Nutraceuticals

Efficacy, Safety and Toxicity

Edited by

Ramesh C. Gupta

NUTRACEUTICALS

EFFICACY, SAFETY AND TOXICITY

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Dedication

This book is dedicated to my daughter Rekha, my wife Denise,
and my parents, the late Chandra and Triveni Gupta.

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Introduction

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INTRODUCTION

According to the *Merriam Webster Dictionary*, the term "nutraceutical" is defined as "a foodstuff (as a fortified food or dietary supplement) that provides health benefits in addition to its basic nutritional value." In 1989, Dr. Stephen DeFelice coined the term "nutraceutical" from the words nutrition and pharmaceutical and defined it as "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease." The dietary supplement industry defines nutraceutical as "any nontoxic food component that has scientifically proven health benefits, including treatment and prevention." According to the North American Veterinary Nutraceutical Council, Inc., a veterinary nutraceutical is defined as "a substance which is produced in a purified or extracted form and administered orally to patients to provide agents required for normal body structure and function and administered with the intent of improving the health and well-being of animals." Based on the definitions for nutraceuticals proposed or discussed here, a more appropriate definition would be the following: a substance that is cultivated/produced/extracted or synthesized under optimal and reproducible conditions and, when administered orally to patients, would provide the nutrient(s) required for bringing altered body structure and function back to normal, thus improving the health and well-being of the patients. Therefore, nutraceuticals fall somewhere between food nutrients and drugs. Nutraceuticals, because they may comprise more than a single food or plant component(s) that may be a contributing active ingredient, have the advantage over foods and drugs because they are not required to be listed in the nutrient profiles. Additionally, regulation of nutraceuticals varies widely around the world. For example, China allows nutraceutical claims to treat and prevent diseases with a formal approval from that country's regulatory body, whereas the United States does not allow any health claims for nutraceuticals because there is no formal review and approval process for the marketing authorization of nutraceuticals. The only major US regulation related to nutraceuticals is the 1994 passage of the Dietary Supplement Health and Education Act by the US Congress. Based on this act, dietary supplements are classified as foods, not drugs, allowing them to be sold without proof of safety and effectiveness (FDA, 1994). It has been estimated that more than 150 million Americans consume either a single or multiple dietary supplements, yet it is not necessary for companies to seek FDA approval before marketing and manufacturing these supplements in compliance with the Dietary Supplement Health and Education Act of 1994. Nutraceuticals can be food or a part of food; however, unlike foods, they are not generally recognized as safe, nor can one assume that all nutraceuticals are safe.

By the turn of the twenty-first century, the use of nutraceuticals became increasingly popular around the world. Currently, the nutraceutical industry earns more than a $200 billion per year. Most herbal supplements are classified as dietary supplements and are not subject to the regulations and safety standards applied to conventional medicine in the United States. Current European Union regulations require evidence that herbal medicinal products meet acceptable standards of quality, safety, and efficacy before a product license can be issued. As the global use of dietary supplements has increased, so have health risks emerging from active and inactive components and potential contaminants of dietary supplements, as well as likely interactions with other medications and/or dietary supplements. Consultations with physicians and pharmacists are essential for patient safety because of the potential for side effects and toxicity that may be associated with some nutraceuticals. An updated understanding of currently available scientific information for nutraceuticals and their potential side effects is therefore crucial in well-informed patient care.

Compared to pure synthetic pharmaceuticals, pharmacological and toxicological evaluation of phytochemicals is complex due to: (i) multiple phytochemicals that can be found in a single plant; (ii) variability in plants and their phytochemical constituents due to geography, soil characteristics, and climate; (iii) use of fertilizers and pesticides; (iv) stress; and (v) diurnal variation during harvesting. Also, unlike pharmaceuticals, there is a need for more than one active component in many cases as well as need for extraction procedures (standardized, normalized, or quantified extract preparation) of the active component(s). Some or all of these factors can influence identity, purity, quality, quantity, composition, potency/strength, and safety of active component(s), thereby causing a wide variability in product effectiveness from batch to batch and from one company to another.

Medicinal plant extracts and phytochemicals have been used since the ancient practices of holistic healing in Chinese, Indian (Ayurveda, Homeopathic, Siddha, or Unani), Middle Eastern, Russian, and many other cultural and traditional systems of medicine for prevention and treatment of various acute and chronic ailments. Thousands of these ancient medicines/products have been documented in Chinese, Indian, Japanese, European, and US herbal pharmacopoeias. Phytochemicals and plant-derived components have been the armamentarium of major health care since ancient human civilization. Even today, approximately 80% of the world's populations living in developing countries rely on traditional medicine for their primary health care. In every traditional system of medicine, nutraceuticals play an important role because they are considered effective in the prevention and treatment of diseases with a wide margin of safety and cost-effectiveness compared to modern synthetic drugs (Nasri et al., 2014).

By having antioxidant, anti-inflammatory, immunomodulatory, adaptogenic, anticancer, and several other health benefits, nutraceuticals are used worldwide for the prevention and treatment of chronic diseases such as diabetes, arthritis, cardiovascular and respiratory disorders, neurodegenerative diseases, and cancer. Nutraceuticals are also used to improve general health and delay the aging process by supporting the structure and function of the body, thus contributing to an increase in life expectancy. While some nutraceuticals (e.g., anthocyanins, caffeine, curcumin, fenugreek, ginseng, melatonin, quercetin, and resveratrol) have been extensively studied for their mode of action, efficacy, and safety via well-characterized animal studies and human clinical trials, many others are still used on an anecdotal basis.

Currently, the rapidly growing nutraceutical industry is facing many challenges such as: (i) lack of authenticity of active principle due to unavailability of reference materials or marker compounds; (ii) lack of understanding of molecular interactions between bioactive phytochemicals within the same plant; (iii) variability in origin of raw material (e.g., Chinese vs American ginseng); (iv) variability in processing raw material; (v) lack of standardization of extraction processes; (vi) inadequacy and inconsistency in quality control standards; (vii) lack of good safety and toxicity data; and (viii) lack of well-established and evidence-based clinical trials. In addition, adulteration of nutraceuticals with other phytochemicals such as pyrrolizidine alkaloids, metals (arsenic, lead, and cadmium), mycotoxins (aflatoxins, ochratoxins, etc.), pesticides (insecticides, herbicides, fungicides, etc.), pharmaceutical drugs, and drugs of abuse due to lack of state and international regulations can contribute to severe adverse effects.

Recently, herb–drug interactions are of the utmost concern to consumers and governmental regulatory agencies. Although the underlying mechanisms for the altered drug effects by concomitant herbal medicines have yet to be determined, both pharmacokinetic and pharmacodynamic mechanisms have been considered to play a role in these interactions (Hu et al., 2005). Often, patients do not inform their physicians about concurrent use of nutraceuticals. This has resulted in incidences of herb–drug interactions, ranging from minor side effects to those as severe as liver or kidney failure or even death (Kupiec and Vishnu, 2005). To address these challenges, systematic studies using integrative approaches, including *in vitro* and *in vivo* assays using animal models and human tissue/cell lines as well as human clinical trials, are warranted to better understand the bioavailability, metabolism, dose-responses, and pharmacological and toxicological actions. Additionally, scientists in the field of nutraceuticals need to develop sensitive and reliable biomarkers to validate toxicity and safety data (Gupta, 2014). The partnership initiated between the US EPA, the FDA, and the National Institutes of Health to establish a framework for mechanism-based toxicological assessment would be of great help in this direction. Above all, a thorough understanding and development of trust between the nutraceutical industry, regulatory agencies, health care provider, and patient is the ultimate need for productive and judicious use of nutraceuticals as a complimentary system of medicine.

Recently, Dr. Margaret Chan, Director-General at the World Health Organization (WHO), stated that traditional medicine holds great potential to improve people's health and wellness in every part of the world (Chan, 2014). She emphasized the need to integrate traditional medicine in existing health systems, to modernize this rich resource and cultural heritage using systems biology and toxicology, "Omics," bioinformatics, and other latest technologies, and to educate consumers about what it can and cannot do (Leshner, 2014). No matter the weight of historical, anecdotal data, the US Food and Drug Administration (FDA) or EFSA will not allow new therapeutics for human treatment without verifiable scientific evidence. Scientists in the field of nutraceuticals are applying the latest technologies in an attempt to standardize traditional treatments, especially through isolation, identification, and purification of bioactive compounds and careful analysis of their levels and activities in various herbal remedies. Both the nutraceutical industry and the FDA acknowledge that many new products have been introduced without any safety assessment.

To meet the challenges of the twenty-first century, the nutraceutical industry needs to follow rigorous quality control, pharmacological and toxicological testing, carefully designed clinical studies, reproducibility of results, safety assessment, and proper regulations applicable to all nutraceuticals. With regard to toxicological testing,

special attention needs to be given to some toxicities that are not detected by conventional nonclinical testing, including evaluation of acute and chronic exposure, genotoxicity, carcinogenicity, and reproductive and developmental toxicity and to close pharmacovigilance during early drug exposure to humans (Williamson et al., 2015). In addition, the current toxicological testing paradigm uses modern tools for predictive toxicology, molecular biology, system biology, high-throughput screening methods, computational toxicology, and bioinformatics. Good agricultural and collection practices, good laboratory practices, good manufacturing practices, and good clinical practices can help assure both practitioners and patients that a nutraceutical is effective, safe, and of high quality, meeting international standards and its acceptance in a global market.

In view of these challenges, *Nutraceuticals: Efficacy, Safety and Toxicity* is prepared to meet the current needs of academia, industry, and regulatory agencies. The book explicitly describes the origin and historical background of common nutraceuticals, underlying pharmacological mechanisms of action at biochemical and molecular levels, models for toxicity and safety evaluation, clinical applications, safety, toxicity, herb–drug interactions, and regulatory guidelines. There are 69 chapters that are logistically arranged in six sections. After a brief introduction, a large section covers chapters on the application of nutraceuticals in system diseases and disorders. Section II deals with various models and mechanisms involved in the evaluation of safety and toxicity of nutraceuticals. More than 20 standalone chapters are devoted to common nutraceuticals in Section III. In Section IV, several chapters cover prebiotics, probiotics, and synbiotics. The last two sections of this book deal in detail with toxicity, herb–drug interactions, and regulatory aspects of nutraceuticals from various countries and continents. Thus, this is presented as the most comprehensive book to date on nutraceuticals.

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SECTION I

APPLICATIONS OF NUTRACEUTICALS IN COMMON DISEASES AND DISORDERS

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CHAPTER

1

Nutraceuticals in CNS Diseases: Potential Mechanisms of Neuroprotection

Lucio G. Costa, Jacqueline Garrick, Pamela J. Roque and Claudia Pellacani

INTRODUCTION

In recent years, there has been increasing attention devoted to the possibility that several nutraceuticals may act as neuroprotective agents (Mecocci et al., 2014). Such protective effects have often been ascribed to a direct antioxidant effect and/or to an anti-inflammatory action (Kelsey et al., 2010). However, the exact mechanisms of neuroprotection are still elusive, and various mechanisms have been proposed (Halliwell et al., 2005; Fraga et al., 2010).

Other chapters in this volume (e.g., Chapters 2 and 3) discuss in more detail the beneficial effects of several nutraceuticals in cognitive disorders and various neurodegenerative diseases. This chapter focuses instead on potential mechanisms of neuroprotection at cellular, biochemical, and molecular levels (Kelsey et al., 2010; Mazzio et al., 2011; Vauzour, 2012). Polyphenols (particularly quercetin) are discussed as model nutraceuticals, although other molecules are mentioned and discussed to illustrate additional potential neuroprotective mechanisms.

POLYPHENOLS

Several thousand molecules with a polyphenol structure have been identified in plants, and several hundred are found in edible plants. These compounds are often classified on the basis of their chemical structure, with flavonoids being one of the major classes (Manach et al., 2004; Del Rio et al., 2013). Among flavonoids, several subclasses can be identified, such as flavonols (e.g., quercetin), flavones, anthocyanidins, and various others. Innumerable studies support the idea that diets rich in polyphenols and/or supplementation with specific compounds are endowed with health benefits. In particular, polyphenols have been shown to exert protective actions in several pathological conditions such as cardiovascular disease, metabolic disorders, obesity, diabetes, infections, and cancer, as well as neurotoxic/neurodegenerative processes (Graf et al., 2005; Arts and Hollman, 2005; Scalbert et al., 2011; Vauzour, 2012; Del Rio et al., 2013; Bhullar and Rupasinghe, 2013).

Quercetin (Figure 1.1) is found in many common fruits and vegetables such as apples, berries, onions, and capers (USDA, 2003). Its estimated dietary intake ranges from 4 to 68 mg/day, but it can increase to 200–500 mg/day in individuals who consume high quantities of fruits and vegetables rich in flavonols. Furthermore, quercetin is also sold as a dietary supplement, with a recommended dosage of 1 g/day (Harwood et al., 2007). The quercetin present in foods is not present as aglycon (i.e., without sugar groups), but rather as quercetin glycosides, which, contrary to previous belief, can be efficiently absorbed. Studies using rats and pigs have shown that quercetin distributes to several tissues, particularly lung, kidney, colon, and liver, and lower levels appear in the brain (DeBoer et al., 2005). Total quercetin derived from the diet is normally present in plasma in the nanomolar range (<100 nM), but it can be increased in the micromolar range after supplementation

Nutraceuticals.
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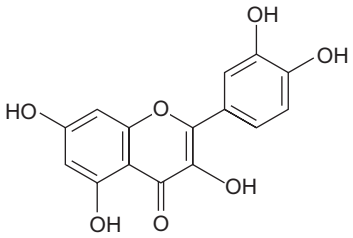

FIGURE 1.1 Structure of quercetin.

(Manach et al., 2005; Conquer et al., 1998). The half-life of quercetin ranges between 11 and 28 h, suggesting the possibility of significantly increasing plasma concentration on supplementation (Manach et al., 2004, 2005). Quercetin has an unremarkable toxicological profile, as evidenced by animal and human studies (Harwood et al., 2007; Russo et al., 2012). Similar to other polyphenols, reported beneficial effects of quercetin include effects on cardiovascular diseases, cancer, infections, inflammatory processes, gastrointestinal tract, diabetes, and nervous system disorders (reviewed in Boots et al., 2008; Kelly, 2011; Russo et al., 2012).

COUNTERACTING OXIDATIVE STRESS AS A MECHANISM OF NEUROPROTECTION

Oxidative stress is recognized as an important factor in a variety of neurodegenerative diseases, as a mediator of the adverse effects of a number of neurotoxicants, and as a mechanism for age-related degenerative processes (Halliwell, 2006; Lin and Beal, 2006; Martin and Grotewiel, 2006; Popa-Wagner et al., 2013). Oxidative stress occurs when reactive oxygen species (ROS) accumulate in cells, either from excessive production or from insufficient neutralization, causing damage to proteins, lipids, and DNA. Mitochondria are a major contributor of cellular ROS; ROS produced in the mitochondria can also target the electron transport chain (e.g., complex I), resulting in a cycle generating more ROS, followed by ATP depletion and ultimately cell death (Ott et al., 2007; Kelsey et al., 2010). Based on these premises, the identification of novel compounds that can counteract oxidative stress as potential therapeutics is a very active area of research (Linseman, 2009). Natural compounds have received much attention in this regard; among these, polyphenols have been most investigated (Dajas et al., 2003; Ossola et al., 2009; Spencer, 2009; Kelsey et al., 2010). Evidence for neuroprotection has been provided by *in vitro* studies showing that various polyphenols protect neuronal cells from damage due to oxidative stress, and by *in vivo* animal studies that have shown their ability to protect neurons against oxidative insults. Furthermore, clinical and epidemiological studies have shown that polyphenols can improve deterioration of brain function due to aging or neurodegenerative diseases (Kelsey et al., 2010; Vauzour, 2012).

Specific evidence exists regarding the neuroprotective effects of quercetin (Ossola et al., 2009). *In vitro* studies of neuronal cell lines and of primary neurons have shown that quercetin antagonizes cell toxicity induced by various oxidants (e.g., hydrogen peroxide) and other neurotoxic molecules believed to act by inducing oxidative stress (e.g., 6-hydroxydopamine and *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridinium) (Dajas et al., 2003; Mercer et al., 2005; Vauzour et al., 2008; Arredondo et al., 2010). Important issues for the potential use of quercetin *in vivo* are whether it passes the blood–brain barrier (BBB) and what concentrations of quercetin and/or its metabolites are present in brain tissue. *In vitro* studies with BBB models consistently indicate that quercetin enters the brain (Faria et al., 2010; Ishisaka et al., 2011; Schaffer and Halliwell, 2012). On administration of quercetin *in vivo* to rats and pigs, low levels (from picomolar to nanomolar) were found in brain tissue (DeBoer et al., 2005; Huebbe et al., 2010; Ishisaka et al., 2011). Of interest in this regard are the recent successful efforts to increase bioavailability of quercetin (Russo et al., 2012). In particular, the formulation of quercetin in lipid nanoparticles significantly increases its penetration in the brain (Das et al., 2008; Dhavan et al., 2011). Several studies show that quercetin can exert neuroprotection and antagonize oxidative stress when administered *in vivo*. For example, oral quercetin (0.5–50 mg/kg) was shown to protect rodents from oxidative stress and neurotoxicity induced by various neurotoxic insults (Hu et al., 2008; Das et al., 2008; Barcelos et al., 2011; Ishisaka et al., 2011; Bavithra et al., 2012; Denny Joseph and Muralidhara, 2013).

DIRECT ANTIOXIDANT ACTION OF QUERCETIN

Quercetin is a potent scavenger of ROS, such as $O_2^{\bullet -}$, and of RNS (reactive nitrogen species), such as NO and ONOO (Boots et al., 2008). The antioxidative capacity of quercetin has been ascribed to the presence of two antioxidant pharmacophores within the molecule that have the optimal configuration for free radical scavenging, such as the catechol group in the B ring and the OH group at position 3 (Boots et al., 2008). Direct scavenging of ROS *in vitro* has been observed with quercetin concentrations ranging from 5 to 50 μM (Saw et al., 2014). However, it has been pointed out that the concentration of quercetin expected to be present in the brain would likely be in the nanomolar range, which is not sufficient to exert an appreciable direct antioxidant effect. In contrast, glutathione and vitamin C are present at

millimolar concentrations (Schaffer and Halliwell, 2012). Thus, despite its potent antioxidant capacity *in vitro*, it is unlikely that neuroprotective effects of quercetin observed *in vivo* are due to direct antioxidant action. Rather, it has been suggested that quercetin and/or its metabolites, as well as other polyphenols, may exert their neuroprotective effects by modulating the antioxidant defense mechanisms of the cell (Halliwell et al., 2005; Fraga et al., 2010; Kay, 2010). In this regard, it has been suggested that the beneficial effects of polyphenols may be due to their "prooxidant," rather than "antioxidant," properties (Halliwell, 2008, 2012). A mild degree of oxidative stress may increase the cell's own antioxidant defenses, resulting in overall cytoprotection. This important aspect of polyphenols' biological activity is being discussed more in depth as it relates to the principle of hormesis.

POTENTIAL ROLE OF HORMESIS IN NEUROPROTECTION

Hormesis is generally defined as a dose-response phenomenon characterized by low-dose stimulation and high-dose inhibition, which may be graphically represented by a J-shaped or U-shaped dose response (Mattson, 2008). Such biphasic dose responses have been shown to occur broadly in the biomedical sciences, and are independent of biological model, end-point measure, and chemical class. Hormesis includes the phenomenon of preconditioning, whereby "exposure to a low dose of an agent that is toxic at high doses induces an adaptive, potentially beneficial effect on the cell or organism if exposed to a subsequent and more massive dose of the same or related stressor agent" (Calabrese et al., 2007). As stated, quercetin may have "prooxidant," rather than "antioxidant," properties (Halliwell, 2008, 2012; Boots et al., 2008). During its antioxidant activities, quercetin becomes oxidized into various oxidation products, including semiquinone radicals and quinones (Boots et al., 2008), which may mediate the toxicity of quercetin observed in certain conditions in what is referred to as the quercetin paradox (Boots et al., 2008; Halliwell, 2012).

Evidence is emerging to support hormetic roles for low increases in membrane oxidative stress (Calabrese et al., 2010). Lipid peroxidation generated during moderate exercise has been shown to play an important role in hormetic effects on muscles (Sachdev and Davies, 2008). Similar considerations also apply to oxidative stress in mitochondria; although high levels of oxidative stress are unquestionably detrimental to mitochondria, low levels of ROS may actually have a protective, hormetic effect, hence the term "mito-hormesis" (Tapia, 2006; Calabrese et al., 2010). There is limited evidence that neurons exposed to subtoxic levels of oxidants may be protected against a subsequent exposure to what would have otherwise been a lethal level of stress (Calabrese et al., 2007, 2010, 2012).

Some recent studies with the marine neurotoxin domoic acid (DomA) (Giordano et al., 2013a) provide additional support for this hypothesis. DomA is a potent human and animal neurotoxin that causes primarily apoptotic cell death of neurons as a consequence of activation of AMPA/kainate receptors (Giordano et al., 2007). DomA-induced apoptosis involves oxidative stress, is inhibited by antioxidants, and is more pronounced in neurons from transgenic mice ($Gclm^{-/-}$ mice), which lack the modifier subunit of glutamate cysteine ligase (GCL) and have very low glutathione (GSH) levels (Giordano et al., 2007). Prolonged exposure of mouse neurons to low, nontoxic levels of DomA (5 nM for 10 days) has been shown to protect cells from a subsequent insult of a high concentration of DomA itself and of other oxidants (Giordano et al., 2013a). The mechanism of such protection was related to the ability of chronic low DomA to increase the levels of the two subunits of GCL (GCLC and GCLM) and those of GCLholo, leading to increased GCL activity and GSH synthesis. Transcription of GCL subunits occurs through the sequence-specific binding of nuclear factor erythroid 2-related factor 2 (Nrf2) to antioxidant response elements (AREs) present in the promoters of these two genes (Moinova and Mulcahy, 1999; Wild et al., 1999). The effect observed with DomA in wild-type CGNs resembles the phenomenon of preconditioning, which is considered part of hormesis (Calabrese et al., 2007). Low levels of DomA may thus elicit a mild degree of oxidative stress, particularly in mitochondria (Giordano et al., 2007), which would lead to increased transcription of *Gclc* and *Gclm*, ultimately leading to increased GSH synthesis and neuroprotection.

MODULATION OF THE Nrf2-ARE PATHWAY AS A MECHANISM OF QUERCETIN NEUROPROTECTION

Nrf2 is a master regulator of cellular defense against oxidative stress (Figure 1.2). Under physiological conditions, Nrf2 is sequestered in the cytoplasm by the protein Keap1 (Kelch-like ECH-associated protein 1) with Cullin 3-base E3 ligase, by which Nrf2 protein is ubiquitinylated and targeted for proteasome degradation (Shih et al., 2005; Calabrese et al., 2012; Liang et al., 2013; Gan and Johnson, 2014). Keap1 has several cysteine residues that make it act as a molecular switch by responding to electrophiles and ROS with a conformational change that releases Nrf2 (Shih et al., 2005). Dissociated Nrf2 translocates into the nucleus, where it binds to small Maf proteins. The formed heterodimer binds to *cis*-acting ARE and thereby promotes the transcription of a broad range of phase II and antioxidant

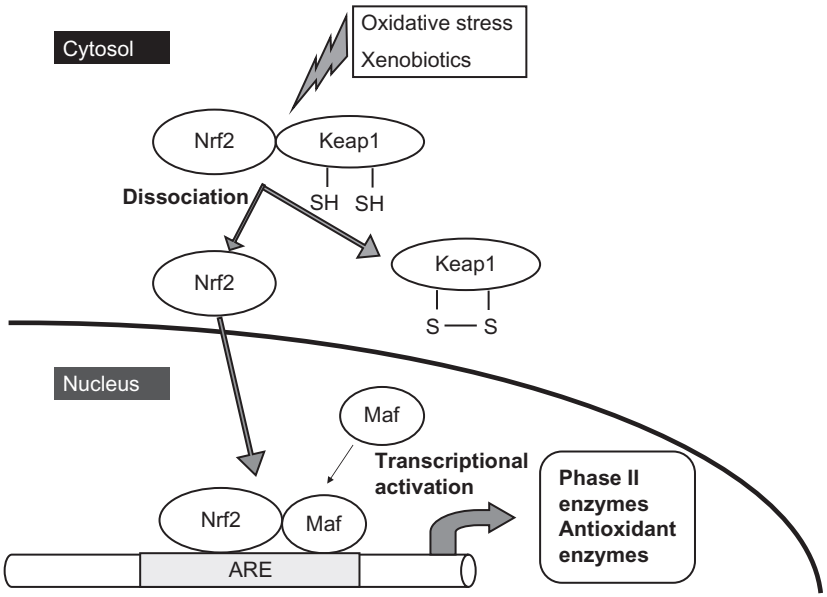

FIGURE 1.2 The Nrf2-ARE pathway. See text for details.

genes (Liang et al., 2013; Gan and Johnson, 2014). Proteins that are under control of the Nrf2-ARE pathway include heme oxygenase-1, GCL, glutathione *S*-transferases, glutathione peroxidase, superoxide dismutase (SOD), catalase, sulforedoxin, thioredoxin, and several others (Shih et al., 2005; Gan and Johnson, 2014). Activation of the Nrf2-ARE pathway provides neuroprotection against oxidative damage and cell death. More recent evidence also suggests that the Nrf2-ARE pathway may modulate the formation and degradation of misfolded protein aggregates that are present in various neurodegenerative diseases (Parkinson's, Alzheimer's, and Huntington's diseases and amyotrophic lateral sclerosis) (Gan and Johnson, 2014). For example, studies with *tert*-butylhydroquinone, a prototype Nrf2 inducer, have shown that activation of the Nrf2-ARE pathway confers protection against neurotoxicity induced by amyloid β and 3-nitropropionic acid (Shih et al., 2005; Nouhi et al., 2011).

Quercetin has been shown to counteract oxidative stress-induced cellular damage by activating the Nrf2-ARE pathway (Arredondo et al., 2010; Granado-Serrano et al., 2012; Saw et al., 2014), and similar effects have been reported for dihydroquercetin (Liang et al., 2013). Additionally, other nutraceuticals (e.g., kaempferol, pterostilbene) have been shown to interact synergistically with quercetin (Saw et al., 2014). The Akt, ERK, and JNK signaling pathway may be involved in the activation of Nrf2 (Liang et al., 2013) and, in turn, they are activated by stress stimuli, including oxidative stress. Thus, quercetin and many other nutraceuticals (e.g., resveratrol, sulforaphane, carnosic acid, dimethyl fumarate) may all act as neurohormetic phytochemicals (Mattson and Cheng, 2006; Calabrese et al., 2012).

MODULATION OF PARAOXONASE 2 (PON2) AS A POTENTIAL NOVEL MECHANISM OF QUERCETIN NEUROPROTECTION

Paraoxonase 2

The paraoxonases (PONs) are a family of three genes (PON1, PON2, PON3) clustered in tandem on the long arm of human chromosome 7q21-22 and on mouse chromosome 6 (Primo-Parmo et al., 1996). Although the name of these enzymes derives from paraoxon, the active metabolite of the organophosphorus (OP) insecticide parathion, which is hydrolyzed by PON1 *in vitro* (Li et al., 2000), the other two PONs do not have OP esterase activity. In contrast, all three PONs are lactonases and they hydrolyze a number of acyl-homoserine lactones (acyl-HCL), molecules that mediate bacterial quorum-sensing signals and are important in regulating expression of virulence factors and in inducing a host inflammatory response (Draganov et al., 2005; Teiber et al., 2008). PON2 also plays a significant role in atherosclerosis, as shown by studies indicating that PON2 overexpression decreases atherosclerotic lesions, although the opposite is true in PON2-deficient mice (Ng et al., 2006a,b). In contrast to PON1 and PON3, which are expressed primarily in the liver, and their protein products associated with high-density lipoproteins in the plasma, PON2 is a ubiquitously expressed intracellular enzyme but is not present in plasma (Ng et al., 2001; Marsillach et al., 2008; Giordano et al., 2011).

In several tissues, PON2 has been shown to exert an antioxidant effect (Ng et al., 2001; Horke et al., 2007),

which is believed to play a major role in preventing the atherosclerotic process, as shown directly in PON2-knockout mice (Horke et al., 2007; Devarajan et al., 2011). Subcellular distribution studies have shown that PON2 is localized primarily in the mitochondria (Giordano et al., 2011; Devarajan et al., 2011). Mitochondria are a major source of free radical-related oxidative stress, and the preponderant localization of PON2 in mitochondria would support a role for this enzyme in protecting cells from oxidative damage. In HeLa cells, PON2 has been shown to bind to coenzyme Q_{10} that associates with complex III in mitochondria, and PON2 deficiency causes mitochondrial dysfunction (Devarajan et al., 2011). In human endothelial cells, PON2 has been shown to reduce, indirectly but specifically, the release of superoxide from the inner mitochondrial membrane, without affecting levels of other radicals such as hydrogen peroxide and peroxynitrite (Altenhofer et al., 2010).

Paraoxonase 2 in the Central Nervous System

PON2 mRNA has been found in mouse and human brain, and PON2 protein has been detected in mouse (Primo-Parmo et al., 1996; Ng et al., 2006a; Marsillach et al., 2008) as well as rat, human (Giordano et al., 2013b), and monkey brain (Costa, de Laat et al., unpublished). In a series of recent studies, the expression of PON2 has been characterized in mouse brain (Giordano et al., 2011, 2013b; Costa et al., 2014). The highest levels of PON2 protein were found in three dopaminergic regions, the substantia nigra, the striatum, and the nucleus accumbens, with lower levels in cerebral cortex, cerebellum, hippocampus, and brainstem. The higher levels of PON2 in dopaminergic areas are of interest because they may be related to the higher level of oxidative stress, due to dopamine metabolism, present in these regions. The regional distribution and gender difference of PON2 were confirmed by measurements of its lactonase activity (measured by dihydrocoumarin (DHC) hydrolysis) and of PON2 mRNA levels (Giordano et al., 2011). At the cellular level, PON2 is significantly higher in astrocytes than in neurons in all brain regions, with the highest levels in cells isolated from the striatum. Striatal neurons and astrocytes isolated from female mice express higher levels of PON2 than the same cells from male animals. PON2 is also present in cortical microglia, at levels similar to those found in neurons (Giordano et al., 2011). At the subcellular level, the highest levels of PON2 are found in mitochondria, followed by membranes (microsomes), in agreement with previous observations in HeLa cells (Devarajan et al., 2011).

To provide a direct indication of whether PON2 exerts a protective effect toward oxidative stress in brain cells, as observed in other tissues and cell types, the cytotoxicity of two known oxidants, hydrogen peroxide (H_2O_2) and 2,3-dimethoxy-1,4-naphtoquinone (DMNQ), was investigated in neurons and astrocytes from different brain regions isolated from wild-type ($PON2^{+/+}$) and $PON2^{-/-}$ mice. In all instances, cells from mice lacking PON2 were more susceptible to the toxicity of both compounds by a factor of 5-fold to 11-fold. The protection afforded by PON2 to neurons and astrocytes was related to its ability to scavenge ROS on exposure to oxidants. For example, DMNQ (10 μM) increased ROS to ~400% of basal levels in cerebellar granule neurons from $PON2^{-/-}$ mice, but only to 170% in the same cells from $PON2^{+/+}$ mice (Giordano et al., 2011).

Gender Differences in Paraoxonase 2 Expression

In every brain region, PON2 levels are higher (by approximately two-fold to three-fold) in female mice than in male mice (Giordano et al., 2011). This may be related to a positive modulatory effect by estrogens. In striatal astrocytes from male mice, 17β-estradiol causes time-dependent and concentration-dependent increases in the levels of PON2 protein. For example, 12 h to 24 h exposure with 200 nM estradiol increases PON2 expression in striatal astrocytes from male mice to the levels found in female striatal astrocytes (Giordano et al., 2013b). Interestingly, in female astrocytes, estradiol can further increase PON2 expression by a factor of approximately 2.5-fold. The estradiol effect is due to transcriptional activation of the PON2 gene and is mediated by activation of estrogen receptors alpha (Giordano et al., 2013b). In ovariectomized mice, PON2 levels (protein and mRNA) are significantly reduced in striatum, cerebral cortex, and liver, approaching the levels found in male mice. Given the findings of enhanced susceptibility to oxidative stress due to lack of PON2 (Giordano et al., 2011), it was of interest to ascertain whether the two-fold to three-fold difference in PON2 levels between genders was sufficient to confer differential susceptibility to oxidants. This was indeed the case, because striatal astrocytes and neurons from male mice were more sensitive to H_2O_2 and DMNQ-induced oxidative stress and ensuing cytotoxicity (Giordano et al., 2013b). Although gender-dependent differences in other cell defense mechanisms cannot be excluded, it is noteworthy that levels of GSH did not differ between genders.

Another important aspect is related to the lack of gender difference in susceptibility in cells from $PON2^{-/-}$ mice. Striatal astrocytes from $PON2^{-/-}$ mice of either gender are highly susceptible to oxidant-induced toxicity, as expected, but there are no significant female/male differences (Giordano et al., 2013b). Further evidence for a central role of PON2 in mediating gender differences in susceptibility to oxidative stress toxicity is provided by experiments with estradiol. In central nervous

system (CNS) cells from PON2$^{+/+}$ male mice, exposure to estradiol (200 nM, 24 h) provided protection against toxicity induced by the two oxidants. This is not surprising because neuroprotective actions of estrogens are well known (Simpkins et al., 2010; Azcoitia et al., 2011). However, the protective effect of estradiol is absent in cells from PON2$^{-/-}$ mice, suggesting that a major mechanism of estrogen neuroprotection may be represented by induction of PON2 (Giordano et al., 2013b).

Modulation of Paraoxonase 2

The studies summarized in the previous paragraph show that the enzyme paraoxonase 2 exerts an antioxidant/anti-inflammatory effect in the CNS, and that levels of expression of PON2 are an important determinant of susceptibility to neurotoxicity. Hence, modulation of PON2 by agents/factors that increase its level of expression may result in neuroprotection.

In macrophages, PON2 expression is increased by oxidative stress (Rosenblat et al., 2003), and in vascular cells an endoplasmic reticulum stress element-like sequence was found to be present in the promoter region of PON2 (Horke et al., 2007). Arachidonic acid (Rosenblat et al., 2010), the licorice phytoestrogen glabridin (Yehuda et al., 2011), and the hypocholesterolemic drug atorvastatin (Rosenblat et al., 2004) also upregulate PON2 expression in various cell types. Urokinase plasminogen activator upregulates PON2 in macrophages via NADPH oxidase and the transcription factor SREBP-2 (Fuhrman et al., 2009). Pomegranate juice was found to increase PON2 in macrophages through activation of the PPARγ and AP-1 pathways (Shiner et al., 2007), whereas extracts of Yerba mate (*Ilex paraguariensis*) have been reported to increase PON2 mRNA and lactonase activity in macrophages *in vitro* and after *in vivo* administration to healthy women (Fernandes et al., 2012).

Modulation of Paraoxonase 2 by Quercetin

Quercetin was reported to increase PON2 mRNA and protein in macrophages *in vitro*, although administration of 150 mg/day to human volunteers for 6 weeks was without effects (Boesch-Saadatmandi et al., 2009). A recent study has examined the induction of PON2 by quercetin *in vitro* (Costa et al., 2013). Quercetin was found to increase PON2 protein expression in mouse striatal astrocytes (mixed gender) by approximately two-fold at concentrations ranging from 1 to 20 μM (Figure 1.3). Similar results were also observed in mouse striatal neurons and in mouse RAW264.7 macrophages (Costa et al., 2013). The effect of quercetin was antagonized by SP600125, an inhibitor of the JNK/AP-1 pathway. In contrast, the PPARγ inhibitor GW9662 did not antagonize quercetin's effect on PON2 while totally

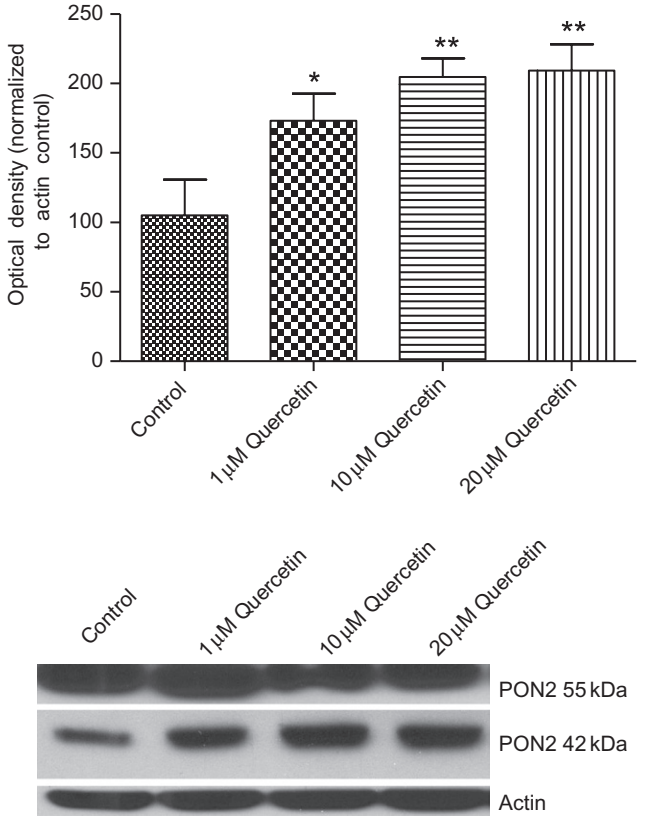

FIGURE 1.3 Induction of PON2 by quercetin. Mixed gender mouse striatal astrocytes were exposed for 24 h to quercetin (1, 10, or 20 μM). Shown are the quantification of the 42 kDa alloform (top, n=3) and a representative blot (bottom). Significantly different from control: *P<0.05, **P<0.01. *Source: From Tait (2011).*

abrogating the induction of PON2 by the PPARγ agonist rosiglitazone (Costa et al., 2013). Quercetin may thus induce a very low level of oxidative stress (Halliwell, 2008; Chang et al., 2009), which in turn would modulate the JNK/AP-1 pathway (Granado-Serrano et al., 2010), causing an increase in PON2 expression. Alternatively, given the effects of estradiol on PON2 expression (Giordano et al., 2013b), quercetin may induce PON2 expression by virtue of its phytoestrogen activity (Galluzzo et al., 2009; Ruotolo et al., 2014), although this would need to be investigated further.

Independent of the underlying mechanism(s), the ability of quercetin to induce PON2 may play a role in the reported neuroprotective actions of this polyphenol, which have been observed *in vitro* as well as *in vivo* (Miodini et al., 1999; Mercer et al., 2005; Boots et al., 2008; Ossola et al., 2009; Barcelos et al., 2011; Selvakumar et al., 2012). In striatal astrocytes from PON2$^{+/+}$ mice (mixed gender), exposure for 24 h to quercetin abolished the increase in ROS levels caused by hydrogen peroxide (H_2O_2) or DMNQ (Costa et al., 2013). This resulted in protection against the toxicity of these oxidants, as shown in Table 1.1; indeed, the IC_{50} values for

TABLE 1.1 Role of PON2 in Quercetin Protection Against Oxidative Stress in Striatal Astrocytes

	IC_{50} (μM)	
	Control	**Quercetin**
PON2$^{+/+}$ MICE		
H_2O_2	38.9 ± 4.5	157.0 ± 8.1*
DMNQ	37.5 ± 5.6	131.3 ± 9.2*
PON2$^{-/-}$ MICE		
H_2O_2	6.3 ± 1.3	11.9 ± 1.2
DMNQ	6.1 ± 1.0	8.3 ± 1.1

Source: Adapted from Costa et al. (2013).
Striatal astrocytes from wild-type (PON2$^{+/+}$) or PON2$^{-/-}$ mice (mixed genders) were exposed for 24 h to 20 μM quercetin. After washout, cells were treated for 24 h with four to five concentrations of hydrogen peroxide (H_2O_2) or 2,3-dimethoxy-1,4-naphtoquinone (DMNQ), and cytotoxicity was measured by the MTT assay. Results indicate the IC_{50} values (μM) and are the mean (± SD) of three separate experiments. *Significantly different from wild-type control ($P < 0.01$). The findings indicate that exposure of wild-type cells to quercetin provides neuroprotection, as evidenced by an approximately four-fold increase in IC_{50} for the two oxidants. Control astrocytes from PON2$^{-/-}$ mice are six-fold more susceptible to the toxicity of H_2O_2 and DMNQ, likely because of the lack of PON2. Additionally, in PON2$^{-/-}$ cells, quercetin-induced protection is small (approximately 1.5-fold).

cytotoxicity of H_2O_2 and DMNQ increased by 3.5-fold to 4-fold after treatment with quercetin, which doubled PON2 expression. This is similar to what is observed in brain cells from male and female mice, in which a two-fold to three-fold difference in PON2 expression resulted in a three-fold to four-fold difference in susceptibility to these two oxidants (Giordano et al., 2013b). Although other neuroprotective pathways (e.g., Nrf2-ARE) may be involved in the observed neuroprotection, experiments performed in cells from PON2$^{-/-}$ mice show that modulation of PON2 expression plays an important role in the neuroprotective effect of quercetin. On quercetin exposure of PON2$^{-/-}$ cells, the IC_{50} for H_2O_2 and DMNQ were increased by only 1.9-fold and 1.4-fold, respectively (Table 1.1). This partial protection may be due to NrF2-ARE induction.

THE ISSUE OF METABOLITES

An important issue to consider as part of the discussion on the beneficial effects of quercetin relates to the potential role played by its metabolites (Del Rio et al., 2010). Quercetin is metabolized to various conjugated metabolites: 3′-*O*-methyl-quercetin (isorhamnetin; MeQ), quercetin-3-*O*-glucuronide (Glu3Q); 3′-*O*-methylquercetin-*O*-glucuronide (Glu3MeQ); and quercetin-3′-*O*-sulfate (Sul3Q) (Day et al., 2001; Harwood et al., 2007). As stated, only limited amounts of quercetin aglycon are found after ingestion of quercetin, although there is some controversy regarding this issue (e.g., Shanely et al., 2010; Kelly, 2011), and methylated, sulfated, and glucuronide metabolites are the most prominent moieties found in plasma. Studies have shown that Glu3Q has antioxidant abilities *in vitro* and *in vivo* (Moon et al., 2001; Shirai et al., 2006; Kawai et al., 2008). Additional biological effects of methylated and sulfate metabolites have been reported (Yeh et al., 2011; Boesch-Saadatmandi et al., 2011; Ruotolo et al., 2014), although some studies have failed to observe an effect of quercetin metabolites (Cho et al., 2012). Of interest is also the observation that conjugated quercetin can enter the cell, where it is converted to its nonconjugated form (Fiorani et al., 2003). Thus, quercetin metabolites should be tested to ascertain, for example, whether they induce the Nrf2-ARE pathways or modulate PON2 expression. One metabolite, Glu3Q, has been recently shown to act as an agonist at estrogen receptor α, and may thus be capable of inducing PON2 (Ruotolo et al., 2014). Furthermore, various other polyphenol catabolites have been shown to exert strong biological activity, particularly in protecting neuronal cells against DMNQ-induced oxidative stress and toxicity (Verzelloni et al., 2011).

CONCLUDING REMARKS AND FUTURE DIRECTIONS

There is still great interest in the mechanisms that may underlie the neuroprotective effects of nutraceuticals. In this chapter, the focus has been on the polyphenol quercetin and on mechanisms related to its ability to counteract oxidative stress-mediated neurotoxicity. However, several other potential mechanisms have been investigated and should be considered. Because nutraceuticals comprise a large and diverse class of compounds with different chemical structures and bioavailability, multiple targets for biological activity are to be expected. For example, in a discussion on the biochemical and cellular bases for nutraceutical strategies to combat Parkinson's disease (PD), the following potential targets were indicated: α-synuclein aggregation; ubiquitin proteasome function; mTOR signaling/lysosomal-autophagy; energy failure; faulty catecholamine trafficking; dopamine oxidation; synthesis of toxic dopamine-quinones; inflammation; methylation; and oxidation of neuromelanin (Mazzio et al., 2011). Dozens of nutraceutical compounds have been identified that would affect at least one of the indicated targets (Mazzio et al., 2011). On a more general basis, neuroinflammation, which is believed to be most relevant in neurodevelopmental and neurodegenerative disorders (Skaper et al., 2014; Baune, 2015), represents a potential important target for nutraceuticals (Baune, 2015; Nabavi et al., 2015). Compounds that may antagonize microglia

activation and reduce the release of pro-inflammatory cytokines would be of much relevance. Soy isoflavones daidzein and genistein are suggested to reduce microglial activation and subsequent release of pro-inflammatory factors (Chinta et al., 2013; Jantaratnotai et al., 2013), although further studies in this area are needed. There is also evidence in this regard that polyphenols or garlic extract may have beneficial anti-inflammatory properties (Vauzour, 2012; Takechi et al., 2013).

An additional field of interest with regard to the mechanisms of neuroprotection provided by nutraceuticals is that of sirtuins. These proteins (in mammals there are seven, SIRT1–SIRT7) are involved in a variety of cellular and molecular processes and pathways with distinct cellular localization and molecular targets (Dang, 2012). Of these, SIRT1 predominantly localizes in the nucleus and acts as a deacetylase for histones and other targets. SIRT1 protects cells from apoptosis and promotes differentiation of stem cells. SIRT2 is prevalent in the cytoplasm and has been found to accumulate in neurons, whereas other SIRTs localize primarily in the mitochondria (Dang, 2012). The neuroprotective effects of the polyphenol resveratrol have been ascribed to activation of SIRT1, leading to inhibition of amyloid-β peptide, suppression of Bax-dependent apoptosis, and repression of multiple pro-apoptotic transcription factors (Kelsey et al., 2010).

One aspect that may deserve more attention relates to the modulation of autophagy. Autophagy (from the Greek "to eat oneself") refers to the cellular degradative pathways that involve delivery of the cytoplasmatic cargo to the lysosomes (Mariño et al., 2011; Gabryel et al., 2012; Giordano et al., 2014). Autophagy (macroautophagy) is a multistep process involving the formation of double membrane structures, the autophagosomes, which then fuse with lysosomes. The content of the resulting autophagolysosomes (misfolded proteins, cellular metabolic waste) is then degraded by hydrolytic enzymes. Autophagy is also important for removal of damaged mitochondria and of normal mitochondria undergoing turnover in a process known as mitophagy. The integrity of the CNS is very dependent on normal basal autophagy because damaged organelles and misfolded proteins would accumulate in neurons unless they are successfully removed (Marino et al., 2011). Deletion of key autophagy genes (*Atg5*, *Atg7*) causes severe neurodegeneration (Komatsu et al., 2006). Rapamycin, an inhibitor of mTOR (mammalian target of rapamycin) activity, is a potent inducer of autophagy and acts as a neuroprotector (Pan et al., 2009; Giordano et al., 2014). Stimulation of autophagy by nutraceuticals would thus lead to neuroprotection, as has been shown, for example, in the case of resveratrol (Lin et al., 2014), a traditional Korean herbal formula (Bae et al., 2015), carnosine (Baek et al., 2014), and other compounds (Giordano et al., 2014). Because the well-being of most CNS cells is dependent on the integrity of mitochondria, these organelles represent the principal target for neuroprotective strategies, including, among several, modulation of mitophagy and oxidative stress (Perez-Pinzon et al., 2012).

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CHAPTER

2

Prevention of Neurodegenerative Disorders by Nutraceuticals

Francesca Pistollato and Magdalini Sachana

INTRODUCTION

Dietetic macronutrients and micronutrients play a crucial role in the control of brain physiology, and food intake is known to stimulate the activity of neurotrophic factors regulating synaptic plasticity. Among micronutrients, vitamins (e.g., B1, B6, folic acid, B12, C, D, K, and α-tocopherol) and minerals (e.g., iron, lithium, magnesium, copper, iodine, and manganese) are known to modulate central nervous system (CNS) functionality, whereas macronutrients (e.g., polyunsaturated fatty acids (PUFAs), essential amino acids, low glycemic index foods, and dietary fibers) are relevant for the maintenance of cognitive functions and the prevention of neurodegeneration (Pistollato and Battino, 2014).

The chronic persistence of neuroinflammation is currently considered the main driving force of the neurodegenerative process. Neuroinflammation is generally characterized by the activation of glia and microglia and the upregulation of inflammatory-related molecules (Morales et al., 2014), and this phenomenon seems to occur especially during the aging process (Michaud et al., 2013). Plant-derived bioactive nutrients, such as antioxidants, *n*-3 and *n*-6 PUFAs, and other anti-inflammatory nutraceuticals, have been shown to prevent neuroinflammation, thus reducing the risk of neurodegeneration (Virmani et al., 2013). The presence of chronic neuroinflammatory status is determined by several factors, such as lifestyle and diet. Importantly, dietetic patterns characterized by high consumption of animal-derived products and a very low or null intake of plant-derived foods can lead to persistent chronic inflammation, the potential onset of metabolic syndrome (MetS)-related dysfunctions, and a high risk of cognitive impairment (Pistollato and Battino, 2014). One of the factors characteristic of both the MetS and neurodegeneration is the presence of a high plasma homocysteine level, a condition known as hyperhomocyteinemia (hHcy). Indeed, hHcy is commonly known as an independent risk factor for cardiovascular disease and stroke; however, it is also correlated to several neurodegenerative diseases (Boldyrev et al., 2013), including Alzheimer's disease (AD) and vascular dementia (Troen et al., 2008). hHcy has also been found in L-DOPA-treated Parkinson's disease (PD) patients (Zoccolella et al., 2005) and is correlated to neuroinflammation (Sudduth et al., 2013) and to loss of central cholinergic neurons (Pirchl et al., 2010). Additionally, a high level of serum homocysteine has been found in the body fluids of autistic children (Kaluzna-Czaplinska et al., 2013). Some nutraceuticals, specifically B vitamins, are fundamental to stabilize homocysteine levels; indeed, vitamin B6 (pyridoxine), B12 (cobalamin), and B9 (folic acid) are cofactors needed to guarantee the physiological functioning of the enzymes specifically involved in homocysteine metabolism.

To counteract neuroinflammation-related processes plant-derived polyphenols, such as resveratrol, sulforaphanes, and curcumin, have been regarded as essential antioxidant factors contributing to the regulation of brain homeostasis. Besides their antioxidant capacity, these neurohormetic phytochemicals are also known to downregulate oxidative/inflammatory stress signaling pathways and to upregulate the expression of genes encoding antioxidant enzymes, phase-2 enzymes, neurotrophic factors, and cytoprotective-related signaling pathways, such as the sirtuin–Forkhead box O (FoxO) pathway, the nuclear factor-kappaB (NF-κB) pathway, the nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant responsive element (ARE) pathway, and the cyclic AMP responsive element binding protein (CREB)-related pathway (Vauzour, 2012).

Nutraceuticals.
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This chapter describes the molecular mechanisms underlying the beneficial effects elicited by well-described nutraceuticals in the prevention and the regression of AD, PD, and autism spectrum disorders (ASDs).

NUTRACEUTICALS FOR THE PREVENTION AND AMELIORATION OF AD

AD is the most common form of dementia, characterized by the extracellular accumulation of beta-amyloid (Aβ) deposits and progressive microtubule disintegration, leading to dysfunctional neuronal communication and neuronal cell death. Moreover, mature long-lasting amyloid plaques appear to be less toxic than the prefibrillar aggregates, which represent their precursors. The early aggregates seem to interact with cell membranes, causing oxidative stress and an increase in free Ca^{2+} levels, eventually leading to apoptotic or necrotic cell death (Stefani and Dobson, 2003). As the disease advances, confusion, irritability, aggression, mood swings, trouble with language, and long-term memory loss often occur, with an average life expectancy upon diagnosis of approximately 7 years (Waldemar et al., 2007). Among risk factors, hyperlipidemia, hHcy, diabetes, alcohol consumption, smoking, and obesity have been found to increase AD risk, whereas consumption of plant-based foods, enriched in bioactive phytocompounds, and also fish, Mediterranean diet, and unsaturated fat or *n*-3 fatty acids, together with physical and social activity, have been described as protective in observational studies (Weih et al., 2007). Currently, given the lack of a definitive and effective treatment for AD, lifestyle changes, including plant-based nutritional interventions and natural nutraceutical supplementations, and also practicing cognitive and social activity and physical exercise are considered alternative measures to prevent AD occurrence (Mecocci et al., 2014). In particular, flavonoids, vitamins, and other natural substances have been studied in relation to AD and have been considered beneficial for the maintenance of cognitive performances (Table 2.1). Polyphenols, which are naturally present in vegetables, fruits, herbs, and nuts, may promote prevention and regression of AD by targeting specific signaling pathways associated with protein folding and neuroinflammation (Essa et al., 2012). Importantly, an effective strategy to prevent and/or reduce protein misfolding and Aβ formation and restoring cellular aggretome might be the use of amyloid-binding polyphenols. These polyphenols seem to act via different mechanisms, either inhibiting fibril formation or steering oligomer formation into unstructured, nontoxic pathways (Ngoungoure et al., 2015).

AD and its consequential neuronal damage seem to occur as a consequence of a sustained neuroinflammatory process, which seems to be caused by a plethora of different damage signals such as trauma, infection, oxidative agents, redox-active iron, and oligomers of tau and Aβ. In this context, astrocytes and microglial cells get progressively activated, leading to overproduction of pro-inflammatory agents (Morales et al., 2014). Several natural anti-inflammatory compounds have been tested in AD models both *in vitro* and *in vivo* and are currently regarded as preventive and coadjuvant treatments for AD. In particular, curcumin, a natural phenolic compound derived from the perennial herb *Curcuma longa* (turmeric), is known to exhibit anti-inflammatory and antioxidant effects (Lu et al., 2014). Curcumin has been found to activate the heat shock response, reducing oxidative damage related to AD (Calabrese et al., 2003). Moreover, curcumin treatment has been reported to attenuate cognitive impairment and stimulate neuroprotection in a mouse model of AD and to inhibit the generation of Aβ by inducing autophagy, as evidenced by analysis of the autophagy-related protein LC3, and this effect was mediated via downregulation of the phosphatidylinositol-3-kinase (PI3K)/Akt and the mammalian target of rapamycin (mTOR) signaling pathway (Wang et al., 2014). Furthermore, an *in vitro* study has shown that both curcumin and its analog Cur1 protect neuroblastoma SK-N-SH cells from the exogenous effects of Aβ1–42 exposure, and the protective effect is elicited via the upregulation of human telomerase reverse-transcriptase (hTERT) expression (Xiao et al., 2014).

Soybean isoflavones, such as genistein, have been regarded as beneficial for the prevention and regression of neurodegenerative diseases (Lee et al., 2005). *In vitro* treatment with a synthetic version of genistein, called GS-14, has been shown to be neuroprotective on SH-SY5Y cells previously exposed to Aβ proteins; GS-14 inhibits acetylcholinesterase (AChE) and also modulates estrogenic activity, suggesting its possible use as an effective agent for the treatment of AD (Shi et al., 2012). Analogously, some genistein derivatives, in particular one called 25d, tested both *in vitro* and *in vivo*, have been found to promote AChE inhibitory activity and to display antioxidative activity, promoting inhibition of Aβ aggregation and exhibiting metal chelating properties. The analog 25d reversed scopolamine-induced memory deficit in mice, suggesting that 25d may be a promising multifunctional agent for the treatment of AD (Qiang et al., 2014). Additionally, treatment with genistein has been found to attenuate the cytotoxicity and inflammatory damage induced by Aβ25–35 exposure *in vitro* by inhibiting the Toll-like receptor 4 (TLR4) and NF-κB upregulation mediated by Aβ25–35 and the DNA binding and transcriptional activities of NF-κB (Zhou et al., 2014).

Among phytochemicals, the isothiocyanate sulforaphane, derived from the hydrolysis of the glucoraphanin, a glucosinolate mainly present in Brassica vegetables, has also demonstrated neuroprotective effects in several

TABLE 2.1 Summary of Nutrients and Nutraceuticals and Their Relevance to AD and Dementia

Nutrient or nutraceutical	Elicited effects	References
Polyphenols, amyloid-binding polyphenols	Target-specific signaling pathways associated with protein folding and neuroinflammation Inhibit fibril formation or steer oligomer formation	Essa et al. (2012), Ngoungoure et al. (2015)
Curcumin, Cur1	Anti-inflammatory and antioxidant effects Activate the heat shock response, reducing oxidative damage Attenuate cognitive impairment and stimulate neuroprotection, inhibit the generation of Aβ by inducing autophagy via downregulation PI3K/Akt and mTOR Protect from exogenous effects of Aβ1–42 via the upregulation of hTERT expression	Calabrese et al. (2003), Lu et al. (2014), Wang et al. (2014), Xiao et al. (2014)
Isoflavones (e.g., genistein, GS-14, 25d)	Elicit neuroprotection from Aβ protein exposure, inhibits AChE, modulates estrogenic activity Antioxidative activity, inhibit Aβ aggregation and exhibit metal chelating properties; reverse scopolamine-induced memory deficit Attenuate cytotoxicity and inflammatory damage induced upon Aβ25–35 exposure by inhibiting TLR4 and NF-κB upregulation	Lee et al. (2005), Qiang et al. (2014), Shi et al. (2012), Zhou et al. (2014)
Isothiocyanates (e.g., sulforaphane)	Activate Nrf2/ARE pathway, promoting the upregulation of GSH Antioxidant potential upon exposure to Aβ25–35, upregulate antioxidant enzymes via activation of Nrf2, prevent Aβ-mediated apoptosis	Lee et al. (2013), Tarozzi et al. (2013)
Folate, vitamin B12, vitamin B6	Prevent tau hyperphosphorylation and memory deficits induced by acute administration of homocysteine Inhibit tau hyper-phosphorylation and accumulation in hippocampus and cortex; downregulate GSK-3β, CDK5, JNK, ERK, and p38MAPK, attenuate memory deficits No improvements of primary and secondary cognitive measurements, depression as adverse effect Ineffective for AD or dementia	Aisen et al. (2008), Nelson et al. (2009), Wei et al. (2011), Zhang et al. (2008)
Fortasyn Connect™ (cocktail of docosahexaenoic acid, eicosapentaenoic acid, uridine-5'-monophosphate, choline, phospholipids, antioxidants, and B vitamins)	Designed to enhance synapse formation and functionality Improve memory performance, positively affect brain functional connectivity	Engelborghs et al. (2014), Mi et al. (2013)
Shilajit, fulvic acid, with/without B vitamins	Contribute to AD prevention	Carrasco-Gallardo et al. (2012)
Vitamin E, vitamin C, α-lipoic acid (E/C/ALA)	Lower CSF F2-isoprostane levels, indicative of oxidative stress reduction in the brain but associated with faster cognitive decline	Galasko et al. (2012)
γ- and α-Tocopherols	High α-tocopherol seems to be associated with higher amyloid load when γ-tocopherol levels were low Conversely, high α-tocopherol seems to be associated with lower amyloid levels when γ-tocopherol levels were high	Morris et al. (2015)
SAM, PUFAs	Neuroprotective (particularly SAM) under conditions of reduced GST activity, diminished SAM, increased accumulation of SAH, and deprived folate	Panza et al. (2009)

in vitro and *in vivo* studies. Sulforaphane, in particular, seems to activate the antioxidant Nrf2/ARE pathway, promoting the upregulation of glutathione (GSH) (Tarozzi et al., 2013). Additionally, *in vitro* treatment with sulforaphane exerts antioxidant potential in SH-SY5Y cells exposed to Aβ25–35, as shown by the upregulation of antioxidant enzymes (i.e., γ-glutamylcysteine ligase, NAD(P)H:quinone oxidoreductase-1, and heme oxygenase-1) via activation of Nrf2, thus preventing Aβ-mediated apoptosis (Lee et al., 2013).

Numerous epidemiological findings, confirmed also by *in vivo* animal studies, show that several MetS-related factors, such as atherosclerosis, diabetes, hHcy, hypertension, and high total and LDL cholesterol levels, may play a role in the development of AD (Ehrlich and Humpel, 2012). For this reason, supplementation with folate and

vitamin B12 has been found to be effective in preventing tau protein hyperphosphorylation and memory deficits induced by acute administration of homocysteine in young rats (Zhang et al., 2008). Folate and vitamin B12 supplementation has also been found to be effective in aged rats with chronic hHcy, promoting inhibition of tau hyperphosphorylation and accumulation in hippocampus and cortex, together with downregulation of glycogen synthase kinases-3β (GSK-3β), cyclin-dependent kinase-5 (CDK-5), c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), and p38 mitogen-activated protein kinase (p38MAPK). As a consequence, a significant attenuation of memory deficits occurs (Wei et al., 2011). Nevertheless, a multicenter, randomized, double-blind, controlled clinical trial of high-dose folate, vitamin B6, and vitamin B12 supplementation in 409 (of 601 screened) individuals with mild-to-moderate AD and normal folic acid, vitamin B12, and homocysteine levels has shown that vitamin supplements effectively reduced homocysteine levels compared to the placebo group, they did not improve primary and secondary cognitive measurements, eventually leading to adverse events such as occurrence of depression (Aisen et al., 2008). For this reason, regimens with high-dose B vitamin supplements might not be advisable. Accordingly, a 2009 study has examined the associations between dietary and supplementation of folate, vitamin B12, and vitamin B6 and the incidence of AD among elderly men and women (Cache County Memory, Health and Aging Study). Interestingly, the authors of that study have not observed differences in risk of AD or dementia by increasing the total intake of folate, vitamin B12, or vitamin B6 (Nelson et al., 2009).

Several nutritional intervention studies in patients affected by AD have been conducted in past years; some of these interventions are based on single nutrient administrations, and others are based on nutrient combination, such as the medical food "Fortasyn Connect™," which is designed to enhance synapse formation and functionality and contains a cocktail of docosahexaenoic acid, eicosapentaenoic acid, uridine-5'-monophosphate, choline, phospholipids, antioxidants, and B vitamins (Mi et al., 2013). In two randomized controlled trials, "Fortasyn Connect™" in its nutrient formulation (Souvenaid®) has resulted in improving memory performance in mild, drug-naïve patients with AD, positively affecting brain functional connectivity (Engelborghs et al., 2014; Mi et al., 2013). Moreover, a clinical trial conducted on patients with mild AD has shown that both shilajit alone, a sticky tar-like substance, and its active principle, fulvic acid, as well as a combination of shilajit and B vitamins, contribute to AD prevention (Carrasco-Gallardo et al., 2012).

Another randomized clinical study has been conducted on subjects with mild to moderate AD and analyzed the effects elicited by a 16-week treatment intervention comparing the administration of vitamin E (α-tocopherol, 800 IU/day), vitamin C (500 mg/day), α-lipoic acid (900 mg/day), globally named the E/C/ALA group, or coenzyme Q (400 mg, three times per day), or simple placebo (Galasko et al., 2012). Importantly, the antioxidants present in the E/C/ALA treatment did not alter any cerebrospinal fluid (CSF) biomarkers related to amyloid or tau pathology, rather than lowering CSF F_2-isoprostane levels, which is indicative of oxidative stress reduction in the brain. However, the E/C/ALA treatment also induced a faster cognitive decline, which represents a possible serious concern, especially if longer-term clinical trials have to be conducted (Galasko et al., 2012).

The correlation between α- and γ-tocopherol brain concentrations and AD neuropathology has been assessed in 115 deceased participants of the prospective Rush Memory and Aging Project by taking into account the following parameters: amyloid load and neurofibrillary tangle severity. In particular, brain concentrations of γ- and α-tocopherols seem associated with AD neuropathology in an interrelated mechanism. High α-tocopherol seems to be associated with higher amyloid load when γ-tocopherol levels were low; conversely, high α-tocopherol seems to be associated with lower amyloid levels when γ-tocopherol levels were high (Morris et al., 2015).

A 2009 review has commented on the positive effects elicited by a combined supplementation with *S*-adenosylmethionine (SAM) and PUFAs for very mild AD subgroups or mild cognitive impairment (Panza et al., 2009). In particular, the use of SAM as a neuroprotective dietary supplement for AD patients seems to be relevant because AD subjects are often characterized by reduced glutathione *S*-transferase (GST) activity, diminished SAM, and increased accumulation of the downstream metabolic product resulting from SAM-mediated transmethylation (i.e., *S*-adenosylhomocysteine (SAH)) under conditions of deprived folate (Panza et al., 2009). These studies globally suggest that appropriately combined, rather than single, nutraceutical supplementations might prevent AD-related symptoms and/or benefit AD patients. Large scale clinical trials will be essential to confirm the efficacy of nutrients and nutraceuticals for the prevention or regression of both mild cognitive impairment and AD.

NUTRACEUTICALS FOR THE PREVENTION AND AMELIORATION OF PD

PD is a degenerative disorder of the CNS characterized by motor symptoms, such as shaking, rigidity, slowness of movement, and difficulty with walking and gait, possibly followed by late-onset dementia. These impairments are a consequence of a progressive degeneration of dopaminergic neurons localized in the substantia nigra. The pathology of this disease is also

characterized by the accumulation of the α-synuclein protein into the Lewy bodies within neurons, and the insufficient formation and activity of dopamine within the midbrain (Shulman et al., 2011).

Current treatments are effective in managing the early motor symptoms, mainly through the administration of L-DOPA and dopamine agonists; however, with the progression of the disease, these drugs eventually become ineffective and often further complications occur, such as dyskinesia, which is characterized by involuntary writhing movements (Shulman et al., 2011). Besides surgery, deep brain stimulation, gene therapy, stem cell transplantations, and novel neuroprotective pharmacologic agents, supplementation with specific plant-based phytocompounds and foods and some forms of rehabilitation have been proven effective in alleviating PD-related symptoms. In particular, nutraceuticals play a critical role in the regulation of energy metabolism and signaling pathways that control neurotransmission and neuroinflammation (Table 2.2). Nutraceuticals have been proven to interfere with several mechanisms related to the PD symptoms manifestation, such as α-synuclein aggregation, ubiquitin-proteasome function, mTOR signaling and lysosomal-mediated autophagy, energy failure, faulty trafficking of catecholamine, dopamine oxidation, hHcy, methylation patterns, neuroinflammation, and irreversible oxidation of neuromelanin (Mazzio et al., 2011). Some of the applied nutraceuticals include vitamins C, D, and E, coenzyme Q10, creatine, unsaturated fatty acids, sulfur-containing

TABLE 2.2 Summary of Nutrients and Nutraceuticals and Their Relevance to PD

Nutrient or nutraceutical	Elicited effects	References
Vitamins C, D, and E, coenzyme Q10, creatine, unsaturated fatty acids, sulfur-containing compounds, polyphenols, stilbenes, and phytoestrogens	Prevent α-synuclein aggregation, ubiquitin-proteasome function, mTOR signaling and lysosomal-mediated autophagy, energy failure, faulty trafficking of catecholamine, dopamine oxidation, hHcy, methylation patterns, neuroinflammation, and irreversible oxidation of neuromelanin Useful for the management of PD	Chao et al. (2012), Mazzio et al. (2011)
Sulforaphane	Reduces motor coordination deficits and rotations induced by 6-OHDA in mice, reduces apoptosis by blocking DNA fragmentation and caspase-3 activation, enhances GSH levels and some neuronal survival pathways (ERK1/2)	Morroni et al. (2013)
Catechins, (−)-epigallocatechin-3-gallate, quercetin	Neutralize stress-related free radicals and inflammation Inhibit L-DOPA methylation and prevents oxidative hippocampal neurodegeneration Inhibit human liver COMT-mediated O-methylation of L-DOPA *in vitro* (only (+)-catechin) Inhibit L-DOPA methylation in both peripheral compartment and striatum in rats, reduce glutamate-induced oxidative cytotoxicity *in vitro* via inactivation of NF-κB signaling, confer neuroprotection against kainic acid *in vivo*	Kang et al. (2010), Kang et al. (2013b), Mandel et al. (2012), Weinreb et al. (2004)
Extracts of tangerine peel (rich in polymethoxylated flavones), cocoa-2 (rich in procyanidins), and red clover (rich in isoflavones)	Attenuate dopaminergic neuron loss, no protection observed *in vivo*	Datla et al. (2007)
Ginkgo biloba extract 761 (EGb 761, containing 24% flavonoids and 6% terpenoids)	Antioxidant, elicits neurorecovery of damaged midbrain DA neurons, improves locomotion	Rojas et al. (2012)
Resveratrol	Activates the SIRT1 enzyme; neuroprotective against oxidative stress Prevents H_2O_2 or 6-OHDA triggered toxicity and the toxic effects elicited by Aβ1-42 and the α-synuclein-A30P Improves mitochondrial activity by activating several metabolic sensors, resulting in the activation of PGC-1α Activates AMPK and SIRT1 and upregulates the expression of several PGC-1α target genes, resulting in enhanced mitochondrial oxidative function	Albani et al. (2009, 2010), Ferretta et al. (2014)

(*Continued*)

TABLE 2.2 (Continued)

Nutrient or nutraceutical	Elicited effects	References
Curcumin, curcuminoids, CNB-001 (a curcumin derivative)	Prevent neuroinflammation Prevent MPTP-mediated depletion of dopamine and tyrosine hydroxylase immunoreactivity, downregulate GFAP, iNOS proteins, pro-inflammatory cytokine, and total nitrite generation in the striatum of MPTP-treated mice; promote increased motor performance and gross behavioral activity Ameliorate behavioral anomalies, increase expression of monoamine transporter, and improve mitochondria functionality Attenuate motor impairments and pathological changes elicited by MPTP administration In combination with DFO elicit neuroprotection in a 6-OHDA-PD rat model, increasing the levels of PCC, SOD, and GSH	Jayaraj et al. (2014a,b), Lv et al. (2014), Ojha et al. (2012), Witkin and Li (2013)
Vitamin D3	Stabilizes PD symptoms without triggering hypercalcemia	Suzuki et al. (2013)
Folate, vitamin B12, vitamin B6	Only vitamin B6 decreases risk of PD	de Lau et al. (2006), Murakami et al. (2010)
Tocopherol, CoQ10, and GSH	CoQ10 and GSH in particular show a small but significant improvement in PD symptoms	Weber and Ernst (2006)
Creatine	Ameliorates PD symptoms	Gualano et al. (2010)

compounds, polyphenols, stilbenes, and phytoestrogens (Chao et al., 2012). Among plant-derived nutraceuticals, the antioxidant sulforaphane has been found to reduce motor coordination deficits and rotations induced by 6-hydroxydopamine (6-OHDA) in mice (Morroni et al., 2013); 6-OHDA is a chemical compound used in some animal models to mimic the effects of PD. Sulforaphane reduces the 6-OHDA-dependent apoptosis by blocking DNA fragmentation and caspase-3 activation and enhances GSH levels and some neuronal survival pathways, such as ERK1/2, in the murine brain (Morroni et al., 2013). These data suggest that sulforaphane might effectively slow the progression of PD by modulating oxidative stress and the apoptotic machinery (Pistollato and Battino, 2014).

Moreover, natural plant polyphenols, such as the green and black flavonoid catechins present in tea, seem to neutralize stress-related free radicals and inflammation (Mandel et al., 2012; Weinreb et al., 2004). It has recently been shown that (−)-epigallocatechin-3-gallate, a tea polyphenol, inhibits L-DOPA methylation and prevents oxidative hippocampal neurodegeneration (Kang et al., 2010). Additionally, tea catechins [(+)-catechin and (−)-epicatechin] and quercetin have been reported to strongly inhibit human liver catechol-*O*-methyltransferase (COMT)-mediated O-methylation of L-DOPA *in vitro*, whereas only (+)-catechin, due to its better bioavailability *in vivo*, has been found to significantly inhibit L-DOPA methylation in both peripheral compartment and striatum in rats. Furthermore, (+)-catechin elicits strong reduction of glutamate-induced oxidative cytotoxicity in HT22 mouse hippocampal neurons cultured *in vitro*, and this occurs via inactivation of the NF-κB signaling pathway. *In vivo* administration of (+)-catechin has been found to be neuroprotective against kainic acid-induced oxidative rat hippocampal neurodegeneration (Kang et al., 2013b).

Another study conducted *in vivo* by using a 6-OHDA-induced PD rat model has reported that short-term presupplementation with plant extracts rich in flavonoids composed of extracts of tangerine peel (rich in polymethoxylated flavones), cocoa-2 (rich in procyanidins), and red clover (rich in isoflavones) significantly attenuates dopaminergic neuron loss, whereas no significant protection has been observed in animals supplemented with red and white grape seeds (rich in catechins) and cocoa-1 (rich in catechins) (Datla et al., 2007).

G. biloba extract 761 (EGb 761), a patented and defined mixture of active compounds extracted from *G. biloba* leaves and containing flavonoids (24%) and terpenoids (6%), has been described as neuroprotective due to its antioxidant function (Rojas et al., 2012). In a rodent model of PD, EGb761 has been found to be neuroprotective and to elicit neurorecovery of damaged midbrain DA neurons, improving locomotion (Rojas et al., 2012).

Resveratrol, a natural phytocompound acknowledged as a potential anticancer drug, has recently been shown

to display neuroprotective actions by activating the sirtuin 1 (SIRT1) enzyme, one of the seven described sirtuin deacetylases involved in many physiologic and pathologic processes including apoptosis, autophagy, diabetes, cancer, cardiovascular disorders, and neurodegeneration (Albani et al., 2010). In this regard, resveratrol has been found to be neuroprotective in a neuroblastoma cell model of oxidative stress, preventing H_2O_2 or 6-OHDA triggered toxicity and also the toxic effects elicited by exposure to two aggregation-prone proteins (i.e., the Aβ1–42 and the α-synuclein-A30P) (Albani et al., 2009). Furthermore, resveratrol has been described to improve mitochondrial activity by activating several metabolic sensors, resulting in the activation of the peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) (Ferretta et al., 2014). Recent data on primary fibroblast cultures obtained from two patients with early-onset PD linked to different Park2 mutations have revealed that resveratrol activates AMPK and SIRT1 and upregulates the expression of several PGC-1α target genes, resulting in enhanced mitochondrial oxidative function, indicative of a shift from glycolytic to oxidative metabolism (Ferretta et al., 2014).

Animal models and human-related studies have indicated curcumin as beneficial for stroke, AD, stress and mood disorders, and also PD and its related neuroinflammation (Witkin and Li, 2013). In particular, oral administration of curcuminoids has been found to significantly prevent 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP)-mediated depletion of dopamine and tyrosine hydroxylase immunoreactivity. Also, downregulation of glial fibrillary acidic protein (GFAP) and inducible nitric oxide synthase (iNOS) protein expression has been observed, and pro-inflammatory cytokine and total nitrite generation in the striatum of MPTP-treated mice have been noted to be significantly reduced by curcuminoid administration. Motor performance and gross behavioral activity have been improved, suggesting curcuminoid compounds as potential therapeutic candidate nutraceuticals for the prevention and/or management of PD (Ojha et al., 2012). Recent studies have described the effects of CNB-001, a novel pyrazole derivative of curcumin known to possess various neuroprotective properties, in a subacute MPTP rodent model of PD. Pretreatment with CNB-001 ameliorates behavioral anomalies, increases expression of monoamine transporter, and improves mitochondria functionality (Jayaraj et al., 2014a). CNB-001 has also been found to significantly attenuate motor impairments and pathological changes elicited by MPTP administration (Jayaraj et al., 2014b). However, it is important to notice that data related to the neuroprotective efficacy of tested agents may depend on the MPTP administration protocol adopted (i.e., acute vs subacute administration) (Anderson et al., 2006). Combined treatment with curcumin and desferrioxamine (DFO), a bacterial siderophore produced by the actinobacteria *Streptomyces pilosus* and used as an iron chelating agent, has been found to be neuroprotective in a 6-OHDA-PD rat model, increasing the levels of propionyl-CoA carboxylase (PCC), superoxide dismutase (SOD), and GSH (Lv et al., 2014). Altogether these *in vitro* and *in vivo* animal studies suggest that plant-derived nutraceuticals may play a protective role in the prevention of PD.

Some clinical studies tried to address the efficacy of nutraceutical administration in PD-affected subjects. A 2013 study assessed whether vitamin D3 supplementation inhibits PD progression in patients with vitamin D receptor FokI CC genotype and found that vitamin D3 supplementation could stabilize PD symptoms for a short period of time without triggering hypercalcemia (Suzuki et al., 2013). Furthermore, in the so-called Rotterdam Study, a prospective population-based cohort study including people aged 55 years and older, the association between dietary intake of folate, vitamin B12, and vitamin B6 and the incidence of PD has been assessed in 5289 participants who were free of dementia and parkinsonism and underwent complete dietary assessment at baseline. Authors of this study have found that a higher dietary intake of vitamin B6 is associated with a significantly decreased risk of PD, whereas no association has been observed for intake of dietary folate and vitamin B12. Thus, supplementation with vitamin B6 might decrease PD risk, probably through mechanisms not related to homocysteine metabolism (de Lau et al., 2006). The results were later confirmed by a Japanese study, where it has been reported that although the intake of folate, vitamin B12, and riboflavin is not associated with the risk of PD, a low intake of vitamin B6 is related to an increased risk of PD, as observed in 249 patients with diagnosed PD and 368 controls without neurodegenerative diseases (Murakami et al., 2010).

A 2006 study revised publications on the effects of the three main antioxidants or supplements used for the prevention and/or treatment of PD: tocopherol, CoQ10, and GSH. Authors have found that, besides some large observational studies focusing on tocopherol administration and one study of CoQ10 that enrolled 80 patients, other retrieved studies included less than 30 patients and were conducted for 3 months or shorter periods of time. Whereas tocopherol supplementation does not alter the course of PD, two of the CoQ10 studies and the study on GSH show a small but significant improvement of PD symptoms (Weber and Ernst, 2006).

Recent findings point to the potential use of creatine, which is implicated in energy provision through a reaction catalyzed by phosphorylcreatine kinase, for the

prevention and amelioration of PD symptoms, and creatine supplementation has been proposed as a therapeutic tool for elderly subjects (Gualano et al., 2010). Further large scale clinical studies would be required to better define the role of nutraceutical supplementations for PD prevention and amelioration of PD symptoms.

NUTRACEUTICALS FOR THE PREVENTION AND AMELIORATION OF ASD

ASD is a cluster of heterogeneous and complex neurodevelopmental disorders. The current revised definition of ASD no longer differentiates the various ASD subtypes such as autistic disorder or Asperger syndrome, but rather includes all of them under the same definition. The common features encountered in ASD are abnormal social interactions, impaired communication, and stereotyped or repetitive behaviors. There is evidence of genetic predisposition to ASD mainly derived from twin studies (Folstein and Rosen-Sheidley, 2001). However, it is believed that autistic phenotypes are attributed to interactions involving not only genetic factors but also environmental factors, such as chemicals, viral infections, and stress (Geier et al., 2009; Gillott and Standen, 2007; Libbey et al., 2005; Tordjman et al., 2014).

Although extensive research in the field of ASD has been performed during the past two decades, the full mechanistic understanding of ASD and the development of appropriate treatments remains limited. Currently, applied behavioral therapies and antipsychotic medications to ASD individuals are not considered effective or safe (Bobo et al., 2013). Due to these limitations, the use of nutraceuticals in ASD management is steadily increasing and a significant number of companies produce various compositions of nutraceuticals available over the counter (Alanazi, 2013). Despite the initial use of nutraceuticals that relied exclusively on anecdotal and empirical evidence, a substantial amount of animal and human data has been generated to provide some support and justification for the beneficial administration of nutraceuticals in ASD treatment protocols (Table 2.3). This section aims to review the metabolic or physiological abnormalities found in ASD patients that form the basis for the development of treatments with nutraceutical origin. The presented data derive from: (i) epidemiological studies in which levels of micronutrients and specific biomarkers associated with ASD phenotype have been measured; (ii) clinical trials in which nutraceuticals have been administered to ASD individuals; and (iii) animal models of ASD that illustrate the mechanisms by which nutraceuticals prevent, ameliorate, or cure neurodevelopmental disorders of this spectrum.

In general, multivitamin intake during periconception has been associated with a lower incidence of ASD in children. A recent study demonstrates that mothers of ASD individuals are less likely to receive vitamin supplements 3 months before and 1 month after conception than mothers with healthy children (Schmidt et al., 2011). Multivitamin and mineral supplementation to ASD patients up to 3 months leads to improvements in sleep, reduction of autistic symptoms, and amelioration in biomarkers of energy production as well as oxidative stress, suggesting that the effectiveness of multivitamins is partially related to better mitochondrial function (Adams et al., 2011). Similarly, a case control study comparing autistic children using conventional medication and micronutrient supplementation that included not only a multivitamin but also minerals, amino acids, and antioxidants has revealed greater improvement in micronutrient-treated group (Mehl-Madrona et al., 2010).

Besides the studies that followed the administration of cocktails containing vitamins in combination with cofactors, some other experimental approaches have been focused on providing evidence through human or animal studies by using individual vitamins or nutraceuticals. The reason behind this is that the levels of only certain vitamins or certain biomarkers have been found to be altered in ASD children.

Vitamin B2 is significantly elevated in children with autism and their siblings compared with controls, but there is no difference in other B vitamins or homocysteine (Main et al., 2015). This study included 35 children with autism, 27 of their siblings without autism, and 25 age- and gender-matched community controls (Main et al., 2015).

Folic acid or folate (Vitamin B9) supplementation during pregnancy has been accused of higher incidence of ASD, whereas it is well-recognized to protect against neural tube defects. However, after reviewing the limited and contradictory studies, Castro et al. (2014) have concluded that further studies are required to determine the critical role of gestational folic acid supplementation in relation to ASD occurrence (Castro et al., 2014). Recently, it has been found that several transcription factors, imprinted genes, neurodevelopmental genes, and genes associated with ASD exhibit altered expression levels in the brain of mice pups that have been exposed to high concentrations of folic acid during gestation (Barua et al., 2015). Folate levels in patients with ASD are lower compared to controls (Ali et al., 2011; Castro et al., 2014). In addition, a number of genetic polymorphisms in enzymes involved in the folate pathway have been identified in ASD individuals (Frye and Rossignol, 2014). These abnormalities result in the impairment of folate transport across the blood–brain barrier (BBB) and into neuronal cells. The most studied abnormality in folate metabolism related to ASD is the formation of

TABLE 2.3 Summary of Nutrients and Nutraceuticals and Their Relevance to ASD

Nutrient or nutraceutical	Elicited effects	References
Multivitamin and mineral supplements	During periconception promote lower incidence of ASD children Improve sleep, reduce autistic symptoms Ameliorate biomarkers of energy production and oxidative stress, improving mitochondrial function	Adams et al. (2011), Mehl-Madrona et al. (2010), Schmidt et al. (2011)
Vitamin B2	Significantly elevated in children with autism	Main et al. (2015)
Folic acid, folinic acid	Supplementation during pregnancy has been associated with higher incidence of ASD Promotes altered expression of transcription factors, imprinted genes, neurodevelopmental genes, and genes associated with ASD when administered during pregnancy in mice Folate levels in patients with ASD are lower compared to controls Impairment of folate transport across the BBB and into neuronal cells Folinic acid can cross the BBB, ameliorating ASD and CFD	Ali et al. (2011), Barua et al. (2015), Castro et al. (2014), Frye and Rossignol (2014)
Vitamin B12	When combined with GSH and special low-fructose and organic diet, it improves social interaction, concentration, spoken and written language, and behavior	Patel and Curtis (2007)
Propionic (propanoic) acid	Together with enteric short-chain fatty acids (SCFAs), it is correlated to some forms of ASD	Macfabe (2013)
n-3 PUFAs	Their levels are decreased in ASD Supplementation of *n*-3 PUFAs in ASD children improves or does not affect main ASD symptoms	Amminger et al. (2007), Bent et al. (2011), Vancassel et al. (2001), Wilczynski-Kwaitek et al. (2009)
Biopterin, tetrahydrobiopterin, sapropterin	Highly concentrated in urine samples and at low levels in CSF of ASD children Supplementation with tetrahydrobiopterin improves language skills, eye contact, communication, and repetitive behavior Sapropterin improves tetrahydrobiopterin metabolism in ASD individuals and ameliorates NO metabolism	Danfors et al. (2005); Frye et al. (2010, 2013), Tani et al. (1994)
Probiotics (e.g., *Bacteroides fragilis*)	Alleviate MIA, anxiety, and stereotyped behaviors, improve GI function and restore serum metabolite levels related to autism (i.e., 4-EPS and indolepyruvate); ameliorate the detoxification capability of ASD individuals and consequently provide protection from environmental chemicals	Alanazi (2013), Hsiao (2014), Parracho et al. (2010)
Flavonoids (e.g., luteolin, quercetin, and rutin)	Antioxidant, anti-inflammatory, and neuroprotective properties; improve ASD symptoms by targeting JAK2/STAT3 signaling Improve GI and allergy symptoms, eye contact, and social interaction in ASD children	Parker-Athill et al. (2009), Taliou et al. (2013), Theoharides et al. (2012)
Ginseng (red and white)	Red ginseng extract improves sociability and social preference paradigms in an ASD mouse model	Kim et al. (2013)
N-acetyl-L-cysteine, methylcobolamine, folic acid, vitamin C, and other antioxidants	Positively modulate GSH levels and metabolism	Frye and Rossignol (2014), Hardan et al. (2012)

autoantibodies to the folate receptor alpha, which leads to cerebral folate deficiency (CFD). A limited number of studies has investigated the beneficial effects of folinic acid, which is a reduced form of folate that can cross the BBB in children with ASD and CFD, revealing promising results (Castro et al., 2014; Frye and Rossignol, 2014). However, two recent reviews concluded that the effects of folate-enhancing interventions on the clinical symptoms of ASD have not been fully explored yet, because only one reduced form of folate has been investigated so far and only with respect to CFD (Castro et al., 2014; Frye and Rossignol, 2014).

The levels of vitamin B12 have been found decreased in a cohort of autistic children (Ali et al., 2011). Improvements in social interaction, concentration, spoken and written language, and behavior have been

recorded after vitamin B12 administration for 3–6 months in combination with GSH and special low-fructose and organic diets in a small group of autistic children (4–10 years old) (Patel and Curtis, 2007).

Enteric short-chain fatty acids and, more specifically, propionic (propanoic) acid seem to be produced from ASD-related gastrointestinal (GI) bacteria, and there is evidence suggesting that they may be the cause behind some forms of ASD (Macfabe, 2013). Propionic acid-exposed rats demonstrate repetitive and antisocial behaviors and similar neurochemical and neuropathological alterations as ASD patients (Macfabe, 2013). The levels of phospholipid fatty acids in the plasma of a population of autistic subjects show a marked reduction that reflects the decrease in the levels of total *n*-3 PUFAs but not of *n*-6 PUFAs (Vancassel et al., 2001).

Two studies that have followed omega-3 fatty acid supplementation in ASD children revealed contradicting results. One study shows improvement in main ASD symptoms (Amminger et al., 2007), whereas the other demonstrates no beneficial effect after omega-3 fatty acid treatment (Bent et al., 2011). However, both studies have failed to include a sufficient number of ASD individuals and to provide data that can be analyzed statistically with confidence. Future assessments that would include not only behavioral performance indexes but also biological indicators and, more specifically, the omega 6/3 ratios could improve the understanding of the role of fatty acids in the management of ASD (Wilczynski-Kwaitek et al., 2009).

Biopterins, which are considered important cofactors of catecholaminergic and several critical metabolic pathways, have been found to be highly concentrated in urine samples and at low levels in CSF of ASD children (Tani et al., 1994). These findings are more pronounced in ASD individuals who are younger than 6 years old. Studies targeting this age group have shown that tetrahydrobiopterin treatment can be beneficial in language skills, eye contact, communication, and repetitive behavior (Danfors et al., 2005). In these controlled and other open-label clinical trials (Frye et al., 2010, 2013), sapropterin, which is a synthetic form of tetrahydrobiopterin, has been administered to ASD children. Interestingly, sapropterin has been found not only to improve tetrahydrobiopterin metabolism in ASD individuals, but also to ameliorate nitric oxide (NO) metabolism because serum biomarkers related to NO have been suggested to have predictive value for ASD children's response to sapropterin (Frye et al., 2013).

GI symptoms, abnormal food cravings, and unique intestinal bacterial populations have been proposed to be implicated in the development and severity of ASD symptoms. More specifically, certain beneficial bacteria are not present in the microbiota of ASD patients (Kang et al., 2013a). Some ASD cases are connected to maternal viral or bacterial infection during pregnancy, also known as maternal immune activation (MIA). It has been suggested that MIA leads to a decrease of gut barrier integrity and an increase of the presence of bacteria metabolites in the blood of embryos. Recently, it has been shown that the treatment of MIA with probiotics alleviates some of the ASD clinical symptoms in a mouse model of ASD, such as anxiety and stereotyped behaviors (Hsiao, 2014; Hsiao et al., 2013). Interestingly, these MIA offspring mice that received *Bacteroides fragilis* show improved GI function and restored the levels of serum metabolites that are considered human autism biomarkers, like 4-ethylphenylsulfate (4-EPS) and indole pyruvate (Hsiao, 2014). Regarding clinical trials assessing the beneficial role of probiotics in ASD children, only one is available that demonstrates significant improvement in behavioral scores, despite the limitations of the study due to high interindividual variability and the high dropout rate of participants (Parracho et al., 2010). Moreover, probiotics improve GI function, and it has also been suggested that they ameliorate the detoxification capability of ASD individuals and consequently provide protection from environmental chemicals, such as mercurial compounds that can be more hazardous to these patients compared to typical developing children (Alanazi, 2013).

Accumulating evidence suggests that inflammation in brain regions related to cognitive function is a hallmark of ASD. The natural flavonoid luteolin possesses antioxidant, anti-inflammatory, and neuroprotective properties. Despite their antithyroid properties, which might possibly impact brain development, flavonoids have been shown to improve ASD symptoms in animal models and humans. In a MIA mouse model of ASD, luteolin inhibits autism-like behaviors by targeting Janus kinase 2 (JAK2)/Signal Transducer and Activator of Transcription 3 (STAT3) signaling (Parker-Athill et al., 2009). A mixture of flavonoids composed of luteolin, quercetin, and the quercetin glycoside rutin has been shown to improve GI and allergy symptoms, eye contact, and social interaction when administered to a group of ASD children (Theoharides et al., 2012). Accordingly, the same formulation has been given to 40 children and seems to be effective in reducing ASD symptoms without causing any major adverse effect (Taliou et al., 2013).

Ginseng is one of the most widely used medicinal plants. Red ginseng compared to white is considered to provide superior pharmacological effects with limited adverse effects. Making use of the prenatal valproic acid-injection rat model of ASD, which produces social impairment and similar neuropathological changes monitored in ASD patients, it has been shown that the chronic administration of red ginseng extract improves sociability and social preference paradigms in a dose-dependent manner (Kim et al., 2013).

Abnormal GSH metabolism is a common finding in ASD children (Frye and Rossignol, 2014). The reduced levels of this major intracellular antioxidant lead to oxidative damage that is usually detected in the cortex of ASD individuals. A supplement that provides a precursor to GSH named *N*-acetyl-L-cysteine has been administered with success to ASD individuals, but the levels of GSH have not been measured (Hardan et al., 2012). Besides *N*-acetyl-L-cysteine, other novel ASD interventions that address oxidative stress are methyl cobalamin in combination with or without folic acid, vitamin C, and other antioxidants (Frye and Rossignol, 2014).

Although the etiology of ASD remains elusive, converging lines of research indicate that mitochondrial dysfunction may play a substantive role in disease pathophysiology. Some of the nutraceuticals presented here target this cellular compartment and appear to be promising ASD treatment approaches. However, without an established causal link, the generation of therapeutic targets for ASD has been relatively unsuccessful and has been focused solely on the amelioration of individual symptoms by certain nutraceuticals without providing mechanistic understanding or reliable clinical data.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

In recent years, nutraceuticals have been proven beneficial for the prevention or amelioration of cognitive impairments in degenerative diseases such as AD and PD, and manifestations of ASD. Some clinical studies have tried to address the role and the molecular mechanisms underlying the effects elicited by bioactive compound supplementations in human subjects; however, the data obtained in these studies are sometimes controversial and often are obtained using small cohorts of subjects and/or for interventions with limited times. Moreover, scientific research on the role of nutraceuticals in relation to neurodegenerative diseases often has been based on observations derived from *in vitro* and/or on animal models reproducing neurodegenerative diseases. These models have often yielded contradictory results, given the immutable interspecies differences (Jucker, 2010) and the obvious limitation of *in vitro* cellular models. Animal models have been useful for improving the understanding of the etiology of these neurodegenerative diseases and for assessing the effects of new treatments. Nevertheless, available animal models often do not resemble the actual pathophysiology of idiopathic diseases such as PD, thus limiting data translatability into clinical practice (Potashkin et al., 2011). Future research will be required to consider the multifaceted nature of neurodegenerative diseases and to assess, through human-based epidemiological and clinical studies, the effectiveness and utility of nutritional multivitamin, mineral, and plant-based mixtures to slow the neurodegenerative progression and their protective/preventive effects (Arab and Sabbagh, 2010). Even though human-based interventional/clinical studies are still scarce, the adoption of a "precautionary principle" in relation to food choices should be highly recommended to prevent cognitive impairment and also MetS and cancer risk. Among the "precautions," limiting or avoiding alcohol, high-fat dairy products, red meat, processed meat, and meat cooked at high temperatures and also increasing the intake of soy products and fruits and vegetables enriched in bioactive phytonutraceuticals should be advised (Gonzales et al., 2014).

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