013e3283312956.

Prasad, A. S. 2014. Zinc: An antioxidant and anti-inflammatory agent: Role of zinc in degenerative disorders of aging. *J Trace Elem Med Biol* 28 (4):364–371. doi: 10.1016/j.jtemb.2014.07.019.

Qu, H., Y. Zhang, X. Chai, and W. Sun. 2012. Isoforsythiaside, an antioxidant and antibacterial phenylethanoid glycoside isolated from Forsythia suspensa. *Bioorg Chem* 40 (1):87–91. doi: 10.1016/j.bioorg.2011.09.005.

Rahigude, A., P. Bhutada, S. Kaulaskar, M. Aswar, and K. Otari. 2012. Participation of antioxidant and cholinergic system in protective effect of naringenin against type-2 diabetes-induced memory dysfunction in rats. *Neuroscience* 226:62–72. doi: 10.1016/j.neuroscience.2012.09.026.

Rani, P. J. and C. Panneerselvam. 2002. Effect of L-carnitine on brain lipid peroxidation and antioxidant enzymes in old rats. *J Gerontol A Biol Sci Med Sci* 57 (4):B134–B137.

Riabchenko, N. I., V. I. Riabchenko, B. P. Ivannik, L. A. Dzikovskaia, R. V. Sin'kova, I. P. Grosheva, E. S. Degtiareva, and T. I. Ivanova. 2010. Antioxidant and prooxidant properties of the ascorbic acid, dihydroquercetine and mexidol in the radical reactions induced by the ionizing radiation and chemical reagents. *Radiats Biol Radioecol* 50 (2):186–194.

Robbins, R. J. 2003. Phenolic acids in foods: An overview of analytical methodology. *J Agric Food Chem* 51 (10):2866–2887. doi: 10.1021/jf026182t.

Rocha, B. A., C. S. Teixeira, J. C. Silva-Filho, R. B. Nobrega, D. B. Alencar, K. S. Nascimento, V. N. Freire et al. 2015. Structural basis of ConM binding with resveratrol, an anti-inflammatory and antioxidant polyphenol. *Int J Biol Macromol* 72:1136–1142. doi: 10.1016/j.ijbiomac.2014.08.031.

Rodriguez-Bies, E., P. Navas, and G. Lopez-Lluch. 2015. Age-dependent effect of every-other-day feeding and aerobic exercise in ubiquinone levels and related antioxidant activities in mice muscle. *J Gerontol A Biol Sci Med Sci* 70 (1):33–43. doi: 10.1093/gerona/glu002.

Rozza, A. L., F. M. de Faria, A. R. S. Brito, and C. H. Pellizzon. 2014. The gastroprotective effect of menthol: Involvement of anti-apoptotic, antioxidant and anti-inflammatory activities. *PLoS One* 9 (1):e86686. doi: 10.1371/journal.pone.0086686.

Russo, A., R. Longo, and A. Vanella. 2002. Antioxidant activity of propolis: Role of caffeic acid phenethyl ester and galangin. *Fitoterapia* 73 (Suppl 1):S21–S29.

Ruttkay-Nedecky, B., L. Nejdl, J. Gumulec, O. Zitka, M. Masarik, T. Eckschlager, M. Stiborova, V. Adam, and R. Kizek. 2013. The role of metallothionein in oxidative stress. *Int J Mol Sci* 14 (3):6044–6066. doi: 10.3390/ijms14036044.

Sadi, G. and D. Konat. 2015. Resveratrol regulates oxidative biomarkers and antioxidant enzymes in the brain of streptozotocin-induced diabetic rats. *Pharm Biol* 54 (7):1156-1163. doi: 10.3109/13880209.2015.1056311.

Sandor, P. S., L. Di Clemente, G. Coppola, U. Saenger, A. Fumal, D. Magis, L. Seidel, R. M. Agosti, and J. Schoenen. 2005. Efficacy of coenzyme Q10 in migraine prophylaxis: A randomized controlled trial. *Neurology* 64 (4):713–715. doi: 10.1212/01.WNL.0000151975.03598.ED.

Santos, I. M., R. Tome Ada, G. B. Saldanha, P. M. Ferreira, G. C. Militao, and R. M. Freitas. 2009. Oxidative stress in the hippocampus during experimental seizures can be ameliorated with the antioxidant ascorbic acid. *Oxid Med Cell Longev* 2 (4):214–221. doi: 10.4161/oxim.2.4.8876.

Sarrou, E., P. Chatzopoulou, K. Dimassi-Theriou, and I. Therios. 2013. Volatile constituents and antioxidant activity of peel, flowers and leaf oils of *Citrus aurantium* L. growing in Greece. *Molecules* 18 (9):10639–10647. doi: 10.3390/molecules180910639.

Sautin, Y. Y., T. Nakagawa, S. Zharikov, and R. J. Johnson. 2007. Adverse effects of the classic antioxidant uric acid in adipocytes: NADPH oxidase-mediated oxidative/nitrosative stress. *Am J Physiol Cell Physiol* 293 (2):C584–C596. doi: 10.1152/ajpcell.00600.2006.

Saygin, M., H. Asci, F. N. Cankara, D. Bayram, S. Yesilot, I. A. Candan, and H. H. Alp. 2015. The impact of high fructose on cardiovascular system: Role of alpha-lipoic acid. *Hum Exp Toxicol*. doi: 10.1177/0960327115579431.

Sedlacek, V., N. Ptackova, P. Rejmontova, and I. Kucera. 2015. The flavoprotein FerB of *Paracoccus denitrificans* binds to membranes, reduces ubiquinone and superoxide, and acts as an *in vivo* antioxidant. *FEBS J* 282 (2):283–296. doi: 10.1111/febs.13126.

Serrano, J., A. Cipak, J. Boada, H. Gonzalo, D. Cacabelos, A. Cassanye, R. Pamplona, N. Zarkovic, and M. Portero-Otin. 2010. Double-edged sword behaviour of gallic acid and its interaction with peroxidases in human microvascular endothelial cell culture (HMEC-1). Antioxidant and pro-oxidant effects. *Acta Biochim Pol* 57 (2):193–198.

Shay, K. P., R. F. Moreau, E. J. Smith, A. R. Smith, and T. M. Hagen. 2009. Alpha-lipoic acid as a dietary supplement: Molecular mechanisms and therapeutic potential. *Biochim Biophys Acta* 1790 (10):1149–1160. doi: 10.1016/j.bbagen.2009.07.026.

Singh, K. and P. Ahluwalia. 2003. Studies on the effect of monosodium glutamate [MSG] administration on some antioxidant enzymes in the arterial tissue of adult male mice. *J Nutr Sci Vitaminol (Tokyo)* 49 (2):145–148.

Singh, P., K. A. Mann, H. K. Mangat, and G. Kaur. 2003. Prolonged glutamate excitotoxicity: effects on mitochondrial antioxidants and antioxidant enzymes. *Mol Cell Biochem* 243 (1–2):139–145.

Skibsted, L. H. 2012. Carotenoids in antioxidant networks. Colorants or radical scavengers. *J Agric Food Chem* 60 (10):2409–2417. doi: 10.1021/jf2051416.

Sohi, K. K., N. Mittal, M. K. Hundal, and K. L. Khanduja. 2003. Gallic acid, an antioxidant, exhibits antiapoptotic potential in normal human lymphocytes: A Bcl-2 independent mechanism. *J Nutr Sci Vitaminol (Tokyo)* 49 (4):221–227.

Song, Y., J. Liu, F. Zhang, J. Zhang, T. Shi, and Z. Zeng. 2013. Antioxidant effect of quercetin against acute spinal cord injury in rats and its correlation with the p38MAPK/iNOS signaling pathway. *Life Sci* 92 (24–26):1215–1221. doi: 10.1016/j.lfs.2013.05.007.

Stahl, W., N. Ale-Agha, and M. C. Polidori. 2002. Non-antioxidant properties of carotenoids. *Biol Chem* 383 (3–4):553–558. doi: 10.1515/BC.2002.056.

Stahl, W. and H. Sies. 2003. Antioxidant activity of carotenoids. *Mol Aspects Med* 24 (6):345–351.

Stojkovski, V., N. Hadzi-Petrushev, V. Ilieski, R. Sopi, I. Gjorgoski, D. Mitrov, N. Jankulovski, and M. Mladenov. 2013. Age and heat exposure-dependent changes in antioxidant enzymes activities in rat's liver and brain mitochondria: Role of alpha-tocopherol. *Physiol Res* 62 (5):503–510.

Suslina, Z. A., T. N. Fedorova, M. Maksimova, and E. K. Kim. 2003. Antioxidant activity of mildronate and L-carnitine in the treatment of patients with cerebrovascular diseases. *Eksp Klin Farmakol* 66 (3):32–35.

Tabata, K., M. Kim, M. Makino, M. Satoh, Y. Satoh, and T. Suzuki. 2012. Phenolic diterpenes derived from *Hyptis incana* induce apoptosis and G_2/M arrest of neuroblastoma cells. *Anticancer Res* 32 (11):4781–4789.

Tamaki, N., R. C. Orihuela-Campos, Y. Inagaki, M. Fukui, T. Nagata, and H. O. Ito. 2014. Resveratrol improves oxidative stress and prevents the progression of periodontitis via the activation of the Sirt1/AMPK and the Nrf2/antioxidant defense pathways in a rat periodontitis model. *Free Radic Biol Med* 75:222–229. doi: 10.1016/j.freeradbiomed.2014.07.034.

Tellone, E., M. C. De Rosa, D. Pirolli, A. Russo, B. Giardina, A. Galtieri, and S. Ficarra. 2014. Molecular interactions of hemoglobin with resveratrol: Potential protective antioxidant role and metabolic adaptations of the erythrocyte. *Biol Chem* 395 (3):347–354. doi: 10.1515/hsz-2013-0257.

Thirunavukkarasu, V., A. T. Anitha Nandhini, and C. V. Anuradha. 2004. Cardiac lipids and antioxidant status in high fructose rats and the effect of alpha-lipoic acid. *Nutr Metab Cardiovasc Dis* 14 (6):351–357.

Thomas, S., C. S. Vieira, M. A. Hass, and L. B. Lopes. 2014. Stability, cutaneous delivery, and antioxidant potential of a lipoic acid and alpha-tocopherol codrug incorporated in microemulsions. *J Pharm Sci* 103 (8):2530–2538. doi: 10.1002/jps.24053.

Tiano, L., L. Padella, P. Carnevali, O. Gabrielli, F. Bruge, F. Principi, and G. P. Littarru. 2008. Coenzyme Q10 and oxidative imbalance in Down syndrome: biochemical and clinical aspects. *Biofactors* 32 (1–4):161–167.

Trevisan, M. T., M. G. Vasconcelos Silva, B. Pfundstein, B. Spiegelhalder, and R. W. Owen. 2006. Characterization of the volatile pattern and antioxidant capacity of essential oils from different species of the genus Ocimum. *J Agric Food Chem* 54 (12):4378–4382. doi: 10.1021/jf060181+.

Tuck, K. L., M. P. Freeman, P. J. Hayball, G. L. Stretch, and I. Stupans. 2001. The in vivo fate of hydroxytyrosol and tyrosol, antioxidant phenolic constituents of olive oil, after intravenous and oral dosing of labeled compounds to rats. *J Nutr* 131 (7):1993–1996.

Ueda, Y., T. Doi, K. Nagatomo, and A. Nakajima. 2007. In vivo activation of N-methyl-D-aspartate receptors generates free radicals and reduces antioxidant ability in the rat hippocampus: Experimental protocol of in vivo ESR spectroscopy and microdialysis for redox status evaluation. *Brain Res* 1178:20–27. doi: 10.1016/j.brainres.2007.08.022.

Ullah, M. F., H. Y. Khan, H. Zubair, U. Shamim, and S. M. Hadi. 2011. The antioxidant ascorbic acid mobilizes nuclear copper leading to a prooxidant breakage of cellular DNA: Implications for chemotherapeutic action against cancer. *Cancer Chemother Pharmacol* 67 (1):103–110. doi: 10.1007/s00280-010-1290-4.

Uluata, S., D. J. McClements, and E. A. Decker. 2015. How the multiple antioxidant properties of ascorbic acid affect lipid oxidation in oil-in-water emulsions. *J Agric Food Chem* 63 (6):1819–1824. doi: 10.1021/jf5053942.

Ushiyama, M., T. Kuramochi, S. Yagi, and S. Katayama. 2008. Antioxidant treatment with alpha-tocopherol improves erectile function in hypertensive rats. *Hypertens Res* 31 (5): 1007–1013. doi: 10.1291/hypres.31.1007.

Vasconcelos, G. S., N. C. Ximenes, C. N. de Sousa, Q. Oliveira Tde, L. L. Lima, D. F. de Lucena, C. S. Gama, D. Macedo, and S. M. Vasconcelos. 2015. Alpha-lipoic acid alone and combined with clozapine reverses schizophrenia-like symptoms induced by ketamine in mice: Participation of antioxidant, nitrergic and neurotrophic mechanisms. *Schizophr Res* 165 (2–3):163–70. doi: 10.1016/j.schres.2015.04.017.

Wagner, A. E., P. Huebbe, T. Konishi, M. M. Rahman, M. Nakahara, S. Matsugo, and G. Rimbach. 2008. Free radical scavenging and antioxidant activity of ascorbigen versus ascorbic acid: Studies in vitro and in cultured human keratinocytes. *J Agric Food Chem* 56 (24):11694–11699. doi: 10.1021/jf802403d.

Wang, Q., Y. Wang, Y. Deng, T. Shi, X. Liu, X. Sun, X. Li, and D. Zhou. 2011. A rapid determination of drug candidate tyrosol galactoside in rat plasma by HPLC and its application to the pharmacokinetics study. *Eur J Drug Metab Pharmacokinet* 35 (3–4):131–136. doi: 10.1007/s13318-010-0015-8.

Yogalakshmi, B., P. Viswanathan, and C. V. Anuradha. 2010. Investigation of antioxidant, anti-inflammatory and DNA-protective properties of eugenol in thioacetamide-induced liver injury in rats. *Toxicology* 268 (3):204–12. doi: 10.1016/j.tox.2009.12.018.

Young, A. J. and G. M. Lowe. 2001. Antioxidant and prooxidant properties of carotenoids. *Arch Biochem Biophys* 385 (1):20–27. doi: 10.1006/abbi.2000.2149.

Yu, L., S. Wang, Y. Kogure, S. Yamamoto, K. Noguchi, and Y. Dai. 2013. Modulation of TRP channels by resveratrol and other stilbenoids. *Mol Pain* 9:3. doi: 10.1186/1744-8069-9-3.

Yu, P., C. Hu, E. J. Meehan, and L. Chen. 2007. X-ray crystal structure and antioxidant activity of salidroside, a phenylethanoid glycoside. *Chem Biodivers* 4 (3):508–513. doi: 10.1002/cbdv.200790043.

Zago, M. P. and P. I. Oteiza. 2001. The antioxidant properties of zinc: Interactions with iron and antioxidants. *Free Radic Biol Med* 31 (2):266–274.

Zeng, H. H., P. F. Tu, K. Zhou, H. Wang, B. H. Wang, and J. F. Lu. 2001. Antioxidant properties of phenolic diterpenes from Rosmarinus officinalis. *Acta Pharmacol Sin* 22 (12):1094–1098.

Zerin, T., Y. S. Kim, S. Y. Hong, and H. Y. Song. 2013. Quercetin reduces oxidative damage induced by paraquat via modulating expression of antioxidant genes in A549 cells. *J Appl Toxicol* 33 (12):1460–1467. doi: 10.1002/jat.2812.

Zhang, H. M. and Y. Zhang. 2014. Melatonin: A well-documented antioxidant with conditional pro-oxidant actions. *J Pineal Res* 57 (2):131–146. doi: 10.1111/jpi.12162.

17

Antioxidant Nutraceuticals and Skincare

Bell Loo, Hiep Nguyen, and Uyen Le

Contents

17.1 Introduction

In recent years, the use of nutraceuticals, a combo of food derived and pharmaceuticals, has remarkably increased in various fields when people are seeking for complementary or alternative beneficial products to replace the expensive disease-treatment approach. Antioxidant nutraceuticals represent a variety of products on their antioxidant activity. An antioxidant is generally defined as a substance that significantly decreases the adverse effects of reactive oxygen species, reactive nitrogen species, or both on normal physiological function in human. Clinically, antioxidant is understood as "a product that inhibits the oxidation *in vitro* and reduces the oxidative stress *in vivo*" (Costantini 2008, Lushchak 2014). Typical antioxidants are vitamins E, C, and polyphenols, which have been well known as scavengers related to different impacts on oxidative stress. In fact, mixtures of compounds, rather than a single product, are highly evaluated for the antioxidant activities.

The nutraceutical industry mainly comprises natural products, dietary supplements, and functional foods. For the skincare, however, the application of nutraceuticals, although still limited, mainly involves in topical delivery. In this chapter, we consider the skincare as *the use of cosmetics to care for the skin* (*Oxford English Dictionary* 2014) and focus on antioxidant nutraceuticals applied for skin's cleansing, massaging, toning, and moisturizing.

17.2 Skin structure and physiology

The skin provides the largest barrier between human body and the outside environment. It contains three layers: epidermis, the dermis, and subcutaneous layer (Nicol 2005). The epidermis contains keratinocytes whose function is to make keratin to protect the skin. The dermis is the middle layer of the skin. It is made of structural protein collagen, which is one of the important building

blocks of human skin that provides strength to the skin. The subcutaneous layer, or hypodermis, is the lowermost layer that is mainly for fat storage.

The skin plays a vital role in many functions including protecting the body from physical, chemical, and biologic attacks from external environment. The stratum corneum is the main barrier of the skin and is located in the outermost layer of the skin. It is also the main barrier for drug absorption into the skin (Nicol 2005). The stratum corneum is composed of proteins and lipids. The lipids are sequestered within the extracellular spaces of stratum corneum. The function of lipids is to both prevent excessive loss of water from the body and block entry of many topical drugs except for the drugs that are lipid soluble or of low molecular weight. This causes a significant challenge to administering drugs through the skin either for local cutaneous effects or as systemic treatment entering through superficial dermal capillaries.

17.3 Aging on skin

Skin aging is a constant process. Skin changes as people age. The skin aging process is determined by our body's natural aging process and environmental factors such as sun exposure, and lifestyle factors such as smoking and menopause. During the skin aging process, both the appearance of the skin and function of the skin are impacted. Changes in the appearance of skin are the most obvious signs of aging. They include wrinkles, age spots, sagging of the skin, dry skin, loss of elasticity, and dilated blood vessels near the surface of the skin (Fisher et al. 1997). Sun damage is the major cause of wrinkling (Helfrich et al. 2008). Sun-exposed areas of the skin such as face, neck, upper chest, hands, and forearms have shown most obvious changes of skin aging. In addition, repeated exposure to the sun or tanning both can destroy collagen, elastin and can make skin aging faster.

17.4 Free radicals and skin health

The skin is directly exposed to the environmental pollutants, sun, and others, which are able to cause free radicals in addition to reactive oxygen species (ROS) (Agarwal et al. 1993) produced by body's metabolism (Fisher et al. 1997, Helfrich et al. 2008). The ROS are commonly generated from exposure to UV radiation, environmental pollution, alcohol intake, and smoking (Ernster et al. 1995, Fisher et al. 1997, Kennedy et al. 2003, Vierkotter et al. 2010, Lademann et al. 2011).

17.4.1 Ultraviolet radiation

Ultraviolet irradiation from the sun has a detrimental effect on the skin such as sunburn and premature skin aging (Fisher et al. 1997). In a study of the

pathophysiology of premature skin and skin aging induced by ultraviolet light, 59 white participants (33 men and 26 women) from age 21 to 58 years were examined (Fisher et al. 1997). Participants had no prior skin disease. The buttock skin of the participants was irradiated with fluorescent ultraviolet lights, and skin specimens were obtained from irradiated and nonirradiated areas by biopsy. The results showed that multiple exposures to ultraviolet irradiation caused an increase in matrix metalloproteinase (MMP), which decreased skin collagen and may lead to skin aging.

In another study, the effect of smoking and sun on the aging skin is evaluated in terms of developing cutaneous elastosis (i.e., premature aging due to prolong exposure to sunlight) and telangiectasia (i.e., small dilated blood vessels near the surface of the skin) (Kennedy et al. 2003). There were 966 participants enrolling in the study. Exposure to sunlight and smoking was collected, and the amount of elastosis and telangiectasia in the face and neck was reported based on a four-graded score ranging from none to severe. The results showed that there was a strong link between increasing age, sun exposure, and premature aging. The amount of telangiectasia was more in men than in women. In addition, smoking was also related to premature aging with both men and women. The study concluded that sunlight exposure and smoking are related to skin aging.

Sunscreens have been well recommended to protect skin from ultraviolet-B-associated skin burning and damage. However, it demonstrated inadequately to protect the skin against ultraviolet-A-induced free radicals, which are implications for skin aging and melanoma (Eberlein-Konig et al. 1998).

17.4.2 Air pollution

Skin aging has been well known to be associated with sun exposure, but growing evidence suggested that environmental factors such as air pollution may also have an impact in this process (Krutmann et al. 2014). In a study of airborne particle exposure and extrinsic skin aging, the impact of air pollution on skin aging in 400 Caucasian women aged 70–80 was evaluated. Skin aging was measured by score of intrinsic and extrinsic skin aging, a validated skin aging score. Traffic particle emissions and soot in fine dust around the area of residence were measured. The results showed that air pollution exposure was markedly related to sign of skin aging, especially associated with pigment spots but less associated with wrinkles. The study reported that increase in soot (per 0.5×10^{-5} per m) particles from busy traffic (per 475 kg per year and square km) was related to 20% more pigment spots on forehead and cheeks.

17.4.3 Alcohol

In a study of interaction between carotenoids and free radicals in human skin, the level of antioxidant network, essential to protect the skin against free radicals, was reported to decrease with alcohol consumption (Lademann et al. 2011).

Similarly, melanoma risk was increased in patients with high consumption of alcohol, who had more than 14 drinks per week as compared to nondrinkers (Freedman et al. 2003). It was stated that alcohol consumption increases free radicals, which greatly contributed to the disease.

17.4.4 Smoking

In a study regarding the impact of smoking on skin health, it was reported that cigarette smoking was associated with skin wrinkle (Ernster et al. 1995). The study evaluated patients' smoking status in terms of pack years of smoking by questionnaire, whereas facial wrinkle category and facial wrinkle score were analyzed by blinded standardized visual assessment. The results showed that the relative risk of moderate-to-severe wrinkling for current smokers was in age 40–69 for women and age 40–59 for men. The study excluded individuals who aged 30–39, as wrinkles are not common among this age group. In addition, the risk of wrinkling was equal to about 1.4 years of aging in both sexes. Similarly, in another study, it was reported that smoking increased skin aging including loss of skin elasticity due to loss of connective tissue and increased skin wrinkle. Interestingly, this loss caused by smoking is similar to UV irradiance (Grant 2008).

17.4.5 Diet

It has been well known that nutrition played an important role in skin functioning. However, the effect of diet on skin aging appearance is not quite clear. In a study of dietary nutrient intakes and skin aging appearance among middle-aged American women, 4025 women aged between 40 and 74 years were examined (Cosgrove et al. 2007). Nutrient intakes were reported from a 24-hour recall, and dermatologists examined the skin appearance. Skin aging was considered as having skin wrinkle, skin dryness due to old age, and skin atrophy. The results showed that taking higher amounts of vitamin C lowered skin wrinkle; in addition, taking higher amounts of linoleic acid decreased skin dryness due to aging and skin atrophy. It was also reported that an increase in 17 g of fat and 50 g of carbohydrate consumption was associated with increased skin wrinkle.

17.5 Antioxidant nutraceuticals beneficial for the skin

17.5.1 Green tea

Green tea polyphenols (GTPs) have been extracted from the leaves of *Camellia sinensis*. Green tea has been well proved to provide a great deal of benefits due to its antioxidant, photochemopreventive, and anti-aging properties. It has been proved to inhibit photoaging process in human skin (Schagen et al. 2012). Its healthy effects may be explained by polyphenols, in particular epicatechin, epicatechin-3-gallate, epigallocatechin,

and epigallocatechin-3-gallate (EGCG). Based on a study on hairless mice, Agarwal et al. (1993) observed a considerable reduction in UV-induced edema and cyclooxygenase activity after the use of GTPs. However, in a more recent clinical work, treatment of topical and oral GTPs did not produce a significant improvement in elastic tissue content (Chiu et al. 2005). Green tea has been widely used either as monotherapy or combined with other ingredients for synergistic effects. Tanase-converted green tea extract was found to provide statistically higher anti-wrinkle activity or significantly decrease the size of wrinkles, as estimated by Skin Visiometer SV 60. In a research with 33 female subjects, Mahmood and Akhtar explored the enhanced effectiveness of green tea with the aid of lotus in rhytides treatment. The combination of green tea and lotus extract proposed a considerably better impact on skin wrinkle than either of them alone (Mahmood and Akhtar 2013). Green tea polyphenols are inherently unstable and are therefore significantly diminished into commercial tea products after short-duration preparation. In order to reduce oxidation rate, butylated hydroxytoluene has been added into topical formulation of polyphenols. It has been noted that various tea extracts provide different level of antioxidant activities. A comprehensive review of Stephen Hsu provides a profound understanding of catechins, polyphenol compounds in green tea, about its chemopreventive, natural healing, and anti-aging properties for human skin (Hsu 2005). Many laboratory researches on animal models have postulated that the use of green tea polyphenols by topical application or oral administration reduces UV-induced skin tumorigenesis. In addition, polyphenolic constituents, especially EGCG, in GTPs are believed to possess anti-inflammatory and antitumor effects (Katiyar and Elmets 2001). The beneficial effects of EGCG on skincare will be clarified in Section 17.5.20.

17.5.2 Mushrooms

Mushrooms have long been used in healthcare due to its potent antioxidants such as flavonoids, tocopherols, ascorbic acid, phenolic acids, and carotenoids. Furthermore, mushrooms exhibit activities against oncogenesis, tumor metastasis. Mushroom species vary in their use; specifically, *Lentinus edodes* and *Ganoderma lucidum* have antioxidant properties, whereas *Cordyceps taii* and *Sparassis cripsa* are employed in photoaging treatment (Bowe 2013). These beneficial activities have been well validated in *in vitro* and *in vivo* studies.

17.5.3 Vitamin E

Tocopherol (vitamin E) is a lipophilic antioxidant found in skin, various vegetables, and meat. Some plants such as wheat germ (*Triticum vulgare*), hazelnut (*Corylus avellana*), sunflower (*Helianthus annuus*), sesame (*Sesamum indicum*) and pumpkin (*Cucurbita pepo*) are particularly rich in vitamin E. It has been used for more than 60 years in both experimental

and clinical studies. Eight active isoforms of vitamin E are classified into two groups: tocopherols and tocotrienols. α-tocopherol has been shown to protect animal and human skin from photoaging activities (Fryer 1993). Topical use of vitamin E helps reduce both light-induced acute and chronic damage. In particular, α-tocopherol and tocotrienol efficiently improve skin roughness, facial lines, and wrinkles. These activities can be attributed to their ability to increase water content and water-binding capacity of the skin stratum corneum. α-tocopherol reduced activities of collagenase, an enzyme digests collagen to cause aging skin. In addition, vitamin E is also employed to scavenge free radical and soothe the skin (Dayan 2008). Studies suggest that a combination of different antioxidants can enhance their potency of photoprotection. This was observed by Lin et al. when they investigated the ultraviolet (Hamilton-Reeves et al. 2010) photoprotection after application of vitamin E and vitamin C (Lin et al. 2003). However, attention needs to be paid on cutaneous adverse effect of vitamin E.

17.5.4 Coenzyme Q10

Coenzyme Q10 (CoQ10) or ubiquinone is a fat-soluble, endogenous, and vitamin-like antioxidant that is found naturally in all human cells, especially in fat tissues. The majority of CoQ10 locates in mitochondria of most eukaryotic cells. CoQ10 is a key factor in electron transport chain in cellular respiration process in which it generates 95% of the human body's energy (Schagen et al. 2012). In skin, CoQ10 is abundant in epidermis to serve as a primary barrier to damaging oxidant. *In vitro* and human studies have been performed to demonstrate the photoprotective properties of CoQ10 by reducing the expression of collagenase after UVA irradiation (Hoppe et al. 1999). In the case of CoQ10 deficiency, it can be taken from oily fish (salmon, tuna), organ meats (Cerundolo et al. 2009), and whole grains through a healthy and balanced diet (Schagen et al. 2012). CoQ10 has been formulated into various dosage forms of supplements such as soft/hard capsule, oral spray or tablets, or several sometic products in brand name of Coenzyme Q10, Co-Q10, elppa CoQ10, LiQSorb, Liquid Co-Q10, NutraDrops, Q-Sorb Co Q-10, and QuinZyme. The intake of CoQ10 effectively increases CoQ10 level in tissues and mitochondria in order to lower oxidative potential (Kwong et al. 2002). An *in vitro* laboratory work has suggested the use of complementary CoQ10 to deal with oxidative stress in case of primary CoQ10 shortage (Lopez et al. 2010). However, CoQ supplementation neither improved main antioxidant defense on mice nor lengthened the life span of mice.

17.5.5 Vitamin C

Vitamin C (*L-ascorbic acid*) is known as a potent hydrophilic, intracellular, and extracellular antioxidant, protecting skin from free radicals. The benefits of vitamin C to skin come from its ability to enhance collagen synthesis and photoprotection (Korac and Khambholja 2011). Vitamin C can be taken from food,

especially citrus fruits, tomatoes, potatoes, and so on. However, the level of vitamin C in skin decreases continuously due to sunlight and environmental factors. Therefore, regular supply of vitamin C is reasonable to maintain its level in skin. It was evident in *in vitro* and *in vivo* studies that topical vitamin C reduces sunburn cells and erythema to prevent skin damage after exposure to both UVA and UVB irradiation. In addition, vitamin C improves photoprotectant activities of sunscreen products. Its anti-inflammatory properties have been employed to heal light-induced damage such as solar lentigines. The enhanced synthesis of collagen and inhibition of MMP-1 lead to skin wrinkle reduction (Dayan 2008). Regarding formulation preparation, oxidation of vitamin C needs to be avoided. Vitamin C is a light and air-sensitive compound and should be prepared in airtight, light-resistant environment.

17.5.6 Selenium

Selenium, an essential trace mineral for human body, protects cells from damaging effects of free radicals. It can be taken from various mushroom species, nuts, cod, tuna, and calf's liver. Selenium plays a key role in the activities of glutathione peroxidase, thioredoxin reductase, and vitamin E regeneration. In topical treatment, selenium sulfide and L-selenomethionine are commonly used. L-selenomethionine has an excellent transdermal delivery and safety profile (Lin et al. 2011). With the aid of vitamin E, selenium was shown to effectively cure blistering, pigmentation, and skin tumor in mice model (Burke et al. 2003).

17.5.7 Vitamin A

Vitamin A is a fat-soluble compound. Provitamin A, most commonly β-carotene, is found in various foods such as meat, fish, poultry, fruits, and vegetables. Vitamin A is used in topical products in the forms of retinoids and carotenoids (Chen et al. 2012). It was observed that UV irradiation causes a reduction in the level of carotenoids, β-carotene, and lycopene in human skin (Ribaya-Mercado et al. 1995). Vitamin A and its derivatives (tretinoin, isotretinoin, and tazarotene) have displayed anti-aging properties. By binding to nuclear receptors, retinoic acid receptors, and retinoid X, vitamin A enhances collagen generation and increases thickness of epidermal layer.

17.5.8 Proanthocyanidin

Proanthocyanidin inhibits DNA mutation and elastase to protect the integrity of skin elastin. It has been used as a potent antioxidant and free radical scavenger (Korac and Khambholja 2011). It was found to have synergistic effects with vitamin C and vitamin E. Its topical application has been used to keep skin away from deleterious effect of UV rays in sunlight. If a thin layer of proanthocyanidin cream is applied on skin, less burning is observed. Regarding skin carcinogenesis, grape seed proanthocyanidin

effectively decreases the size of UV radiation-induced skin tumor in hairless mice, prevents the tumor formation, and increases tumor cell death rate (Baliga and Katiyar 2006).

17.5.9 Anthocyanins

Anthocyanins are frequently consumed as various foods to protect the body against oxidants. An *in vivo* study has been conducted to prove the potent antioxidant effect of anthocyanins (Tsuda et al. 2000). A possible mechanism of oxidant elimination of anthocyanins belongs to their capacity to interfere the hydroxyl radical generation process. Moreover, cyanidin's antioxidant activity is four times more potent than vitamin E (Rice-Evans et al. 1995). In addition, amino acid tyrosine can be kept away from reactive oxidant peroxynitrite by anthocyanin pelargonidin.

17.5.10 Resveratrol

Resveratrol is classified into natural polyphenol group that is rich in the skin of red grapes. Supply of resveratrol is from wine, grape juice, cranberries, peanuts, peanut products, root of *Polygonum cuspidatum*, leaves of *Veratrum grandiflorum*, roots, and rhizomes of *Veratrum formosanum* (Sanders et al. 2000, Wang et al. 2002, Counet and Collin 2003). It is a lipophilic compound with trans and cis configuration. It has been investigated for unique antiaging, antioxidant, antimutagen, and anti-inflammatory properties. Resveratrol exhibits the characteristics of a chelating agent, a radical scavenger, and inflammation inhibitor (Giardina et al. 2010). It was found to inhibit tumorigenesis in mouse skin (Haidarali et al. 2014). Regarding skin protection, an *in vitro* study on HaCaT cells was conducted to prove that resveratrol offered protective activities against nitric oxide free radical donor sodium nitroprusside (Bastianetto et al. 2010). Giardina et al. used resveratrol in skin fibroblast treatment and figured out that an increase in the drug dose resulted in an increase in cell proliferation (human promyelocytic leukemia cell differentiation) rate and a decrease in collagenase activity (Giardina et al. 2010). A review of Baxter offers an insight into the effectiveness of prevention of photoaging as compared with other antioxidants in skincare formulation (Baxter 2008).

17.5.11 Curcumin

Curcumin, also known as diferuloylmethane, has been utilized as anti-inflammatory, antiproliferative, and cancer-preventive agent. It has been extracted from dried root of Indian spice turmeric (*Curcuma longa*), which belongs to ginger family (Zingiberaceae). Curcumin has displayed action against oxidative stress and inflammation to reduce deleterious consequences of injury. Several laboratory researches have validated its anti-inflammatory, antitumoral, and antioxidant properties (Saraf and Kaur 2010). The effectiveness of curcumin on mouse model has been well documented in the literature.

UVA-induced ornithine decarboxylase activity in epidermis of mouse skin was found to remarkably decrease after topical treatment of curcumin. This observance can be explained by its potential to scavenge reactive oxygen species. In addition, curcumin is effective in treating UV-induced apoptosis in human epidermal carcinoma cell. Regarding anti-aging properties, mechanism of action of curcumin in human fibroblast is carried out through phosphatidylinositol 3-kinase/Akt pathways to provoke cellular antioxidant response (Schagen et al. 2012).

17.5.12 Silymarin

Silymarin (Silibinin) is a polyphenol that is abundant in milk thistle (*Silybum marianum*). Silymarin's photopreventive activities exhibit in silybin, silidianin, and silicristin; among them silybin has been found the most active photochemical compound. Topical use of silymarin demonstrated a significant antitumor effect. In a hairless mice skin study, the number of UVB-induced tumor was decreased by 92% (Korac and Khambholja 2011). Antioxidant effects of silymarin were successfully utilized to inhibit sunburn cell formation and apoptosis due to UV exposure. In another work, Katiyar et al. showed the effectiveness of silymarin application on mouse skin to scavenge reactive oxygen species, to prevent UV-induced immune suppression and oxidative stress, and to decrease pyrimidine dimers and skin tumor (Katiyar 2002).

17.5.13 Quercetin

Quercetin plays a key role as a building block for the flavonoid family. It exists in many vegetables such as mayapple (*Podophyllum*), apple (*Malus domestica*), tea (*Camellia sinensis*), onion (*Allium cepa*), nuts, cauliflower, cranberry (*Vaccinium macrocarpon*), and cabbage. The highest level of quercetin is found in onion. Anti-inflammatory and antioxidant properties of quercetin have been reported to have anticarcinogenic effects on rat mammary cancer, colonic neoplasia, oral carcinogenesis, and mice skin carcinogenesis (Nishino et al. 1984). For skincare, quercetin helps protect human skin from UVA and UVB range (Choquenet et al. 2008).

17.5.14 Apigenin

Apigenin is a commonly known flavonoid that is rich in a great deal of plants including citrus fruit, parsley, yarrow, horsetail, lemon, peppermint, marigold, *Artemisia*, cumin, carrot, agrimony, amica, purple coneflower, and eyebright. Svobodova et al. found out that apigenin has protective properties against UV-induced skin carcinogenesis in mouse model (Svobodova et al. 2003). Apigenin displays anti-inflammatory activities in several studies.

17.5.15 Flavonoids

Flavonoids have been found ubiquitously in plants. Schroeter et al. have studied the use of these antioxidant and chelating agents to prevent chronic and aging diseases (Schroeter et al. 2002). In food or diet, flavonoids exist in two major forms as glycosides and polymers. Flavonoids can serve as antioxidant, enzyme modulator, and antimutagenic or cytotoxic compounds. These biological effects are recognized based on the extent, nature, and position of the substituents and hydroxyl groups. The most widely validated property of flavonoids is their protective activity from oxidative stress. It can be explained by the fact that flavonoids can eliminate peroxyl radicals, can chelate redox-active metals, and can inhibit lipid peroxidation.

17.5.16 Licorice

Licorice extract contains several active agents such as licochalcone A, glabridin, liquiritin, and glabrene and is found in *Glycyrrhiza glabra*. Each compound displays different properties. Liquirtin has been utilized in hyperpigmentation treatment, whereas glabridin and licochalcone A possess anti-inflammatory effects (Fowler et al. 2010). In human studies, licochalcone A was concluded to considerably reduce erythema and heal irritated skin sites, which is confirmed by a reduction of prostaglandin E2, leukotriene B4, IL-6, and TNF-α (Kolbe et al. 2006).

17.5.17 Soy isoflavones

Several researches have shown soy and its components as powerful antioxidants for skincare. Isoflavones are present in soybeans in the forms of daidzein and genistein. A study on postmenopausal women found that soy isoflavone treatment increased the epidermis' thickness by 9.46% in 23 subjects, reduced wrinkle, and enhanced desired properties of skin such as the amount of collagen, number of elastic fibers, and number of blood vessels (Accorsi-Neto et al. 2009). Kim et al. conducted an *in vivo* study to investigate the effects of topical dietary soy isoflavones on (Hamilton-Reeves et al. 2010) photoaged hairless mouse model. Anti-aging property of isoflavones can be explained via the inhibition of UV-induced metalloproteinase and prevention of collagen degradation (Kim et al. 2004). Soy isoflavone aglycone was not only used in topical administration but also (40 mg per day) was taken orally to improve fine wrinkle and skin elasticity of the aged skin of women volunteer (Izumi et al. 2007).

17.5.18 Polyphenols

Polyphenols are characterized as structures of a number of phenol units. They can be classified into a number of groups including phenolic acids,

flavonoids (anthocyanins, catechins, flavones, flavonols), stibenes, isoflavones, proanthocyanins, tannins, and lignans based on the amount of phenol rings and the ring linkage. They are plentiful in fruit juices, red wine, grape skin, grape seeds, tea, coffee olive pulp, and maritime pine bark and formulated into functional foods, cosmetic products, or dietary supplements. Source and content of polyphenols in foods are intensively presented in Manach's review work (Manach et al. 2004). Multiple researches have been performed to validate the Anti-aging effect of plant-derived polyphenols over recent years. They can be used to suppress oxidative stress in cancer, cardiovascular, and neurodegenerative treatment and can offer defense against diabetes and osteoporosis diseases. Polyphenol structural molecules possess activities against UV radiation and pathogen (Pandey and Rizvi 2009). *In vivo* laboratory researches using animal skin model investigated the effectiveness of various polyphenols including genistein, silymarin, grape seed proanthocyanidins, and green tea polyphenols in treating UV-induced skin damage, possibly skin cancer (Nichols and Katiyar 2010). In order to understand the mechanism of action of polyphenols, several pathways have been thoroughly proposed and studied. The most widely accepted theory is of direct, redox-dependent interaction between polyphenols and cell's receptors or enzymes in signal transduction pathway (Halliwell et al. 2005).

17.5.19 Carotenoids

Carotenoids play a key role in pigmentation in plants, algae, microorganisms, and photosynthetic bacteria. Fruits and vegetables are the abundant sources of carotenoid in human diet. Carotenoid-rich diet has been found to lower the risk of age-associated diseases (Rahman 2007). In the group, lycopene and β-carotene help protect skin from harmful effects of UV and short visible radiation (Ermakov et al. 2005). Regarding chemical structure, the number of conjugated double bonds accounts for antioxidant activities by different mechanism such as extinguishing singlet oxygen, delocalizing unpaired electrons, and chemically reacting with free radicals. In addition, antioxidant potency of carotenoids depends on their concentration and oxygen partial pressure. In particular, β-carotene is used as an antioxidant agent at either low concentration or low oxygen partial pressure, whereas it exhibits properties of prooxidants at high concentration or high partial pressure of oxygen. Carotenoid levels, as measured by noninvasive method using resonance Raman spectroscopy, indicate the antioxidant status in the body. The level of carotenoid antioxidant substances in the skin has a strong correlation to lifestyles including dietary supplementations and stress factors. The amount of carotenoid decreases due to high oxidative stress factors such as illness, smoking, alcohol consumption, and sunlight overexposure, whereas a healthy diet with fruits and vegetables provides a high level of carotenoids in skin to treat furrows and wrinkles. A study has been conducted to investigate the effect of fresh orange juice consumption on skin carotenoid level. Skin carotenoid score was used as a means to compare two types of orange juice that

are harvested from regularly and partially irrigated trees. The score was found to increase by regular consumption of orange juice (Massenti et al. 2015). In addition, Darvin et al. found an increase in the level of carotenoids during summer and autumn seasons (Darvin et al. 2008).

17.5.20 Epigallocatechin-3-Gallate

There are a vast variety of polyphenolic compounds or catechins in green tea including epicatechin-3-gallate (ECG), epigallocatechin (EGC), epicatechin (EC), (+)-catechin, and EGCG. The major component in green tea responsible for its beneficial effects is EGCG that contribute to more than 50% of the mass of catechin in green tea (Nagle et al. 2006). Anti-inflammatory and anticarcinogenic properties of green tea have been validated in numerous studies and medical practice. It is evident that EGCG interferes with multiple MMP including MMP-2, MMP-9, and MMP-12 and inhibits the activities of leukocyte elastase in cancer treatment.

17.6 Skincare routine and products

17.6.1 Skincare routine

It has been well known that a daily routine to care the skin generally includes four steps: cleansing, toning, specific targeting, and moisturizing. It is recommended that sunscreen should be applied during the daytime (**Figure 17.1**).

17.6.2 Cleanser

A cleanser is a facial care product that is used to remove makeup, dead skin cells, oil, dirt, and other types of pollutants from the face. This helps to unclog pores and prevent skin condition such as acne. It is best to use a cleanser in the morning and at night to remove all the impurities and oils that the face

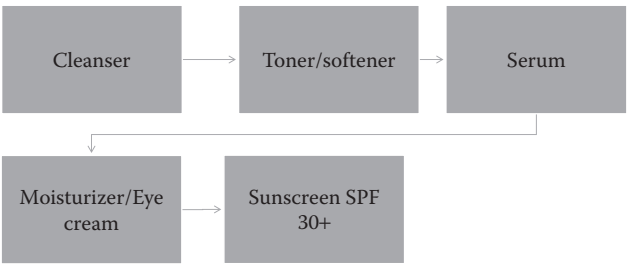


Figure 17.1 *General routine skincare regimen.*

has collected during the day. In order to use the cleanser, splash the face with warm water, apply a small amount of cleanser onto the palm, put the cleanser to the face, and rinse of the cleanser thoroughly with lukewarm water.

17.6.3 Toner

A toner removes the traces of makeup, dirt, and oil that a cleanser may have missed. It also reduces pores, eliminates oil, and refines the skin. The toner makes skin feel tighter and prepares the skin for the moisturizer. Once a person has cleansed her face, it should begin with the next step—tone a person's skin. Toner is applied by dampening a cotton ball with toner and wiping it across the face. Remember to avoid the eye area while applying a toner and do not use a toner more than once a day, especially a person who has dry skin.

17.6.4 Softener

The toner and softener can be used interchangeably. Skin softener helps to protect, nourish, and moisturize very dry, itchy, or irritated skin. It enhances the benefits of moisturizer. Softener should be used after cleansing; if the skin is little dry, apply softener to hydrate the skin. Softener is used by applying a small amount of softener to a cotton ball and by gently patting over the face. Wait a minute or two before moving on to next steps.

17.6.5 Serum

Face serums are lightweight moisturizers that penetrate deeper to deliver active ingredients such as antioxidants into the skin. Using serum regularly can give skin a firmer smoother texture, can make pores appeal smaller, and can increase moisture level. Serum can be applied in the morning, at night, or both. It is used by applying tiny dots of serum all over the face and by blending lightly. Gently tap the surface of the face with fingers. The tapping action helps the serum to sink fully into the deeper layers of the skin.

17.6.6 Moisturizer

Although lotions and creams are both moisturizers and can make the skin feel soft and smooth, they can be applied to different skin types. Both lotions and creams can be oil-based or water-based. If they are oil-based, they are an emulsion, or mixture, of water in oil. If they are water-based, they are an emulsion of oil in water. Most lotions and creams are water-based. Lotions are of low viscosity, so they are lighter and less oily than creams. A person with normal skin can apply lotion to keep skin moisturized. Creams work well on dry skin, as creams are thicker and can provide extra protection. Moisturizer should be applied onto a clean and toned face, which is recommended after applying toner/softener and serum.

17.6.7 Eye cream

An eye cream is a specially formulated moisturizer that is used to reduce the signs of aging: fine lines, wrinkles, and dark circles. It should be applied specifically to the skin around eyes daily after applying serum.

17.6.8 Sunscreen

Sunscreen prevents the sun's ultraviolet radiation from reaching the skin. Two types of ultraviolet radiation, UVA and UVB, can damage the skin, age it prematurely, and can increase the risk of skin cancer. It can be applied daily for anyone above the age of six months. Sunscreen should be applied 15–30 minutes before going outdoors to give the sunscreen enough time to be absorbed into the skin. Reapply every 2 hours if a person is staying outdoor and apply every 30 minutes if a person is engaging in outdoor activities causing a sweat.

17.6.9 Facial mask

A facial mask can help hydrate skin, can remove excess oils, and can help improve the appearance of the pores. It can also help to pull out impurities. A facial mask can be used once a week, depending on a person's skin and skincare concerns. It is used by applying facial mask directly onto the damp skin after applying cleanser. Be sure to avoid the eye area. Let the facial mask stay on the skin for 10–15 minutes. Then use a washcloth to gently wash away the mask. Pat, do not rub, the skin and dry the skin with a towel.

17.7 Nutraceuticals in skincare products

According to different cosmetic manufacturers, skincare products are typically categorized into cleanser, toner, softener, serum, mask, and moisturizer. Sunscreen is classified as another type of skin product. In this chapter, we included sunscreen to discuss the use of antioxidant nutraceuticals for the health of skin. More than 100 products were searched and recorded from the Internet as well as from famous beauty websites. Below are the common nutraceuticals found in the skincare products that we explored (**Table 17.1**).

Aloe vera is most frequently found in the formulation of cleanser, softener/toner, facial mask, moisturizer, and sunscreen. It has been well known to soothe the skin and help prevent wrinkles and premature aging by keeping the skin hydrated and by boosting the skin's own natural regenerative abilities (Tanaka et al. 2015).

Similar to aloe, *chamomile* has also been used on the skin for minor burns as it contains flavonoids and essential oils that possess significant anti-inflammatory activity. Specifically, chamomile was reported to improve the

Table 17.1 Common Skincare Products Containing Antioxidant Nutraceuticals

Type	Brand Name	Manufacturer	Dosage Form	Active Ingredient
Sunscreen	Yes to Cucumbers SPF 30	Soothing	Lotion	Cucumber, aloe vera, and green tea
Sunscreen	Aveeno Active Naturals Natural Protection, SPF 50	Aveeno	Lotion	Oat
Sunscreen	Jason Natural Sunscreen SPF 30	Jason	Lotion	Acai, jojoba, grape seed oil, aloe vera, green tea, shea butter, sunflower seed oil, and matricaria
Sunscreen	Goddess Garden Organic SPF 30	Goddess Garden	Lotion	Shea butter, green tea, coconut oil, sunflower oil, aloe, and lavender oil
Sunscreen	Nature's Gate Aqua Vegan Sunscreen SPF 50	Nature's Gate	Lotion	Chamomile, aloe, shea butter, matricaria flower extract, jojoba, aloe, and white rosemary
Sunscreen	Burt's Bees Sunscreen SPF 30	Burt's Bees	Cream	Hemp seed oil, soybean oil, sunflower oil, avocado oil, and tocopherol
Sunscreen	Alba Botanica Hawaiian Sunscreen SPF 45	Alba Botanica	Lotion	Aloe vera, green tea, sunflower seed oil, palm kernel oil, matricaria, ginkgo leaf, glycerin, and tocopherol
Sunscreen	Alba Botanica Natural Emollient Sunscreen SPF 30	Alba Botanica	Lotion	Aloe vera, jojoba, sunflower seed oil, grape seed oil, matricaria, and glycerin
Sunscreen	Coola Suncare Sport Sunscreen SPF 30	Coola	Spray	Algae, cucumber, strawberry extract, glycerin, aloe, and red raspberry seed
Sunscreen	Suntegrity 5 in 1 Natural Moisturizing Face Sunscreen SPF 30	Suntegrity Skincare	Lotion	Green tea, red algae, sunflower, cucumber, hyaluronic acid, aloe, jojoba, coconut oil, and pomegranate extracts
Sunscreen	Lavanila The Healthy Sunscreen SPF 40 Face Cream	Lavanila	Cream	Aloe, shea butter, coconut oil, palm oil, noni juice, and green tea extract
Sunscreen	Amore Pacific Natural Protector SPF 30	Amore Pacific	Lotion	Bamboo, green tea, Korean red ginseng, hyaluronic acid, and tocopherol
Sunscreen	Hang Ten-Dark Tanning Oil Natural Sunscreen SPF8 Spray	Hang Ten	Spray	Kukui seed oil, argan oil, coconut oil, acai fruit extract sunflower seed oil, and rosemary leaf extract
Sunscreen	Juice Beauty Sport Moisturizer SPF 30	Juice Beauty	Lotion	Organic white grape juice, jojoba, aloe, tocopherol, lemon extract, mandarin, sunflower seed oil, apple juice, coconut oil, and castor seed oil

(*Continued*)

Table 17.1 (*Continued*) Common Skincare Products Containing Antioxidant Nutraceuticals

Type	Brand Name	Manufacturer	Dosage Form	Active Ingredient
Sunscreen	Supergoop! Skin Soothing Mineral Sunscreen SPF 40	Supergoop!	Lotion	Olive polyphenols, Sunflower seed oil, safflower seed oil, and citric acid
Sunscreen	Aubrey Organics Natural Sun SPF 30 Green Tea	Aubrey Organics	Lotion	Green tea, aloe, glycerin, and citric acid
Sunscreen	Yes To Carrots Nourishing Daily Facial Moisturizer SPF 15	Yes To	Lotion	Carrots extract, chamomile, aloe, dead sea water, shea butter, tocopherol, pumpkin seed oil, and melon fruit extract
Sunscreen	Vichy Silkscreen Dry-Finish Sunscreen Lotion SPF 45	Vichy	Lotion	Vitamin E and white grape polyphenols, grapefruit extract
Sunscreen	Jason Kids Natural Sunscreen SPF 45	Jason Natural Products	Lotion	Aloe, calendula flower extract, matricaria flower extract, palm kernel oil, and tocopherol
Sunscreen	Chantecaille Just Skin Moisturizer SPF 15	Chantecaille	Lotion	Honey suckle, edelweiss, green tea, tocopherol, algae, and rose water
Cleanser	Premier Dead Sea Luxury Facial Cleanser with Micro Grains	Premier Dead Sea	Foam	Bergamot oil, jojoba oil, neroli oil, dead sea minerals, chamomile, oat, witch hazel extracts, and aloe vera
Cleanser	Emma Hardie Amazing Face Natural Lift and Sculpt Moringa Cleansing	Emma Hardie	Foam	Wild sea fennel, orange, neroli and mandarin extracts, jasmine, cedar wood and rose, grape seed oil, sweet almond oil, and moringa seed extract
Cleanser	Hyaluronic Acid Facial cleanser 99% Natural	Swanson Premium	Foam	Aloe vera, oregano leaf, thyme leaf, cinnamon bark, rosemary leaf, lemon peel, golden seal root, olive leaf extracts, and lavender flower extract
Cleanser	Purity Made Simple	Philosophy	Gel	Meadowfoam seed oil, coconut, rosewood oil pepper seed extract, and carrot seed oil
Cleanser	Paula's Choice Moisture Boost One Step Face Cleanser	Paula's Choice	Gel	Aloe vera, matricaria flower extract
Cleanser	Alba Botanica Hawaiian Facial Cleanser Pineapple Enzyme	Alba Botanica	Gel	Jojoba seed oil pineapple fruit extract, papaya fruit, aloe, allantoin, and ginger root extract
Cleanser	Goldfaden MD Pure Start Gentle Detoxifying Facial Cleanser	Goldfaden MD	Foam	Wheat protein, grapefruit extract, and lycopene

(*Continued*)

Table 17.1 (*Continued*) Common Skincare Products Containing Antioxidant Nutraceuticals

Type	Brand Name	Manufacturer	Dosage Form	Active Ingredient
Cleanser	Mad Hippie Cream Cleanser Normal to Dry Skin	Mad Hippie Advanced Skincare	Gel	Jojoba oil, orchid extract, macadamia oil, algae extract, sesame oil, and green tea
Cleanser	Laboratoire Remede Hydra Therapy Cleansing Crème	Laboratoire Remede	Cream	Cotton seed oil, algae extract, rosemary leaf oil, sunflower seed oil, lemon peel oil, peppermint oil, coconut oil, red seed weed extract, and tocopherol
Cleanser	Port Products Detoxifying Daily Cleanser	Port Products	Gel	Yucca root extract, oat kernel extract, aloe, tea tree oil, lavender oil, peppermint oil, and mandarin orange peel oil
Moisturizer	Face & Body Moisturizing Lotion	The Honest Company	Lotion	Aloe, primrose oil, olive fruit oil, jojoba seed oil, chamomile flower oil, shea butter, and safflower seed oil
Moisturizer	Cocoa Butter Lotion Natural Moisturizer	Wonder Labs	Lotion	Cocoa butter, sweet almond oil, soy lecithin, cocoa, aloe vera, grapefruit seed extract, allantoin, citric acid, and cedar bark extract
Moisturizer	Nature's Gate Colloidal Oatmeal Moisturizing Lotion	Nature's Gate	Lotion	Colloidal oatmeal, shea butter, sweet almond oil, apricot kernel oil, soybean oil, glycerin, vitamin E, aloe, ginkgo, echinacea, sunflower seed oil, sesame seed oil, tocopherol, jojoba, allantoin, and panthenol
Moisturizer	Nourish Organic Face Lotion, Lightweight Moisturizing, Argan + Rosewater	Nourish Organics	Lotion	Aloe, argan oil, soy lecithin, sweet orange essential oil, sweet almond oil, acai fruit oil, shea butter, rice bran extract, tocopherol, vitamin C, and glycerin
Moisturizer	Cucumber Melon Nourishing Lotion for Face & Body, Coconut Oil Moisturizer	Organic Fiji	Lotion	Coconut oil, apricot kernel oil, avocado oil, sweet almond oil, cucumber, melon, sun flower seed oil, and citric acid
Moisturizer	Organic Hand & Body Lotion	Puracy	Lotion	Aloe vera, sunflower oil, palm oil, jojoba oil, green tea, citric acid, shea butter, and tocopherol
Moisturizer	Natural Body Lotion	Just Natural	Lotion	Aloe vera, blackcurrant seed oil, meadow foam oil, broccoli seed oil, andiroba oil, camellia seed oil, almond oil, grape seed oil, macadamia nut oil, and cocoa butter
Moisturizer	Shikai Borage Dry Skin Therapy Lotion	Shikai	Lotion	Vitamin E, shea butter, borage oil, aloe, safflower seed oil, jojoba seed oil, and vitamin C
Moisturizer	Nubian Heritage Lotion, Raw Shea Butter	Nubian Heritage	Lotion	Cocoa butter, shea butter, soymilk, castor seed oil, mango seed butter, glycerin, jojoba, tocopherol, allantoin, and honey suckle flower extract

(*Continued*)

Table 17.1 (*Continued*) Common Skincare Products Containing Antioxidant Nutraceuticals

Type	Brand Name	Manufacturer	Dosage Form	Active Ingredient
Moisturizer	Natural Body Lotion, Orange Petalooza	Gud	Lotion	Sunflower seed oil, black walnut leaf extract, raspberry, soybean oil, shea butter, and tocopherol
Moisturizer	Moisturizing Body Lotion, Vanilla	Everyday Shea	Lotion	Lemongrass extract, shea leaf extract, shea butter, and red palm oil
Moisturizer	Body Lotion, Almond Vanilla	Nourish Organic	Lotion	Aloe, shea butter, rice bran oil, sweet almond oil, rice bran extract, acai fruit oil, soy lecithin, maca root extract, comfrey root extract, lemon peel oil, and tocopherol
Moisturizer	Body Lotion, Hydrating, Citrus	Weleda	Lotion	Sesame seed oil, coconut oil, aloe, olive oil, shea butter, and glycerin
Moisturizer	Raw Shea Butter Lotion	Shea Moisture	Lotion	Shea butter, castor seed oil, mango seed butter, cocoa seed butter, jojoba oil, soy milk, comfrey root extract, honeysuckle flower and Japanese honeysuckle flower extract, tocopherol, and palm kernel oil
Moisturizer	Moisturizing Lotion Olive Oil & Wheat Proteins	Canus	Lotion	Soybean oil, goat milk, wheat protein, and aloe
Moisturizer	Organic Hand & Body Lotion	Herbal Choice Mari	Lotion	Olive oil, rose hip oil, argan oil, benzoin oil, sweet orange oil, bilberry leaf, and lavender
Moisturizer	Body Lotion, Intensive Therapy, Vitamin E	Derma E	Lotion	Safflower seed oil, cranberry seed oil, olive fruit oil, jojoba seed oil, green tea leaf extract, aloe, red palm oil, allantoin, tocopherol, hyaluronic acid, lavender, and neroil
Moisturizer	Skin Trip Moisturizer Coconut	Mountain Ocean	Lotion	Coconut oil, aloe juice, and safflower oil
Moisturizer	Sebamed Moisturizing Lotion	Sebamed	Lotion	Chamomile extract and allantoin
Moisturizer	Pure Natural Hand & Body Lotion 70% Aloe Vera Gel	Jason Natural	Lotion	Aloe, sunflower seed oil, avocado oil, panax ginseng root extract, and tocopherol
Moisturizer	Glotherapeutics Moisturizing Oil Control Emulsion	Glotherapeutics	Cream	Hyaluronic acid, milk thistle, totarol, aloe, allantoin, and witch hazel
Moisturizer	La Roche-Posay Toleriane Fluide Protective Non-Oily Emulsion	La Roche-Posay	Cream	Thermal spring water and glycerin

(*Continued*)

Table 17.1 (*Continued*) Common Skincare Products Containing Antioxidant Nutraceuticals

Type	Brand Name	Manufacturer	Dosage Form	Active Ingredient
Moisturizer	Philosophy Sea of Love Firming Body Emulsion	Philosophy	Cream	Shea butter, olive oil, macadamia oil, and citric acid
Moisturizer	All in One Snail Repairing Cream	JIGOTT	Cream	Snail secretion, hyaluronic acid, and glycerin
Moisturizer	Lavera Men Sensitiv Nourishing Moisturizing Day Cream	Lavera	Cream	Ginkgo, bamboo, shea butter, jojoba, sweet almond oil, aloe, soybean oil, sunflower seed, tocopherol, lecithin, and which hazel
Moisturizer	Borghese Age-Defying Cellular Complex Hydrate Night Renewal Emulsion	Borghese	Emulsion	Grape seed oil, soybean, sea salt, hyaluronic acid, and caffeine
Moisturizer	Jason Ultra-Comforting Moisturizing Crème	Jason Natural Products	Cream	Aloe, sweet almond oil, sunflower seed oil, and avocado oil
Moisturizer	Atopalm MLE Daytime Under make up Moisture Cream	NeoPharm Co. LTD	Cream	Grape seed oil, olive fruit oil, vegetable oil, allantoin, glycerin, and green tea seed oil
Moisturizer	Olivella Virgin Olive Oil body Cream	Olivella	Cream	Beta-carotene, virgin olive oil, bergamot fruit oil, orange oil, allantoin, and lavender oil
Moisturizer	Linoleic Line Lifting Face Cream	North American Hemp Co	Cream	Hemp seed oil, green tea extract, ginseng extracts, shea butter, olive fruit oil, sesame oil, chamomile extract, algae extract, echinacea extract, and licorice extract
Toner/softener	Witch Hazel skin Softening Facial Toner Lilac	Humphreys	Solution	Witch hazel with lilac, aloe, papaya and pineapple extract, panthenol, allantoin, red and black tea, green tea, and hyaluronic acid
Toner/softener	Innisfree Wine Peeling Jelly Softener	Innisfree	Solution	Red wine
Toner/softener	Benefiance Wrinkle Resist 24 Balancing Softener	Shiseido	Solution	Wild thyme extract, mukurossi extract, glycerin, and tocopherol
Toner/softener	Deoproce Hydro Essential Whitening Softener	Deoproce	Solution	Green tea, arbutin, macadamia oil, jojoba oil, licorice root extract, witch hazel, and matricaria
Toner/softener	Organic Neroli-rose Hydrosol Exceptional Skin Toner and Softener	Jamilah Aromatherapy	Solution	Bitter orange tree, rose, and orange bloom
Toner/softener	Age Defense Essential Softener	Etude House	Solution	Evening primrose flower extract, glycerin

(*Continued*)

Table 17.1 (*Continued*) Common Skincare Products Containing Antioxidant Nutraceuticals

Type	Brand Name	Manufacturer	Dosage Form	Active Ingredient
Toner/softener	Smooth E White Baby Face Foam 4 in 1 Cleanser Toner Moisturizer	Smooth E	Solution	Natural vitamin E
Toner/softener	Isa Knox X2D2 Whitening Secret Skin Softener	Isa Knox	Solution	Palm skin oil and snow drop extract
Toner/softener	Coconut Oil 100% Pure Natural Skin Softener	Ultrapure Labs	Solution	Coconut oil
Toner/softener	Witch Hazel Redness Reducing Cucumber Melon Toner	Humphrey's	Solution	Witch hazel, honeydew melon, cucumber, cloudberry, sea buckthorn, aloe, Canadian willow herb, white and green tea, and chamomile
Toner/softener	Simple Soothing Facial Toner	Simple	Solution	Chamomile, witch hazel, panthenol, and matricaria
Toner/softener	Burt's Bees Garden Tomato Toner	Burt's Bees	Solution	Tomato, cucumber, parsley, green tea, sugarcane, and bilberry
Toner/softener	Paula's Choice Skin Balancing Pore-Reducing Toner	Paula's Choice	Solution	Chamomile flower extract, burdock, root extract, jojoba, glycerin, and vegetable protein
Toner/softener	Derma E Toner Soothing Anti-aging Pycnogenol	Derma E	Solution	Pycnogenol (bark extract), green tea, aloe, chamomile, glycerin, allantoin, and panthenol
Toner/softener	Yes to Tomatoes Clear Skin Acne Clearing Facial Toner	Yes To	Solution	Tomatoes, witch hazel, fruit extract, watermelon fruit extract, and red pepper
Toner/softener	Kiehl Cucumber Herbal Alcohol Free Toner	Kiehl	Solution	Cucumber, fruit extract, aloe, and allantoin
Toner/softener	Acure Organics Facial Toner Combo to Oily Skin	Acure Organics	Solution	Witch hazel, vegetable glycerin, rose water, and chamomile
Toner/softener	Alba Botanica Advanced Facial Toner Sea Kelp	Alba Botanica	Solution	Sea kelp extract, aloe vera, grapefruit extract, witch hazel, and allantoin
Toner/softener	Alaffia Coconut Water Face Toner	Alaffia	Solution	Coconut water, papaya, glycerin, lavender, and citric acid

(*Continued*)

Table 17.1 (*Continued*) Common Skincare Products Containing Antioxidant Nutraceuticals

Type	Brand Name	Manufacturer	Dosage Form	Active Ingredient
Toner/softener	L'occitane Angelica Hydra Vital Face Toner	L'occitane	Emulsion	Angelica essential oil and angelica water
Serum	Life Extension Rejuvenex Factor Firming Serum	Life Extension	Emulsion	Pomegranate, grape seed extract, panthenol, CoQ10, tocopherol, green tea, and white tea
Serum	Lancer Fade Serum Intense Brightening Complex	Lancer Skincare	Emulsion	Sugarcane, licorice root extract, red algae extract, apple, orange, and lemon
Serum	Andalou Naturals Luminous Eye Serum	Andalou Naturals	Emulsion	Aloe, fruit stem cells, goji, green coffee, bioactive 8 berry complex, green tea, hyaluronic acid, and tocopherol
Serum	bareMinerals BareSkin Pure Brightening Serum Foundation	bareMinerals	Emulsion	Lilac plant stem cell, bare minerals extracts, lecithin, glycerin, jojoba, and tocopherol
Serum	Nourish Organic Argan Face Serum Pure Hydrating Apricot + Rosehip	Nourish Organic	Emulsion	Apricot kernel oil, rosehip oil, argan oil, jojoba, sweet orange oil, safflower oil, and tocopherol
Serum	Andalou Naturals Enlighten Serum Turmeric Plus C Brightening	Andalou Naturals	Emulsion	Apple and grape fruit stem cell cultures, aloe vera, tocopherol, hyaluronic acid, rosehip, grape seed, sunflower oil, and glycerin, bioactive 8 berry complex
Serum	Goldfaden MD Radical Difference Advanced Antioxidant Serum	Goldfaden MD	Emulsion	Red tea extract, cherry extract, and hyaluronic acid
Serum	Nature Secrète with Pure Argan Oil Lightening Serum Anti-Ageing	Nature Secrète	Emulsion	Snail slime, argan oil, argan tree, and CoQ10
Serum	Blum Naturals Face Serum	Blum Naturals	Emulsion	Aloe vera, glycerin, olive fruit oil, jojoba, tocopherol, and citric acid
Serum	Dead Sea Mineral Mud & Water Eye Serum Elite Line	Sea Vital	Cream	Dead sea mineral mud, extract of witch hazel and rose, ginkgo extract, musk rose extract, and vitamin E
Serum	Always Mineral Face Serum	Always Mineral	Emulsion	Argan oil, jojoba oil, tea tree oil, grape seed oil, lavender oil, clary sage oil, and grapefruit seed extract

(*Continued*)

Table 17.1 (*Continued*) Common Skincare Products Containing Antioxidant Nutraceuticals

Type	Brand Name	Manufacturer	Dosage Form	Active Ingredient
Serum	Glymed Plus Retinol Restart Rejuvenation Serum	Glymed	Emulsion	Olive oil and retinol
Serum	ILike Organic Skincare Ilike Age Defense Bioflavonoid Serum	Ilike	Emulsion	Black currant, elder berry, cantaloupe, grape, algae, and polyphenols
Serum	Regenerative Facial Serum Normal to Dry Skin Natural Organic Antiaging Oil	Lila Botanicals	Emulsion	Hemp oil, jojoba oil, pomegranate oil, evening primrose oil, rose hip seed oil, tamanu oil, and sea buckthorn
Serum	Babytime Skincare Massage Serum	Babytime	Emulsion	Sunflower oil, coconut oil, aloe, jojoba, and olive fruit oil safflower oil
Serum	Life extension Cosmesis Skincare Youth Serum	Life Extension	Emulsion	Seaweed, tea blend extract, and hyaluronic acid
Serum	Aura Cacia Organic Facial Oil Serum	Aura Cacia	Emulsion	Tamanu, lavender, tea tree, sunflower oil, argan kernel oil, and jojoba
Serum	Andalou Naturals Fruit Stem Cell Science Pure Pore Serum Clarifying Willow Bark	Andalou Naturals	Emulsion	Fruit stem cell complex, willow bark extract, aloe vera, bioactive 8 berry complex, sun flower oil, white tea, tocopherol, witch hazel, peppermint oil, and thyme
Serum	Hyaluronic Acid Serum	Reviva Labs	Solution	Green tea extract, hyaluronic acid, and aloe
Serum	Farmhouse Fresh Wine Down Serum	Farmhouse Fresh	Solution	Grape extract, lentils, apples, watermelon, resveratrol extract, green tea, rice extract, and chamomile
Facial mask	Sephora Collection Lotus Face Mask	Sephora	Suspension	Lotus flower extract Aloe vera extract Rice extract
Facial mask	Sephora Collection Lingzhi Face Mask	Sephora	Gel	Lingzhi extract, broccoli extract, and aloe
Facial mask	Sandalwoood Face Mask	Uncle Harry's Natural Products	Gel	Bentonite clay, sandalwood, oatmeal, turmeric, Neem: Vegan

(*Continued*)

Table 17.1 (*Continued*) Common Skincare Products Containing Antioxidant Nutraceuticals

Type	Brand Name	Manufacturer	Dosage Form	Active Ingredient
Facial mask	Fresh Rose Face Mask	Fresh	Gel	Rose water, rose petals, cucumber extract, aloe vera, and green tea
Facial mask	WEI Golden Root Purifying Mud Mask	WEI	Gel	Golden root and pure China clay
Facial mask	Karuna Hydrating+ Face Mask	Karuna	Gel	Aloe vera, chamomile, chinese licorice, soybean, and matricaria flower extract
Facial mask	Andalou Naturals Skin food Mask, Age Defying Avo Cocoa	Andalou Naturals	Cream	Fruit stem cell complex, dark cocoa, avocado, sunflower oil, tocopherol, CoQ10, jojoba oil, bioactive 8 berry complex, glycerin, shea butter, sugarcane, and aloe
Facial mask	Acne Solutions Clarifying Colloidal Sulfur Mask	Dr Dennis Gross Skincare	Gel	Prickly pear extract, willow bark, rosemary, licorice root, bentonite, and retinol
Facial mask	Yes To Tomatoes Clear Skin Clearing Facial Mask	Yes To	Gel	Tomatoes, sweet almond oil, aloe vera, red pepper fruit extract, pumpkin fruit extract, oat kernel meal, dead sea mud, tocopherol, jojoba, avocado, ginkgo, matricaria flower extract, and watermelon
Facial mask	Andalou Naturals Pumpkin Honey Glycolic Mask	Andalou Naturals	Gel	Fruit stem cells, pumpkin, aloe, sunflower oil, glycerin, tocopherol, pineapple, apple, grape, bioactive 8 berry complex, sugarcane, meadow foam oil, and CoQ10
Facial mask	Outrageously Organic Clay Mask	Outrageously Organic	Gel	Activated charcoal, tea tree oil, apple cider vinegar, and rosemary water
Facial mask	Korres Wild Rose + Vitamin C Advanced Brightening Sleeping Facial	Korres	Gel	Wild rose oil, rose water, aloe, and jojoba
Facial mask	Freeman Feeling Beautiful Facial Clay Mask Avocado & Oatmeal	Freeman	Gel	Avocado, oatmeal, tocopherol, and bentonite
Facial mask	Andalou Naturals Enzyme Mask Age-Defying Bioactive 8 Berry	Andalou Naturals	Gel	Bioactive 8 berry complex, advanced fruit stem cells, resveratrol Q10, aloe, lemon, sugarcane, sea buckthorn, rose hip, meadowfoam oil, lecithin, white tea, orange oil, cranberry juice, apple juice, papaya, and sun flower oil
Facial mask	Ole Henriksen Blue/Black Berry Enzyme Mask	Ole Henriksen	Gel	Blueberry, blackberry, papaya enzyme, aloe, panthenol, and Lavender extract
Facial mask	Splendid Dirt Nutrient Mud Mask with Organic Pumpkin Purée	FarmHouse Fresh	Gel	Pumpkin puree, yogurt cultures, glycerin, and bentonite

(*Continued*)

Table 17.1 (*Continued*) Common Skincare Products Containing Antioxidant Nutraceuticals

Type	Brand Name	Manufacturer	Dosage Form	Active Ingredient
Facial mask	Elemis Exotic Cream Moisturizing Mask	Elemis	Cream	Sea Rocket Extract, orange and bergamot, honey, glycerin, jojoba, sunflower, bitter orange oil, and mandarin orange peel
Facial mask	Acure Argan Stem Cell + CGF Cell Stimulating Facial Mask	Acure	Gel	Argan stem cells, chlorella growth factor, green clay, argan oil, CoQ10, sea buckthorn oil, glycerin, cocoa butter, algae, black berry, rose hip, pomegranate, and aloe
Facial mask	Astara Skincare Activated Sea Mineral Mask	Astara	Gel	Oatmeal, lemongrass, citrus bioflavonoids, algae and kelp, jojoba, cucumber, rosemary extract, oat kernel protein, tocopherol, and lemon oil
Facial mask	Andalou Naturals 1000 Roses Rosewater Mask Sensitive	Andalou Naturals	Gel	Rose and fruit stem cells, pomegranate, aloe vera, cranberry juice, rose hip, hyaluronic acid, panthenol, and white tea
Eye cream	Derma E Hydrating Eye Crème, with Hyaluronic Acid & Pycnogenol	Derma E	0.5 oz tube	Witch hazel extract, green tea, aloe, jojoba oil, allantoin, panthenol, pycnogenol bark extract, hyaluronic acid, tocopherol, and vitamin A
Eye cream	Just Natural Anti-Aging Eye Cream	Just Natural Products	2 oz jar	Black currant seed oil, rose hip, aloe, borage seed oil, evening primrose oil, camellia seed oil, olive oil, sweet orange bloom essential oil, glycerin, and vitamin E
Eye cream	Time to Hydrate Gentle Eye Cream	AHAVA	0.51 oz jar	Aloe, chamomile, calendula, shea, dead sea water, panthenol, allantoin, carob bean, date fruit extract, obliphica fruit oil, glycerin, and butcher broom extract
Eye cream	Sibu Beauty Sea Buckthorn Age Defying Eye Cream	Sibu	0.5 oz tube	Sea buckthorn seed oil, pumpkin olive oil, aloe, brussels sprout extract, broccoli, cauliflower, cabbage, glycerin, lecithin, rice bran protein, and soybean protein
Eye cream	Mad Hippie 104403 Eye Cream	Mad Hippie	0.5 oz pump bottle	Peptides, ceramides, pomegranate, white tea, chamomile flower extract, argan oil, vitamin C, and Vitamin E
Eye cream	Q 10 Lifting Eye Cream	Sebamed	0.5 oz tube	Shea butter, avocado, almond oil, bisabolol, and coenzyme Q-10
Eye cream	Burt's Bees Naturally Ageless Line Smoothing Eye Cream with Pomegranate & Magnolia	Burt's Bees	0.5 oz tube	Pomegranate, magnolia, sunflower seed oil, olive oil, evening primrose seed extract, willow bark extract, tocopherol, and soybean oil
Eye cream	3LAB The Eye Cream Anti-Aging Treatment	3 LAB	0.7 oz jar	Korean ginseng stem cell extract, fermented 7 complex, rice bran oil, jojoba, licorice root extract, cucumber extract, algae extract, sunflower seed oil, sandalwood oil, and tocopherol

(*Continued*)

Table 17.1 (*Continued*) Common Skincare Products Containing Antioxidant Nutraceuticals

Type	Brand Name	Manufacturer	Dosage Form	Active Ingredient
Eye cream	GlyMed Plus Eye and Lip Renewal complex	GlyMed	1 oz tube	Aloe, hyaluronic acid, lactic acid, glycolic acid, YianYiang oil, olive oil, glycerin, vegetable oil, tocopherol, castor oil, and allantoin
Eye cream	100% Pure Coffee bean Caffeine Eye Cream	100% Pure	0.3 oz tube	Green coffee, green and white tea, chamomile and lavender wax, Vitamin C and E, black currant oil, extract of rosemary, orange, thyme, grapefruit, goldenseal, and vanilla
Eye cream	Cane + Austin Corrective Treatment Eye Cream	Cane + Austin	0.5 oz bottle	Retinol, peptides, amica and vitamin K, E, C, green tea polyphenol, cucumber extract, and hyaluronic acid
Eye cream	Home Health Products Goji Berry Eye Cream	Home Health Products	1 oz jar	Goji berry, glycerin, aloe, bisabocol, soybean oil, matricaria flower extract, oat extract, panthenol, tocopherol, vitamin A, and green tea
Eye cream	Merlot Grape Seed Eye Cream	Merlot	1 oz jar	Grape seed extract, panthenol, horse chestnut seed extract, evening primrose, tocopherol, vitamin H, and vitamin A
Eye cream	Renew Eye Lift Renewal Cream	Suki	0.5 oz jar	Jojoba seed oil, sugarcane extract, lavender oil, bees wax, acai oil, berry oil, caffeine, hibiscus flower extract, hyaluronic acid, coenzyme Q10, licorice extract, resveratrol, shea butter, glycerin, green tea, lecithin, and gooseberry fruit extract
Eye cream	Pure Marula Eye Cream	John Paul Selects	0.5 oz jar	Aloe, marula seed oil, glycerin, shea butter, rosemary leaf extract, sunflower seed oil, and lactic acid
Eye cream	Nourish Organic Eye Treatment Avocado + Argan	Nourish Organic	0.5 oz tube	Aloe, vitamin E, shea butter, avocado, argan oil, sunflower seed oil, glycerin, soy lecithin, rice bran extract, rose flower, rose hip oil, corn starch, witch hazel, tocopherol, and vitamin C
Eye cream	Weleda Eye Cream	Weleda	0.34 oz tube	Pomegranate seed oil, sesame oil, jojoba oil, macadamia oil, shea butter, wheat germ oil, olive oil, sunflower seed oil, butcher's broom, and vitamin A
Eye cream	Origins GiZing Refreshing Eye Cream	Origins	0.5 oz jar	Coffee bean, panax ginseng, and magnolia extract
Eye cream	Queen Bee 100% All-Natural, Organic Under Eye Cream - Removes Dark Circles, Facial Lines and Wrinkles Naturally	Best Health Nutrition	1 oz jar	Olive oil, beeswax, shea butter, coconut oil, and vanilla essential oil vitamin K
Eye cream	Kinerase Advanced Eye Cream	Kinerase	0.7 oz tube	Kinetin, glycerin safflower seed oil, shea butter, aloe, and panthenol

appearance of the skin due to its antioxidant activity by improving skin's texture and elasticity, as well as reduces signs of photodamaging (Srivastava et al. 2010).

Jojoba oil is also frequently found in the formulation of cleanser, serum, sunscreen, and facial mask as it offers various benefits for the skin. It can be used as a skin moisturizer as the oil is light and easily absorbed by the skin. Jojoba oil has been reported to keep the skin hydrated to prevent early signs of aging (Kapoor and Saraf 2010).

Witch hazel is found most frequently in skin softener/toner due to its astringent properties. It can help to remove excess oil from the skin, making it effective at treating blackheads or other blemishes caused by an accumulation of dried sebum in the pores (Graf 2000).

Grape seed extract contains potent antioxidants such as flavonoids and polyphenols that help to protect the skin from the sun damage and free radicals. Topical application of grape seed extract was reported to provide protection from the sun and prevent aging (Bogdan Allemann and Baumann 2008).

Topical *green tea* has been found to provide a photoprotective effect. Specifically, it reduces the number of sunburn cells, protects epidermal Langerhans cells from UV damage, and reduces the DNA damage that is formed after UV radiation.

Sunflower seed oil is used as an emollient in cosmetics. It is rich in fatty acids such as linoleic acid. It is an excellent source for dry skin. It has the ability to help repair skin's barrier and reduce inflammation.

Olive oil contains three major antioxidants: vitamin E, polyphenols, and phytosterols. When applied topically, it helps protect the skin from premature aging, restores skin smoothness, and prevents free radical damage to the skin (Cicerale et al. 2010).

Both *coconut oil* and *sweet almond oil* contain saturated fat and vitamin E. When applied on the skin, they keep the skin smooth to touch. They also retain the moisture content of the skin as fats eliminate moisture loss through the pores on skin. Above all, they prevent premature aging and wrinkling of the skin. They can also reverse the damages that are brought about by sun exposure due to their antioxidant activities (Sultana et al. 2007, Nevin and Rajamohan 2010).

Shea butter is a triglyceride derived mainly from stearic acid and oleic acid. It is widely used in cosmetics as a moisturizer. Shea butter contains vitamin A, which is important for improving a number of skin conditions such as sunburn.

17.8 Conclusion

The use of topically applied antioxidants for the skincare is promising as the tendency toward natural products has been increasing. Further profound studies are needed in examining the potential adverse effects of antioxidants because many natural ingredients may potentially cause allergies, irritation, and skin sensitivities. In addition, more controlled clinical trials in humans are essential in examining the efficacy of antioxidant in preventing skin aging.

References

Accorsi-Neto, A., M. Haidar, R. Simoes, M. Simoes, J. Soares-Jr, and E. Baracat. 2009. Effects of isoflavones on the skin of postmenopausal women: A pilot study. *Clinics* (*Sao Paulo*) 64 (6):505–510.

Agarwal, R., S. K. Katiyar, S. G. Khan, and H. Mukhtar. 1993. Protection against ultraviolet B radiation-induced effects in the skin of SKH-1 hairless mice by a polyphenolic fraction isolated from green tea. *Photochem Photobiol* 58 (5):695–700.

Allemann, I. B. and L. Baumann. 2008. Antioxidants used in skin care formulations. *Skin Ther Lett* 13 (7):5–9.

Baliga, M. S. and S. K. Katiyar. 2006. Chemoprevention of photocarcinogenesis by selected dietary botanicals. *Photochem Photobiol Sci* 5 (2):243–253. doi:10.1039/b505311k.

Bastianetto, S., Y. Dumont, A. Duranton, F. Vercauteren, L. Breton, and R. Quirion. 2010. Protective action of resveratrol in human skin: Possible involvement of specific receptor binding sites. *PLoS One* 5 (9):e12935. doi:10.1371/journal.pone.0012935.

Baxter, R. A. 2008. Anti-aging properties of resveratrol: Review and report of a potent new antioxidant skin care formulation. *J Cosmet Dermatol* 7 (1):2–7. doi:10.1111/j.1473-2165.2008.00354.x.

Bowe, W. P. 2013. Cosmetic benefits of natural ingredients: Mushrooms, feverfew, tea, and wheat complex. *J Drugs Dermatol* 12 (9 Suppl):s133–s136.

Burke, K. E., J. Clive, G. F. Combs, Jr., and R. M. Nakamura. 2003. Effects of topical L-selenomethionine with topical and oral vitamin E on pigmentation and skin cancer induced by ultraviolet irradiation in Skh:2 hairless mice. *J Am Acad Dermatol* 49 (3):458–472.

Cerundolo, R., K. E. Michel, M. H. Court, B. Shrestha, K. R. Refsal, J. W. Oliver, V. Biourge, and F. S. Shofer. 2009. Effects of dietary soy isoflavones on health, steroidogenesis, and thyroid gland function in dogs. *Am J Vet Res* 70 (3):353–360. doi:10.2460/ajvr.70.3.353.

Chen, L., J. Y. Hu, and S. Q. Wang. 2012. The role of antioxidants in photoprotection: A critical review. *J Am Acad Dermatol* 67 (5):1013–1024. doi:10.1016/j.jaad.2012.02.009.

Chiu, A. E., J. L. Chan, D. G. Kern, S. Kohler, W. E. Rehmus, and A. B. Kimball. 2005. Double-blinded, placebo-controlled trial of green tea extracts in the clinical and histologic appearance of photoaging skin. *Dermatol Surg* 31 (7 Pt 2):855–860; discussion 860.

Choquenet, B., C. Couteau, E. Paparis, and L. J. Coiffard. 2008. Quercetin and rutin as potential sunscreen agents: Determination of efficacy by an in vitro method. *J Nat Prod* 71 (6):1117–1118. doi:10.1021/np7007297.

Cicerale, S., L. Lucas, and R. Keast. 2010. Biological activities of phenolic compounds present in virgin olive oil. *Int J Mol Sci* 11 (2):458–479. doi:10.3390/ijms11020458.

Cosgrove, M. C., O. H. Franco, S. P. Granger, P. G. Murray, and A. E. Mayes. 2007. Dietary nutrient intakes and skin-aging appearance among middle-aged American women. *Am J Clin Nutr* 86 (4):1225–1231.

Costantini, D. 2008. Oxidative stress in ecology and evolution: Lessons from avian studies. *Ecol Lett* 11 (11):1238–1251. doi:10.1111/j.1461-0248.2008.01246.x.

Counet, C. and S. Collin. 2003. Effect of the number of flavanol units on the antioxidant activity of procyanidin fractions isolated from chocolate. *J Agric Food Chem* 51 (23):6816–6822. doi:10.1021/jf030349g.

Darvin, M. E., A. Patzelt, F. Knorr, U. Blume-Peytavi, W. Sterry, and J. Lademann. 2008. One-year study on the variation of carotenoid antioxidant substances in living human skin: Influence of dietary supplementation and stress factors. *J Biomed Opt* 13 (4):044028. doi:10.1117/1.2952076.

Dayan, N. 2008. *Skin Aging Handbook: An Integrated Approach to Biochemistry and Product Development. Norwich, NY: William Andrew.*

Eberlein-Konig, B., M. Placzek, and B. Przybilla. 1998. Protective effect against sunburn of combined systemic ascorbic acid (vitamin C) and d-α-tocopherol (vitamin E). *J Am Acad Dermatol* 38 (1):45–48.

Ermakov, I. V., M. Sharifzadeh, M. Ermakova, and W. Gellermann. 2005. Resonance raman detection of carotenoid antioxidants in living human tissue. *J Biomed Opt* 10 (6):064028. doi:10.1117/1.2139974.

Ernster, V. L., D. Grady, R. Miike, D. Black, J. Selby, and K. Kerlikowske. 1995. Facial wrinkling in men and women, by smoking status. *Am J Public Health* 85 (1):78–82.

Fisher, G. J., Z. Q. Wang, S. C. Datta, J. Varani, S. Kang, and J. J. Voorhees. 1997. Pathophysiology of premature skin aging induced by ultraviolet light. *N Engl J Med* 337 (20):1419–1428. doi:10.1056/NEJM199711133372003.

Fowler, J. F., Jr., H. Woolery-Lloyd, H. Waldorf, and R. Saini. 2010. Innovations in natural ingredients and their use in skin care. *J Drugs Dermatol* 9 (6 Suppl):S72–S81.

Freedman, D. M., A. Sigurdson, M. M. Doody, R. S. Rao, and M. S. Linet. 2003. Risk of melanoma in relation to smoking, alcohol intake, and other factors in a large occupational cohort. *Cancer Causes Control* 14 (9):847–857.

Fryer, M. J. 1993. Evidence for the photoprotective effects of vitamin E. *Photochem Photobiol* 58 (2):304–312.

Giardina, S., A. Michelotti, G. Zavattini, S. Finzi, C. Ghisalberti, and F. Marzatico. 2010. Efficacy study in vitro: Assessment of the properties of resveratrol and resveratrol + N-acetyl-cysteine on proliferation and inhibition of collagen activity. *Minerva Ginecol* 62 (3):195–201.

Graf, J. 2000. Herbal anti-inflammatory agents for skin disease. *Skin Therapy Lett* 5 (4):3–5.

Grant, W. B. 2008. Skin aging from ultraviolet irradiance and smoking reduces risk of melanoma: Epidemiological evidence. *Anticancer Res* 28 (6B):4003–4008.

Haidarali, S., C. R. Patil, S. Ojha, R. Mohanraj, D. S. Arya, and S. N. Goyal. 2014. Targeting apoptotic pathways in myocardial infarction: Attenuated by phytochemicals. *Cardiovasc Hematol Agents Med Chem* 12 (2):72–85.

Halliwell, B., J. Rafter, and A. Jenner. 2005. Health promotion by flavonoids, tocopherols, tocotrienols, and other phenols: Direct or indirect effects? Antioxidant or not? *Am J Clin Nutr* 81 (1 Suppl):268S–276S.

Hamilton-Reeves, J. M., G. Vazquez, S. J. Duval, W. R. Phipps, M. S. Kurzer, and M. J. Messina. 2010. Clinical studies show no effects of soy protein or isoflavones on reproductive hormones in men: Results of a meta-analysis. *Fertil Steril* 94 (3):997–1007. doi:10.1016/j.fertnstert.2009.04.038.

Helfrich, Y. R., D. L. Sachs, and J. J. Voorhees. 2008. Overview of skin aging and photoaging. *Dermatol Nurs* 20 (3):177–183.

Hoppe, U., J. Bergemann, W. Diembeck, J. Ennen, S. Gohla, I. Harris, J. Jacob et al. 1999. Coenzyme Q10, a cutaneous antioxidant and energizer. *Biofactors* 9 (2–4):371–378.

Hsu, S. 2005. Green tea and the skin. *J Am Acad Dermatol* 52 (6):1049–1059. doi:10.1016/j.jaad.2004.12.044.

Izumi, T., M. Saito, A. Obata, M. Arii, H. Yamaguchi, and A. Matsuyama. 2007. Oral intake of soy isoflavone aglycone improves the aged skin of adult women. *J Nutr Sci Vitaminol* (*Tokyo*) 53 (1):57–62.

Kapoor, S. and S. Saraf. 2010. Assessment of viscoelasticity and hydration effect of herbal moisturizers using bioengineering techniques. *Pharmacogn Mag* 6 (24):298–304. doi:10.4103/0973-1296.71797.

Katiyar, S. K. 2002. Treatment of silymarin, a plant flavonoid, prevents ultraviolet light-induced immune suppression and oxidative stress in mouse skin. *Int J Oncol* 21 (6):1213–1222.

Katiyar, S. K. and C. A. Elmets. 2001. Green tea polyphenolic antioxidants and skin photoprotection (Review). *Int J Oncol* 18 (6):1307–1313.

Kennedy, C., M. T. Bastiaens, C. D. Bajdik, R. Willemze, R. G. Westendorp, J. N. Bouwes Bavinck, and Study Leiden Skin Cancer. 2003. Effect of smoking and sun on the aging skin. *J Invest Dermatol* 120 (4):548–554. doi:10.1046/j.1523-1747.2003.12092.x.

Kim, S. Y., S. J. Kim, J. Y. Lee, W. G. Kim, W. S. Park, Y. C. Sim, and S. J. Lee. 2004. Protective effects of dietary soy isoflavones against UV-induced skin-aging in hairless mouse model. *J Am Coll Nutr* 23 (2):157–162.

Kolbe, L., J. Immeyer, J. Batzer, U. Wensorra, K. Tom Dieck, C. Mundt, R. Wolber et al. 2006. Anti-inflammatory efficacy of Licochalcone A: Correlation of clinical potency and in vitro effects. *Arch Dermatol Res* 298 (1):23–30. doi:10.1007/s00403-006-0654-4.

Korac, R. R. and K. M. Khambholja. 2011. Potential of herbs in skin protection from ultraviolet radiation. *Pharmacogn Rev* 5 (10):164–173. doi:10.4103/0973-7847.91114.

Krutmann, J., W. Liu, L. Li, X. Pan, M. Crawford, G. Sore, and S. Seite. 2014. Pollution and skin: From epidemiological and mechanistic studies to clinical implications. *J Dermatol Sci* 76 (3):163–168. doi:10.1016/j.jdermsci.2014.08.008.

Kwong, L. K., S. Kamzalov, I. Rebrin, A. C. Bayne, C. K. Jana, P. Morris, M. J. Forster, and R. S. Sohal. 2002. Effects of coenzyme Q(10) administration on its tissue concentrations, mitochondrial oxidant generation, and oxidative stress in the rat. *Free Radic Biol Med* 33 (5):627–638.

Lademann, J., S. Schanzer, M. Meinke, W. Sterry, and M. E. Darvin. 2011. Interaction between carotenoids and free radicals in human skin. *Skin Pharmacol Physiol* 24 (5):238–244. doi:10.1159/000326074.

Lin, C. H., C. L. Fang, S. A. Al-Suwayeh, S. Y. Yang, and J. Y. Fang. 2011. In vitro and in vivo percutaneous absorption of seleno-L-methionine, an antioxidant agent, and other selenium species. *Acta Pharmacol Sin* 32 (9):1181–1190. doi:10.1038/aps.2011.89.

Lin, J. Y., M. A. Selim, C. R. Shea, J. M. Grichnik, M. M. Omar, N. A. Monteiro-Riviere, and S. R. Pinnell. 2003. UV photoprotection by combination topical antioxidants vitamin C and vitamin E. *J Am Acad Dermatol* 48 (6):866–874. doi:10.1067/mjd.2003.425.

Lopez, L. C., C. M. Quinzii, E. Area, A. Naini, S. Rahman, M. Schuelke, L. Salviati, S. Dimauro, and M. Hirano. 2010. Treatment of CoQ(10) deficient fibroblasts with ubiquinone, CoQ analogs, and vitamin C: Time-and compound-dependent effects. *PLoS One* 5 (7):e11897. doi:10.1371/journal.pone.0011897.

Lushchak, V. I. 2014. Free radicals, reactive oxygen species, oxidative stress and its classification. *Chem Biol Interact* 224C:164–175. doi:10.1016/j.cbi.2014.10.016.

Mahmood, T. and N. Akhtar. 2013. Combined topical application of lotus and green tea improves facial skin surface parameters. *Rejuvenation Res* 16 (2):91–97. doi:10.1089/rej.2012.1380.

Manach, C., A. Scalbert, C. Morand, C. Remesy, and L. Jimenez. 2004. Polyphenols: Food sources and bioavailability. *Am J Clin Nutr* 79 (5):727–747.

Massenti, R., A. Perrone, M. A. Livrea, and R. Lo Bianco. 2015. Regular consumption of fresh orange juice increases human skin carotenoid content. *Int J Food Sci Nutr* 66 (6):718–721. doi:10.3109/09637486.2015.1077794.

Nagle, D. G., D. Ferreira, and Y. D. Zhou. 2006. Epigallocatechin-3-gallate (EGCG): Chemical and biomedical perspectives. *Phytochemistry* 67 (17):1849–1855. doi:10.1016/j.phytochem.2006.06.020.

Nevin, K. G. and T. Rajamohan. 2010. Effect of topical application of virgin coconut oil on skin components and antioxidant status during dermal wound healing in young rats. *Skin Pharmacol Physiol* 23 (6):290–297. doi:10.1159/000313516.

Nichols, J. A. and S. K. Katiyar. 2010. Skin photoprotection by natural polyphenols: Anti-inflammatory, antioxidant and DNA repair mechanisms. *Arch Dermatol Res* 302 (2):71–83. doi:10.1007/s00403-009-1001-3.

Nicol, N. H. 2005. Anatomy and physiology of the skin. *Dermatol Nurs* 17 (1):62.

Nishino, H., A. Iwashima, H. Fujiki, and T. Sugimura. 1984. Inhibition by quercetin of the promoting effect of teleocidin on skin papilloma formation in mice initiated with 7,12-dimethylbenz[a]anthracene. *Gan* 75 (2):113–116.

Oxford English Dictionary. 2014. *Oxford English Dictionary*. Oxford, UK: Oxford University Press.

Pandey, K. B. and S. I. Rizvi. 2009. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid Med Cell Longev* 2 (5):270–278. doi:10.4161/oxim.2.5.9498.

Rahman, K. 2007. Studies on free radicals, antioxidants, and co-factors. *Clin Interv Aging* 2 (2):219–136.

Ribaya-Mercado, J. D., M. Garmyn, B. A. Gilchrest, and R. M. Russell. 1995. Skin lycopene is destroyed preferentially over beta-carotene during ultraviolet irradiation in humans. *J Nutr* 125 (7):1854–1859.

Rice-Evans, C. A., N. J. Miller, P. G. Bolwell, P. M. Bramley, and J. B. Pridham. 1995. The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radic Res* 22 (4):375–383.

Sanders, T. H., R. W. McMichael, Jr., and K. W. Hendrix. 2000. Occurrence of resveratrol in edible peanuts. *J Agric Food Chem* 48 (4):1243–1246.

Saraf, S. and C. D. Kaur. 2010. Phytoconstituents as photoprotective novel cosmetic formulations. *Pharmacogn Rev* 4 (7):1–11. doi:10.4103/0973-7847.65319.

Schagen, S. K., V. A. Zampeli, E. Makrantonaki, and C. C. Zouboulis. 2012. Discovering the link between nutrition and skin aging. *Dermatoendocrinol* 4 (3):298–307. doi:10.4161/derm.22876.

Schroeter, H., C. Boyd, J. P. Spencer, R. J. Williams, E. Cadenas, and C. Rice-Evans. 2002. MAPK signaling in neurodegeneration: Influences of flavonoids and of nitric oxide. *Neurobiol Aging* 23 (5):861–880.

Srivastava, J. K., E. Shankar, and S. Gupta. 2010. Chamomile: A herbal medicine of the past with bright future. *Mol Med Rep* 3 (6):895–901. doi:10.3892/mmr.2010.377.

Sultana, Y., K. Kohli, M. Athar, R. K. Khar, and M. Aqil. 2007. Effect of pre-treatment of almond oil on ultraviolet B-induced cutaneous photoaging in mice. *J Cosmet Dermatol* 6 (1):14–19. doi:10.1111/j.1473-2165.2007.00293.x.

Svobodova, A., J. Psotova, and D. Walterova. 2003. Natural phenolics in the prevention of UV-induced skin damage. A review. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 147 (2):137–145.

Tanaka, M., E. Misawa, K. Yamauchi, F. Abe, and C. Ishizaki. 2015. Effects of plant sterols derived from Aloe vera gel on human dermal fibroblasts in vitro and on skin condition in Japanese women. *Clin Cosmet Investig Dermatol* 8:95–104. doi:10.2147/CCID.S75441.

Tsuda, T., F. Horio, and T. Osawa. 2000. The role of anthocyanins as an antioxidant under oxidative stress in rats. *Biofactors* 13 (1–4):133–139.

Vierkotter, A., T. Schikowski, U. Ranft, D. Sugiri, M. Matsui, U. Kramer, and J. Krutmann. 2010. Airborne particle exposure and extrinsic skin aging. *J Invest Dermatol* 130 (12):2719–2726. doi:10.1038/jid.2010.204.

Wang, Y., F. Catana, Y. Yang, R. Roderick, and R. B. van Breemen. 2002. An LC-MS method for analyzing total resveratrol in grape juice, cranberry juice, and in wine. *J Agric Food Chem* 50 (3):431–435.

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

18

Catechins as Potential Therapeutic Agents for Ocular Diseases

Radouil Tzekov, Shannon Kelly, and Anjali Hirani

Contents

18.1 Introduction

Catechins are a type of natural phenol compounds with potent antioxidant activities. They belong to the group of hydroxyflavans (flavonols), a subgroup within the large flavonoid family with more than 5000 members identified to date, which are 15-carbon molecules, plant secondary metabolites (**Figure 18.1**). The name of the catechin chemical family is derived from catechu, which is the juice or extract of the Asian plant Mimosa catechu (Acacia catechu).

Although there are more than 30 different types of catechins, one form, the epigallocatechin 3-gallate (EGCG) (**Figure 18.2**), has been studied most extensively in medicinal chemistry and pharmacology. This is the most abundant and active constituent among tea polyphenols, mostly found in green and white teas, and in smaller quantities in black teas (Cabrera et al. 2003, 2006). Another three major catechins, (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), and (−)-epicatechin (EC), have also been studied, mostly due to their abundance in different types of tea leaves.

18.2 Natural occurrences

Catechins are present in several food items. Chocolate and tea leaves have a particularly high catechin content. Unroasted cocoa beans contain (−)-epicatechin (EC) and (+)-catechin (C) (Robinson et al. 1961, Hurst et al. 2011), producing a bitter taste. Unprocessed cocoa beans contain 21.9–43.3 mg EC and 21.51 mg (+)-C per gram (Kim and Keeney 2006, Bhagwat et al. 2014). The high temperatures required to ferment and roast cocoa results in epimerization of EC to (−)-catechin (Robinson et al. 1961, Kofink et al. 2007). Thus, roasted cocoa products contain (−)-catechin as well as EC and (+)-C in smaller amounts than unroasted beans (Kofink et al. 2007, Hurst et al. 2011). Fermentation results in a nearly 80% loss of C and EC (Payne et al. 2010), resulting in a much sweeter flavor than that of fresh beans.

Catechins constitute up to 30% of the dry weight of green tea leaves (Graham 1992, Lin 2006). Once processed, approximately 10% of the dry weight of green tea is comprised of (+) C, EC, ECG, EGC, EGCG, and (+)-gallocatechin (GC) (Juneja et al. 2000). Nearly 50% of the catechin content of green tea is made up of EGCG (Izawa et al. 2010). About 5% of the dry weight of black tea consists of catechins because many catechins and other polyphenols are lost in the processing of black tea due to oxidation (Juneja et al. 2000).

18.3 Physicochemical properties

The main physicochemical properties of the five types of catechins mostly used in research are summarized in **Table 18.1**.

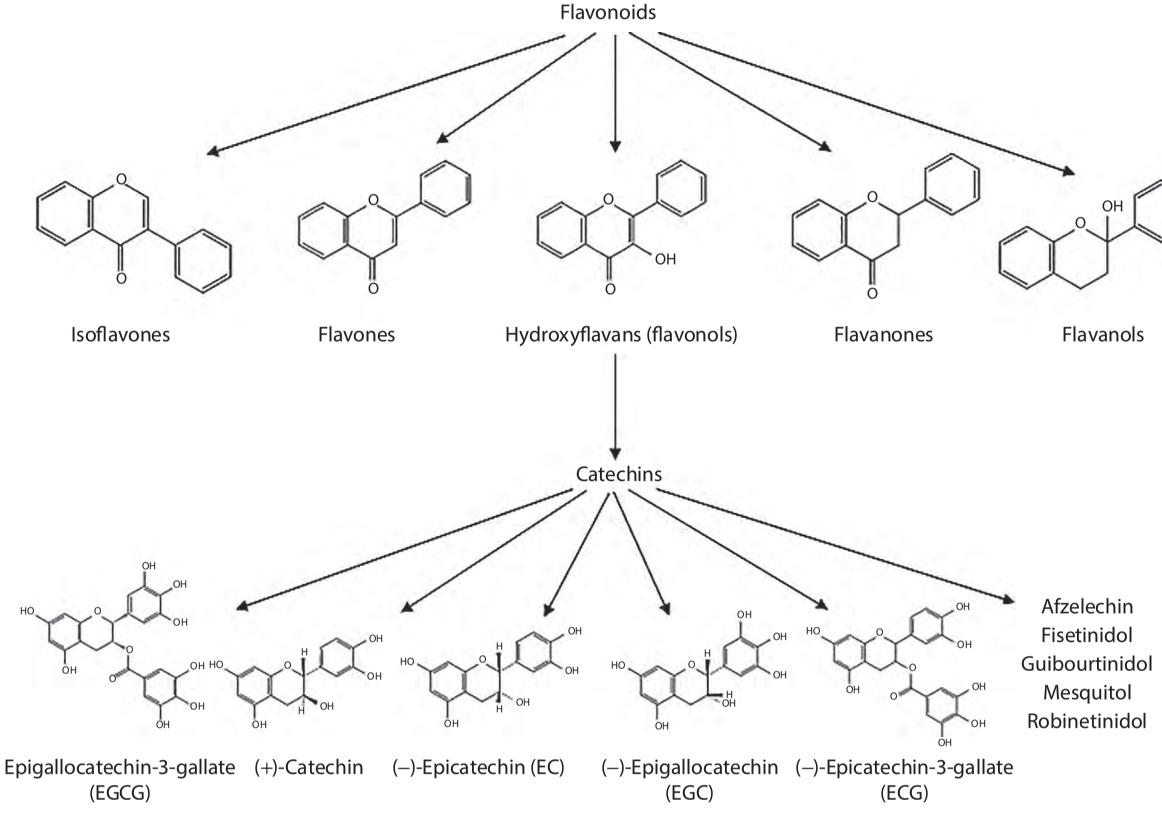


Figure 18.1 *The polyphenolic family and different types of catechins.*

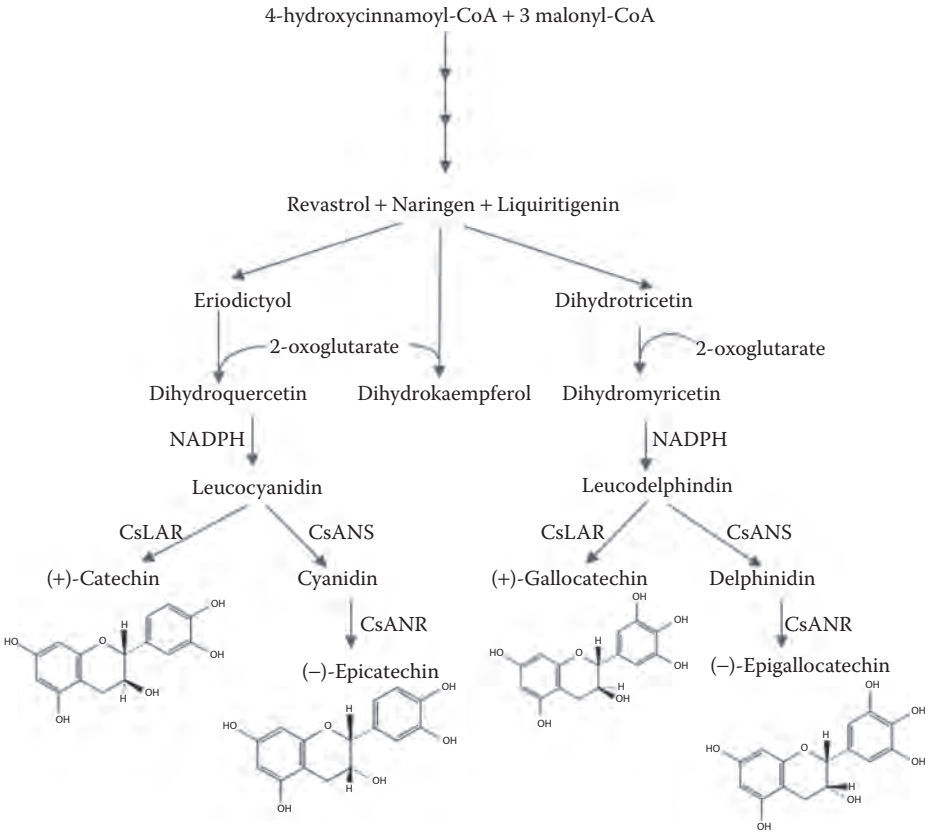


Figure 18.2 *Biosynthesis of catechins.*

Table 18.1 Physicochemical Properties of Main Catechins

	Catechin	EC	GC	EGC	EGCG
Chemical formula	$C_{15}H_{14}O_6$	$C_{15}H_{14}O_6$	$C_{15}H_{14}O_7$	$C_{15}H_{14}O_7$	$C_{22}H_{18}O_{11}$
Molar mass (g/mol)	290.3	290.3	306.3	306.3	458.4
Appearance	Colorless solid	Colorless solid	Colorless solid	Colorless solid	Colorless solid
Melting point (°C)	176	242	188	218	254
Water solubility	Slightly soluble	Slightly soluble	Slightly soluble	Slightly soluble	Slightly soluble

18.3.1 Stability

Catechins can undergo various transformations during food processing: degradation, oxidation, epimerization, and polymerization, depending on different factors such as ambient temperature, presence of oxygen, pH, and so on. Even under stable and carefully monitored storage conditions, the

content decreases over time as it was demonstrated for green tea catechins during 6 months of storage, where overall catechin content decreased ~30% (Friedman et al. 2009).

18.4 Metabolism

18.4.1 Biosynthesis

Catechins are biosynthesized in plants as part of the shikimate pathway. This is a metabolic pathway that links metabolism of carbohydrates to the biosynthesis of aromatic compounds and is used by bacteria, fungi, and plants, but not animals for the biosynthesis of aromatic amino acids (Herrmann and Weaver 1999). Thus, catechins must be consumed from external sources, mainly plants, to enter the human body.

In the process of catechins plant biosynthesis (Figure 18.2), one molecule of 4-hydroxycinnamoyl-CoA reacts with 3 units of malonyl-CoA to produce polyketide. This polyketide can then follow one of the three paths. One of these paths results in the production of naringenin, a flavanone (Dewick 2002). Enzymatic catalysis by anthocyanidin synthase (ANS), leucoanthocyanidin reductase (LAR), and anthocyanidin reductase (ANR) leads to the production of (+)-C, EC, GC, and EGC (Wu et al. 2014).

18.4.2 Biodegradation

There are several natural processes by which catechins can be degraded. EC, for example, can be reduced by lithium aluminum hydride and aluminum chloride to produce propanol (Bokadia 1962, Hathway 2014). However, this process is not found in nature because these compounds do not naturally exist in their pure form. Some bacteria and fungi contain catechin oxygenase, the enzyme necessary for the oxidation of catechins (Arunachalam et al. 2003). Studies have shown that catechins can be oxidized by *P. campestris* through polyphenoloxidase oxidation (Hathway and Seakins 1957, Mahadevan and Sivaswamy 1985). Lewis and Starkey (1969) found that the fungi *Aspergillus fumigatus* and some *Fusarium* species decomposed cultures of catechins after 8 days. The same study showed that the fungus *Aspergillus niger* decomposed 100% of the catechin present in a sample (Lewis and Starkey 1969, Singh 2002). The bacterial species *Pseudomonas* and *Rhizobium* have also been shown to degrade catechins (Singh 2002). Specifically, Muthukumar et al. (1982) found that *Rhizobium japonicum* efficiently decomposed catechins.

The mechanisms associated with the aerobic biodegradation of catechins by fungi and bacteria are very similar (Arunachalam et al. 2003). A summary of this process is shown in **Figure 18.3**. Catechin oxygenase breaks catechin into phloroglucinol carboxylic acid and protocatechuic acid. Phloroglucinol

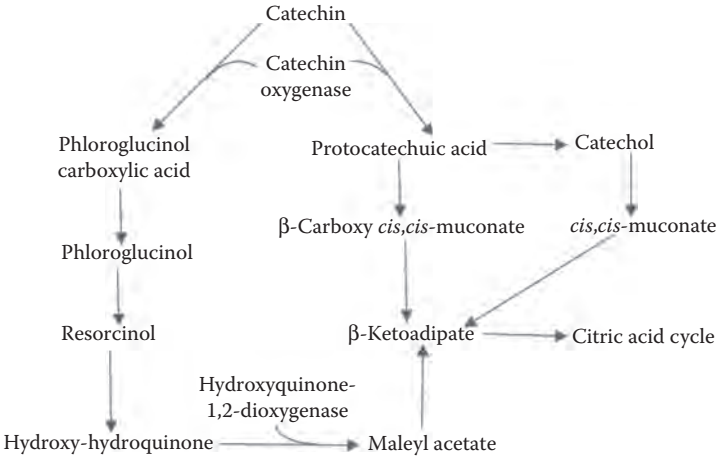


Figure 18.3 *Aerobic biodegradation pathway of catechin. (From Arunachalam, M. et al., Proc. Ind. Nat. Sci. Acad. B., 69, 353–370, 2003.)*

carboxylic acid metabolizes further into phloroglucinol, then resorcinol, and then hydroxy-hydroquinone. Hydroxyquinon-1,2-dioxygenase cleaves hydroxyl-hydroquinone into maleyl acetate, which becomes β-ketoadipate. Protocatechuic acid is converted into both β-carboxy-*cis,cis*-muconate and catechol. β-Carboxy-*cis,cis*-muconate forms ketoadipate. Catechol cleaves to form *cis,cis*-muconate and ketoadipate. The ketoadipate formed then enters the citric acid cycle, forming acetyl CoA (Arunachalam et al. 2003).

In addition to the aerobic pathway shown in **Figure 18.3**, catechin and epicatechin can also be metabolized anaerobically. This has been seen in the microflora found in the colons of humans and rats (Arunachalam et al. 2003, Lhoste et al. 2003). The anaerobic degradation of catechins proceeds through a different mechanism. Catechin is first converted into diaryl propanol, which is then broken into acetate and phenylvalerolactone. Phenylvalerolactone is then transformed into phenylpropionate derivatives and may form phenylacetate (Arunachalam et al. 2003).

Taken orally, (+)-catechin is metabolized and excreted by humans within 24 hours. This process produces eleven metabolites found in urine, including *m*-hydroxyphenylpropionic acid, δ-(3,4-dihydroxyphenyl)-γ-valerolactone, and δ-(3-hydroxyphenyl)-γ-valerolactone (Das 1971). Rats metabolize catechins in the small intestine and liver, and catechins are excreted primarily through bile (Donovan et al. 2001). Green tea catechins (0.6%) were consumed by rats and mice in a study conducted by Kim et al. (2000). The concentrations of EGCG, EC, and EGC present in the blood plasma of these rats and mice peaked after 14 days and returned to day 1 levels after 28 days (Kim et al. 2000). The amounts of EGCG found in the rat plasma were consistently lower than the EC and EGC concentrations. The concentrations

of EGCG found in mice plasma, lung tissue, and liver tissue were much higher than the concentration found in rats (Kim et al. 2000). Mata-Bilbao et al. (2008) studied the metabolism of green tea catechins in beagles over 24 hours. They found that peak catechin concentrations in blood plasma occurred 1 hour after oral intake. Catechin derivatives were found in urine samples, indicating that, similar to rats, beagles excrete EGCG and ECG through bile (Mata-Bilbao et al. 2008).

18.4.3 Biotransformation

A natural part of catechin metabolism is biotransformation. This can take different forms. The condensation of (+)-C and EC, for example, results in the formation of proanthocyanidins, a group of antioxidants found in plants (Park et al. 2011, Zaprometov 2012). Catechin metabolism in rats involves methylation and sulfonation in the liver (Donovan et al. 2001). This study also demonstrated that methylation and glucuronidation occur in the small intestine of rats (Donovan et al. 2001). Meselhy et al. (1997) found that EC and EGC are extensively metabolized by human intestinal bacteria within 24 hours. Schantz et al. (2010) later determined that EGCG and ECG were cleaved during this process. Rat fecal suspensions, on the other hand, resulted in no degradation of EC or EGC (Meselhy et al. 1997). This suggests a distinct difference in the bacteria found in the gastrointestinal tracts of rats and humans.

Oxidation is another form of biotransformation experienced by catechins and has been studied extensively. Shibuya et al. (2005) found that (+)-C and EC can be transformed by the *Diaporthe* species of fungus into (+)-(2*R*,3*S*,4*S*)-3,4,5,7,3′,4′-hexahydroxyflavan and (–)-(2*R*,3*R*,4*R*)-3,4,5,7,3′,4′-hexahydroxyflavan. The bacterial species *Burkholderia* KTC-1 was discovered by Matsuda et al. (2007) to oxidize (+)-catechin into taxifolin in two steps. Osman et al. (2007) investigated the oxidation of (+)-catechin by laccase. They found that dimers of dehydrocatechin types A and B were formed as well as other oligomers. The addition of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) in combination with laccase produced a higher proportion of the hydrophobic oligomers. Low concentrations of ascorbic acid combined with the addition of laccase resulted in a higher proportion of hydrophilic products. High concentrations of ascorbic acid, however, inhibited the oxidation of catechin by laccase (Osman et al. 2007). Chen et al. (1998) studied the effects of ascorbic acid and citric acid on EC, ECG, EGC, and EGCG *in vitro*. They noted that, although EC and ECG are somewhat stable in sodium phosphate buffer, EGCG and EGC are both unstable. The addition of citric acid had no effect on the stability of these catechins. The presence of ascorbic acid, however, resulted in an increased stability of all four catechins (Chen et al. 1998). In addition, Peters et al. (2010) found that catechins demonstrate a higher bioavailability in the presence of ascorbic acid and sucrose. This discovery could play an important role in catechin drug development.

18.4.4 Bioactivity

18.4.4.1 Bioavailability

Each variety of catechins demonstrates low bioavailability in humans and in rodents of which EGCG is the least bioavailable (Chen et al. 1997, Zhu et al. 2000, Lipinski et al. 2001, Yashin et al. 2012). Only 1.6% of an oral dose of EGCG was reportedly absorbed by rats, and a 26.5% bioavailability of EGCG was recorded in mice (Chen et al. 1997, Lambert et al. 2003). This low bioavailability is caused by several factors. Much of the catechin degradation that occurs in the body occurs in the small intestine, resulting in a significant loss of catechins (Peters et al. 2010). The transport of catechins is also limited due to their low affinity for efflux transport systems (Peters et al. 2010). The combination of their instability in the small intestine and their poor ability to transport result in a low absorption of catechins (Peters et al. 2010). In addition, catechins are metabolized and excreted by humans very quickly, typically within 24 hours (Peters et al. 2010). Thus, the concentrations of catechins that are absorbed typically after oral administration are lower compared to an estimated therapeutic plasma concentration (Chow et al. 2005).

The oral bioavailability of EGCG and other catechins may be affected by combination with different foods or water, vitamin C, fish oil, and so on (Mereles and Hunstein 2011). Catechins demonstrate a very low solubility in water (Cuevas-Valenzuela et al. 2014), which also leads to poor absorption of catechins in the gastrointestinal tract. Studies have shown that using a water-ethanol mixture and increased temperatures result in a higher solubility of catechins (Srinivas et al. 2010, Cuevas-Valenzuela et al. 2014). Peters et al. (2010) found that a combination of ascorbic acid and sucrose results in an increased bioavailability of EGC, EGCG, and ECG. Milk, however, has been shown to have no effect on the bioavailability of tea catechins (Van het Hoff et al. 1998). Chow et al. (2005) discovered a higher bioavailability of catechins when consumed after fasting overnight.

Methylation, sulfonation, ring fission, and other biotransformations occur during the absorption of catechins (Meng et al. 2002; Yashin et al. 2012), resulting in a low absorption of raw catechins. This has been reflected in several studies. Del Rio et al. studied the bioavailability of catechins from ready-to-drink (RTD) tea by analyzing urine samples of healthy human volunteers. They found that 7.2% of the catechins originally consumed were recovered in the urine samples (Del Rio et al. 2010). Warden et al. (2001) measured the peak amounts of EGC, EC, EGCG, and ECG in plasma, urine, and fecal samples. The results showed 0.16%, 1.1%, and 0.42% recovery of catechins in the plasma, urine, and fecal samples, respectively. Conjugated and methylated forms of catechins reportedly make up approximately 80% of tea catechins in plasma and urine (Baba et al. 2001, Yashin et al. 2012). However, it is difficult to analyze the absorption of catechins from urine and fecal samples due to metabolism by the liver and conjugation in the gastrointestinal tract (Warden et al. 2001).

18.5 Effects on ocular tissues

18.5.1 Effects on cornea

Even after oral administration, generally considered to be very ineffective because of low bioavailability, the levels of some catechins such as EGC can remain elevated in the corneal tissue in high concentrations for up to 10 hours (Chu et al. 2010). This finding prompted additional research, which showed that EGCG acts as an anti-inflammatory and antioxidant agent *in vitro* on human corneal epithelial cells (Cavet et al. 2011). Catechins were also effective in quenching reactive oxygen species (ROS) in a similar *in vitro* model (Stoddard et al. 2013).

Catechin efficacy in *in vivo* models confirmed an anti-inflammatory and anti-neovascular potential but did not support an antioxidative action. Thus, in a murine model of dry eye, EGCG treatment was able to reduce the clinical signs in the mouse model and to suppress the cytokine expression in the cornea (Lee et al. 2011). Similar results were obtained in a mouse model with corneal ulceration and severe epithelial exfoliation as a result of ultraviolet irradiation of the cornea, where EGCG exhibited potent protective effects (Chen et al. 2014). *In vivo* efficacy for EGCG has been demonstrated also in a rabbit model of corneal neovascularization, where its topical administration inhibited the neovascular process (Koh et al. 2014). This *in vivo* efficacy is likely not mediated by an increase in the antioxidative capacity, as recent work demonstrated either no difference or even an increase in oxidative stress in the cornea (measured by concentration of 8-isoprostane) at certain time points, for example, 6 and 20 hours after oral administration of green tea extract containing a mixture of various catechins in rats (Chu et al. 2015).

18.5.2 Effects on trabecular meshwork, intraocular pressure, and glaucoma

Presently the local concentration of catechins in the trabecular meshwork tissue in animals or humans after administration of individual catechin species or a mixture (e.g., green tea extract) remains unknown. Thus, the effect of catechins on the trabecular meshwork has to be deduced indirectly by measuring the consternation in the aqueous humor. Studies have shown that this concentration increases and remains elevated for several hours in the rat after oral administration of green tea extract (Chu et al. 2010, 2015). Surprisingly, this is not mirrored by a decrease in local oxidative stress levels assessed by 8-isoprostane; to the contrary, these levels increased significantly at 10 and 15 hours post-administration (Chu et al. 2015). Despite the lack of antioxidative enhancement, individual catechin species such as EGCG showed a neuroprotective effect in a mouse model of glaucoma using microbeads injection to induce elevated intraocular pressure (Shen et al. 2015) by reducing the loss of retinal ganglion cells (RGC).

To date, only one clinical trial reported effects of catechins in glaucoma in humans. This was a 3-month, randomized, placebo-controlled, double-blind, cross-over design clinical trial involving 18 patients with ocular hypertension (OHT) and 18 patients with primary open angle glaucoma (POAG) who received either oral placebo or EGCG. Pattern electroretinography (ERG) increased in amplitude in POAG patients taking EGCG, and this increase was inversely correlated with baseline amplitudes (Falsini et al. 2009). Although this was a short-term study, this confirms the potential for catechins as therapeutic agents in POAG.

18.5.3 Effects on lens

Opacification of the lens, also known as cataract, is a major cause of visual loss and blindness in the developing world. One of the causes for cataract development is likely oxidative damage to the lens proteins. Thus, powerful antioxidants such as catechins could play an important role in preventing oxidative damage to lens proteins. This, indeed, was found to be the case for EGCG *in vitro* by several studies (Ye et al. 1992, Huang et al. 2000, Yao et al. 2009, Dubey et al. 2015).

Furthermore, studies using animal models of cataract formation support the effectiveness of catechins. Thus, cataract formation induced by intraperitoneal injection of *N*-methyl-*N*-nitrosurea (MNU) in young rats was reduced by catechin treatment (Lee et al. 2010). Similarly, cataract induced by intraperitoneal injection of sodium selenite in young rats was suppressed by administration of the flavonoid fraction of Moringa oleifera leaves containing a high amount of flavonoids, including catechins (Sasikla et al. 2010). Finally, administration of procyanidin-B2, a catechin dimer, delayed cataract formation in diabetic rats (Muthenna et al. 2013). In contrast to other eye tissues discussed previously (cornea and aqueous humor), recent evidence suggests that levels of oxidative stress in the lens monitored by measuring the levels of 8-isoprostane were decreased after administration of green tea extract in rats (Chu et al. 2015).

18.5.4 Effects on vitreous and retina

The retina is a highly metabolically active tissue and consumes a considerable amount of oxygen, especially in the outer retina, located close to the choroid. The high oxygen tension (70–90 mm Hg) (Ahmed et al. 1993, Wang et al. 2010) in the outer segments of the photoreceptors leads to constant oxidation of phospholipids (Birkle and Bazan 1989, Sun et al. 2006, Lei et al. 2013), which make a substantial portion of the photoreceptor membranes.

18.5.4.1 In vitro *effects of catechins on neural retina*

Catechins reversed the increase in retinal malondialdehyde (indicating lipid peroxidation) after glutamate administration on porcine retinal culture and

reversed the changes in four out of the seven retinal proteins dysregulated after excessive glutamate injury (Siu et al. 2008). Another study demonstrated that EGCG potently inhibits *in vitro* the aggregation of mutant HTT exon 1 protein, involved in the development of Huntington disease (HD) and improves photoreceptor degeneration in transgenic HD flies (Ehrnhoefer et al. 2006). This is an important indicator of potential therapeutic usability, as protein misfolding plays a significant role in the pathogenesis of several inherited retinal degenerations (Tzekov et al. 2011).

18.5.4.2 In vivo *effects of catechins on neural retina*

Injection of sodium nitroprusside (SNP) in the vitreous of adult rats causes a dramatic increase in lipid peroxidation and a decrease in the electrical activity of the retina as measured by ERG. In this model, an intravitreal application of EGCG attenuated the detrimental influence of SNP to retinal photoreceptors as measured by significant amplitude ERG preservation 3 days after SNP administration (Zhang and Osborne 2006). Similar results were obtained in an ischemia-reperfusion model (Zhang et al. 2008) and in a rat model of retinal degeneration induced by MNU intraperitoneal injection (Emoto et al. 2014). Survival of RGC after glutamate injury *in vivo* after N-Methyl-D-aspartic acid (NMDA)-induced excitotoxicity in the retina was established in a rat model (Chen et al. 2012).

18.5.4.3 *Effects on retinal pigment epithelium*

The retinal pigment epithelium (RPE) is a unique cellular layer, exposed to high oxygen levels from the neighboring choroid and to high metabolic stress from constant digestion of photoreceptor outer segments, rich in oxidized phospholipid material. There have been many studies in the last 15 years, all confirming the beneficial effects of catechins, mostly EGCG, on RPE cells *in vitro* in terms of reducing Vascular Endothelial Growth Factor (VEGF) levels, cell proliferation, UV damage, oxidative stress, and so on (Chida et al. 1999, Hanneken et al. 2006, Yang et al. 2007, Chan et al. 2008, Li et al. 2013, Chen et al. 2014, Cia et al. 2014, Lee et al. 2014). How much of this *in vitro* efficacy translates into *in vivo* protective and beneficial effects remains to be determined in future studies as *in vivo* and human data are lacking.

18.5.4.4 *Effects on vitreous*

To the best of our knowledge, there have been no reported studies on the effects of catechins on models of vitreous diseases. What is known to date is that some catechins, such as GC, can reach the vitreous and accumulate in it up to 20 hours after oral administration in rats (Chu et al. 2010). The vitreous has high oxygen content in the layer closest to the retina (vitreous cortex). This gradient can extend further anteriorly and affect the lens after vitreous removal when vitrectomy is performed (Kleinberg et al. 2011). One form of catechin, GC, was selectively absorbed in the vitreous humor, whereas other

catechins such as EGCG remained at high levels but showed classic pharmacokinetic elimination in a recent study (Chu et al. 2015). Thus, catechins have the potential to exert antioxidative action in the vitreous and the surrounding tissues. However, the same study by Chu et al. showed that the oxidative stress marker 8-isoprostane levels were increased in the vitreous at several time points after treatment, suggesting that, under some combinations and dosages *in vivo*, catechins may also have prooxidative properties in ocular tissues in general and in the vitreous in particular.

18.5.5 Effects on optic nerve

The optic nerve is comprised of the axons of the RGC and plays a crucial role in the transmission of visual information to the brain. Currently, there is a lack of studies on the effect of catechins on RGC axons in vitro. However, application of EGCG on hippocampal axons in an outgrowth assay showed regenerative effects in support of the studies demonstrating neuroprotective properties in neural tissue (Herges et al. 2011).

The neuroprotective properties of catechins were investigated *in vivo* in models of optic nerve diseases. One of the most used models for optic nerve degeneration is the optic nerve crush model. A study by Xie et al. indicated protective effects of EGCG on RGCs after optic nerve crush in rats 7 to 28 days after injury as measured by the counts of surviving RGCs and the expression of neurofilament light protein (Xie et al. 2010). At a similar short time period after retinal and optic nerve injury induced by elevating the intraocular pressure in rats, EGCG was effective in increasing the levels of two proteins in the optic nerve: tubulin and neurofilament light (Zhang et al. 2007). Surprisingly, similar protective results were obtained after a more severe type of injury—optic nerve axotomy in rats, where EGCG treatment reduced retinal ganglion cell loss by 12% at 7 days post injury (Peng et al. 2010).

18.6 Current developments as candidates for ocular therapeutics

Several challenges remained before catechins could be used widely for treatment of ocular diseases. One of the key issues as an obstacle for application as a topical agent or for intraocular administration is the rate of compound oxidation, which for catechins is very high. Another issue is low water solubility. Therefore, formulation work is necessary to make catechins suitable for widespread clinical development in ophthalmology.

Currently, formulation efforts for catechins are focused in two directions—micro and nanoparticles. A recent review summarized these and some additional efforts, which include colloidal complexes, cyclodextrin complexes, liposomes, and so on (Rodrigues et al. 2013). However, very few of these formulation efforts are tailored for topical or intraocular ophthalmic drug delivery, which has a number of specific requirements related to delivery to

the anterior or posterior segment of the eye, such as water solubility, stability, rate of release in the vitreous, uptake by the retina, and so on (Kompella et al. 2010, Achouri et al. 2013, Molokhia et al. 2013).

18.6.1 Lipid nanoparticles

Encapsulating catechins in lipid nanoparticles could be a suitable approach to avoid drug oxidation, which is currently one of the major challenges in formulating catechins for topical or intraocular drug delivery. One of the very few examples of formulation efforts using lipid nanoparticles was reported recently. In this work, EGCG was successfully encapsulated in lipid nanoparticles with good physicochemical stability and compatibility to ocular administration (Fangueiro et al. 2014), which undoubtedly will stimulate further ophthalmic clinical development.

18.7 Perspectives

If confirmed by further work *in vivo*, the neuroprotective, anti-inflammatory, and neuroregenerative properties of the catechins described earlier would expand their potential application in ocular diseases into additional directions beyond more narrow approaches that are based solely on their antioxidative capacity. Thus, catechins can be used either as monotherapy or in combination with established treatments for a variety of ocular conditions of considerable public health importance, such as age-related macular degeneration, diabetic retinopathy, glaucoma, and so on.

18.8 Conclusion

Many studies in the last two decades have demonstrated a variety of important and potentially beneficial properties of catechins in ocular tissues *in vitro* and *in vivo* models. However, the original concept that their beneficial anti-inflammatory and neuroprotective activities are due mostly, if not exclusively, to their antioxidant properties is currently undergoing revision. Apparently, the mechanisms behind the positive action of catechins in animal models of several degenerative inflammatory ocular diseases are more complex and still poorly understood. Nevertheless, the overall impression from the current literature describing the potential use of these compounds in ocular conditions is positive and will stimulate further work.

References

Achouri, D., K. Alhanout, P. Piccerelle, and V. Andrieu. 2013. Recent advances in ocular drug delivery. *Drug Dev Ind Pharm* 39 (11):1599–1617.

Ahmed, J., R. D. Braun, R. Dunn Jr., and R. A. Linsenmeier. 1993. Oxygen distribution in the macaque retina. *Invest Ophthalmol Vis Sci* 34 (3):516–521.

Arunachalam, M., M. M. Raj, N. Mohan, and A. Mahadevan. 2003. Biodegradation of catechin. *Proc—Ind Nat Sci Acad B* 69 (4):353–370.
Baba, S., N. Osakabe, M. Natsumi, Y. Muto, T. Takizawa, and J. Terao. 2001. In vivo comparison of the bioavailability of (+)-catechin, (–)-epicatechin and their mixture in orally administered rats. *J Nutr* 131 (11):2885–2891.
Bhagwat, S., D. B. Haytowitz, and J. M. Holden. 2014. *USDA Database for the Flavonoid Content of Selected Foods, Release 3.1.* Washington, DC: U.S. Department of Agriculture, Agricultural Research Service.
Birkle, D. L. and N. G. Bazan. 1989. Light exposure stimulates arachidonic acid metabolism in intact rat retina and isolated rod outer segments. *Neurochem Res* 14 (2):185–190.
Bokadia, M. M., B. R. Brown, D. Cobern, A. Roberts, and G. A. Somerfield. 1962. 318. Polymerisation of flavans. Part VI. Reduction of flavanoids and chalcones with lithium aluminium hydride in the presence of aluminium chloride. *J Chem Soc* 1962:1658–1666.
Cabrera, C., R. Artacho, and R. Gimenez. 2006. Beneficial effects of green tea—A review. *J Am Coll Nutr* 25 (2):79–99.
Cabrera, C., R. Gimenez, and M. C. Lopez. 2003. Determination of tea components with antioxidant activity. *J Agric Food Chem* 51 (15):4427–4435.
Cavet, M. E., K. L. Harrington, T. R. Vollmer, K. W. Ward, and J. Z. Zhang (2011). Anti-inflammatory and anti-oxidative effects of the green tea polyphenol epigallocatechin gallate in human corneal epithelial cells. *Mol Vis* 17:533–542.
Chan, C. M., J. H. Huang, H. H. Lin, H. S. Chiang, B. H. Chen, J. Y. Hong, and C. F. Hung. 2008. Protective effects of (–)-epigallocatechin gallate on UVA-induced damage in ARPE19 cells. *Mol Vis* 14:2528–2534.
Chen, F., L. Jiang, C. Shen, H. Wan, L. Xu, N. Wang, and J. B. Jonas. 2012. Neuroprotective effect of epigallocatechin-3-gallate against N-methyl-D-aspartate-induced excitotoxicity in the adult rat retina. *Acta Ophthalmol* 90 (8):e609–e615.
Chen, L., M. Lee, H. Li, and C. S. Yang. 1997. Absorption, distribution, and elimination of tea polyphenols in rats. *DMD* 25 (9):1045–1050.
Chen, M. H., C. F. Tsai, Y. W. Hsu, and F. J. Lu. 2014. Epigallocatechin gallate eye drops protect against ultraviolet B-induced corneal oxidative damage in mice. *Mol Vis* 20:153–162.
Chen, R., M. Hollborn, A. Grosche, A. Reichenbach, P. Wiedemann, A. Bringmann, and L. Kohen. 2014. Effects of the vegetable polyphenols epigallocatechin-3-gallate, luteolin, apigenin, myricetin, quercetin, and cyanidin in primary cultures of human retinal pigment epithelial cells. *Mol Vis* 20:242–258.
Chen, Z., Q. Y. Zhu, Y. F. Wong, Z. Zhang, and H. Y. Chung. 1998. Stabilizing effect of ascorbic acid on green tea catechins. *J Agric Food Chem* 46 (7):2512–2516.
Chida, M., K. Suzuki, T. Nakanishi-Ueda, T. Ueda, H. Yasuhara, R. Koide, and D. Armstrong. 1999. In vitro testing of antioxidants and biochemical end-points in bovine retinal tissue. *Ophthalmic Res* 31 (6):407–415.
Chow, H. S., I. A. Hakim, D. R. Vining, J. A. Crowell, J. Ranger-Moore, W. M. Chew, C. A. Celaya et al. 2005. Effects of dosing condition on the oral bioavailability of green tea catechins after single-dose administration of polyphenon E in healthy individuals. *Clin Cancer Res* 11:4627.
Chu, K. O., K. P. Chan, C. C. Wang, C. Y. Chu, W. Y. Li, K. W. Choy, M. S. Rogers, and C. P. Pang. 2010. Green tea catechins and their oxidative protection in the rat eye. *J Agric Food Chem* 58 (3):1523–1534.
Chu, K. O., K. P. Chan, Y. P. Yang, Y. J. Qin, W. Y. Li, S. O. Chan, C. C. Wang, and C. P. Pang. 2015. Effects of EGCG content in green tea extract on pharmacokinetics, oxidative status and expression of inflammatory and apoptotic genes in the rat ocular tissues. *J Nutr Biochem* 26 (11):1357–1367.
Cia, D., J. Vergnaud-Gauduchon, N. Jacquemot, and M. Doly. 2014. Epigallocatechin gallate (EGCG) prevents H2O2-induced oxidative stress in primary rat retinal pigment epithelial cells. *Curr Eye Res* 39 (9):944–952.

Cuevas-Valenzuela, J., A. Gonzales-Rojas, J. Wisniak, A. Apelblat, and J. P. Perez-Correa. 2014. Solubility of (+)-catechin in water and water-ethanol mixtures within the temperature range 277.6–331.2 K: Fundamental data to design polyphenol extraction processes. *Fluid Phase Equilibr* 382:279–285.

Das, N. P. 1971. Studies on flavonoid metabolism: Absorption and metabolism of (+)-catechin in man. *Biochem Pharmacol* 20 (12):3435–3445.

Del Rio, D., L. Calani, F. Scazzina, L. Jechiu, C. Cordero, and F. Brighenti. 2010. Bioavailability of catechins from ready-to-drink tea. *Nutrition* 26 (5):528–533.

Dewick, P. 2002. Chapter 4: The shikimate pathway: Aromatic amino acids and phenylpropanoids. In: *Medicinal Natural Products: A Biosynthetic Approach*, (2nd ed.). New York: John Wiley & Sons, pp. 121–166.

Donovan, J. L., V. Crespy, C. Manach, C. Morand, C. Besson, A. Scalbert, and C. Rémésy. 2001. Catechin is metabolized by both the small intestine and liver of rats. *J Nutr* 131 (6):1753–1757.

Dubey, S., P. Deep, and A. K. Singh. 2015. Phytochemical characterization and evaluation of anticataract potential of seabuckthorn leaf extract. *Vet Ophthalmol* 19 (2):144–148.

Ehrnhoefer, D. E., M. Duennwald, P. Markovic, J. L. Wacker, S. Engemann, M. Roark, J. Legleiter et al. 2006. Green tea (–)-epigallocatechin-gallate modulates early events in huntingtin misfolding and reduces toxicity in Huntington's disease models. *Hum Mol Genet* 15 (18):2743–2751.

Emoto, Y., K. Yoshizawa, Y. Kinoshita, T. Yuri, M. Yuki, K. Sayama, N. Shikata, and A. Tsubura. 2014. Green tea extract suppresses N-methyl-N-nitrosourea-induced photoreceptor apoptosis in sprague-dawley rats. *Graefes Arch Clin Exp Ophthalmol* 252 (9):1377–1384.

Falsini, B., D. Marangoni, T. Salgarello, G. Stifano, L. Montrone, S. Di Landro, L. Guccione, E. Balestrazzi, and A. Colotto. 2009. Effect of epigallocatechin-gallate on inner retinal function in ocular hypertension and glaucoma: A short-term study by pattern electroretinogram. *Graefes Arch Clin Exp Ophthalmol* 247 (9):1223–1233.

Fangueiro, J. F., T. Andreani, L. Fernandes, M. L. Garcia, M. A. Egea, A. M. Silva, and E. B. Souto. 2014. Physicochemical characterization of epigallocatechin gallate lipid nanoparticles (EGCG-LNs) for ocular instillation. *Colloids Surf B Biointerfaces* 123:452–460.

Friedman M., C. E. Levin, S. U. Lee, and N. Kozukue. 2009. Stability of green tea catechins in commercial tea leaves during storage for 6 months. *J Food Sci* 74 (2):H47–H51.

Graham, H. 1992. Green tea composition, consumption, and polyphenol chemistry. *Prev Med* 21 (3):334–350.

Hanneken, A., F. F. Lin, J. Johnson, and P. Maher. 2006. Flavonoids protect human retinal pigment epithelial cells from oxidative-stress-induced death. *Invest Ophthalmol Vis Sci* 47 (7):3164–3177.

Hathway, D. E. 2014. The condensed tannins. In: Hillis, W. E. (Ed.). *Wood Extractives and their Significance to the Pulp and Paper Industries*, (1st ed.). New York, NY: Academic Press. pp. 191–228.

Hathway, D. E. and J. W. T. Seakins. 1957. Enzymic oxidation of catechin to a polymer structurally related to some phlobatannins. *Biochem J* 67 (2):239–245.

Herges, K., J. M. Millward, N. Hentschel, C. Infante-Duarte, O. Aktas, and F. Zipp. 2011. Neuroprotective effect of combination therapy of glatiramer acetate and epigallocatechin-3-gallate in neuroinflammation. *PLoS One* 6 (10):e25456.

Herrmann, K. and L. Weaver. 1999. The shikimate pathway. *Plant Biol* 50:473–503.

Huang, W., S. Li, J. Zeng, Y. Liu, M. Wu, and M. Zhang. 2000. Growth inhibition, induction of apoptosis by green tea constituent (–)-epigallocatechin-3-gallate in cultured rabbit lens epithelial cells. *Yan Ke Xue Bao* 16 (3):194–198.

Hurst, J. W., S. Krake, S. Bergmeier, M. Payne, K. Miller, and D. Stuart. 2011. Impact of fermentation, drying, roasting and Dutch processing on flavan-3-ol stereochemistry in cacao beans and cocoa ingredients. *Chem Cent J* 5:53.

Izawa, K., Y. Amino, M. Kohmura, Y. Ueda, and M. Kuroda. 2010. *Comprehensive Natural Products II: Chemistry and Biology,* Vol. 4, Chemical Ecology, Amsterdam: Elsevier.

Juneja, L. R., T. Okubo, and P. Hung. 2000. Catechins. In: Naidu, A.S. (Ed.). *Natural Food Antimicrobial Systems*, (1st ed.). New York: CRC, pp. 381–398.
Kim, S., M. J. Lee, J. Hong, C. Li, T. J. Smith, G. Y. Yang, D. N. Seril, and C. S. Yang. 2000. Plasma and tissue levels of tea catechins in rats and mice during chronic consumption of green tea polyphenols. *Nutr Cancer* 37 (1):41–48.
Kleinberg, T. T., R. T. Tzekov, L. Stein, N. Ravi, and S. Kaushal. 2011. Vitreous substitutes: A comprehensive review. *Surv Ophthalmol* 56 (4):300–323.
Kofink, M., M. Papagiannopoulos, and R. Galensa. 2007. (–)-Catechin in cocoa and chocolate: Occurrence and analysis of an atypical flavan-3-ol enantiomer. *Molecules* 12 (7): 1274–1288.
Koh, C. H., H. S. Lee, and S. K. Chung. 2014. Effect of topical epigallocatechin gallate on corneal neovascularization in rabbits. *Cornea* 33 (5):527–532.
Kompella, U. B., R. S. Kadam, and V. H. Lee. 2010. Recent advances in ophthalmic drug delivery. *Ther Deliv* 1 (3):435–456.
Lambert, J. D., M. Lee, H. Lu, X. Meng, J. J. J. Hong, D. N. Seril, M. C. Sturgill, and C. S. Yang. 2003. Epigallocatechin-3-gallate is absorbed but extensively glucuronidated following oral administration to mice. *J Nutr* 133 (12):4172–4177.
Lee, H. S., S. K. Chauhan, A. Okanobo, N. Nallasamy, and R. Dana. 2011. Therapeutic efficacy of topical epigallocatechin gallate in murine dry eye. *Cornea* 30 (12):1465–1472.
Lee, H. S., J. H. Jun, E. H. Jung, B. A. Koo, and Y. S. Kim. 2014. Epigalloccatechin-3-gallate inhibits ocular neovascularization and vascular permeability in human retinal pigment epithelial and human retinal microvascular endothelial cells via suppression of MMP-9 and VEGF activation. *Molecules* 19 (8):12150–12172.
Lee, S. M., I. G. Ko, S. E. Kim, D. H. Kim, and B. N. Kang. 2010. Protective effect of catechin on apoptosis of the lens epithelium in rats with N-methyl-N-nitrosourea-induced cataracts. *Korean J Ophthalmol* 24 (2):101–107.
Lei, L., R. Tzekov, J. H. McDowell, W. C. Smith, S. Tang, and S. Kaushal. 2013. Formation of lipofuscin-like material in the RPE Cell by different components of rod outer segments. *Exp Eye Res* 112:57–67.
Lewis, J. and R. Starkey. 1969. Decomposition of plant tannins by some soil microorganisms. *Soil Sci* 107 (4):235–241.
Lhoste, E. F., V. Ouriet, S. Bruel, J. P. Flinois, C. Brézillon, J. Magdalou, C. Chéze, and L. Nugon-Baudon. 2003. The human colonic microflora influences the alterations of xenobiotic-metabolizing enzymes by catechins in male F344 rats. *Food Chem Toxicol* 41 (5):695–702.
Li, C. P., J. Yao, Z. F. Tao, X. M. Li, Q. Jiang, and B. Yan. 2013. Epigallocatechin-gallate (EGCG) regulates autophagy in human retinal pigment epithelial cells: A potential role for reducing UVB light-induced retinal damage. *Biochem Biophys Res Commun* 438 (4):739–745.
Lin, J. 2006. Catechins and inhibitory activity against carcinogenesis. In: Awad, A. B. and Bradford, P. G. (Eds.). *Nutrition and Cancer Prevention*, (1st ed.). Boca Raton, FL: CRC Press, pp. 351–368.
Lipinski, C. A., F. Lombardo, B. W. Dominy, and P. J. Feeney. 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* 46 (1–3):3–26.
Mahadevan, A. and S. N. Sivaswamy. 1985. In: Mukerji, K. G. and Singh, V. P. (Eds.). *Frontiers in Applied Microbiology*, (1st ed.). Lucknow, India: Print House India, pp. 327–347.
Mata-Bilbao, M. L., C. Andrés-Lacueva, E. Roura, O. Jáuregui, E. Escribano, C. Torre, and R. M. Lamuela-Raventós. 2008. Absorption and pharmacokinetics of green tea catechins in beagles. *Br J Nutr* 100 (3):496–502.
Matsuda, M., Y. Otsuka, S. Jin, J. Wasaki, J. Watanabe, T. Watanabe, and M. Osaki. 2007. Biotransformation of (+)-catechin into taxifolin by a two-step oxidation: Primary stage of (+)-catechin metabolism by a novel (+)-catechin-degrading bacteria, *Burkholderia* sp. KTC-1, isolated from tropical peat. *Biochem Biophys Res Commun* 366 (2):414–419.

Meng, X., S. Sang, N. Zhu, H. Lu, S. Sheng, M. J. Lee, C. T. Ho, and C. S. Yang. 2002. Identification and characterization of methylated and ring-fission metabolites of tea catechins formed in humans, mice, and rats. *Chem Res Toxicol* 15 (8):1042–1050.

Mereles, D. and W. Hunstein. 2011. Epigallocatechin-3-gallate (EGCG) for clinical trials: More pitfalls than promises? *Int J Mol Sci* 12 (9):5592–5603.

Meselhy, M. R., N. Nakamura, and M. Hattori. 1997. Biotransformation of (–)-epicatechin 3-O-gallate by human intestinal bacteria. *Chem Pharm Bull* (*Tokyo*) 45 (5):888–893.

Molokhia, S. A., S. C. Thomas, K. J. Garff, K. J. Mandell, and B. M. Wirostko. 2013. Anterior eye segment drug delivery systems: Current treatments and future challenges. *J Ocul Pharmacol Ther* 29 (2):92–105.

Muthenna, P., G. Raghu, C. Akileshwari, S. N. Sinha, P. Suryanarayana, and G. B. Reddy. 2013. Inhibition of protein glycation by procyanidin-B2 enriched fraction of cinnamon: Delay of diabetic cataract in rats. *IUBMB Life* 65 (11):941–950.

Muthukumar, G., A. Arunakumari, and A. Mahadevan. 1982. Degradation of aromatic compounds by *Rhizobium* spp. *Plant Soil* 69:163–169.

Osman, A. M., K. K. Y. Wong, and A. Fernyhough. 2007. The laccase/ABTS system oxidizes (+)-catechin to oligomeric products. *Enzyme Microb Technol* 40 (5):1272–1279.

Park, Y., M. Jeon, H. Hwang, M. Park, S. Lee, S. Kim, and M. Kim. 2011. Antioxidant activity and analysis of proanthocyanidins from pine (Pinus densiflora) needles. *Nutr Res Pract* 5 (4):281–287.

Payne, M. J., W. J. Hurst, K. B. Miller, C. Rank, and D. A. Stuart. 2010. Impact of fermentation, drying, roasting, and Dutch processing on epicatechin and catechin content of cacao beans and cocoa ingredients. *J Agric Food Chem* 58 (19):10518–10527.

Peng, P. H., L. F. Chiou, H. M. Chao, S. Lin, C. F. Chen, J. H. Liu, and M. L. Ko. 2010. Effects of epigallocatechin-3-gallate on rat retinal ganglion cells after optic nerve axotomy. *Exp Eye Res* 90 (4):528–534.

Peters, C. M., R. J. Green, E. M. Janle, and M. G. Ferruzzi. 2010. Formulation with ascorbic acid and sucrose modulates catechin bioavailability from green tea. *Food Res Int* 43 (1): 95–102.

Robinson, T., A. W. Ranalli, and A. W. Phillips. 1961. Cocoa polyphenols, changes in cocoa tannins during processing. *J Agric Food Chem* 9 (4):295–298.

Rodrigues, C. F., K. Ascencao, F. A. Silva, B. Sarmento, M. B. Oliveira, and J. C. Andrade. 2013. Drug-delivery systems of green tea catechins for improved stability and bioavailability. *Curr Med Chem* 20 (37):4744–4757.

Sasikala, V., B. N. Rooban, S. G. Priya, V. Sahasranamam, and A. Abraham. 2010. Moringa oleifera prevents selenite-induced cataractogenesis in rat pups. *J Ocul Pharmacol Ther* 26 (5):441–447.

Schantz, M., T. Erk, and E. Richling. 2010. Metabolism of green tea catechins by the human small intestine. *Biotechnol J* 5 (10):1050–1059.

Shen, C., L. Chen, L. Jiang, and T. Y. Lai. 2015. Neuroprotective effect of epigallocatechin-3-gallate in a mouse model of chronic glaucoma. *Neurosci Lett* 600:132–136.

Shibuya, H., A. Agusta, K. Ohashi, S. Maehara, and P. Simanjuntak. 2005. Biooxidation of (+)-catechin and (–)-epicatechin into 3,4-dihydroxyflavan derivatives by the endophytic fungus *diaporthe* sp. isolated from a tea plant. *Chem Pharm Bull* 53 (7): 866–867.

Singh, V. P. 2002. Biotransformations of tannery wastes. In: Stapleton, R. D. and Singh, V. P. (Eds.). *Biotransformations: Bioremediation Technology for Health and Environmental Protection*, (1st ed.). New York, NY: Elsevier, pp. 279–290.

Siu, A. W., M. K. Lau, J. S. Cheng, C. K. Chow, W. C. Tam, K. K. Li, D. K. Lee, T. S. To, C. H. To, and C. W. Do. 2008. Glutamate-induced retinal lipid and protein damage: The protective effects of catechin. *Neurosci Lett* 432 (3):193–197.

Srinivas, K., J. W. King, L. R. Howard, and J. K. Monrad. 2010. Olubility of gallic acid, catechin, and protocatechuic acid in subcritical water from (298.75 to 415.85) K. *J Chem Eng Data* 55 (19):3101–3108.

Stoddard, A. R., L. R. Koetje, A. K. Mitchell, M. P. Schotanus, and J. L. Ubels. 2013. Bioavailability of antioxidants applied to stratified human corneal epithelial cells. *J Ocul Pharmacol Ther* 29 (7):681–687.

Sun, M., S. C. Finnemann, M. Febbraio, L. Shan, S. P. Annangudi, E. A. Podrez, G. Hoppe et al. 2006. Light-induced oxidation of photoreceptor outer segment phospholipids generates ligands for CD36-mediated phagocytosis by retinal pigment epithelium: A potential mechanism for modulating outer segment phagocytosis under oxidant stress conditions. *J Biol Chem* 281 (7):4222–4230.

Tzekov, R., L. Stein, and S. Kaushal. 2011. Protein misfolding and retinal degeneration. *Cold Spring Harb Perspect Biol* 3 (11):a007492.

Van het Hoff, K. H., G. A. Kivits, J. A. Weststrate, and L. B. Tijburg. 1998. Bioavailability of catechins from tea: The effect of milk. *Eur J Clin Nutr* 52 (5):356–359.

Wang, S., G. Birol, E. Budzynski, R. Flynn, and R. Linsenmeier. 2010. Metabolic responses to light in monkey photoreceptors. *Curr Eye Res* 35 (6):510–518.

Warden, B., L. S. Smith, G. R. Beecher, D. A. Balentine, and B. A. Clevidence. 2001. Catechins are bioavailable in men and women drinking black tea throughout the day. *J Nutr* 131 (6):1731–1737.

Wu, Z., X. Li, Z. Liu, Z. Xu, and J. Zhuang. 2014. De novo assembly and transcriptome characterization: Novel insights into catechins biosynthesis in Camellia sinensis. *BMC Plant Biology* 14:277.

Xie, J., L. Jiang, T. Zhang, Y. Jin, D. Yang, and F. Chen. 2010. Neuroprotective effects of Epigallocatechin-3-gallate (EGCG) in optic nerve crush model in rats. *Neurosci Lett* 479 (1):26–30.

Yang, S. W., B. R. Lee, and J. W. Koh. 2007. Protective effects of epigallocatechin gallate after UV irradiation in cultured human retinal pigment epithelial cells. *Korean J Ophthalmol* 21 (4):232–237.

Yao, J., Y. Liu, X. Wang, Y. Shen, S. Yuan, Y. Wan, and Q. Jiang. 2009. UVB radiation induces human lens epithelial cell migration via NADPH oxidase-mediated generation of reactive oxygen species and up-regulation of matrix metalloproteinases. *Int J Mol Med* 24 (2):153–159.

Yashin, A., B. Nemzer, and Y. Yashin. 2012. Bioavailability of tea components. *J Food Res* 1 (2):281–290.

Ye, J. J., K. Frenkel, and J. A. Zadunaisky. 1992. Lens opacification and H2O2 elevation induced by a tumor promoter. *Lens Eye Toxic Res* 9 (1):37–48.

Zaprometov, M. N. 2012. Proanthocyanidins and catechins. In: Vasil, I. and Constabel, F. (Eds.). *Cell Culture and Somatic Cell Genetics of Plants*, Vol. 5, San Diego, CA: Elsevier. pp. 77–88.

Zhang, B. and N. N. Osborne. 2006. Oxidative-induced retinal degeneration is attenuated by epigallocatechin gallate. *Brain Res* 1124 (1):176–187.

Zhang, B., R. Safa, D. Rusciano, and N. N. Osborne. 2007. Epigallocatechin gallate, an active ingredient from green tea, attenuates damaging influences to the retina caused by ischemia/reperfusion. *Brain Res* 1159:40–53.

Zhang, B., D. Rusciano, and N. N. Osborne. 2008. Orally administered epigallocatechin gallate attenuates retinal neuronal death in vivo and light-induced apoptosis in vitro. *Brain Res* 1198:141–152.

Zhu, M., Y. Chen, and R. C. Li. 2000. Oral absorption and bioavailability of tea catechins. *Planta Med* 66 (5):444–447.

19

Antioxidant Nutraceuticals for the Prevention and Treatment of Acute and Chronic Lung Diseases

*Current Trends and Future Prospects**

Manish Bodas, Garrett Pehote, and Neeraj Vij†

Contents

* Manish Bodas and Garrett Pehote have equally contributed to this chapter.

† Correspondence: Dr. Neeraj Vij, Email: vijlaboratory@gmail.com.

19.1 Introduction

The respiratory system is one of the critical organ systems in our body that performs the life-supporting role of providing oxygen and eliminating the toxic carbon dioxide (Santus et al. 2014). The extraordinarily large surface area of the lungs renders them highly prone to being exposed to a variety of prooxidants and toxic agents (Santus et al. 2014). The airways are knowingly or unknowingly being bombarded with environmental gaseous and particulate matter present in air pollution (Liu et al. 2009; Valavanidis et al. 2013), tobacco smoke (Valavanidis et al. 2009), or occupational settings (Kaushik et al. 2012) leading to generation of inflammation and oxidative stress. Thankfully, our lungs are fairly robust, as they are capable of managing a certain threshold level of inflammatory–oxidative stress that prevents or delays the onset of acute and chronic pulmonary diseases *via* a healthy antioxidant defense mechanism (Comhair and Erzurum 2002). A breach of this critical threshold leads to an imbalance of prooxidants and antioxidant factors resulting in unabated inflammatory–oxidative stress that forms the basis of numerous debilitating lung conditions such as acute lung injury (ALI), acute respiratory distress syndrome (ARDS), bronchopulmonary dysplasia (BPD), asthma, chronic bronchitis, chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), and idiopathic pulmonary fibrosis (IPF) (Park et al. 2009; Holguin 2013). The leading deleterious agents known to cause oxidative damage are the reactive oxygen species (ROS) and reactive nitrogen species (RNS), both contributing to initiation and progression of various lung diseases (Holguin 2013). Moreover, numerous types of inflammatory cells that infiltrate the pulmonary vasculature upon any external insult are activated by the cell surface receptors–ligand interaction (Lao et al. 2014). These ligands include secreted factors such as cytokines (Lao et al. 2014), chemokines (Lao et al. 2014), and the proteases–antiproteases (Bodas et al. 2012) that regulate pulmonary immune responses. The activated immune cells are also the major source of the ROS/RNS (Domej et al. 2014; Lao et al. 2014). Once generated, these ROS/RNS undergo complex chemical interactions with biomolecules and inflict oxidative damage to proteins, DNA, and/or lipids (Domej et al. 2014), thus targeting key cellular homeostatic processes such as cell proliferation (Matés et al. 2008), protease/antiprotease balance (Bodas et al. 2012), inflammation and proteostasis/autophagy (Holguin 2013), and so on. The perturbations in these critical cellular

mechanisms contribute to initiation and progression of pathophysiological changes leading to lung diseases (Comhair and Erzurum 2002; Holguin 2013). Thus, subduing the elevated inflammatory–oxidative stress forms the basis of numerous therapeutic strategies utilized for management of several lung disorders, where there is a clear and urgent need to address the progression of acute and chronic lung diseases (Lao et al. 2014). Although various therapeutic compounds are being developed, they have significant side effects (Kawada et al. 2012). Thus, bringing major transformations in our regular diet (lifestyle) supplemented with *nutraceuticals*—based on prevention and treatment of lung diseases—seems promising (Sharafkhaneh et al. 2007; Sorkness 2009). This strategy may well become the choice of both medical fraternity and the common man, aptly supported by the intriguing understanding of the gut–lung axis and its role in modulating immune responses (Marsland et al. 2015).

Although the term *nutraceutical* was coined by Stephen DeFelice in 1989 (Chauhan et al. 2013), the fundamental idea comes from ancient traditional knowledge where *food* was considered to possess immense preventive and therapeutic values. A quote from an ancient Indian text, Charaka Samhita says that "*It is the wholesome use of food that promotes the health of a person, and that which is unwholesome is the cause of disease*." Intriguingly, the same text also highlights the currently neglected fact that majority of our health problems arise due to the kind of food stuff we put inside our bodies. Thus, in Ayurveda, the traditional Indian science of medicine, food is considered divine and more powerful than just nourishment, and forming the essence of your body and mind, whether *sick* or *healthy*, is entirely one's choice. During the last century, the scarcity of time to prepare or consume wholesome meals has led to the exponential rise of *fast/convenience foods* that is leading to diminished use of foods with high nutritional value resulting in an exponential increase in poor lifestyle-related diseases (Wickens et al. 2005). In addition, the unrestricted use of antibiotics and chemical compounds (with a longer list of side effects than its benefits) for medical treatment was like adding *fuel to the fire*! (Dahl 2006; Carter et al. 2014; Barnes 2015; Blair et al. 2015). After years of suffering, we are slowly realizing the benefits of maintaining a healthy and disease-free lifestyle, which has led to the advent of *natural dietary supplements* and *nutraceuticals*. The term *nutraceutical* is an amalgamation of *nutrition* (indicating a nourishing-food or food-component) and *pharmaceutical* (with reference to a drug; Chauhan et al. 2013). Any product of either animal or plant origin that has a beneficial pharmaceutical effect apart from its nutritional value is identified as a *nutraceutical*. On the other hand, dietary supplements may provide beneficial health effects if included as a part of the daily diet, albeit they lack any proven pharmacological health effect (Kofoed et al. 2015). There is a worldwide escalation in the use of nutraceuticals in prevention (proactive medicine) and treatment of various chronic disease conditions (Santini et al. 2016). On the other hand, an alarming increase in the global burden of acute and chronic respiratory diseases, such as COPD, asthma, lower respiratory tract infections, and so on

(Ferkol and Schraufnagel 2014; Burney et al. 2015), suggests an urgent need to assess the currently available and prospective nutraceuticals that can assist in the prevention and treatment of these life-threatening respiratory conditions. Although the currently available nonnatural drugs for the treatment of airway diseases are quite effective, they have adverse side effects that prohibit their extended use for treating prolonged or lifelong chronic conditions (Dahl 2006; Karbasi-Afshar et al. 2014). Thus, this chapter aims to compile the current knowledge on the application of nutraceuticals for prevention and treatment of inflammatory–oxidative stress-mediated acute and chronic lung disease states. Our long-term goal should be to drastically reduce or eliminate the use of nonnatural drugs or chemicals as human therapeutics.

19.2 Role of poor lifestyle-related inflammatory–oxidative stress and pulmonary disease pathogenesis

19.2.1 Generation of inflammatory–oxidative stress in the lungs

There are many factors that may lead to generation of inflammatory–oxidative stress in the lungs with poor lifestyle and environmental exposures being the prominent one (Barnes et al. 2003). The major mediator of this inflammatory–oxidative stress is ROS that can be either generated endogenously within the cell or introduced into our airways upon exposure to external agents such as cigarette smoke (CS; Valavanidis et al. 2009) or air pollutants, which contain high levels of gaseous oxidants. Within the lung or deep airway, the stimulus-induced activation of inflammatory and/or other cells such as macrophages, neutrophils, eosinophils, lymphocytes, and epithelial/endothelial cells can lead to the production of superoxide ($O_2^{\bullet -}$), which is quickly converted to H_2O_2 by the superoxide dismutase (SOD) enzyme (Park et al. 2009). While this happens, the hydroxyl radical ($^{\bullet}OH$) is formed as a secondary reaction with ferrous ion (Fe^{2+}) (Park et al. 2009). These reactive molecules produced by these cells are a major cause of cellular oxidative stress. The major intracellular sources of ROS include mitochondrial respiration, cytochrome P-450, and the NADPH oxidase enzyme complex, and so on, which can all be modulated by poor lifestyle or environmental exposures further exacerbating the lung condition (Park et al. 2009), all of which contribute to the pathogenesis of inflammatory–oxidative stress-related lung diseases.

19.2.2 Tobacco smoking as the primary cause of pulmonary oxidative stress

Exposure to tobacco smoke is the primary cause of inflammatory–oxidative stress that is evident in a variety of chronic inflammatory and/or obstructive lung conditions such as asthma, IPF, and COPD. CS is composed of numerous chemicals and toxins that trigger inflammatory–oxidative stress (Church and Pryor 1985; D'hulst et al. 2005; Rahman and Adcock 2006; Faux et al. 2009;

Wu et al. 2014; Zuo et al. 2014). Briefly, CS exposure-induced ROS activates proinflammatory cytokines and chemokines that help with the recruitment of inflammatory cells, further increasing the inflammatory response in the airways (Zuo et al. 2014). It is well known that lungs of cigarette smokers have high levels of oxidative stress than their nonsmoking counterparts as a result of the large amounts of free radicals found in the gaseous components of CS (Zuo et al. 2014). Both acute and chronic CS exposures have also been shown to activate leukocytes that further increase ROS production in the airways (Zuo et al. 2014). Thus, excessive ROS produced through CS exposure cannot be neutralized by the lungs antioxidant-system due to the oxidant/antioxidant imbalance, which has been implicated in the pathogenesis of inflammatory–oxidative stress-related lung disorders such as COPD-emphysema (Zuo et al. 2014).

19.2.3 Environmental and occupational exposures affecting airway health

Similar to first-hand CS exposure, other environmental or occupational exposures are the important sources of toxicant-induced airway diseases (Hnizdo 2010; Jordan et al. 2011; Goldklang et al. 2013; Kostikas et al. 2013; Poljšak and Fink 2014; Banderali et al. 2015; Bono et al. 2015; Vieira 2015).

As described earlier, CS is the primary cause of oxidative stress in the lungs. Even if a person is not an active smoker, he or she can be at a significant risk of developing CS-induced inflammatory–oxidative stress-related lung dysfunction by being passively exposed to second-hand cigarette smoke (SHS). In fact, SHS is a prominent risk factor for the development of COPD in people who do not smoke (Goldklang et al. 2013; Kostikas et al. 2013). In a study by Kostikas et al. (2013) on the effects of SHS on the airway, it was found that there was an increased amount of oxidative stress in the airway presented by an increase in H_2O_2 levels in the exhaled breath of participants. Along with the increase in oxidative stress, SHS exposure was shown to induce the inflammatory processes by recruiting and activating proinflammatory immune cells (Kostikas et al. 2013). These inflammatory cells generate ROS and thus augment the oxidative burden in the lungs leading to COPD-emphysema initiation and progression.

Acute and chronic exposures to environmental pollution also promote oxidative stress in the lungs. Various noxious particles in the polluted air have been shown to exhibit physiochemical and toxicological properties that can have negative effects on human health (Bono et al. 2015). These pollutants have been shown to trigger ROS-mediated oxidative stress (Poljšak and Fink 2014), which is shown to exacerbate a variety of respiratory conditions (**Figure 19.1**). These results displayed that there was increased signs of oxidative stress in the airways of nonsmoking youths in urban environments suggesting the impact of air pollutants on airway.

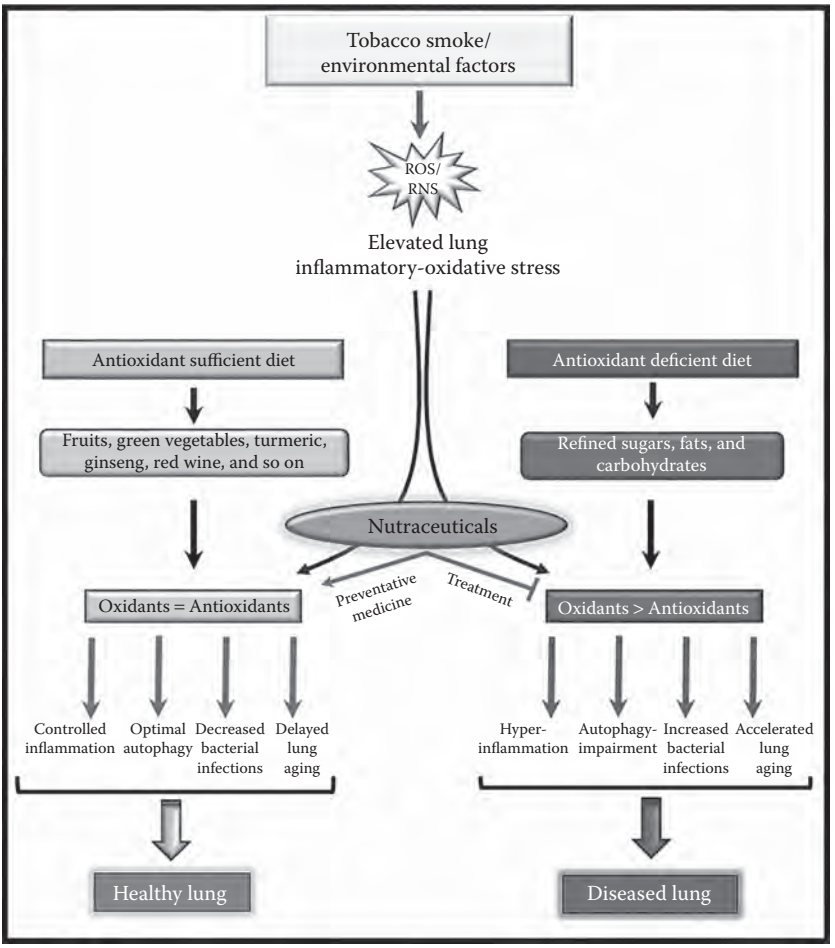


Figure 19.1 *Role of balanced diet and nutraceuticals in lung health. Our lungs are constantly being exposed to toxic agents from several environmental factors or tobacco smoke. This leads to generation of reactive molecules, which collectively trigger the inflammatory–oxidative stress response in the airways. An antioxidant sufficient diet helps in maintaining the balance of oxidants and antioxidants and thus protects the lung from inflammatory–oxidative stress-related outcomes, thus maintaining a healthy lung. Moreover, consumption of antioxidant nutraceuticals can be of additional advantage in maintaining the oxidant–antioxidant balance and thus acting as preventive medicine to protect the lung against inflammatory–oxidative stress. Conversely, intake of an antioxidant insufficient diet shifts the balance toward higher oxidant levels leading to inflammatory–oxidative stress-related pathologies and a diseased lung. Thus, nutraceuticals could be beneficial in treating acute and chronic lung diseases, but the consumption of a balanced antioxidant sufficient diet along with a watchful intake of essential nutraceuticals is the best way to prevent the advent of inflammatory–oxidative stress-related respiratory disorders and thus sustaining optimal lung function.*

Occupational exposure to toxic agents can similarly promote airway oxidative stress. Briefly, agricultural workers are repeatedly exposed to germicides and pesticides that have a role in increasing oxidative stress through increased ROS production (Poljšak and Fink 2014). This suggests that agricultural, industrial, or housekeeping workers have the increased risk for oxidative stress-related lung conditions. It has been shown that pesticides, cleaning supplies, or industrial resins can produce an increased oxidative burden through the production of ROS and lipid peroxidation (Hnizdo 2010; Poljšak and Fink 2014; Vieira 2015), thus acting as an occupational initiator of lung disease.

19.2.4 Poor lifestyle-related accelerated lung aging and airway dysfunction

Toxic inhalants are not the only source of pulmonary oxidative stress but they can also accelerate the normal aging process that is linked to proteostasis/autophagy decline-related imbalance in the redox mechanisms, which exacerbate oxidative stress (Chandrasekaran et al. 2016; Davalli et al. 2016; Kurundkar and Thannickal 2016). Thus, these mechanisms lead to the age-related increases in ROS (Stadtman 2002; Chandrasekaran et al. 2016; Davalli et al. 2016; Kurundkar and Thannickal 2016), and progressive lung function declines. It is now well appreciated that COPD is a disease of accelerated lung aging (Ito and Barnes 2009; Mercado et al. 2015; Kuwano et al. 2016), and there is an increased prevalence of COPD in the aging population (Ito and Barnes 2009; Mercado et al. 2015; Fragoso 2016). Both aging and CS exposure are implicated in the generation of higher than normal amount of ROS that eventually impacts lung health through its widespread deleterious effects on the lung homeostatic machinery such as proteostasis or autophagy (Chandrasekaran et al. 2016; Kurundkar and Thannickal 2016; Kuwano et al. 2016; Yue and Yao 2016). In fact, ROS can trigger cellular senescence mechanisms that facilitate the progression of chronic lung diseases (Choudhury and MacNee 2016; Kurundkar and Thannickal 2016; Kuwano et al. 2016). Mechanistically, the poor lifestyle-related accelerated aging process can lead to ROS-mediated DNA damage (Choudhury and MacNee 2016; Davalli et al. 2016), mitochondrial dysfunction (Hoffmann et al. 2013; Aravamudan et al. 2014; Davalli et al. 2016), proteostasis/autophagy impairment (Mercado et al. 2015; Li et al. 2016; Tai et al. 2017), and cellular senescence (Hwang et al. 2013; Mercado et al. 2015). In fact, ROS induces a decrease in SIRT1 expression and/or activity suggesting accelerated aging (Hwang et al. 2013; Poulose and Raju 2015). Briefly, SIRT1 is a histone deacetylase that regulates the expression of genes involved in the antioxidant response, inflammation, and autophagy, and ROS-mediated decreased activity of SIRT1 provokes inflammatory–oxidative stress and autophagy impairment that accelerates lung aging (Hwang et al. 2013). The aging-associated increase in ROS is also considered to play a crucial role in the pathogenesis of other chronic lung diseases such as ALI, ARDS, and IPF (Leung et al. 2015; Schouten et al. 2015; Kling et al. 2016).

19.2.5 Improper diet and oxidative stress

Eating food is essential for living or survival; thus, the food we eat has a direct impact on our health. In general, poor eating habits or lifestyle can initiate acute inflammatory–oxidative or even apoptotic responses. Moreover, our lungs can also be affected by our dietary habits, and studies have shown that diets with high levels of refined starches, sugars (especially high-fructose corn syrup), and fats or diets that are low in fruits and vegetables promote systemic inflammatory–oxidative stress (Figure 19.1) that can result in the initiation and/or aggravation of chronic lung diseases such as asthma, IPF, and COPD (Yu et al. 2013; Agrawal and Prakash 2014; Singh et al. 2015; Baffi et al. 2016; Li et al. 2016). Improper diet is also directly correlated with an alarming increase in the incidences of obesity and metabolic syndrome (MetS) (Bjorklund and Chirumbolo 2017; Rani et al. 2016), both of which are now also considered as important risk factors for asthma in children and adults (Agrawal and Prakash 2014; Garmendia et al. 2014; Serafino-Agrusa et al. 2015; Baffi et al. 2016). In mice, a maternal high-fat diet during lactation induces early-onset obesity, hyperinsulinemia, and asthma-like disease in the offsprings (Dinger et al. 2016; Johnson et al. 2017). Moreover, mice fed with a high-fat or high-fructose diet also demonstrate features of MetS along with increased oxo-nitrative stress and asthma-like complications in the lungs (Singh et al. 2015), thus providing experimental evidence to the clinical findings that correlate MetS with decreased lung function and asthma-like phenotype (Thuesen et al. 2009; Singh et al. 2016).

Another thought-provoking aspect of diet and lung health comes from the recent studies on the *gut–lung axis*, which demonstrates that the gut microbiota could impact lung immunity and the pulmonary response to environmental allergens or toxins (Samuelson et al. 2015). Thus, oxidative stress-mediated gut microbiota impairment can influence the pathogenesis of MetS and lung diseases such as asthma, pulmonary infections, septic shock, ALI, ARDS, and COPD (Noval Rivas et al. 2016; Young et al. 2016). Thus, the maintenance of a healthy gut microbiota seems to be crucial for prevention of acute and chronic lung conditions, and the best approach to achieve this is to maintain a healthy antioxidant/inflammatory-rich diet or utilizing nutraceuticals when disease is developed.

19.3 Pathogenesis of lifestyle-related respiratory diseases and their control by antioxidant nutraceuticals

19.3.1 Role of nutraceuticals in acute lung injury and acute respiratory distress syndrome

A poor lifestyle may hamper normal lung functions such as gaseous exchange that leads to an inadequate amount of oxygen reaching the peripheral organ systems, which forms the basis of ALI/ARDS pathogenesis. In the event of a

severe insult such as infections, major injuries, and trauma, the pulmonary edema fluid starts to build up in the air sacs or alveoli that impairs the respiration process, leading to low oxygen levels in the blood and eventually multiple organ failure or death in very severe cases. Although both these diseases have similar pathology, ARDS is considered a more severe form of ALI (Lesur et al. 1999; Ware and Matthay 2000; Chow et al. 2003; Castillo et al. 2015), as it results in severe endothelial damage resulting from pulmonary edema fluid that enters the airways as a consequence of the increased capillary permeability (Goldenberg et al. 2011; Castillo et al. 2015). This, in conjunction with epithelial damage, prevents the fluid from being expelled from the alveoli and thus results in elevated levels of cytokines/chemokines that promote inflammation and recruitment of neutrophils (Tyrrell et al. 2012; Castillo et al. 2015). The activation of these proinflammatory cells leads to intense inflammation and oxidative stress in the airway that leads to the pathogenesis of ALI and ARDS (Chow et al. 2003; Bhatia et al. 2012; Castillo et al. 2015). Briefly, these activated immune cells generate potentially harmful metabolites, such as proteolytic enzymes (neutrophil elastase) and reactive oxygen/nitrogen (ROS/RNS) species, that eventually lead to pulmonary epithelial and endothelial cell damage. Moreover, heightened oxidative stress further activates NF-κB that augments the expression of several proinflammatory cytokines (Chow et al. 2003; Guo and Ward 2007; Castillo et al. 2015). In addition, the reaction of nitric oxide (NO) and superoxide (O_2^-) leads to the formation of peroxynitrite, the most notorious oxidative species that mediates the oxidation and nitration of crucial amino acids in numerous lung proteins such as surfactant protein A. This posttranslational modification diminishes the functions of these critical proteins, thus contributing to the pulmonary dysfunction associated with ALI and ARDS (Park et al. 2009). As anticipated, decreased levels of key protective antioxidants such as glutathione (GSH), superoxide dismutase (SOD), and catalase are reported in ALI/ARDS subjects, which is indicative of an imbalance in prooxidant and antioxidant systems.

Therefore, antioxidant nutraceuticals are being utilized in improving the suffering of critically ill ALI and ARDS patients. Briefly, Berberine is an active component of the Chinese herb *Rhizoma Coptidis* and is known to possess both antioxidant and anti-inflammatory activities (Gao et al. 2014). It was recently reported that Berberine has the potential to protect against ALI/ARDS through its inhibitory activity on tissue factor (TF) protein that is involved in the pathogenesis of ALI/ARDS (Gao et al. 2014). Similarly, fisetin (3,7,3′,4′-tetrahydroxyflavone) is another potent anti-inflammatory/antioxidant molecule, which is a flavonol, a structurally different chemical that belongs to the flavonoid group of polyphenols (Geraets et al. 2009; Feng et al. 2016). A recent study shows that lipopolysaccharide (LPS)-induced ALI could be controlled by fisetin treatment (Geraets et al. 2009), thus warranting its further evaluation in protecting ALI/ARDS in human subjects. Another promising nutraceutical candidate for ALI/RDS treatment is Tanreqing (TRQ), a water-soluble extract from five traditional Chinese medicines (Rogerio et al. 2016).

Table 19.1 Nutraceuticals for Prevention and Treatment of Respiratory Diseases

Respiratory Disease	Nutraceuticals Tested	Models Used[a]	Prospective Nutraceuticals That Hold Promise
ARDS	Berberine	IV	Broccoli
	Emodin	AM (mice)	Curcumin
	Fisetin	AM (rat, mice)	*Picrorhiza kurroa*
	Magnolia	IV	*Tylophora asthmatica*
	Sanguinarine	AM (mice)	
	Tylophora asthmatica	IV	
Asthma	ASHMI	IV, AM (mice), CT	Broccoli
	Ding Chuan Tang (DCT)	CT	Curcumin
	Fisetin	IV, AM (mice)	Emodin
	Kanakasava	AM (rat)	*Tylophora asthmatica*
	Magnolia	IV	
	Picrorhiza kurroa	IV	
	Pycnogenol	CT	
	Resveratol	IV	
	Saiboku-to (TJ-96)	CT	
Bronchopulmonary dysplasia	Vitamin A	CT	
	Zinc	CT	
Cystic fibrosis	Curcumin	IV	Garlic
	Ginseng	IV, AM (rat)	
	Piceatannol	IV	
COPD	Berberine	AM (mice)	Curcumin
	Emodin	AM (mice)	*Tylophora asthmatica*
	Fisetin	CT	
	Ginseng	CT	
	Magnolia	IV	
	Picrorhiza kurroa	IV	
	Resveratol	IV	
	Sulforaphane	IV, AM (mice)	
Pulmonary fibrosis	Berberine	AM (rat)	Broccoli
	Emodin	AM (rat, mice)	Curcumin

[a] IV, *in vitro*; AM, animal; CT, clinical trial.

TRQ protects rats from LPS-induced ALI by reducing the levels of inflammatory–oxidative stress mediators, thus showing potential benefits in the treatment of ALI/ARDS (Rogerio et al. 2016). Although these studies indicate that some nutraceuticals are currently being tested for ALI/ARDS as shown in **Table 19.1**, they are not very successful in some groups of patients (Rice et al. 2011; Zhu et al. 2014), and thus more stringent multicenter clinical studies are warranted to devise strategies for proper dosing and administration to attain widespread application in a diverse population.

19.3.2 Poor maternal nutrition and bronchopulmonary dysplasia: Can nutraceuticals help?

Bronchopulmonary dysplasia (BDP) is a chronic lung disease that affects premature infants who undergo artificial ventilation (Saugstad 2003; Perrone et al. 2012). It is well accepted that maternal nutrition plays a crucial role in overall fetal development, including the lungs, and an unhealthy diet during pregnancy may lead to premature birth, inviting the onset of BPD in the newborn (Bloomfield 2011). The characteristic feature of BDP is impaired lung development and the remodeling of lung tissue that involves distorted alveolarization and impaired vasculogeneis (Shahzad et al. 2016). Briefly, exposure to high oxygen levels during mechanical ventilation (oxygen toxicity) leads to heightened inflammatory–oxidative stress that plays a prominent role in the pathogenesis of BDP (Saugstad 2003; Perrone et al. 2012). The developing immature lung is highly prone to oxidative insults due to the following primary reasons: hypoxic–hyperoxic challenge at birth, infections related to incomplete development of immune system, lack of antioxidants, and high levels of free iron that leads to production of highly reactive species such as superoxide (O_2^-) and hydroxyl radical (OH) (Gitto et al. 2009; Ozsurekci and Aykac 2016). This elevated oxidative stress leads to activation of NF-κB (Saugstad 2003), which triggers the induction of proinflammatory mediators. The inflammatory response in BPD is mediated by an increase in proinflammatory cytokines such as IL-1β, IL-6, IL-8, TNFα, and monocyte chemoattractant proteins and a parallel decrease in anti-inflammatory factors such as IL-10 and normal alveolar and vascular growth factors (Shahzad et al. 2016). These inflammatory cytokines along with continuing oxidative stress recruit and activate the inflammatory cells, primarily alveolar macrophages and neutrophils (Perrone et al. 2012; Shahzad et al. 2016), which further release proinflammatory cytokines and proteases that result in severe tissue damage *via* lipid peroxidation, increased vascular permeability, surfactant inactivation, and pulmonary edema in the airways along with alveolar cell death (Perrone et al. 2012; Shahzad et al. 2016; Ozsurekci and Aykac 2016).

Mechanistically, an imbalance of proteinases and antiproteinases is reported in BPD patients, which when coupled with high oxygen level-induced oxidative stress increases the expression of tissue destructive enzymes such as matrix metalloproteins (MMPs), elastase, cysteine protease, and trypsin that play a pathogenic role in the BPD-related epithelial and alveolar damage leading to pulmonary-dysfunction (Saugstad 2003). Moreover, inflammatory–oxidative stress also induces transforming growth factor-β (TGFβ) levels, which is an important mediator of fibroblast/myofibroblast proliferation and resulting tissue remodeling observed in BPD subjects (Shahzad et al. 2016).

The pathophysiology of BPD indicates that prevention and treatment of BPD through nutritional supplements may protect the infants from developing chronic lung disease in later life (Ma et al. 2016; Shahzad et al. 2016), although intense research and large multicenter clinical trials preclude the widespread

use of nutrition-based improvement in this complex and chronic lung disease (Poindexter 2015). A recent study showed that maternal polyunsaturated fatty acid (PUFA) ω-3 supplementation protects rats from hyperoxia-induced lung injury, thus highlighting the importance of maternal nutrition in preventing the onset of BPD (Sharma et al. 2015). Moreover, postnatal nutrient supplementation strategies are also implemented to protect against BPD (Ma et al. 2016) but lack enough clinical studies to support their widespread application. One of the few clinical studies suggesting the utility of nutraceuticals in infants with BPD showed a combinatorial therapeutic approach where infants who received inhaled nitric oxide (iNO) plus vitamin A were protected from BPD symptoms, although the effect was significant only in 750–999 g birth weight group (Gadhia et al. 2014; Darlow et al. 2016). Similarly, another clinical trial showed that the application of Zinc supplementation on extremely low-birth weight (ELBW) infants with chronic lung disease improves infant growth, although no lung injury-related parameters were studied (Terrin et al. 2014), indicating toward the need for more specific research and large-scale clinical trials to support the use of Zinc in the treatment of BPD.

19.3.3 Antioxidant nutraceuticals for poor lifestyle-related asthma

Globally, around 300 million people are afflicted with asthma (Yoon et al. 2016), which is a chronic obstructive lung disease that is characterized by airway inflammatory–oxidative stress, increased airway responsiveness, and irregular airflow obstruction (Ercan et al. 2006; Park et al. 2009; Yoon et al. 2012; Yoon et al. 2016). It is believed that improper diet lacking sufficient antioxidants plays a decisive role in the initiation and progression of asthmatic changes in the airways (Garcia-Larsen et al. 2016; Schneider et al. 2006). Maternal dietary supplementation of antioxidants during pregnancy has been linked to decreased incidence of childhood asthma, which may be either due to reduction in the genetic predisposition of the offspring toward developing childhood asthma or transfer of protective antioxidants through breastfeeding (Schneider et al. 2006). Although, in adults with asthma, nutrient supplementation with antioxidants and anti-inflammatory compounds has not shown much promise in clinical trials (Smith et al. 2015; Litonjua et al. 2016), thus suggesting the need for more experimental and clinical investigations. The causal relationship between diet and asthma has become more complex with the identification of a population with *obese–asthma* phenotype, which confirms the role of nutritional imbalance in the development of asthma-like features (Agrawal et al. 2013; Agrawal and Prakash 2014).

Thus, exposure to environmental and/or food allergens coupled with poor lifestyle leads to production of several endogenous hyperreactive free radicals such as ROS, hydrogen peroxide, and peroxynitrite, which can initiate transcription and translation of proinflammatory cytokines and chemokines that attract inflammatory cells to the airways. The activation of inflammatory cells such as eosinophils, alveolar macrophages, and neutrophils and also the

resident structural cells such as epithelial and smooth muscle cells leads to further generation of oxidant species, thus maintaining continued inflammatory–oxidative stress in the asthmatic airways (Sahiner et al. 2011). Moreover, a direct exposure to exogenous oxidants from toxic gases in air pollution or the gaseous phase of CS also contributes to asthma exacerbations (Sahiner et al. 2011). These pathophysiology of asthma include an increase in airway smooth muscle contraction, epithelial cell death, and mucus production and a concomitant decrease in mucociliary clearance of pathogenic microorganisms from the upper airways (Sahiner et al. 2011). Further evidence for oxidative stress playing a role in the pathology of asthma comes from a study by (Ercan et al. 2006) in which they selected a few hundred asthmatic children in Turkey to study for oxidants and antioxidants to determine the role of oxidative stress in the disease. They used malondialdehyde as a marker for oxidative stress and glutathione as a marker for antioxidant levels. They found that malondialdehyde levels were increased in the asthmatic children over the controls and that the levels of glutathione decreased, which suggests that there are more oxidants than antioxidants in the asthmatic airways leading to the observed oxidative stress. Thus, similar to other inflammatory–oxidative stress-related airway diseases, an imbalance of prooxidants and antioxidants forms the principal orchestrator of asthma pathogenesis, although whether this imbalance is the cause of disease or an outcome of ongoing inflammatory signaling in the airways is still not clear (Sahiner et al. 2011).

As asthma is a global disease afflicting people of all ages that confronts us with a significant economic burden in treatment costs, it is not a surprise that there are several antioxidant nutraceuticals and natural products currently available for the prevention and treatment of asthma (Table 19.1). Some of the most widely studied antioxidant nutraceuticals used for asthma control are resveratrol (Murcia and Martínez-Tomé 2001; Stojanović et al. 2001; Culpitt et al. 2003; Shen et al. 2003; Olas et al. 2005; Richard et al. 2005; Nam 2006; Sharafkhaneh et al. 2007; Donnelly et al. 2015), *anti-asthma simplified herbal medicine intervention* (ASHMI) (Wen et al. 2005; Sorkness 2009), Saiboku-to (Nakajima et al. 1993; Urata et al. 2002; Sorkness 2009), Kanakasava (Arora et al. 2017), vitamin D (Fischer et al. 2016), Ding Chuan Tang (DCT) (Chan et al. 2006; Sorkness 2009), curcumin (Chong et al. 2014), Adathoda vasica Nees (Nilani et al. 2010), *Picrorhiza kurroa* extract (Dorsch et al. 1991; Stolk et al. 1994; Peters et al. 2001; Sharafkhaneh et al. 2007), and *Tylophora asthmatica* (Ganguly et al. 2001; Yang et al. 2006; Sharafkhaneh et al. 2007). Briefly, numerous studies have proven the effectiveness of resveratrol in controlling airway inflammation and oxidative stress in both eosinophilic and noneosinophilic asthma phenotypes, which cannot be treated by general anti-asthmatic glucocorticoids (Murcia and Martínez-Tomé 2001; Stojanović et al. 2001; Culpitt et al. 2003; Shen et al. 2003; Donnelly et al. 2004; Olas et al. 2005; Richard et al. 2005; Nam 2006; Sharafkhaneh et al. 2007). Intriguingly, resveratrol is recently shown to protect mice against high-fat diet (obesity)-associated allergic airway inflammation (Andre et al. 2016), thus demonstrating its

broad therapeutic potential in asthma. Even after all these beneficial effects, consumption of resveratrol as a dietary supplement is restricted by the lack of proper clinical research about the dosing and potential reactions with other common medications (Murcia and Martínez-Tomé 2001; Stojanović et al. 2001; Culpitt et al. 2003; Shen et al. 2003; Donnelly et al. 2004; Olas et al. 2005; Richard et al. 2005; Nam 2006; Sharafkhaneh et al. 2007). Another extensively investigated therapeutic candidate is the *ASHMI* that has shown substantial promise as an antioxidant/anti-inflammatory nutraceutical by controlling critical disease parameters associated with asthma such as airway hyperresponsiveness (AHR), Th2-cytokines, neutrophilic inflammation, and mucus production (Wen et al. 2005; Sorkness 2009). In a recent interesting study, the offsprings of mice treated with ASHMI showed reduced airway inflammation post ova challenge (Lopez-Exposito et al. 2015), and this reduction was superior to that seen with a parallel treatment with dexamethasone (DEX), one of the most common anti-asthmatic drugs (Lopez-Exposito et al. 2015). Apart from the nutraceuticals cited earlier, there are other prospective candidates that are also used to improve asthma symptoms but are not yet commercialized such as the herb *thyme* (Engelbertz et al. 2008) and ferulic acid (Rogerio et al. 2016), a compound found in many fruits and vegetables (Rogerio et al. 2016). Thus, future studies are necessary to characterize these and other new nutraceutical options with special emphasis on prevention rather than the treatment after the occurrence of disease.

19.3.4 Nutraceuticals to control poor lifestyle-related pathology in cystic fibrosis

Cystic fibrosis (CF) is an autosomal recessive disorder mostly found in Caucasians and is the most common fatal disease caused by a single genetic defect in Europe and North America (Cantin et al. 2007; Voisin et al. 2014). The disease is caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (cftr) and is characterized by chronic airway inflammation and infection (Jungas et al. 2002; Cantin et al. 2007; Luciani et al. 2010; Voisin et al. 2014). The dysfunction in CFTR protein due to gene mutations may render the airways of CF patients to be more susceptible to oxidative stress because experiments have shown that CFTR-deficient mice have lower levels of glutathione, an antioxidant, in the lung epithelial lining fluid than control mice (Jungas et al. 2002; Cantin et al. 2007). Moreover, we and others have shown that CFTR deficiency leads to elevated inflammatory–oxidative stress *via* ROS activation (Luciani et al. 2010; Bodas et al. 2011), and rescuing the misfolded CFTR to plasma membrane or potentiating its function ameliorates the CF-related lung pathologies (Rowe and Verkman 2013; De Stefano et al. 2014), thus confirming the mechanistic role of CFTR in regulating inflammatory–oxidative stress response. As CF is a genetic condition, nutrition mostly plays a role in managing disease outcomes, rather than preventing them. Poor dietary habits lacking sufficient antioxidant/anti-inflammatory factors may exacerbate the already existing inflammatory–oxidative stress in the

CF airways and also render the person more susceptible to lung infections, further worsening disease pathology. Mechanistically, the elevated inflammatory–oxidative stress in CF airways leads to the activation of NF-κB pathway, which induces the expression of proinflammatory mediators (Cantin et al. 2007; Bodas and Vij 2010; Luciani et al. 2010; Nichols and Chmiel 2015). Once activated, NF-κB further involves other transcription factors such as Nuclear factor of activated T cells (NFAT), Activator protein-1 (AP-1), Activator protein-2 (AP-2), and Mitogen activated protein kinase (MAPK) to act as transcriptional regulators of inflammation to initiate a hyperinflammatory response (Cantin et al. 2007; Bodas and Vij 2010; Nichols and Chmiel 2015).

Moreover, the NF-κB pathway can also be activated by bacterial infection that is very common in CF, with *Pseudomonas aeruginosa* (*Pa*) being the foremost pathogen prevalent in the CF airways (Bodas and Vij 2010). Bacterial infection exacerbates the inflammatory–oxidative stress response by activating neutrophils that produce reactive species such as superoxide, hydrogen peroxide, and hydroxyl radical and neutrophil-specific enzymes such as myeloperoxidase (MPO), which collectively potentiate the proinflammatory response in CF airways (Bodas and Vij 2010; Galli et al. 2012). The inflammatory response in the CF lungs is mediated by cytokines and chemokines such as IL-6 and IL-8 that constantly expose the airway with copious amounts of inflammatory–oxidative stress, resulting in lung function decline. Apart from sustained inflammatory–oxidative stress, another classical feature of CF airways that potentiates the infection-related CF pathology is the massive production of thick sticky mucus that is attributed to lack of functional CFTR-mediated ion transport and resulting hypohydration of the airways (Kreda et al. 2012; Vij et al. 2013). It is interesting to note that CFTR may be directly or indirectly associated with mucus hypersecretion or altered composition of mucus (Kreda et al. 2012), although both these hypotheses are unequivocally associated with elevated inflammatory–oxidative stress. For example, GSH, one of the crucial antioxidants in the CF airways, regulates mucus viscosity by modulating the disulfide bond-mediated aggregation of mucins (Kreda et al. 2012), and increased oxidant levels are associated with induction of mucin secretion (Takeyama et al. 2000; Kreda et al. 2012). In addition, oxidative damage also affects the lung surfactant proteins that form the fluid coating inside the alveoli to maintain robust alveolar/lung function (Kreda et al. 2012).

Thus, any antioxidant nutraceutical for CF treatment should be coupled with an ability to correct the genetic defect so as to rescue the functional CFTR and to subdue the pulmonary dysfunction in CF subjects. The current pharmacological treatments available for rescuing the mutant CFTR are quite potent, and one of them was recently approved by the FDA (Schmidt et al. 2016). The biggest caveat of these synthetic compounds is their severe side effects and also the humongous cost of long-term treatment. Thus, a large population of CF subjects is desiring for alternative treatment strategies to fight CF although it seems to be a far cry seeing the currently available options (Table 19.1). Genistein, a plant-derived isoflavonone with antioxidant properties has been shown to act

as a CFTR potentiator for both G551D and ΔF508 CFTR mutations, thus gaining attention as a potential CF nutraceutical. Even though genistein shows promise as it is also reported to increase expression of mutant CFTR protein upon long-term *in vitro* treatment (Schmidt et al. 2008), there is lack of any clinical studies to support its current use as a nutraceutical for CF. One of the most talked about antioxidant compound, curcumin, is the active ingredient of the Asian spice, turmeric (Berger et al. 2005; Braga and Almgren 2013). *In vitro* and murine studies using curcumin showed that it can correct the processing defect and can induce trafficking of ΔF508 CFTR to the plasma membrane (Cartiera et al. 2010) along with beneficial effects on reducing intestinal obstruction observed in CF mouse models (Egan et al. 2004). The translation of curcumin to a CF nutraceutical is crippled by confounding preclinical studies, poor plasma bioavailability, and a rapid metabolism rate (Mall and Kunzelmann 2005). The application of nanotechnology could be useful in designing curcumin delivery systems that could improve its bioavailability and allow targeted and sustained release of this potential CF nutraceutical drug candidate.

19.3.5 Antioxidant nutraceuticals for combating the inflammatory–oxidative stress in chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is one of the most common preventable albeit fatal diseases in the world and is the leading cause of morbidity and mortality (Kirkham et al. 2011; Kirkham and Barnes 2013; Barnes 2016). COPD is characterized by chronic inflammation of the airways primarily caused by exposure to tobacco/biomass smoke, which leads to irreversible airway obstruction (chronic bronchitis) and alveolar destruction (emphysema). Although there is a plethora of evidence suggesting the prominent role of CS-induced oxidative stress in the initiation and progression of COPD (Barnes 2016), nonetheless poor nutrition-related insufficiency of crucial antioxidants also correlates with COPD-emphysema disease severity (Heulens et al. 2015; Kodama et al. 2016; Odler et al. 2015), suggesting the protective role of a balanced antioxidant-rich diet in reducing airway inflammatory–oxidative stress in COPD subjects. Briefly, poor dietary habits will worsen the deleterious impact of CS and/or other oxidants leading to severe inflammatory–oxidative stress that has been observed in the epithelial lining fluid, exhaled breath, and urine of persons with COPD-emphysema (Park et al. 2009). Mechanistically, the CS-mediated activation of airway epithelial cells and alveolar macrophages in COPD patients results in ROS generation (Kirkham et al. 2011), which activates NF-κB that in turn facilitates transcription of many proinflammatory cytokine genes such as IL-8 and TNF-α, as well as chemokines, which are considered to be important in COPD pathogenesis (Barnes 2016). Moreover, ROS has been shown to play an important role in the recruitment of inflammatory cells such as monocytes, neutrophils, and lymphocytes (Park et al. 2009) into the airways, which once activated produce more ROS resulting in a notorious cycle of oxidative stress

and inflammation in the lungs. This elevated ROS reacts with various biological molecules to hamper key cellular homeostatic processes such as the protease–antiprotease balance, tissue repair mechanisms, autophagy, and the resulting viability of airway epithelial/endothelial cells that eventually leads to irreparable damage to the airways of COPD subjects (Park et al. 2009). Moreover, the crucial role of the redox sensitive transcription factor, nuclear factor erythroid-2-related factor 2 (Nrf2), is widely acclaimed in regulating the inflammatory–oxidative stress response in COPD subjects (Boutten et al. 2010). Activation of Nrf2 leads to transcriptional activation of a battery of antioxidant cytoprotective genes that defend the lungs against CS–ROS-mediated cellular damage and resulting emphysema (Boutten et al. 2011). The decreased levels of Nrf2 protein in COPD subjects further highlight its significance in controlling redox homeostasis in CS–ROS-induced chronic obstructive lung disease (Malhotra et al. 2008).

As discussed earlier, autophagy is another vital cellular homeostatic mechanism that protects the cell by mediating the clearance of damaged/misfolded proteins that accumulate into perinuclear bodies termed aggresomes (Tran et al. 2015; Bodas et al. 2017a). We and others have suggested the crucial role of CS-induced oxidative/nitrative stress in autophagy impairment (Bodas et al. 2017a). It is clear that formation of aggresomes mediates deleterious lung pathologies and correlates with the severity of emphysema in COPD subjects (Bodas et al. 2017a). Mechanistically, we and others have shown that CS exposure leads to diminished expression and function of CFTR protein that potentiates the inflammatory–oxidative stress and thus hampers the autophagy process. Intriguingly, we have recently described that CS–ROS-induced acquired CFTR dysfunction by virtue of its perinuclear accumulation into aggresome bodies correlates with the pathogenesis of COPD emphysema (Bodas et al. 2017b). Moreover, we recently identified that CS exposure leads to perinuclear accumulation of transcription factor EB (TFEB), the master transcriptional regulator of several key autophagy and lysosome biogenesis genes (Pena-Llopis and Brugarolas 2011; Bodas et al. 2017a), resulting in its functional unavailability and thus autophagy impairment (Bodas et al. 2017b). We also found that the nuclear accumulation of TFEB decreases while its perinuclear aggregation increases with the severity of emphysema in COPD subjects, further highlighting the role of CS-ROS mediated autophagy-impairment as the key mechanism of COPD-emphysema pathogenesis. We have successfully utilized some FDA-approved antioxidant drugs with autophagy inducing properties, such as cysteamine, gemfibrozil, and fisetin to nullify the deleterious effects of CS exposure-mediated lung injury and COPD-emphysema (Bodas et al. 2017b).

Thus, the use of nutraceuticals and natural compounds with potent antioxidant properties is warranted and has been implemented in the prevention and treatment of chronic obstructive lung diseases such as COPD-emphysema (Figure 19.1). Fisetin is a nutraceutical flavanol belonging to the flavonoid group of polyphenols and an *over the counter drug* to improve brain health (Weseler et al. 2009). We have recently shown the efficacy of Fisetin

in controlling cigarette smoke extract (CSE)-induced oxidative stress, autophagy-impairment and resulting emphysema (Bodas et al. 2017a), thus providing experimental evidence for its candidature as a prospective nutraceutical for controlling COPD-emphysema. In fact, Fisetin was reported to control LPS-induced proinflammatory cytokine production from peripheral blood of COPD patients (Weseler et al. 2009), further indicating its COPD restricting potential only after a detailed clinical investigation. There are numerous other natural compounds known to suppress oxidative stress in COPD (Table 19.1) or to enhance the endogenous antioxidant levels, which is always a more judicious strategy (Biswas et al. 2013). Briefly, after the discovery of sulforaphane, a potent antioxidant natural compound derived from broccoli sprouts (Morimitsu et al. 2002; Barnes 2008), its efficacy in controlling CS-induced inflammatory–oxidative stress was tested in several studies (Jiao et al. 2013). Sulforaphane activates the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), a critical regulator of antioxidant response genes, and controls diverse pathogenic aspects of CS-induced oxidative stress (Morimitsu et al. 2002; Barnes 2008). Although its future use as a COPD-targeting nutraceutical was set back as a recent clinical trial showed that sulforaphane failed to activate the expression of key Nrf2 target genes (Wise et al. 2016). Another potential nutraceutical candidate, the red wine wonder drug, resveratrol is an antioxidant which activates SIRT1 protein that is implicated in premature lung aging in COPD subjects (Hwang et al. 2010, 2013). A plethora of studies have cited the potential utility of resveratrol in controlling CS-induced inflammatory–oxidative stress, albeit the lack of supportive clinical studies still prevents its use in COPD-emphysema subjects. A few other natural antioxidant compounds such as berberine (Xu et al. 2015) and emodin (Nemmar et al. 2015; Xue et al. 2015) were tested in murine models of CS-induced COPD-emphysema; and consumption of soy which is rich in the antioxidant Genistein, improved lung function in COPD subjects (Hirayama et al. 2009), although future clinical studies preclude their human application.

19.3.6 Role of inflammatory–oxidative stress in idiopathic pulmonary fibrosis and prospects of antioxidant nutraceuticals

Idiopathic pulmonary fibrosis (IPF) is a chronic lung condition caused by exposure to various toxins, infections and the resulting inflammatory–oxidative stress (Cantin et al. 1987; Gao et al. 2008; Park et al. 2009). In IPF, there is an accumulation of alveolar macrophages and neutrophils in the lower respiratory tract which mediates inflammation, apoptosis and lung fibrosis also called scarring (Cantin et al. 1987; Gao et al. 2008). For most people that suffer from PF, the disease continuously progresses and ultimately leads to death (Cantin et al. 1987; Gao et al. 2008). Although the precise mechanisms that cause the onset of this disease are not fully understood, oxidative stress and inflammation are suggested to be the major

orchestrators of lung injury observed in pulmonary fibrosis (Gao et al. 2008; Park et al. 2009), thus maintaining an antioxidant-rich diet may assist in delaying disease progression.

As described previously, the inflammatory cells recruited into the airways release ROS that plays a role in increasing oxidative stress in IPF subjects (Cantin et al. 1987). Along with releasing ROS, macrophages also recruit other proinflammatory cells that secrete cytokines and chemokines (Cantin et al. 1987; Gao et al. 2008; Park et al. 2009). The major cytokine attributed to lung tissue injury is TGF-β (Gao et al. 2008; Park et al. 2009), whose expression and/or activation is induced by ROS and conversely TGF-β can trigger mitochondrial ROS generation, thus maintaining a continuous vicious cycle of inflammatory–oxidative stress. Moreover, TGF-β also decreases the antioxidant responses in the airway (Park et al. 2009; Liu and Desai 2015) and also promotes fibrotic changes in the lungs of IPF subjects (Park et al. 2009; Liu and Desai 2015). Mechanistically, ROS-induced TGF-β mediates profibrotic changes in the airways by promoting fibroblast proliferation and collagen production, inducing epithelial to mesenchymal transition (EMT), overproducing extracellular matrix (ECM) proteins, and triggering epithelial cell death (Rupp et al. 2008; Liu and Desai 2015). Although it is clear that oxidative stress plays a crucial role in the pathogenesis of PF, there are scarce reports of antioxidant therapeutics being successful in IPF management (Chen et al. 2009; Chitra et al. 2013; Guan et al. 2016) pointing toward the complexity of the underlying mechanisms. Thus, there is an urgent need to devise new prevention and/or treatment strategies based on nutraceuticals that may control ROS-mediated disease pathogenesis in IPF subjects.

19.4 Conclusion and future perspectives

Dietary nutraceuticals now form a major constituent of several ongoing studies for prevention and treatment of acute and chronic respiratory diseases. These basic and translational investigations have sprouted considerable interest in the common man to embrace antioxidant nutraceuticals to complement their therapeutic strategies against airway diseases apart from the presently used nonnatural drugs. The future will depend on whether the translational studies can help in setting up proper guidelines for the dosage or can improve the bioavailability of some of the promising nutraceutical candidates. Moreover, it will be a daunting task to pursue large pharma companies to include these slightly less potent but much safer compounds into their testing/production repertoire. We think that nutraceuticals would be a better choice to *treat* acute and chronic lung diseases, but the consumption of a balanced antioxidant sufficing diet is the healthiest way to *prevent* the generation of excessive oxidative stress, rather than suppressing it!

References

Agrawal, A. and Y. S. Prakash. 2014. Obesity, metabolic syndrome, and airway disease: A bioenergetic problem? *Immunol Allergy Clin North Am* 34:785–796.

Agrawal, A., A. Sood, A. Linneberg, and B. Ghosh. 2013. Mechanistic understanding of the effect of obesity on asthma and allergy. *J Allergy (Cairo)* 2013:598904.

Andre, D. M., M. C. Calixto, C. Sollon, E. C. Alexandre, L. O. Leiria, N. Tobar, G. F. Anhe, and E. Antunes. 2016. Therapy with resveratrol attenuates obesity-associated allergic airway inflammation in mice. *Int Immunopharmacol* 38:298–305.

Aravamudan, B., A. Kiel, M. Freeman, P. Delmotte, M. Thompson, R. Vassallo, G. C. Sieck, C. M. Pabelick, and Y. S. Prakash. 2014. Cigarette smoke-induced mitochondrial fragmentation and dysfunction in human airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 306:L840–L854.

Arora, P., S. H. Ansari, V. Anjum, R. Mathur, S. Ahmad. 2017. Investigation of anti-asthmatic potential of Kanakasava in ovalbumin-induced bronchial asthma and airway inflammation in rats. *J Ethnopharmacol* 197:242–249.

Baffi, C. W., L. Wood, D. Winnica, P. J. Strollo, Jr., M. T. Gladwin, L. G. Que, and F. Holguin. 2016. Metabolic syndrome and the lung. *Chest* 149:1525–1534.

Banderali, G., A. Martelli, M. Landi, F. Moretti, F. Betti, G. Radaelli, C. Lassandro, and E. Verduci. 2015. Short and long term health effects of parental tobacco smoking during pregnancy and lactation: A descriptive review. *J Transl Med* 13:327.

Barnes, P. J. 2008. Defective antioxidant gene regulation in COPD: A case for broccoli. *Am J Respir Crit Care Med* 178:552–554.

Barnes, P. J. 2015. Identifying molecular targets for new drug development for chronic obstructive pulmonary disease: What does the future hold? *Semin Respir Crit Care Med* 36:508–522.

Barnes, P. J. 2016. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. *J Allergy Clin Immunol* 138:16–27.

Barnes, P. J., S. D. Shapiro, and R. A. Pauwels. 2003. Chronic obstructive pulmonary disease: Molecular and cellular mechanisms. *Eur Respir J* 22:672–688.

Berger, A. L., C. O. Randak, L. S. Ostedgaard, P. H. Karp, D. W. Vermeer, and M. J. Welsh. 2005. Curcumin stimulates cystic fibrosis transmembrane conductance regulator Cl-channel activity. *J Biol Chem* 280:5221–5226.

Bhatia, M., R. L. Zemans, and S. Jeyaseelan. 2012. Role of chemokines in the pathogenesis of acute lung injury. *Am J Respir Cell Mol Biol* 46:566–572.

Biswas, S., J. W. Hwang, P. A. Kirkham, and I. Rahman. 2013. Pharmacological and dietary antioxidant therapies for chronic obstructive pulmonary disease. *Curr Med Chem* 20:1496–530.

Bjorklund, G. and S. Chirumbolo. 2017. Role of oxidative stress and antioxidants in daily nutrition and human health. *Nutrition* 33:311–321.

Blair, J. M., M. A. Webber, A. J. Baylay, D. O. Ogbolu, and L. J. Piddock. 2015. Molecular mechanisms of antibiotic resistance. *Nat Rev Microbiol* 13:42–51.

Bloomfield, F. H. 2011. How is maternal nutrition related to preterm birth? *Annu Rev Nutr* 31:235–261.

Bodas, M. and N. Vij. 2010. The NF-kappaB signaling in cystic fibrosis lung disease: Pathophysiology and therapeutic potential. *Discov Med* 9:346–356.

Bodas, M., I. Tran, and N. Vij. 2012. Therapeutic strategies to correct proteostasis-imbalance in chronic obstructive lung diseases. *Curr Mol Med* 12:807–814.

Bodas, M., N. Patel, D. Silverberg, K. Walworth, and N. Vij. 2017a. Master autophagy regulator Transcription factor-EB (TFEB) regulates cigarette smoke induced autophagy-impairment and COPD-emphysema pathogenesis. *Antioxid Redox Signal* 27:150–167.

Bodas, M., D. Silverberg, K. Walworth, K. Brucia, and N. Vij. 2017b. Augmentation of S-Nitrosoglutathione Controls cigarettesmoke-induced inflammatory-oxidative stress and COPD-emphysema pathogenesis by restoring CFTR function. *Antioxid Redox Signal* 27:433–451.

Bodas, M., T. Min, S. Mazur, and N. Vij. 2011. Critical modifier role of membrane-cystic fibrosis transmembrane conductance regulator-dependent ceramide signaling in lung injury and emphysema. *J Immunol* 186:602–613.

Bono, R., R. Tassinari, V. Bellisario, G. Gilli, M. Pazzi, V. Pirro, G. Mengozzi, M. Bugiani, and P. Piccioni. 2015. Urban air and tobacco smoke as conditions that increase the risk of oxidative stress and respiratory response in youth. *Environ Res* 137:141–146.

Boutten, A., D. Goven, E. Artaud-Macari, J. Boczkowski, and M. Bonay. 2011. NRF2 targeting: A promising therapeutic strategy in chronic obstructive pulmonary disease. *Trends Mol Med* 17:363–371.

Boutten, A., D. Goven, J. Boczkowski, and M. Bonay. 2010. Oxidative stress targets in pulmonary emphysema: Focus on the Nrf2 pathway. *Expert Opin Ther Targets* 14:329–346.

Braga, S. F. and M. M. Almgren. 2013. Complementary therapies in cystic fibrosis: Nutritional supplements and herbal products. *J Pharm Pract* 26:14–17.

Burney, P., D. Jarvis, and R. Perez-Padilla. 2015. The global burden of chronic respiratory disease in adults. *Int J Tuberc Lung Dis* 19:10–20.

Cantin, A. M., S. L. North, G. A. Fells, R. C. Hubbard, and R. G. Crystal. 1987. Oxidant-mediated epithelial cell injury in idiopathic pulmonary fibrosis. *J Clin Invest* 79:1665–16673.

Cantin, A. M., T. B. White, C. E. Cross, H. J. Forman, R. J. Sokol, and D. Borowitz. 2007. Antioxidants in cystic fibrosis. Conclusions from the CF antioxidant workshop, Bethesda, Maryland, November 11–12, 2003. *Free Radic Biol Med* 42:15–31.

Carter, G. T., V. Duong, S. Ho, K. C. Ngo, C. L. Greer, and D. L. Weeks. 2014. Side effects of commonly prescribed analgesic medications. *Phys Med Rehabil Clin N Am* 25:457–470.

Cartiera, M. S., E. C. Ferreira, C. Caputo, M. E. Egan, M. J. Caplan, and W. M. Saltzman. 2010. Partial correction of cystic fibrosis defects with PLGA nanoparticles encapsulating curcumin. *Mol Pharm* 7:86–93.

Castillo, R. L., R. Carrasco Loza, and C. Romero-Dapueto. 2015. Pathophysiological approaches of acute respiratory distress syndrome: Novel bases for study of lung injury. *Open Respir Med J* 9:83–91.

Chan, C. K., M. L. Kuo, J. J. Shen, L. C. See, H. H. Chang, and J. L. Huang. 2006. Ding Chuan Tang, a Chinese herb decoction, could improve airway hyper-responsiveness in stabilized asthmatic children: A randomized, double-blind clinical trial. *Pediatr Allergy Immunol* 17:316–322.

Chandrasekaran, A., M. D. Idelchik, and J. A. Melendez. 2016. Redox control of senescence and age-related disease. *Redox Biol* 11:91–102.

Chauhan, B., G. Kumar, N. Kalam, and S. H. Ansari. 2013. Current concepts and prospects of herbal nutraceutical: A review. *J Adv Pharm Technol Res* 4:4–8.

Chen, X. H., R. S. Sun, J. M. Hu, Z. Y. Mo, Z. F. Yang, G. Y. Jin, W. D. Guan, and N. S. Zhong. 2009. Inhibitory effect of emodin on bleomycin-induced pulmonary fibrosis in mice. *Clin Exp Pharmacol Physiol* 36:146–153.

Chitra, P., G. Saiprasad, R. Manikandan, and G. Sudhandiran. 2013. Berberine attenuates bleomycin induced pulmonary toxicity and fibrosis via suppressing NF-κB dependant TGF-β activation: A biphasic experimental study. *Toxicol Lett* 219:178–193.

Chong, L., W. Zhang, Y. Nie, G. Yu, L. Liu, L. Lin, S. Wen, L. Zhu, and C. Li. 2014. Protective effect of curcumin on acute airway inflammation of allergic asthma in mice through Notch1-GATA3 signaling pathway. *Inflammation* 37:1476–1485.

Choudhury, G. and W. MacNee. 2016. Role of Inflammation and oxidative stress in the pathology of ageing in COPD: Potential therapeutic interventions. *COPD* 14:122–135.

Chow, C. W., M. T. Herrera Abreu, T. Suzuki, and G. P. Downey. 2003. Oxidative stress and acute lung injury. *Am J Respir Cell Mol Biol* 29:427–431.

Church, D. F. and W. A. Pryor. 1985. Free-radical chemistry of cigarette smoke and its toxicological implications. *Environ Health Perspect* 64:111–126.

Comhair, S. A. and S. C. Erzurum. 2002. Antioxidant responses to oxidant-mediated lung diseases. *Am J Physiol Lung Cell Mol Physiol* 283:L246–L255.
Culpitt, S. V., D. F. Rogers, P. S. Fenwick, P. Shah, C. De Matos, R. E. Russell, P. J. Barnes, and L. E. Donnelly. 2003. Inhibition by red wine extract, resveratrol, of cytokine release by alveolar macrophages in COPD. *Thorax* 58:942–946.
D'hulst, A. I., K. Y. Vermaelen, G. G. Brusselle, G. F. Joos, and R. A. Pauwels. 2005. Time course of cigarette smoke-induced pulmonary inflammation in mice. *Eur Respir J* 26:204–213.
Dahl, R. 2006. Systemic side effects of inhaled corticosteroids in patients with asthma. *Respir Med* 100:1307–1317.
Darlow, B. A., P. J. Graham, and M. X. Rojas-Reyes. 2016. Vitamin A supplementation to prevent mortality and short- and long-term morbidity in very low birth weight infants. *Cochrane Database Syst Rev* 10:CD000501.
Davalli, P., T. Mitic, A. Caporali, A. Lauriola, D. D'Arca. 2016. ROS, Cell senescence, and novel molecular mechanisms in aging and age-related diseases. *Oxid Med Cell Longev* 2016:3565127.
De Stefano, D., V. R. Villella, S. Esposito, A. Tosco, A. Sepe, F. De Gregorio, L. Salvadori. et al. 2014. Restoration of CFTR function in patients with cystic fibrosis carrying the F508del-CFTR mutation. *Autophagy* 10:2053–2074.
Dinger, K., P. Kasper, E. Hucklenbruch-Rother, C. Vohlen, E. Jobst, R. Janoschek, I. Bae-Gartz et al. 2016. Early-onset obesity dysregulates pulmonary adipocytokine/insulin signaling and induces asthma-like disease in mice. *Sci Rep* 6:24168.
Domej, W., K. Oettl, and W. Renner. 2014. Oxidative stress and free radicals in COPD—Implications and relevance for treatment. *Int J Chron Obstruct Pulmon Dis* 9:1207–1224.
Donnelly, L. E., R. Newton, G. E. Kennedy, P. S. Fenwick, R. H. Leung, K. Ito, R. E. Russell, and P. J. 2004. Barnes. Anti-inflammatory effects of resveratrol in lung epithelial cells: Molecular mechanisms. *Am J Physiol Lung Cell Mol Physiol* 287:L774–L783.
Dorsch, W., H. Stuppner, H. Wagner, M. Gropp, S. Demoulin, and J. Ring. 1991. Antiasthmatic effects of Picrorhiza kurroa: Androsin prevents allergen- and PAF-induced bronchial obstruction in guinea pigs. *Int Arch Allergy Appl Immunol* 95:128–133.
Egan, M. E., M. Pearson, S. A. Weiner, V. Rajendran, D. Rubin, J. Glockner-Pagel, S. Canny, K. Du, G. L. Lukacs, and M. J. Caplan. 2004. Curcumin, a major constituent of turmeric, corrects cystic fibrosis defects. *Science* 304:600–602.
Engelbertz, J., T. Schwenk, U. Kinzinger, D. Schierstedt, and E. J. Verspohl. 2008. Thyme extract, but not thymol, inhibits endothelin-induced contractions of isolated rat trachea. *Planta Med* 74:1436–1440.
Ercan, H., E. Birben, E. A. Dizdar, O. Keskin, C. Karaaslan, O. U. Soyer, R. Dut, C. Sackesen, T. Besler, and O. Kalayci. 2006. Oxidative stress and genetic and epidemiologic determinants of oxidant injury in childhood asthma. *J Allergy Clin Immunol* 118:1097–1104.
Faux, S. P., T. Tai, D. Thorne, Y. Xu, D. Breheny, and M. Gaca. 2009. The role of oxidative stress in the biological responses of lung epithelial cells to cigarette smoke. *Biomarkers* 14 (Suppl 1):90–96.
Feng, G., Z. Y. Jiang, B. Sun, J. Fu, and T. Z. Li. 2016. Fisetin alleviates lipopolysaccharide-induced acute lung injury via TLR4-mediated NF-κB signaling pathway in rats. *Inflammation* 39:148–157.
Ferkol, T. and D. Schraufnagel. 2014. The global burden of respiratory disease. *Ann Am Thorac Soc* 11:404–406.
Fischer, K. D., S. C. Hall, and D. K. Agrawal. 2016. Vitamin D supplementation reduces induction of epithelial-mesenchymal transition in allergen sensitized and challenged mice. *PLoS One* 11:e0149180.
Fragoso, C. A. 2016. Epidemiology of chronic obstructive pulmonary disease (COPD) in aging populations. *COPD* 13:125–129.

Gadhia, M. M., G. R. Cutter, S. H. Abman, and J. P. Kinsella. 2014. Effects of early inhaled nitric oxide therapy and vitamin A supplementation on the risk for bronchopulmonary dysplasia in premature newborns with respiratory failure. *J Pediatr* 164:744–748.

Galli, F., A. Battistoni, R. Gambari, A. Pompella, A. Bragonzi, F. Pilolli, L. Iuliano, M. Piroddi, M. C. Dechecchi, and G. Cabrini. 2012. Working group on inflammation in cystic F. Oxidative stress and antioxidant therapy in cystic fibrosis. *Biochim Biophys Acta* 1822:690–713.

Ganguly, T., L. PBadheka, and K. B. Sainis. 2001. Immunomodulatory effect of *Tylophora indica* on Con A induced lymphoproliferation. *Phytomedicine* 8:431–437.

Gao, F., V. L. Kinnula, M. Myllärniemi, and T. D. Oury. 2008. Extracellular superoxide dismutase in pulmonary fibrosis. *Antioxid Redox Signal* 10:343–354.

Gao, M. Y., L. Chen, L. Yang, X. Yu, J. P. Kou, and B. Y. Yu. 2014. Berberine inhibits LPS-induced TF procoagulant activity and expression through NF-κB/p65, Akt and MAPK pathway in THP-1 cells. *Pharmacol Rep* 66:480–484.

Garcia-Larsen, V., S. R. Del Giacco, A. Moreira, M. Bonini, D. Charles, T. Reeves, K. H. Carlsen et al. 2016. Asthma and dietary intake: An overview of systematic reviews. *Allergy* 71:433–442.

Garmendia, J. V., D. Moreno, A. H. Garcia, and J. B. De Sanctis. 2014. Metabolic syndrome and asthma. *Recent Pat Endocr Metab Immune Drug Discov* 8:60–66.

Geraets, L., A. Haegens, K. Brauers, J. A. Haydock, J. H. Vernooy, E. F. Wouters, A. Bast, and G. J. Hageman. 2009. Inhibition of LPS-induced pulmonary inflammation by specific flavonoids. *Biochem Biophys Res Commun* 382:598–603.

Gitto, E., S. Pellegrino, S. D'Arrigo, I. Barberi, and R. J. Reiter. 2009. Oxidative stress in resuscitation and in ventilation of newborns. *Eur Respir J* 34:1461–1469.

Goldenberg, N. M., B. E. Steinberg, A. S. Slutsky, and W. L. Lee. 2011. Broken barriers: A new take on sepsis pathogenesis. *Sci Transl Med* 3:88ps25.

Goldklang, M. P., S. M. Marks, and J. M. D'Armiento. 2013. Second hand smoke and COPD: Lessons from animal studies. *Front Physiol* 4:30.

Guan, R., X. Zhao, X. Wang, N. Song, Y. Guo, X. Yan, L. Jiang, W. Cheng, and L. Shen. 2016. Emodin alleviates bleomycin-induced pulmonary fibrosis in rats. *Toxicol Lett* 262:161–172.

Guo, R. F. and P. A. Ward. 2007. Role of oxidants in lung injury during sepsis. *Antioxid Redox Signal* 9:1991–2002.

Heulens, N., H. Korf, N. Cielen, E. De Smidt, K. Maes, C. Gysemans, E. Verbeken, G. Gayan-Ramirez, C. Mathieu, and W. Janssens. 2015. Vitamin D deficiency exacerbates COPD-like characteristics in the lungs of cigarette smoke-exposed mice. *Respir Res* 16:110.

Hirayama, F., A. H. Lee, C. W. Binns, Y. Zhao, T. Hiramatsu, Y. Tanikawa, K. Nishimura, and H. Taniguchi. 2009. Soy consumption and risk of COPD and respiratory symptoms: A case-control study in Japan. *Respir Res* 10:56.

Hnizdo, E. 2010. Lung function loss associated with occupational dust exposure in metal smelting. *Am J Respir Crit Care Med* 181:1162–1163.

Hoffmann, R. F., S. Zarrintan, S. M. Brandenburg, A. Kol, H. G. de Bruin, S. Jafari, F. Dijk et al. 2013. Prolonged cigarette smoke exposure alters mitochondrial structure and function in airway epithelial cells. *Respir Res* 14:97.

Holguin, F. 2013. Oxidative stress in airway diseases. *Ann Am Thorac Soc* 10 (Suppl):S150–S157.

Hwang, J. W., S. Chung, I. K. Sundar, H. Yao, G. Arunachalam, M. W. McBurney, and I. Rahman. 2010. Cigarette smoke-induced autophagy is regulated by SIRT1-PARP-1-dependent mechanism: Implication in pathogenesis of COPD. *Arch Biochem Biophys* 500:203–209.

Hwang, J. W., H. Yao, S. Caito, I. K. Sundar, and I. Rahman. 2013. Redox regulation of SIRT1 in inflammation and cellular senescence. *Free Radic Biol Med* 61:95–110.

Ito, K. and P. J. Barnes. 2009. COPD as a disease of accelerated lung aging. *Chest* 135:173–80.

Jiao, Z., Q. Zhang, J. Chang, D. Nie, M. Li, Y. Zhu, C. Wang, Y. Wang, and F. Liu. 2013. A protective role of sulforaphane on alveolar epithelial cells exposed to cigarette smoke extract. *Exp Lung Res* 39:379–386.

Johnson, S. A., A. B. Javurek, M. S. Painter, C. R. Murphy, C. M. Conard, K. L. Gant, E. C. Howald et al. 2017. Effects of a maternal high-fat diet on offspring behavioral and metabolic parameters in a rodent model. *J Dev Orig Health Dis* 8:75–88.

Jordan, R. E., K. K. Cheng, M. R. Miller, and P. Adab. 2011. Passive smoking and chronic obstructive pulmonary disease: Cross-sectional analysis of data from the health survey for England. *BMJ Open* 1:e000153.

Jungas, T., I. Motta, F. Duffieux, P. Fanen, V. Stoven, and D. M. Ojcius. 2002. Glutathione levels and BAX activation during apoptosis due to oxidative stress in cells expressing wild-type and mutant cystic fibrosis transmembrane conductance regulator. *J Biol Chem* 277:27912–27918.

Karbasi-Afshar, R., J. Aslani, and M. Ghanei. 2014. Efficacy and safety of inhaler steroids in COPD patients: Systematic review and meta-analysis of randomized placebo-controlled trials. *Caspian J Intern Med* 5:130–136.

Kaushik, R., F. Khaliq, M. Subramaneyaan, and R. S. Ahmed. 2012. Pulmonary dysfunctions, oxidative stress and DNA damage in brick kiln workers. *Hum Exp Toxicol* 31:1083–10891.

Kawada, N., T. Moriyama, H. Kitamura, R. Yamamoto, Y. Furumatsu, I. Matsui, Y. Takabatake et al. 2012. Towards developing new strategies to reduce the adverse side-effects of nonsteroidal anti-inflammatory drugs. *Clin Exp Nephrol* 16:25–29.

Kirkham, P. A. and P. J. Barnes. 2013. Oxidative stress in COPD. *Chest* 144:266–273.

Kirkham, P. A., G. Caramori, P. Casolari, A. A. Papi, M. Edwards, B. Shamji, K. Triantaphyllopoulos et al. 2011. Oxidative stress-induced antibodies to carbonyl-modified protein correlate with severity of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 184:796–802.

Kling, K. M., E. Lopez-Rodriguez, C. Pfarrer, C. Muhlfeld, and C. Brandenberger. 2016. Aging exacerbates acute lung injury induced changes of the air-blood barrier, lung function and inflammation in the mouse. *Am J Physiol Lung Cell Mol Physiol*. doi:10.1152/ajplung.00347.2016.

Kodama, Y., Y. Kishimoto, Y. Muramatsu, J. Tatebe, Y. Yamamoto, N. Hirota, Y. Itoigawa et al. 2016. Antioxidant nutrients in plasma of Japanese patients with chronic obstructive pulmonary disease (COPD), asthma-COPD overlap syndrome, and bronchial asthma. *Clin Respir J*. doi:1111/crj12436.

Kofoed, C. L., J. Christensen, L. O. Dragsted, A. Tjonneland, and N. Roswall. 2015. Determinants of dietary supplement use—Healthy individuals use dietary supplements. *Br J Nutr* 113:1993–2000.

Kostikas, K., M. Minas, E. Nikolaou, A. I. Papaioannou, P. Liakos, S. Gougoura, K. I. Gourgoulianis et al. 2013. Secondhand smoke exposure induces acutely airway acidification and oxidative stress. *Respir Med* 107:172–179.

Kreda, S. M., C. W. Davis, and M. C. Rose. 2012. CFTR, mucins, and mucus obstruction in cystic fibrosis. *Cold Spring Harb Perspect Med* 2:a009589.

Kurundkar, A. and V. J. Thannickal. 2016. Redox mechanisms in age-related lung fibrosis. *Redox Biol* 9:67–76.

Kuwano, K., J. Araya, H. Hara, S. Minagawa, N. Takasaka, S. Ito, K. Kobayashi, and K. Nakayama. 2016. Cellular senescence and autophagy in the pathogenesis of chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF). *Respir Investig* 54:397–406.

Lao, X., S. Chen, Y. Dai, and Y. Song. 2014. Cellular stress response and pulmonary inflammation. *Microbes Infect* 16:871–876.

Lesur, O., Y. Berthiaume, G. Blaise, P. Damas, E. Deland, J. G. Guimond, and R. P. Michel. 1999. Acute respiratory distress syndrome: 30 years later. *Can Respir J* 6:71–86.

Leung, J., Y. Cho, R. F. Lockey, and N. Kolliputi. 2015. The role of aging in idiopathic pulmonary fibrosis. *Lung* 193:605–610.
Li, F., F. Lang, H. Zhang, L. Xu, Y. Wang, and E. Hao. 2016. Role of TFEB mediated autophagy, oxidative stress, inflammation, and cell death in endotoxin induced myocardial toxicity of young and aged mice. *Oxid Med Cell Longev* 2016:5380319.
Li, Q., K. J. Baines, P. G. Gibson, and L. G. Wood. 2016. Changes in expression of genes regulating airway inflammation following a high-fat mixed meal in asthmatics. *Nutrients* 8:30.
Litonjua, A. A., V. J. Carey, N. Laranjo, B. J. Harshfield, T. F. McElrath, G. T. O'Connor, M. Sandel et al. 2016. Effect of prenatal supplementation with vitamin D on asthma or recurrent wheezing in offspring by age 3 years: The VDAART randomized clinical trial. *JAMA* 315:362–370.
Liu, L., R. Poon, L. Chen, A. M. Frescura, P. Montuschi, G. Ciabattoni, A. Wheeler, and R. Dales. 2009. Acute effects of air pollution on pulmonary function, airway inflammation, and oxidative stress in asthmatic children. *Environ Health Perspect* 117:668–674.
Liu, R. M. and L. P. Desai. 2015. Reciprocal regulation of TGF-β and reactive oxygen species: A perverse cycle for fibrosis. *Redox Biol* 6:565–577.
Lopez-Exposito, I., K. D. Srivastava, N. Birmingham, A. Castillo, R. L. Miller, and X. M. Li. 2015. Maternal antiasthma simplified herbal medicine intervention therapy prevents airway inflammation and modulates pulmonary innate immune responses in young offspring mice. *Ann Allergy Asthma Immunol* 114:43–51.
Luciani, A., V. R. Villella, S. Esposito, N. Brunetti-Pierri, D. Medina, C. Settembre, M. Gavina et al. 2010. Defective CFTR induces aggresome formation and lung inflammation in cystic fibrosis through ROS-mediated autophagy inhibition. *Nat Cell Biol* 12:863–875.
Ma, L., P. Zhou, J. Neu, and H. C. Lin. 2016. Potential nutrients for preventing or treating bronchopulmonary dysplasia. *Paediatr Respir Rev* 22:83–88.
Malhotra, D., R. Thimmulappa, A. Navas-Acien, A. Sandford, M. Elliott, A. Singh, L. Chen et al. 2008. Decline in NRF2-regulated antioxidants in chronic obstructive pulmonary disease lungs due to loss of its positive regulator, DJ-1. *Am J Respir Crit Care Med* 178:592–604.
Mall, M. and K. Kunzelmann. 2005. Correction of the CF defect by curcumin: Hypes and disappointments. *Bioessays* 27:9–13.
Marsland, B. J., A. Trompette, and E. S. Gollwitzer. 2015. The gut-lung axis in respiratory disease. *Ann Am Thorac Soc* 12(Suppl 2):S150–S156.
Matés, J. M., J. A. Segura, F. J. Alonso, and J. Márquez. 2008. Intracellular redox status and oxidative stress: Implications for cell proliferation, apoptosis, and carcinogenesis. *Arch Toxicol* 82:273–299.
Mercado, N., K. Ito, and P. J. Barnes. 2015. Accelerated ageing of the lung in COPD: New concepts. *Thorax* 70:482–489.
Morimitsu, Y., Y. Nakagawa, K. Hayashi, H. Fujii, T. Kumagai, Y. Nakamura, T. Osawa et al. 2002. A sulforaphane analogue that potently activates the Nrf2-dependent detoxification pathway. *J Biol Chem* 277:3456–3463.
Murcia, M. A. and M. Martínez-Tomé. 2001. Antioxidant activity of resveratrol compared with common food additives. *J Food Prot* 64:379–384.
Nakajima, S., Y. Tohda, K. Ohkawa, J. Chihara, and Y. Nagasaka. 1993. Effect of saiboku-to (TJ-96) on bronchial asthma. Induction of glucocorticoid receptor, beta-adrenaline receptor, IgE-Fc epsilon receptor expression and its effect on experimental immediate and late asthmatic reaction. *Ann N Y Acad Sci* 685:549–560.
Nam, N. H. 2006. Naturally occurring NF-kappaB inhibitors. *Mini Rev Med Chem* 6:945–951.
Nemmar, A., S. Al-Salam, P. Yuvaraju, S. Beegam, and B. H. Ali. 2015. Emodin mitigates diesel exhaust particles-induced increase in airway resistance, inflammation and oxidative stress in mice. *Respir Physiol Neurobiol* 215:51–57.

Nichols, D. P. and J. F. Chmiel. 2015. Inflammation and its genesis in cystic fibrosis. *Pediatr Pulmonol* 50(Suppl 40):S39–S56.
Nilani, P., B. Duraisamy, P. Dhamodaran, S. Ravichandran, and K. 2010. Elango. Effect of selected antiasthmatic plant constituents against micro organism causing upper respiratory tract infection. *Anc Sci Life* 29:30–32.
Odler, B., I. Ivancso, V. Somogyi, K. Benke, L. Tamasi, G. Galffy, B. Szalay, and V. Muller. 2015. Vitamin D deficiency is associated with impaired disease control in asthma-COPD overlap syndrome patients. *Int J Chron Obstruct Pulmon Dis* 10:2017–2025.
Olas, B. and B. Wachowicz. 2005. Resveratrol, a phenolic antioxidant with effects on blood platelet functions. *Platelets* 16:251–260.
Ozsurekci, Y. and K. Aykac. 2016. Oxidative stress related diseases in newborns. *Oxid Med Cell Longev* 2016:2768365.
Park, H. S., S. R. Kim, and Y. C. Lee. 2009. Impact of oxidative stress on lung diseases. *Respirology* 14:27–38.
Pena-Llopis, S. and J. Brugarolas. 2011. TFEB, a novel mTORC1 effector implicated in lysosome biogenesis, endocytosis and autophagy. *Cell Cycle* 10:3987–3988.
Perrone, S., M. L. Tataranno, and G. Buonocore. 2012. Oxidative stress and bronchopulmonary dysplasia. *J Clin Neonatol* 1:109–114.
Peters, E. A., J. T. Hiltermann, and J. Stolk. 2001. Effect of apocynin on ozone-induced airway hyperresponsiveness to methacholine in asthmatics. *Free Radic Biol Med* 31:1442–1447.
Poindexter, B. B. and C. R. Martin. 2015. Impact of nutrition on bronchopulmonary dysplasia. *Clin Perinatol* 42:797–806.
Poljšak, B. and R. Fink. 2014. The protective role of antioxidants in the defence against ROS/RNS-mediated environmental pollution. *Oxid Med Cell Longev* 2014:671539.
Poulose, N. and R. Raju. 2015. Sirtuin regulation in aging and injury. *Biochim Biophys Acta* 1852:2442–2455.
Rahman, I. and I. M. Adcock. 2006. Oxidative stress and redox regulation of lung inflammation in COPD. *Eur Respir J* 28:219–242.
Rani, V., G. Deep, R. K. Singh, K. Palle, and U. C. Yadav. 2016. Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. *Life Sci* 148:183–193.
Rice, T. W., A. P. Wheeler, B. T. Thompson, B. P. deBoisblanc, J. Steingrub, and P. Rock. 2011. Enteral omega-3 fatty acid, gamma-linolenic acid, and antioxidant supplementation in acute lung injury. *JAMA* 306(14):1574–1581.
Richard, N., D. Porath, A. Radspieler, and J. Schwager. 2005. Effects of resveratrol, piceatannol, tri-acetoxystilbene, and genistein on the inflammatory response of human peripheral blood leukocytes. *Mol Nutr Food Res* 49:431–442.
Rivas, M. N., T. R. Crother, and M. Arditi. 2016. The microbiome in asthma. *Curr Opin Pediatr* 28:764–771.
Rogerio, A. P., T. Carlo, and S. R. Ambrosio. 2016. Bioactive natural molecules and traditional herbal medicine in the treatment of airways diseases. *Evid Based Complement Alternat Med* 2016:9872302.
Rowe, S. M. and A. S. Verkman. 2013. Cystic fibrosis transmembrane regulator correctors and potentiators. *Cold Spring Harb Perspect Med* 3(7):a009761.
Rupp, P. A., R. P. Visconti, A. Czirok, D. A. Cheresh, and C. D. Little. 2008. Matrix metalloproteinase 2-integrin alpha(v)beta3 binding is required for mesenchymal cell invasive activity but not epithelial locomotion: A computational time-lapse study. *Mol Biol Cell* 19:5529–5540.
Sahiner, U. M., E. Birben, S. Erzurum, C. Sackesen, and O. Kalayci. 2011. Oxidative stress in asthma. *World Allergy Org J* 4:151–158.
Samuelson, D. R., D. A. Welsh, and J. E. Shellito. 2015. Regulation of lung immunity and host defense by the intestinal microbiota. *Front Microbiol* 6:1085.
Santini, A., G. C. Tenore, and E. Novellino. 2016. Nutraceuticals: A paradigm of proactive medicine. *Eur J Pharm Sci* 96:53–61.

Santus, P., A. Corsico, P. Solidoro, F. Braido, F. Di Marco, and N. Scichilone. 2014. Oxidative stress and respiratory system: Pharmacological and clinical reappraisal of N-acetylcysteine. *COPD* 11:705–717.

Saugstad, O. D. 2003. Bronchopulmonary dysplasia-oxidative stress and antioxidants. *Semin Neonatol* 8:39–49.

Schmidt, A., L. K. Hughes, Z. Cai, F. Mendes, H. Li, D. N. Sheppard, and M. D. Amaral. 2008. Prolonged treatment of cells with genistein modulates the expression and function of the cystic fibrosis transmembrane conductance regulator. *Br J Pharmacol* 153:1311–1323.

Schmidt, B. Z., J. B. Haaf, T. Leal, and S. Noel. 2016. Cystic fibrosis transmembrane conductance regulator modulators in cystic fibrosis: Current perspectives. *Clin Pharmacol* 8:127–140.

Schneider, A. P., R. T. Stein, and C. C. Fritscher. 2007. The role of breastfeeding, diet and nutritional status in the development of asthma and atopy. *J Bras Pneumol* 33:454–462.

Schouten, L. R., M. J. Schultz, A. H. van Kaam, N. P. Juffermans, A. P. Bos, and R. M. Wosten-van Asperen. 2015. Association between maturation and aging and pulmonary responses in animal models of lung injury: A systematic review. *Anesthesiology* 123:389–408.

Serafino-Agrusa, L., M. Spatafora, and N. Scichilone. 2015. Asthma and metabolic syndrome: Current knowledge and future perspectives. *World J Clin Cases* 3:285–292.

Shahzad, T., S. Radajewski, C. M. Chao, S. Bellusci, and H. Ehrhardt. 2016. Pathogenesis of bronchopulmonary dysplasia: When inflammation meets organ development. *Mol Cell Pediatr* 3:23.

Sharafkhaneh, A., S. Velamuri, V. Badmaev, C. Lan, and N. Hanania. 2007. The potential role of natural agents in treatment of airway inflammation. *Ther Adv Respir Dis* 1:105–120.

Sharma, D., A. S. Nkembi, E. Aubry, A. Houeijeh, L. Butruille, V. Houfflin-Debarge, R. Besson, P. Deruelle, and L. Storme. 2015. Maternal PUFA omega-3 supplementation prevents neonatal lung injuries induced by hyperoxia in newborn rats. *Int J Mol Sci* 16:22081–22093.

Shen, F., S. J. Chen, X. J. Dong, H. Zhong, Y. T. Li, and G. F. Cheng. 2003. Suppression of IL-8 gene transcription by resveratrol in phorbol ester treated human monocytic cells. *J Asian Nat Prod Res* 5:151–157.

Singh, M., N. Gupta, and R. Kumar. 2016. Effect of obesity and metabolic syndrome on severity, quality of life, sleep quality and inflammatory markers in patients of asthma in India. *Pneumonol Alergol Pol* 84:258–264.

Singh, V. P., R. Aggarwal, S. Singh, A. Banik, T. Ahmad, B. R. Patnaik, G. Nappanveettil et al. 2015. Metabolic syndrome is associated with increased oxo-nitrative stress and asthma-like changes in lungs. *PLoS One* 10:e0129850.

Smith, L. J., R. Kalhan, R. A. Wise, E. A. Sugar, J. J. Lima, C. G. Irvin, A. J. Dozor, and J. T. Holbrook. 2015. Effect of a soy isoflavone supplement on lung function and clinical outcomes in patients with poorly controlled asthma: A randomized clinical trial. *JAMA* 313(20):2033–2043.

Sorkness, R. L. 2009. CAM and respiratory disease. *Nutr Clin Pract* 24:609–615.

Stadtman, E. R. 2002. Importance of individuality in oxidative stress and aging. *Free Radic Biol Med* 33:597–604.

Stojanović, S., H. Sprinz, and O. Brede. 2001. Efficiency and mechanism of the antioxidant action of trans-resveratrol and its analogues in the radical liposome oxidation. *Arch Biochem Biophys* 391:79–89.

Stolk, J., T. J. Hiltermann, J. H. Dijkman, and A. J. 1994. Verhoeven. Characteristics of the inhibition of NADPH oxidase activation in neutrophils by apocynin, a methoxy-substituted catechol. *Am J Respir Cell Mol Biol* 11:95–102.

Tai, H., Z. Wang, H. Gong, X. Han, J. Zhou, X. Wang, X. Wei et al. 2017. Autophagy impairment with lysosomal and mitochondrial dysfunction is an important characteristic of oxidative stress-induced senescence. *Autophagy* 13:99–133.

Takeyama, K., K. Dabbagh, J. Jeong Shim, T. Dao-Pick, I. F. Ueki, and J. A. Nadel. 2000. Oxidative stress causes mucin synthesis via transactivation of epidermal growth factor receptor: Role of neutrophils. *J Immunol* 164:1546–1552.

Terrin, G., R. B. Canani, A. Passariello, F. Messina, M. G. Conti, S. Caoci, A. Smaldore, E. Bertino, amd M. De Curtis. 2013. Zinc supplementation reduces morbidity and mortality in very-low-birth-weight preterm neonates: A hospital-based randomized, placebo-controlled trial in an industrialized country. *Am J Clin Nutr* 98:1468–1474.

Thuesen, B. H., L. L. Husemoen, L. G. Hersoug, C. Pisinger, and A. Linneberg. 2009. Insulin resistance as a predictor of incident asthma-like symptoms in adults. *Clin Exp Allergy* 39:700–707.

Tran, I., C. Ji, I. Ni, T. Min, D. Tang, and N. Vij. 2015. Role of cigarette smoke-induced aggresome formation in chronic obstructive pulmonary disease-emphysema pathogenesis. *Am J Respir Cell Mol Biol* 53:159–173.

Tyrrell, C., S. R. McKechnie, M. F. Beers, T. J. Mitchell, and M. C. McElroy. 2012. Differential alveolar epithelial injury and protein expression in pneumococcal pneumonia. *Exp Lung Res* 38:266–276.

Urata, Y., S. Yoshida, Y. Irie, T. Tanigawa, H. Amayasu, M. Nakabayashi, and K. Akahori. 2006. Treatment of asthma patients with herbal medicine TJ-96: A randomized controlled trial. *Respir Med* 96:469–474.

Valavanidis, A., T. Vlachogianni, and K. Fiotakis. 2009. Tobacco smoke: Involvement of reactive oxygen species and stable free radicals in mechanisms of oxidative damage, carcinogenesis and synergistic effects with other respirable particles. *Int J Environ Res Public Health* 6:445–462.

Valavanidis, A., T. Vlachogianni, K. Fiotakis, and S. Loridas. 2013. Pulmonary oxidative stress, inflammation and cancer: Respirable particulate matter, fibrous dusts and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms. *Int J Environ Res Public Health* 10:3886–3907.

Vieira, S. E. 2015. The health burden of pollution: The impact of prenatal exposure to air pollutants. *Int J Chron Obstruct Pulmon Dis* 10:1111–1121.

Vij, N. and G. P. Downey. 2013. The yin and yang of cystic fibrosis transmembrane conductance regulator function: Implications for chronic lung disease. *Am J Respir Crit Care Med* 187:120–122.

Voisin, G., G. F. Bouvet, P. Legendre, A. Dagenais, C. Massé, and Y. Berthiaume. 2014. Oxidative stress modulates the expression of genes involved in cell survival in ΔF508 cystic fibrosis airway epithelial cells. *Physiol Genomics* 46:634–646.

Ware, L. B. and M. A. Matthay. 2000. The acute respiratory distress syndrome. *N Engl J Med* 342:1334–1349.

Wen, M. C., C. H. Wei, Z. Q. Hu, K. Srivastava, J. Ko, S. T. Xi, D. Z. Mu et al. 2005. Efficacy and tolerability of anti-asthma herbal medicine intervention in adult patients with moderate-severe allergic asthma. *J Allergy Clin Immunol* 116:517–524.

Weseler, A. R., L. Geraets, H. J. Moonen, R. J. Manders, L. J. van Loon, H. J. Pennings, E. F. Wouters, A. Bast, and G. J. Hageman. 2009. Poly (ADP-ribose) polymerase-1-inhibiting flavonoids attenuate cytokine release in blood from male patients with chronic obstructive pulmonary disease or type 2 diabetes. *J Nutr* 139:952–957.

Wickens, K., D. Barry, A. Friezema, R. Rhodius, N. Bone, G. Purdie, and J. Crane 2005. Fast foods—Are they a risk factor for asthma? *Allergy* 60:1537–1541.

Wise, R. A., J. T. Holbrook, G. Criner, S. Sethi, S. Rayapudi, K. R. Sudini, E. A. Sugar et al. 2016. Broccoli Sprout Extract Trial Research G. Lack of effect of oral sulforaphane administration on Nrf2 expression in COPD: A randomized, double-blind, placebo controlled trial. *PLoS One* 11:e0163716.

Wu, J., H. Xu, P. F. Wong, S. Xia, J. Xu, and J. Dong. 2014. Icaritin attenuates cigarette smoke-mediated oxidative stress in human lung epithelial cells via activation of PI3K-AKT and Nrf2 signaling. *Food Chem Toxicol* 64:307–313.

Xu, D., C. Wan, T. Wang, P. Tian, D. Li, Y. Wu, S. Fan, L. Chen, Y. Shen, and F. Wen. 2015. Berberine attenuates cigarette smoke-induced airway inflammation and mucus hypersecretion in mice. *Int J Clin Exp Med* 8:8641–8647.

Xue, W. H., X. Q. Shi, S. H. Liang, L. Zhou, K. F. Liu, and J. Zhao. 2015. Emodin attenuates cigarette smoke induced lung injury in a mouse model via suppression of reactive oxygen species production. *J Biochem Mol Toxicol* 29:526–532.

Yang, C. W., W. L. Chen, P. L. Wu, H. Y. Tseng, and S. J. Lee. 2006. Anti-inflammatory mechanisms of phenanthroindolizidine alkaloids. *Mol Pharmacol* 69:749–758.

Yoon, S. Y., T. B. Kim, S. Baek, S. Kim, H. S. Kwon, Y. S. Lee, T. Lee et al. 2012. The impact of total antioxidant capacity on pulmonary function in asthma patients. *Int J Tuberc Lung Dis* 16:1544–1550.

Yoon, S. Y., G. H. Hong, H. S. Kwon, S. Park, S. Y. Park, B. Shin, T. B. Kim, H. B. Moon, and Y. S. Cho. 2016. S-adenosylmethionine reduces airway inflammation and fibrosis in a murine model of chronic severe asthma via suppression of oxidative stress. *Exp Mol Med* 48:e236.

Young, R. P., R. J. Hopkins, and B. Marsland. 2016. The gut-liver-lung axis. Modulation of the innate immune response and its possible role in chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* 54:161–169.

Yu, H. Z., Q. Wu, Z. Z. Du, X. Sun, C. Z. Wang, and L. Li. 2013. High-fat diet induces pulmonary fibrosis in rats and inhibitory effects of N-acetylcysteine. *Zhonghua Yi Xue Za Zhi* 93:3547–3550.

Yue, L. and H. Yao. 2016. Mitochondrial dysfunction in inflammatory responses and cellular senescence: Pathogenesis and pharmacological targets for chronic lung diseases. *Br J Pharmacol* 173:2305–2318.

Zhu, D., Y. Zhang, S. Li, L. Gan, H. Feng, and W. Nie. 2014. Enteral omega-3 fatty acid supplementation in adult patients with acute respiratory distress syndrome: A systematic review of randomized controlled trials with meta-analysis and trial sequential analysis. *Intensive Care Med* 40:504–512.

Zuo, L., F. He, G. G. Sergakis, M. S. Koozehchian, J. N. Stimpfl, Y. Rong, P. T. Diaz, and T. M. Best. 2014. Interrelated role of cigarette smoking, oxidative stress, and immune response in COPD and corresponding treatments. *Am J Physiol Lung Cell Mol Physiol* 307:L205–L218.

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Index

Note: Page numbers followed by f and t refer to figures and tables respectively.

B

C

D

E

F

G

H

I

J

K

L

M

N

Q

R

S

T

U

V

W

Y

Z