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ANTIOXIDANT THERAPY AND ITS EFFECTIVENESS IN OXIDATIVE STRESS-MEDIATED DISORDERS

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15.1 INTRODUCTION

Antioxidants are chemical compounds that give an electron to free radical molecules and convert them into a harmless configuration. This prevents damage to chain reaction and can involve lipids, proteins, enzymes, carbohydrates, DNA, cell, and nuclear membranes up to the death of the cell. When the body's scavenging ability is not able to deal with free radical species, that is, reactive oxygen species (ROS) and reactive nitrogen species (RNS), they cause oxidative damage in all the body's tissues, leading to disease. The effect of oxidative stress at the cellular level is illustrated in Figure 15.1. This oxidation-induced damage may be prevented by exogenous or endogenous antioxidants. ROS seem to be an important factor involved in endothelial dysfunction, diabetes, atherosclerosis, and ischemia, while RNS have been associated with arthritis, diabetes, degenerative neuronal diseases, cancer, and atherosclerosis. Under physiological conditions, the overproduction of ROS and RNS and their neutralization are prevented by the activity of the endogenous antioxidative defense system (AOS). It includes enzymes like superoxide dismutase, catalase, glutathione peroxidase, and other antioxidant regenerating enzymes such as glutathione reductase, dehydroascorbate reductase, and glucose-6 phosphate dehydrogenase that maintains reduced NADPH, hydrophilic scavengers like urate ascorbate glutathione, flavonoids, and lipophilic scavengers, like tocopherols, carotenoids, and ubiquinone. Antioxidants are generally supplied in the diet and include polyphenols, lipoic and ascorbic acid, carotenoids, resveratrol, epigallocathechin-3-0-gallate, lycopene, quercetin, genistein, ellagic acid, ubiquinone, and indole-3 carbinole. The properties of these compounds are involved in the physiological redox balance as they can prevent damage to the tissues due to the oxidation typical of all the biological systems and characterized by the production of highly reactive free radicals [1]. There is growing evidence that ROS play a key role in several pathological conditions and in the aging process. No final conclusion about possible therapy protocols based on the administration of antioxidant compounds has been reached yet, but it can be hypothesized that, in the near future, biochemical investigations, able to detect an oxidative imbalance, may become routine tests necessary to restore the antioxidant natural protective barrier, avoiding irreversible damage. An example of the power of antioxidant supplementation to prevent excessive oxidative stress is given by a study conducted in 400 healthy subjects to assess the effect of physical activity combined with antioxidant treatment (330 ml/day of Funciona™). This study shows that, after 10 months, the oxidative stress caused by exercise was prevented by the antioxidant treatment [2]. There is also a lack of agreement concerning the parameters of oxidative stress or antioxidant state in specific

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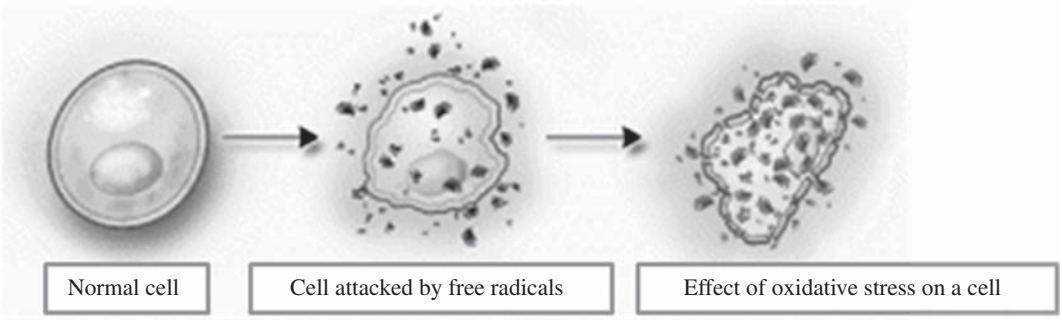

Fig. 15.1 Effect of oxidative stress at cellular level.

patients or diseases, and researchers are trying to find new techniques to monitor oxidative stress in humans. Among other techniques are high-performance liquid chromatography (HPLC) and immunochemical determinations, which have become the common standard. The number of centers using Points of Care is growing among the scientific community because of their low invasiveness, reduced costs, practicality, and ease of use. In particular, Free Oxygen Radicals Testing (FORT) and Free Oxygen Radicals Defence (FORD) have proven their reliability and ease of use [3]. Among the main techniques for free detection is electron spin resonance (ESR), which can provide valuable information on free radicals in solution chemistry, while to examine free radicals in biological materials, spin trapping is the most appropriate technique, which may allow investigators to detect radicals that are normally present at very low concentrations or whose lifetime is too short to be detected by direct ESR analysis. Specific products of DNA oxidation have been proposed as potential general markers of this process. For example Bruce Ames's laboratory, at the Berkeley campus of the University of California (USA), has proposed that thymine glycol and 8-hydroxydeoxyguanosine might be appropriate markers of DNA base oxidation. Analysis of 8-hydroxydeoxyguanosine in extracted DNA is a straightforward method of studying DNA base oxidation in biological tissues, and this substance is also excreted in urine and hence might provide a noninvasive means of assessing whole-body DNA base oxidation. GSH is the major intracellular reductant, and analysis of GSSG:GSH can provide a valid index of cellular oxidative stress. Assays relying on the ability of bleomycin to bind Fe, facilitating the oxidation of DNA (the bleomycin-Fe assay), and of phenanthroline to undertake the same role with Cu (the phenanthroline-Cu assay) have also been developed. Data indicate that small amounts of this "free" or "catalytic" Fe or Cu may be found in biological fluids such as cerebrospinal fluid (CSF) and that these levels increase in some disease states (e.g., Cu or Fe overload). They provide strong support, as increased free radical production may occur in these situations [4]. An evaluation of 67 randomized trials including participants who were either healthy (primary prevention trials) or affected by different diseases (secondary prevention trials; gastrointestinal, cardiovascular, neurological, ocular, dermatological, rheumatoid, renal, endocrinological, or unspecified) analyzed the effect of several antioxidant supplements, such as beta-carotene, vitamin A, vitamin C, vitamin E, and selenium versus placebo or no intervention, concluding that only vitamin A, beta-carotene, and vitamin E seem to increase mortality [5]. α-Lipoic acid (ALA), also named 1,2-dithiolane-3-pentanoic acid or thioctic acid, is synthesized in humans by the liver, heart, and kidney and has also been widely studied for its antioxidative properties. It is water- and fat soluble and is widely distributed in both cellular membranes and cytosol. A number of experimental and clinical studies point to the usefulness of ALA as a therapeutic agent for several medical conditions, namely, diabetes, atherosclerosis, insulin resistance, neuropathy, neurodegenerative diseases, and ischemia-reperfusion injury [6]. ALA exerts an antioxidant effect in biological systems through direct ROS quenching and via transition metal chelation [7]. Carpal tunnel syndrome (CTS) is the most common peripheral mononeuropathy. One study [8] has compared the efficacy of ALA (600 mg/day) and γ-linolenic acid (GLA; 360 mg/day) to that of a multivitamin B preparation (Vit B6 150 mg, Vit B1 100 mg, Vit B12 500 μg daily) administered for 90 days in 112 subjects with moderately severe CTS. The following results were observed:

1. A significant reduction in both symptom scores and functional impairment in the ALA/GLA group, while the multivitamin group experienced a slight improvement in symptoms and a deterioration in functional scores.
2. Electromyography showed a statistically significant improvement in the ALA/GLA group, but not in the multivitamin group.

3. ALA/GLA improved symptoms and functional impairment. The improvement was significant in the multivitamin group but less marked than in the ALA/GLA group.

Many compounds have been studied in relationship to their antioxidant properties. Shark liver oil (SLO) contains both alkylglycerols (AKG) and squalene and is an ancient remedy among the fishermen along the west coast of Norway and Sweden. It has been used for wound healing, treatment of irritations of the respiratory and alimentary tracts, and lymphadenopathy. Squalene is the main component of skin surface polyunsaturated lipids as an emollient and antioxidant and has hydration and antitumor activities [9]. SLO supplementation in high doses €3.6 g of squalene, 3.6 g of AKG, and 750 mg of n-3 polyunsaturated fatty acids (PUFA) per day, for 4 weeks] in 13 volunteers showed an increased response of neutrophils toward bacteria, an increased level of C4 component of complement in blood, the rise of the total antioxidant status of serum, and predominance of type 1 cytokine IFN-γ, TNF-α, and IL-2 production by peripheral blood mononuclear cells. The same study has shown that SLO supplementation also markedly affected lipid metabolism and cholesterol balance [10]. Oxidative stress has also been related to lipotoxicity, that is, the process leading to end organ damage and/or dysfunction following excess overload that results not only from unoxidized FAs but also from endogenous lipids synthesized from excess glucose through the process of the de novo lipogenesis. It has been demonstrated that this can occur in the presence of excess lipid accumulation in nonadipose tissues such as liver [nonalcoholic fatty liver disease (NAFLD)], pancreas (diabetes), muscle (insulin resistance), and heart (diabetic cardiomyopathy) [11]. An interesting hypothesis has suggested that oxidative stress in obesity may result partly from the accumulation of intracellular triglycerides that, in turn, may elevate superoxide radical generation within the electron transport chain by inhibiting the mitochondrial adenosine nucleotide transporter. This inhibition leads to a diminution in intramitochondrial adenosine diphosphate (ADP) that, in turn, reduces the proton flux through the adenosine triphosphate-synthase reaction (i.e., the adenosine triphosphate-synthase reaction requires ADP as substrate [3]). Another naturally occurring compound, attracting the attention of many researchers and clinicians, is curcumin, a constituent of the spice turmeric and one of the principal ingredients in curry powder. Its active ingredient is diferuloylmethane, a hydrophobic polyphenol with a peculiar yellow color, and it is prepared from the root of the *Curcuma longa* plant, a member of the ginger family [12]. Curcumin's clinical use has been associated with several pathological conditions because of its antiinflammatory properties, namely, the reduction of NF-κB, COX2, and proinflammatory cytokines such as IL-1, IL-6, and TNF-α. In particular it seems to improve rheumatoid arthritis, psoriasis, postoperative inflammation, chronic anterior uveitis, and orbital inflammatory pseudotumors [13–18]. Moreover, clinical improvements have been observed in irritable bowel syndrome, tropical pancreatitis (PEP), gall bladder and biliary motility, gastric ulceration, and familial adenomatous polyposis coli [19–25]. It also seems to improve endothelial function in type 2 diabetes mellitus [26] and to lower serum cholesterol [27]. In an open-label study [5 patients with Crohn's disease (CD) and 5 with ulcerative proctitis] curcumin improved clinical and laboratory parameters, with a reduction in need for concomitant medications observed in nine of 10 cases [28]. A larger multicenter randomized, double-blind controlled trial of 89 patients with quiescent ulcerative colitis (UC) showed that two of 43 patients taking curcumin per os had relapsed by 6 months, compared with eight of 39 in the placebo group [29]. A trial involving 25 patients with various different premalignant or high-risk lesions suggested that oral curcumin may have chemopreventive effects in progression of these lesions [30]. In an uncontrolled study of 15 patients with advanced colorectal cancer refractory to standard treatments, 440 mg/day of curcuma extract administered per os showed a 59% reduction in activity of the lymphocytic biomarker glutathione *S*-transferase. Five patients maintained radiologically stable disease over the 2- to 4-month study period [31]. An uncontrolled study involving 62 patients with cancerous oral lesions showed that a topical curcumin application reduced symptoms in 70% of the patients and caused tumor shrinkage in 10% [32]. A study performed in 21 patients with advanced pancreatic cancer treated with high-dose oral curcumin showed a disease stability or regression in four cases [33]. Curcumin is virtually able to affect every stage of carcinogenesis from cell proliferation to angiogenesis and metastasis, and it directly acts on ROS scavenging and production and the NF-κB/mTOR signaling pathways. This spice seems to be a safe and beneficial agent that may be useful to counteract, even if consumed at low doses, a great number of pathological conditions associated with age [34].

Lately, many experimental studies have investigated berries for their possible beneficial effects on health, namely, prevention of certain types of cancer, cardiovascular diseases, type 2 diabetes, obesity, neurodegenerative diseases associated with aging, and infections. In particular, the research community have focused on red raspberries, which contain a variety of beneficial

compounds including essential minerals, vitamins, FAs, and dietary fiber, as well as a wide range of polyphenolic phytochemicals such as flavonoids, phenolic acids, lignans, and tannins (anthocyanins and ellagitannins) [35].

As to black raspberries, a study [36] showed that freeze-dried blackberries, given to patients diagnosed with Barrett's esophagus, resulted in a significant decrease in oxidative DNA damage. Another study [37] showed, after 6 weeks of treatment consisting of four times daily application of a bioadhesive black raspberry gel on premalignant oral lesions, a significant reduction in COX-II protein levels, suppression of genes associated with RNA processing and growth factor recycling, and inhibition of apoptosis. In the same study a subset of patients displayed posttreatment decrease in lesion microvascular density (MVD). A 4-week intervention [38] with a red berry juice containing red raspberry juice improved the levels of glutathione and reduced DNA oxidative damage in healthy adult men, while when a dessert made from a similar juice was given to elderly subjects for 2 weeks, no effect was observed as to oxidative stress status [39]. Compared with preexercise and control levels, postexercise levels of protein and DNA oxidation in cyclists were significantly decreased in the treatment group receiving an antioxidant-rich beverage containing raspberry, black grape, and red currant concentrates [40]. A 90-day study involving the supplementation of healthy participants with a sea buckthorn berry extract found a significant reduction in C-reactive protein (CRP) level [41], which is associated with inflammation and, in particular, cardiovascular risk.

15.2 AGING

The mitochondria [from the Greek *mitos* ("thread") + *khondrion* ("little granule"), diminutive of *khondros* ("granule, lump of salt")] are oval-shaped, membrane-enclosed intracellular organelles containing their own DNA. They exert several functions, namely, the generation of adenosine triphosphate (ATP) and the regulation of cellular proliferation and apoptosis. They are associated with the aging process since they represent the main intracellular source of ROS, produced by the mitochondrial respiratory chain and the major target of free radical action. ROS may attack mitochondrial proteins, lipids, and mitochondrial DNA (mtDNA), which can lead to mtDNA mutations and, in turn, to impairment of the respiratory chain complexes, increased mitochondrial ROS production, and increasing mitochondrial DNA mutations. Therefore oxidative damage to mtDNA represents a stepping stone for protein mutation, generation of additional free radicals, and altered energy production [42]. From a chemical perspective, melatonin, together with its metabolites, can act as an endogenous free radical scavenger and broad-spectrum antioxidant. It is characterized by a small size and amphiphilic nature that make it able to reach all the cellular and subcellular compartments. The highest melatonin intracellular concentration seems to be in mitochondria [43]. Melatonin exerts beneficial consequences that have been observed after melatonin administration, and they may be due to its effect on mitochondrial physiology [44–46], namely, antioxidant and free-radical scavenging properties that preserve the stability, integrity, and function of the mitochondrial membrane. ROS-induced alterations to mitochondrial membrane may be a contributory factor in a variety of pathological conditions including heart ischemia-reperfusion, aging, and age-related cardiovascular and neurodegenerative diseases [47]. Experimental studies have shown that serum levels of melatonin significantly decrease in aged animals compared with young animals [48, 49], and it has been suggested that, in humans, melatonin contributes to the total antioxidant capability of serum [50]. It has been hypothesized [51] that the increase of oxidants may lead to alteration of physical and cognitive functions that are typical features of the aging process. Oxidants accumulate in the body, leading to an increase in oxidative stress that, in turn, can also imply the induction of protective and survival functions.

Centenarians, a widely used model of successful aging, are less prone to oxidative stress and have been found to be in possession of better antioxidant defenses than younger elderly cohorts [52]. These findings have also been confirmed in another report stating that this particular category of individuals show differences in antioxidant defenses (mainly plasma vitamin E) and fasting plasma glucose that seem to provide a contribution to the genesis of oxidative stress and to the differences between other elderly people and centenarians. The same authors report that it is possible that the difference in antioxidant defense between aged subjects and centenarians is due to a particular diet composition, since centenarians have demonstrated an elevated daily protein intake and a diet consisting mainly of vegetables, which are the main natural source of antioxidants, for example, vitamins C and E [53]. A higher antioxidant activity has also been observed in a study comparing chronic diseases and other related health indicators of centenarians with other age groups in longevity areas in China [54]. Okinawan people are famous for their long average life expectancy, high numbers of centenarians, and low risk of age-associated diseases. Their diet is heavy with fruit, that is, rich in phytonutrients and antioxidants, but reduced in meat, refined grains, saturated fat, sugar, salt, and full-fat dairy products. The traditional Mediterranean diet and the modern DASH

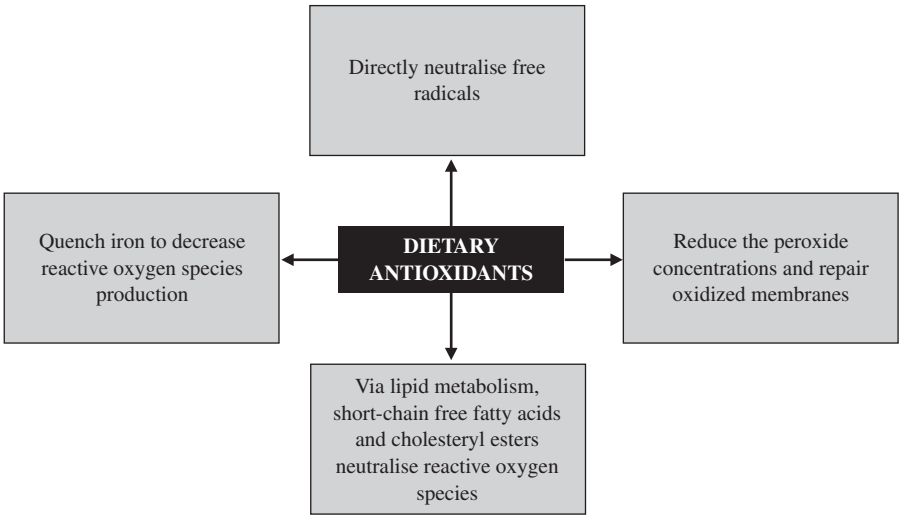

Fig. 15.2 Dietary antioxidants' main functions.

(Dietary Approaches to Stop Hypertension) diet share many characteristics with the Okinawan diet. Features such as the low levels of saturated fat, high antioxidant intake, and low glycemic load in these diets are likely to contribute to a decreased risk of cardiovascular disease, some cancers, and other chronic diseases through multiple mechanisms, including reduced oxidative stress. A comparison of the nutrient profiles of the three dietary patterns shows that the traditional Okinawan diet is the lowest in fat intake, particularly in terms of saturated fat, and the highest in carbohydrate intake, in keeping with the very high intake of antioxidant-rich yet calorie-poor orange-yellow root vegetables, such as sweet potatoes, and green leafy vegetables. The high longevity that characterizes this population is thought to be related to a healthy lifestyle, particularly to the traditional diet that is low in calories but rich in nutrients, especially with regard to phytonutrients in the form of antioxidants and flavonoids [55]. The main function of dietary antioxidants are summarized in Figure 15.2, while the main antioxidants, their mechanisms of action, and their main dietary sources are summarized in Table 15.1.

15.3 CARDIOVASCULAR DISEASE, RISK, AND ISCHEMIA-REPERFUSION INJURY

Coronary heart disease and stroke have been associated to several risk factors, namely, tobacco use, alcohol use (having 1–2 alcohol drinks a day may lead to a 30% reduction in heart disease, but above this level alcohol consumption will damage the heart muscle), high blood pressure (hypertension), obesity and related comorbidities (metabolic syndrome, diabetes, and so on), physical inactivity (increases the risk of heart disease and stroke by 50%), and unhealthy diet (excessive consumption of saturated fat increases the risk of heart disease and stroke; it is estimated to cause about 31% of coronary heart disease and 11% of stroke worldwide). Generally it has been pointed out that abnormal blood lipid levels (hyperlipemia), in the form of high total cholesterol, high levels of triglycerides, and high levels of low-density lipoprotein or low levels of high-density lipoprotein (HDL) cholesterol, increase the risk of heart disease and stroke. Moreover, the contraceptive pill and hormone replacement therapy (HRT) may increase the risk of heart disease [56]. Nowadays, oxidative species seem to play a key role in these pathological conditions. Relevant evidence has supported the theory that free radical-mediated oxidative processes and specific related products play a key role in atherogenesis [3].

Aging has also been related to an increased risk of stroke, in fact the latter doubles every decade after the age of 55. Family history of cardiovascular disease is also a risk indicator. A man is at greater risk of heart disease than a premenopausal woman. Ethnic origin plays a role, too. People with African or Asian ancestry are at higher risk of developing cardiovascular disease than other racial groups [56]. Several studies have linked a high consumption of plant polyphenols with a decreased risk of cardiovascular disease and hypertension [57–61]. Vitamin C (500 mg twice a day) and vitamin E (400 IU twice a day), administered for 1 year, reduce the progression of cardiac transplant-associated arteriosclerosis (TxAA) in patients with normal or abnormal endothelial function, with a particular benefit in patients with endothelial dysfunction [62]. Antioxidant vitamin intake [vitamins A, C, and E, 1683 mg (±1245), 371 mg (±375), and 97 mg (±165) respectively] does not seem to be significantly related to coronary artery calcification, implying that

TABLE 15.1 Common antioxidants, their action, and main dietary sources

Antioxidant	Action	Foods containing antioxidant
Vitamin A (beta-carotene)	Protection against lipid oxidation	Variety of dark orange, red, yellow, and green vegetables and fruits such as broccoli, kale, spinach, sweet potatoes, carrots, red and yellow peppers, apricots, cantaloupe, and mangos
Vitamin C (ascorbic acid)	Inhibition of reactive oxygen species. Stimulation of the vitamin E antioxidant power and selenium. Protection against damages caused by LDL-ox	Citrus fruits and their juices, berries, dark green vegetables (spinach, asparagus, green peppers, brussels sprouts, broccoli, watercress), red and yellow peppers, tomatoes and tomato juice, pineapple, cantaloupe, mangos, papaya, and guava
Vitamin E (α-tocopherol)	Protection of membrane polyunsaturated fatty acids LDL peroxidation	Vegetable oils such as olive, soybean, corn, cottonseed and safflower, nuts and nut butters, seeds, whole grains, wheat, wheat germ, brown rice, oatmeal, soybeans, sweet potatoes, legumes (beans, lentils, split peas), and dark leafy green vegetables
Cu, Zn, Mn, Se	Cofactors of antioxidant enzymes SOD-Cu/Zn, SOD-Mn and glutathione peroxidase	Brazil nuts, brewer's yeast, oatmeal, brown rice, chicken, eggs, dairy products, garlic, molasses, onions, salmon, seafood, tuna, wheat germ, whole grains, and most vegetable.
Other carotenoids (lycopene)	Protection against lipid oxidation, LDL, proteins, and DNA; elimination and inactivation of free radicals	Tomato, ketchup, hot pepper, fishes, watermelon, grapefruit
Resveratrol, catechin, quercetin, phenolic acid (phytochemics)	Protection against lipid and DNA oxidation	Grapes, berries, peanuts, pine nuts

there is no effect on the development of early coronary atherosclerosis. Vitamin E, according to this study, is positively associated with an increased risk of calcified atherosclerosis [63]. Intravenous infusion of Edaravone, a powerful neuroprotective free radical scavenger, has been investigated in 141 patients with cardioembolic stroke, and it was concluded that it may be only effective in patients with mild cardioembolic stroke [64]. Supplementation with antioxidant vitamins and B-group vitamins, separately or together [96 acute ischemic stroke patients randomized to receive either daily oral 727 mg vitamin E and 500 mg vitamin C ($n = 24$), B-group vitamins (5 mg folic acid, 5 mg vitamin B2, 50 mg vitamin B6, and 0.4 mg vitamin B12; $n = 24$), both vitamins together ($n = 24$), or no supplementation ($n = 24$) for 14 days], enhance antioxidant capacity, mitigate oxidative damage, and may have an antiinflammatory effect immediately after an acute ischemic stroke [65]. Administration of 15 g *N*-acetylcysteine (NAC) infused over 24 h, in combination with streptokinase, significantly diminishes oxidative stress and improves left ventricular (LV) function in patients with acute myocardial infarction (MI) [66]. In addition, serum carotenoids have been associated, some interactively with smoking, with some benefits such as markers of inflammation, oxidative stress, and endothelial dysfunction [67]. It has also been proposed that antioxidant supplementation (vitamins E and C) may be able to counteract the progressive oxidative stress associated with Chagas disease, underlining that future perspectives for treatment of Chagas disease might include an antioxidant therapy in order to attenuate the consequences of oxidative insult related to this disease [68]. Administration of vitamin C (1 g) and vitamin E (400 IU) for 8 weeks in untreated essential hypertensive patients improves flow-mediated dilation (FMD), significantly reduces central pulse wave velocity (PWV), and decreases augmentation index (Aix). This treatment also increases plasma vitamin levels and antioxidant capacity and levels of oxidative stress. The decrease and changes in central PWV have been related to changes in levels of oxidative stress. Therefore this treatment has beneficial effects on endothelium-dependent vasodilation and arterial stiffness in these particular patients [69]. In a study involving 19 patients with heart failure (HF) after MI, administration of vitamin C has proven to enhance the contractile response to

dobutamine and improve myocardial efficiency [70]. A double-blind parallel study, consisting of five weeks of 100 mg/day or 200 mg/day gamma-T supplementation, has shown that gamma-T supplementation seems to have a permissive role in decreasing the risk of thrombotic events by improving lipid profile and reducing platelet activity [71]. Interestingly, it has been reported that edaravone can salvage the boundary zone of the infarct and is a useful cytoprotective antiedema agent, as shown by magnetic resonance imaging in six patients with extensive hemispheric ischemic stroke [72]. Three months of lipoic acid supplementation seems to relieve exercise pain according to a randomized, double-blind controlled study in 28 participants with peripheral arterial disease (PAD) [73]. Vitamin E (400 U/day) supplementation appears to reduce cardiovascular events in individuals with diabetes mellitus (DM) and Haptaglobulin (Hp) 2-2 genotype, a subgroup that comprises 2–3% of the general population [74]. A randomized placebo-controlled study in 26 healthy male subjects and eight male patients with PAD has shown that ischemia-reperfusion (IR)-induced vascular injury can be prevented by administration of vitamin C [75]. A randomized double-blind placebo-controlled clinical trial conducted in 110 men with grade 1 essential hypertension (EH) (35–60 years of age without obesity, dyslipidemia, and diabetes mellitus, nonsmokers, not undergoing vigorous physical exercise, without the use of any medication and/or high consumption of fruit and vegetables) who were randomly assigned to receive either vitamins C + E [vitamin C (1 g/day) plus vitamin E (400 IU/day)] or placebo for 8 weeks has shown that enhancement of antioxidant status by supplementation with vitamins C and E in patients with EH is associated with lower blood pressure [76]. The effects of AGI-1067 on coronary atherosclerosis has been investigated in a placebo-controlled, randomized trial. Atherosclerosis regression was observed, although it was not significantly different from placebo. The antiinflammatory effect of AGI-1067 was supported by reduced levels of myeloperoxidase [77]. An extensive review [78] has shown that dietary advice reduces total serum cholesterol and LDL cholesterol after 3–24 months. It also reduces blood pressure and 24-hour urinary sodium excretion after 3–36 months. Furthermore, dietary advice increases fruit and vegetable intake by 1.25 servings/day. Dietary fiber intake increases with advice by 5.99 g/day, while total dietary fat, as a percentage of total energy intake, falls by 4.49% with dietary advice. In addition, saturated fat intake falls by 2.36%. This study points out that dietary advice may be an effective tool to achieve modest beneficial changes in diet and cardiovascular risk factors over approximately 10 months. Melatonin has significant protective action against the cardiac damage and altered physiology that occur during IR injury [79–81]. Furthermore, it has been demonstrated that melatonin, at pharmacological concentrations, strongly protects against IR myocardial damage [82]. This protective effect of melatonin may be explained through its action at the mitochondrial level.

15.4 ROS AND NEURODEGENERATIVE DISORDERS

Oxidative stress is involved in the pathogenesis of neurodegenerative diseases and, in particular, the central nervous system (CNS) can be targeted by free radicals because of its high metal content, leading to the formation of ROS and to the relatively low content of antioxidant defenses. The brain seems to be particularly targeted by ROS since it consumes approximately 20% of total body oxygen. It contains high levels of PUFA and may contain low proportions of endogenous antioxidants. Moreover, iron accumulates in brain-specific regions, and iron-binding proteins, such as ferritin, may be relatively deficient in them [83]. Specifically, PUFA are easily targeted by lipid peroxidation because of the ROS interaction with them. This can be explained by the fact that the increase in the number of the molecule double bonds is related to the ease of the hydrogen atom removal. It is not clear yet whether oxidative damage causes neurodegeneration or what its consequences are.

Unregulated metal metabolism may lead to oxidative stress that, in turn, leads to neuronal cell death, but metals play a key role in cellular metabolism and cell signaling. Mutations in mtDNA or metal overload in aged brain lead to oxidative stress and free radical-mediated pathological changes in neurons. Neuronal proteins and structural components are modified because of oxidative stress in different neurological disorders, leading to neuroinflammation and loss of cognitive function in Alzheimer disease (AD), Parkinson disease (PD), multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS) [84]. Increased oxidative stress has been linked to AD, MS, Batten disease, PD, and brain tumors [85]. In particular, AD is characterized by memory loss with age, a symptom that is linked to ROS-induced neuron damage. AD has been first reported by Alois Alzheimer, a German psychiatrist and neuropathologist, a hundred years ago, and it is the most common course of dementia in the elderly above 65 years of age. It is characterized by two distinct pathological features, namely, intracellular neurofibrillary tangles (NFTs) and extracellular amyloid plaques in the brain, which are both related to neuronal demise and consequential onset of dementia symptoms. NFTs contain abnormally hyperphosphorylated forms of the microtubule-binding

protein tau. Amyloid plaques are made up of insoluble aggregates of amyloid β (Aβ) peptides [86]. Clinical characteristics are memory dysfunction, loss of lexical access, spatial and temporal disorientation, and impairment of judgment. The neurotransmitter acetylcholine, necessary for cognition and memory, and oxidative stress may play an important role in AD development [87]. The complex nature and genesis of oxidative damage in AD can be partly due to mitochondrial and redox-active metal anomalies. AD is essentially an acceleration of the aging mechanism in affected brain regions that progressively become more damaged by free radicals. Neurodegeneration, occurring in specific brain areas, namely, substantia nigra and striatum, plus dopamine depletion is considered the key feature. In addition, increased oxidative stress, abnormal mitochondrial function, and excitotoxicity are among the most relevant initiators or mediators of neuronal damage. The disease is inherited and familiar (FALS) in 10% of all ALS cases. About 20% of FALS cases are associated with mutations and lowered activity of CuZnSOD, which is known to catalyze the formation of hydrogen peroxide through the dismutation of superoxide radical anions, playing a relevant role in regulating oxidative damage to cells. Oxidative damage to DNA, protein, and lipids has been observed [83]. The oxidation of arachidonic and docosahexanoic acids, which are the main constituents of the brain membrane phospholipids, leads to the production of malondialdehyde (MDA) and 4-hydroxynonenal, which increase in autopsied specimens from multiple brain regions and in CSF in AD subjects [88–90]. Advanced glycation end products have been found present in AD brains and closely associated with the senile plaques [91].

Vitamin E is a lipid-soluble, chain-breaking, natural antioxidant that is able to cross the blood-brain barrier and accumulate in the CNS, where it reduces markers of oxidative stress [92; see Fig. 15.3 for other properties and its interaction with vitamin C]. A study performed in more than 4000 elderly patients has shown that decreased circulating levels of vitamin E are consistently associated with decreasing memory levels, while the same association has not been observed for plasma levels of vitamins

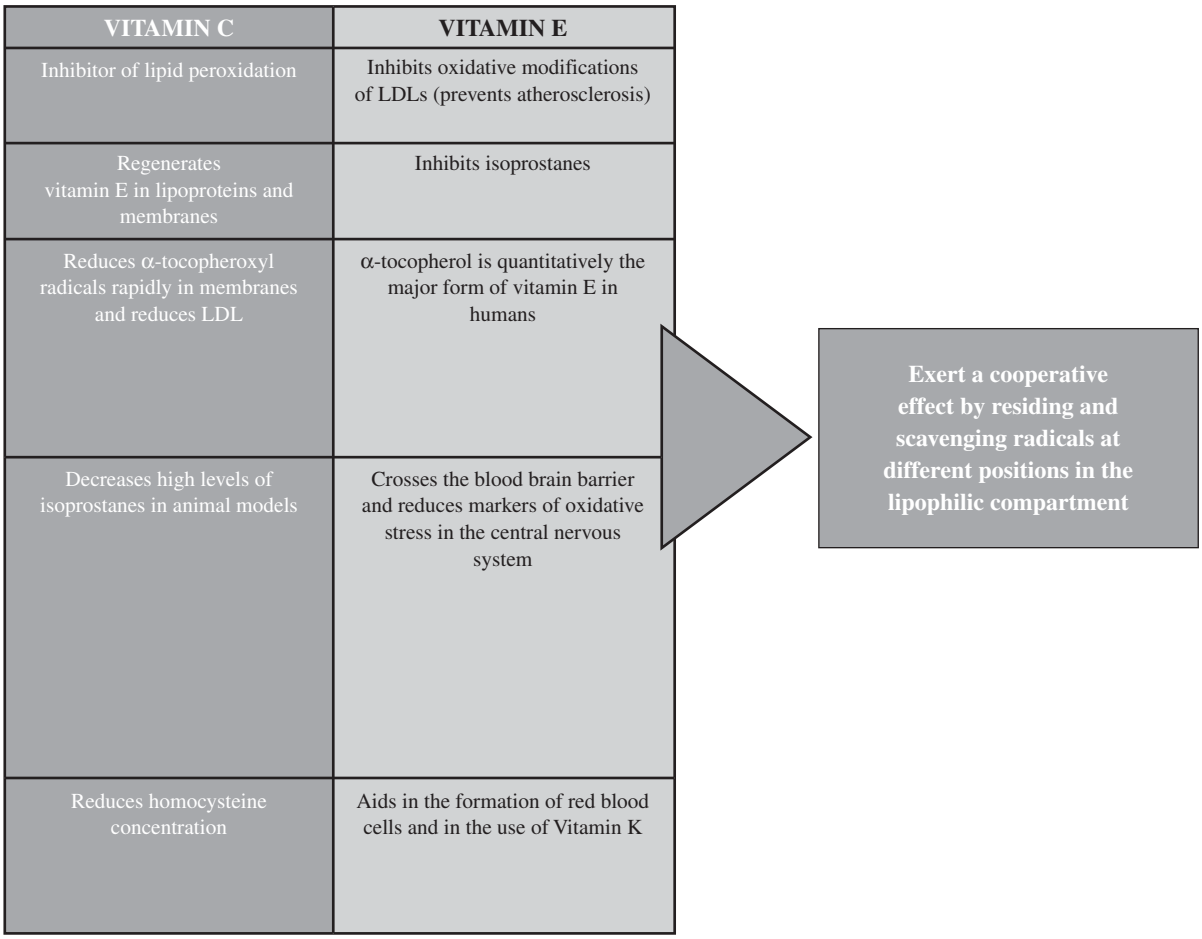

Fig. 15.3 Vitamin C and vitamin E main characteristics and functions [227–229].

A and C and β-carotene [93]. The Honolulu–Asia Aging Study has shown that supplementary intake of vitamins E and C are both associated with better cognitive performance [94]. The Chicago Health and Aging Project, which has analyzed almost 3000 subjects aged 65 to 102 years, has shown that supplementary or dietary intake of vitamin E, but not C, is inversely correlated with cognitive decline [95]. In addition, the Nurses' Health Study, which includes almost 15,000 women aged 70–79 years, has shown that long-term use of vitamin C and E supplements results in a better cognitive status [96]. A lower risk of developing AD [97] has been associated with dietary intake of vitamin E. Another study found that dietary and not supplement-derived vitamin E intake is associated with a lower risk of AD only in individuals who were not carriers of apolipoprotein E4 [98]. Another report showed no association between dietary or supplementary vitamin E intake and a decrease in the risk for AD [99]. The Cache County study also reported no benefit or significant reduction in the risk of AD from supplementation of vitamin E alone. However, the beneficial effect of a reduced risk of developing AD is seen in the combination of vitamin E and vitamin C [100]. A subgroup of the Honolulu–Asia Aging Study, which examined dietary intake of antioxidants and risk of late-life dementia, showed that vitamin C, vitamin E, β-carotene, and flavonoids are not associated with a reduced risk of dementia [101]. In 1997 the results of a double-blind, placebo-controlled 2-year randomized multicenter trial in 341 patients with moderate AD who received the selective monoamine oxidase inhibitor selegiline (10 mg/day), α-tocopherol (vitamin E, 2000 IU/day), both selegiline and α-tocopherol, or placebo were reported. This study shows that treatment with selegiline or α-tocopherol slows the progression of disease [102]. Recently, in a double-blind 3-year-follow-up study, the same high doses of vitamin E had no benefit in subjects with a clinical diagnosis of mild cognitive impairment (MCI). Consequently, the subjects receiving the antioxidants did not show any difference from placebo in preventing the progression of MCI to AD [103]. The same authors have also shown that in a subgroup of these patients the MRI-annualized percent changes of the volumes for some areas of the brain (hippocampus, enthorinal cortex) are less evident in the group receiving the vitamin E than in the placebo group [104].

Among other antioxidants, selenium (Se) seems to be involved in the maintenance of brain function and is particularly present in the brain, especially in the gray matter (area responsible for chemical synaptic communication), even with prolonged Se dietary deficiency [105]. Cognitive impairment, depression, anxiety, and hostility have been associated with low body levels of Se in humans [106]. Levels of Se are elevated in amygdala [107] and microsomes in the temporal lobe of patients with AD [108]. Selenoproteins, such as GPX1, GPX4, SelP, thioredoxin reductases, selenoproteinW (SelW), and selenoprotein M (SelM), have been implicated in brain maintenance and may play a protective role in several neurodegenerative conditions [109]. Patients affected by AD have shown a significant age-dependent decrease in the Se-dependent glutathione peroxidase activity in plasma and red blood cells [110], while another study has shown an increase in thioredoxin reductase activity in patients affected by the same disease [111]. Interestingly, another study has shown that the levels of Se in plasma and CSF from patients affected by AD are comparable to normal individuals [112]. In the future it would be interesting to investigate the role of selenoproteins in neurological aging and their potential interaction with telomeres. In fact, the length of the telomeres in cells, derived from the hippocampus, has been proven to be longer in AD patients than in control individuals, while in buccal and white blood cells from the same AD patients the telomere lengths are significantly shorter than those of the control groups [113]. This shows that AD patients have evolved a unique mechanism for telomere maintenance. Tanaka and colleagues [114] have shown that age-dependent telomere shortening in human brain microvascular endotheliocytes is negated by phosphorylated α-tocopherol, possibly through α-tocopherol's ability to decrease intracellular oxidative stress. Se, in the form of selenite, prevents telomere length shortening during cellular aging in the normal human L-02 hepatocyte [115].

PD is a chronic, progressive neurodegenerative disorder that affects at least 1% of people by the age of 70. This disease was first described by James Parkinson in his 1817 monograph "An Essay on the Shaking Palsy." After that, Charcot described the cardinal clinical features of PD, namely, rest tremor, rigidity, balance impairment, and slowness of movement [116]. The majority of the movement-related symptoms of PD are caused by a lack of dopamine due to the loss of dopamine-producing cells in the substantia nigra. As a consequence, communication between the substantia nigra and corpus striatum becomes impaired, and so does movement. Among others, flavonoids possess potential neuroprotective mechanisms. Their antinflammatory properties added to their ability to act as scavengers for ROS to maintain the correct glutathione levels and to inhibit Ca^{2+} influx, which represents the end of the cell death cascade, make them important candidates for treatment of AD and PD [117, 118].

We have previously described melatonin's antioxidant properties. PD, together with other age-related neurodegenerative disorders, have been associated with a malfunction of mitochondrial complex I [119, 120],

strongly suggesting that melatonin, through the prevention of complex I deficiency, may improve mitochondrial physiology in brain aging and age-associated brain diseases. The effects of melatonin in the field of cognitive impairment or dementia have been summarized in a review that collected all randomized controlled trials involving the use of orally administered melatonin in any dosage compared with a control group. Unfortunately, the conclusion was that there is insufficient evidence to support the effectiveness of melatonin in managing the cognitive and noncognitive sequelae of dementia [121]. Experimental studies have suggested that mitochondrial decay is a major contributor to brain tissue alterations associated with aging [122], and a potential role of melatonin in mitigation of changes associated with brain aging has been observed [123, 124], but conclusive clinical data are not available yet. A randomized controlled trial involving 16 patients receiving 1 hour of morning light exposure Monday to Friday for 10 weeks and 5 mg melatonin (LM) compared to a placebo group receiving usual indoor light has been performed. The following results were reported: significant improvement in daytime somnolence consisting in a reduction in daytime sleep duration, an increase in daytime activity, an improvement in day-night sleep ratio, and a significant increase in rest-activity rhythm amplitude. Unfortunately, this study does not confirm that the improvements can be attributed to melatonin [125]. A large study has shown that the use of vitamins E and C, alone or in combination, did not reduce the risk of AD or overall dementia over 5.5 years of follow-up, and therefore their use is not recommended for prevention of dementia in older adults [126]. Tocopherol, CoQ_{10}, and glutathione have been used in the prevention or treatment of PD, and their effectiveness has been related to their potential to alter the course of two common theories of PD pathogenesis, namely, free radical generation and mitochondrial complex I deficiency. A collection of three large clinical studies involving the use of tocopherol, four trials of CoQ_{10}, and a study of glutathione for the prevention and treatment of PD has shown that antioxidant supplementation, in particular tocopherol, does not alter the course of PD. CoQ_{10} and glutathione, according to the studies analyzed, have shown a statistically significant improvement in PD symptoms [127]. A randomized, double blind, placebo-controlled study evaluated the effect of melatonin on sleep and motor dysfunction in PD. This study involved 18 patients with motor dysfunction randomized to receive melatonin (3 mg) or placebo 1 hour before bedtime, for 4 weeks. Melatonin significantly improved the subjective quality of sleep, but polysomnography (PSG) abnormalities were not changed. Motor dysfunction was not improved by the use of melatonin [128].

15.5 COMPLEX REGIONAL PAIN SYNDROME

Complex regional pain syndrome (CRPS) was described for the first time more than a hundred years ago, and it is characterized by chronicity and relapses that can result in significant disability over time. Fractures and surgical insult are considered the main triggers for CRPS, but it can also develop after a seemingly benign trauma. We still do not fully understand how this condition works. It goes by many names, "reflex Sympathetic Dystrophy," "causalgia," "Sudeck atrophy," "algodystrophy," "neurodystrophy," and "posttraumatic dystrophy." The term CRPS was adopted in 1995 by the International Association for the Study of Pain (IASP) to standardize the taxonomy [129–131]. On the basis of signs and symptoms of CRPS, four subgroups have been created: (1) a unique set of signs and symptoms indicating abnormalities in pain processing, for example, allodynia and hyperalgesia; (2) a different set of clinical signs characterized by skin color and temperature changes, which are indicative of vasomotor dysfunction; (3) a third group characterized by edema and sudomotor dysfunction, for example, sweating changes; and (4) a fourth subgroup including motor and trophic signs and symptoms [132]. The progression of CRPS involves three stages: (1) acute, warm, or inflammatory; (2) dystrophic; and (3) atrophic. MRI findings of diffuse bone marrow edema on both sides of the joint, accompanied by periarticular soft tissue edema, with or without a joint effusion, are present in about half of all patients in CPRS stage 1 [133]. It has been hypothesized that ROS may contribute to CRPS. Experimental studies have shown that *N*-acetyl-L-cysteine and 4-hydroxy-2,2,6,6-tetramethylpiperidine reduce the signs of hyperalgesia and allodynia in animal models of CRPS [134]. In addition, topical treatment with 50% dimethyl sulfoxide cream can be effective in decreasing the hypoxia-related production of free oxygen radicals [135]. The use of vitamin C in CRPS has been widely investigated. A double-blind, prospective multicenter trial involving 416 patients with 427 wrist fractures randomly allocated to placebo or treatment with 200, 500, or 1500 mg of vitamin C daily for 50 days showed that vitamin C reduces the prevalence of CRPS after wrist fractures, and a daily dose of 500 mg for 50 days is recommended [136]. It has been reported that a 5-year-old girl with clinical and radiographic evidence of scurvy who developed features of CRPS 2 years after a left ankle fracture improved with the administration of vitamin C [137].

Zollinger and colleagues [138] performed 32 arthroplasties for first carpometacarpal arthritis in 27 patients, using a cementless total trapeziometacarpal joint prosthesis that may be complicated by CRPS. All their patients took vitamin C 500 mg/day starting 2 days

before surgery and continuing for 50 days. There were no cases of CRPS under vitamin C prophylaxis. Besse et al. [139] studied a group of patients having surgery on the foot or ankle with the exception of diabetic foot cases. Four hundred and twenty feet were included in this study: 185 in Group 1(no Vitamin C treatment), and 235 in Group II (1 g/day of preventative oral vitamin C treatment). CRPS I occurred in 18 cases in Group I (9.6%) and 4 cases in Group II (1.7%) (57). The authors conclude that vitamin C is effective in preventing CRPS I of the foot and ankle since it was not an infrequent complication in their control group (9.6%). It has been hypothesized that both inflammatory and neural mechanisms may contribute to CRPS type I (CRPS-I). The levels of antioxidants in the serum and saliva of 31 patients with CRPS-I and in a control group of 21 healthy volunteers have been investigated, showing that serum lipid peroxidation products (MDA) and all antioxidative parameters analyzed were significantly elevated in CRPS-I patients. Median salivary peroxidase and superoxide dismutase (SOD) activity values, uric acid (UA) concentration, and total antioxidant status (TAS) values were higher in CRPS I patients by 150%, 280%, 60%, and 200%, respectively, as compared with control subjects. Oxidative changes were also found in the serum, namely, mean serum UA and MDA concentrations, and TAS value in the CRPS-I patients were higher by 16%, 25%, and 22%, respectively, than in the control group. Median salivary albumin concentration and median salivary LDH activities in the patients were 2.5 times and 3.1 times higher than in the control group. The accumulated data show that free radicals are involved in the pathophysiology of CRPS I, which is reflected both in serum and salivary analyses [140].

15.6 CANCER

The Hippocratic "corpus" consists of 70 books written by Hippocrates of Cos (460 BCE–370 BCE) and other physicians. It was the first text to use the words *karkinos* and *karkinoma* to describe a nonhealing swelling or ulceration and a malignant nonhealing tumor, respectively. Hippocrates also introduced the word *scirrhus* to describe hard tumors [141]. The word "tumor" derives from the Latin word *tumere* meaning "to swell." It is synonymous with the term "neoplasm" and can be defined as a lesion resulting from the autonomous or relatively autonomous abnormal growth of cells that persists after the initiating stimulus has been removed [142]. ROS formed in vivo are powerful oxidizing agents, capable of damaging DNA and other biomolecules. An increased formation of the molecules can promote the development of malignancy [143]. Histone deacetylase inhibitors are important regulators of many oxidative stress pathways, including those involved in the cellular response to oxidative stress. Aberrant regulation of these pathways by histone deacetylases may play a critical role in cancer progression [144]. The relationship among lipid soluble antioxidant vitamins, lipid peroxidation, disease stage, and systemic inflammatory response has been studied in 14 healthy subjects, 20 patients with benign prostate hyperplasia (BPH), and patients with localized ($n = 40$) and metastatic ($n = 38$) prostate cancer. This study suggests that in patients with prostate cancer lower concentrations of carotenoids, in particular, lycopene, reflect a disease progression rather than a systemic inflammatory response [145].

An interesting study evaluated the association between the intake of antioxidants from foods and supplements and the risk of prostate cancer among men in the screening arm of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. This study does not provide strong support for population-wide increase of high-dose antioxidant supplementation for the prevention of prostate cancer. However, vitamin E supplementation in male smokers and beta-carotene supplementation in men with low dietary beta-carotene intake are associated with a reduced risk of this disease [146]. The relationship between Se supplementation and prevalent and incident colorectal adenomas and colorectal cancer (CRC), detected during the Nutritional Prevention of Cancer trial follow-up, has been studied in 1312 recipients randomized to 200 mcg of selenized yeast [only 598 underwent endoscopic screening (flexible sigmoidoscopy or colonoscopy) for CRC sometime during the follow-up period]. This study suggests that Se supplementation is associated with a significantly reduced risk of prevalent adenomas, but only among subjects with a low baseline Se level or among current smokers [147]. A study performed in French adults (7876 women and 5141 men) who were randomized to take an oral daily capsule of antioxidants (120 mg vitamin C, 30 mg vitamin E, 6 mg beta-carotene, 100 μg Se, and 20 mg zinc) or a matching placebo has shown that antioxidant supplementation affects the incidence of skin cancer (SC) differentially in men and women. In fact, in women the incidence of SC was higher in the antioxidant group, while in men the incidence did not differ between the two treatment groups [148]. A study in 1732 Finnish male smokers who were diagnosed with incident prostate cancer has evidenced that higher prediagnostic serum concentrations of α-tocopherol, but not dietary vitamin E, are associated with a lower risk of developing prostate cancer, particularly advanced prostate cancer [149]. A systematic review, searching multiple electronic databases from their dates of inception until August 2005, has evidenced that beta-carotene

supplementation increases cancer incidence and cancer mortality among smokers, whereas vitamin E supplementation has no effect. Moreover, Se supplementation might have anticarcinogenic effects in men, and therefore it requires further research [150]. A review including all the randomized-controlled trials of Se monosupplements in cancer patients undergoing tumor specific therapy, namely, chemotherapy, radiotherapy, or surgery, identified only two trials. The first of these investigated secondary lymphedema, while the second one investigated radiotherapy-induced diarrhea as a secondary outcome. The trial on secondary lymphedema reported a decreased number of recurrent erysipela infections in the Se supplementation group compared to placebo. The ongoing trial on radiotherapy-associated diarrhea preliminarily reported a lower incidence of diarrhea in patients receiving Se supplementation concomitant to pelvic radiation. According to this review, at present there is insufficient evidence that Se supplementation alleviates the side effects of tumor-specific chemotherapy or radiotherapy treatments [151].

15.7 ASSOCIATION BETWEEN ROS AND VARIOUS DISEASES

Oxidative stress is one of the potential biochemical mechanisms involved in the pathogenesis of NASH [152], and it seems to play a key role in alcoholic steatohepatitis [153]. Thyroid specimens from patients with Graves disease, follicular adenoma, and papillary and follicular carcinomas contain significantly higher concentrations of xanthine oxidase (XOD) and GSH-PX, compared to those in normal thyroid tissue [3]. Redox status imbalance and a change in the lipid profile are the final result of a lack of estrogens that characterizes the menopause. Moreover, the symptoms accompanying the menopause, for example, hot flushes, suggest an increased metabolic activity [154] that may lead to redox status imbalance toward oxidative processes. Oxidative damage, because of the testicular venous backflow, may represent one of the causes of gonad injury and seems to precede the histological alteration typical of varicocele and consequent male infertility [155, 156]. The lung is continuously exposed to a relatively high oxygen tension, pollutants, and metabolic products derived from them. The inhalation of cigarette smoke, ozone, carcinogens and other chemicals, and dust particles is able to further increase ROS and RNS in the lung and may lead, in time, to depletion of endogenous antioxidants [157]. Oxidative stress also plays a relevant pathogenetic role in human immunodeficiency virus (HIV) infections. In fact, in the early phase of the disease, it has been observed that serum and tissue antioxidants levels are low, while peroxidation products are elevated. In addition, high plasma levels of MDA, reduced plasma GSH, decreased GSHPx (glutathione peroxidase) and SOD activities are also found. HIV infection also results in considerably reduced vitamin E and C concentrations and very low plasma Zn and Se levels. In particular, Se deficiency is related to the occurrence, virulence, and disease progression of some virus infections, including HIV progression to AIDS [158].

Several factors are involved in the development of oxidative stress in the joints of rheumatoid arthritis patients. ROS generation from locally activated leukocytes is followed by a pressure increase in the synovial cavity [159], a capillary density reduction, vascular changes, and an increased metabolic rate of synovial tissue. A randomized prospective study was conducted to compare patients receiving α-tocopherol and ascorbate to those receiving standard care. Five hundred ninety-five patients were enrolled and analyzed (91% were victims of trauma). It was concluded that the early administration of α-tocopherol and ascorbic acid reduces the incidence of organ failure and shortens ICU length of stay in this cohort of critically ill surgical patients [160]. Endometriosis is associated with a general inflammatory response in the peritoneal cavity, and oxidative stress may represent a potential factor involved in its pathophysiology. Endothelial nitric oxide synthase, the enzyme that produces NO, is also overexpressed in endometriosis and adenomyosis. The endometrium shows altered expression of enzymes such as SOD and GSHPx involved in defense against oxidative stress. Also, vitamin E levels are also significantly lower in the peritoneal fluid of women with endometriosis in the presence of redox-active metal ions, as estrogens are established oxidants. This mechanism might be due to the estrogen proinflammatory effect; in fact, the results of hormone therapy are correlated with an increased amount of CRP, a known marker of inflammation [161]. Therefore, estrogens and their metabolites have both prooxidant and antioxidant properties depending on the availability of metal ions and/or their dose and formulation. *Helicobacter pylori* is an important agent in the pathogenesis of active chronic gastritis, peptic ulcer, and low-grade gastric mucosa-associated lymphoid tissue (MALT) lymphoma and in gastric carcinogenesis [162]. Generation of cytotoxins, urease, ammonia, T cell-mediated damage and a mainly humoral reaction are among the events that involve mucosal integrity following *H. pylori* infection [163]. Moreover, reactive oxygen metabolites (ROMs) have been found to play a relevant role in gastroduodenal inflammatory damage [164]. In particular, the acute mucosal inflammatory infiltrate (e.g., polymorphonuclear cells, PMNs) that characterizes *H. pylori*-related chronic active gastritis could

be an important source of free radicals [165], as both in vivo and in vitro studies have reported a positive relation between *H. pylori* infection and ROM generation [164, 166]. A review [167] including six randomized clinical trials has examined the effects of cysteine, cystine, or *N*-acetylcysteine supplementation of neonatal growth under parenteral nutrition (PN), showing that:

- Nitrogen retention is significantly increased by cysteine supplementation.
- Plasma levels of cysteine are significantly increased by cysteine supplementation but not by *N*-acetylcysteine supplementation.
- There is insufficient evidence to assess the risks of cysteine supplementation, especially regarding metabolic acidosis, which has been reported during the first 2 weeks of cysteine chloride administration.

An analysis of 23 studies based on the use of antioxidant supplements in amyotrophic lateral sclerosis (SLA) has shown no significant effect for vitamin E 500 mg/twice a day, vitamin E 1 g/five times a day, acetylcysteine 50 mg/kg daily subcutaneous infusion, or a combination of L-methionine 2 g, vitamin E 400 IU, and Se 0.03 mg administered three times a day [168].

PUFA seem to have no major effect on the main clinical outcome in MS (disease progression) and do not substantially affect the risk of clinical relapses over 2 years [169].

15.8 PREGNANCY AND PREECLAMPSIA

High blood pressure and proteinuria occurring after 20 weeks of pregnancy and affecting both the mother and the unborn baby are the main features of preeclampsia. From a clinical perspective this pathological condition is characterized by swelling, sudden weight gain, headaches, changes in vision, generalized vasoconstriction, increased vasoactivity, reduced perfusion to organs, and platelet activation. Changes of the maternal vascular endothelium may play an important role in the development of the condition, and they have been correlated to a dysfunction in the antioxidant defenses. Reduced total omega-3 fatty acids, increased omega-6-to-omega-3 ratio, higher oxidative stress, and lower antioxidant levels have been observed in preeclamptic women. Similar characteristics have also been observed in cord samples. Therefore, a role for oxidative stress, leading to impaired essential PUFA levels, has been hypothesized in this condition, and antioxidant supplementation, along with PUFA, particularly omega-3 fatty acids, may be helpful in the management of preeclampsia [170]. According to a review dated 2008, no evidence for an antioxidant routinely used in the treatment of preeclampsia has been observed [171]. Supplementation with 1000 mg vitamin C and 400 IU vitamin E (α-tocopherol) daily from the second trimester of pregnancy until delivery does not prevent preeclampsia in women at risk [172]. These findings have been confirmed by another randomized, controlled double-blind clinical trial showing that an antioxidant supplementation consisting of both vitamin C (1000 mg) and vitamin E (400 IU) does not reduce the rate of preeclampsia among patients with chronic hypertension or prior preeclampsia [173].

15.9 ASTHMA

Asthma is a chronic relapsing inflammatory disease of the airways. In adult patients, oxidative and nitrosative stress accompany acute asthma [174]. In an attempt to investigate the effects of antioxidant supplementation, it has been observed that patients who consumed more than 46.3 g/day of citrus fruit had a reduced risk of diagnosed and symptomatic asthma. In addition, the same study pointed out that dietary vitamin C and manganese were inversely and independently associated with symptomatic asthma, but only manganese was independently associated with diagnosed asthma. Moreover, plasma vitamin C levels were significantly lower in symptomatic cases than in control subjects [175]. The possible influx of diet modification in asthma has been addressed in a study involving 32 asthmatic adults who underwent a low-antioxidant diet for 10 days and then started a randomized crossover trial involving 3×7-day treatment arms [placebo, tomato extract (45 mg lycopene/day), and tomato juice (45 mg lycopene/day)]. In asthmatic patients following a low-antioxidant diet, it was observed that plasma carotenoid concentrations decreased, Asthma Control Score worsened, %FEV and %FVC decreased, and %sputum neutrophils increased. After the 10 days of low-antioxidant diet, the patients who were treated with both tomato juice and extract showed a reduction in airway neutrophil influx, and treatment with tomato extract reduced sputum neutrophil elastase activity [176].

15.10 CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Chronic obstructive pulmonary disease (COPD) is a chronic disease characterized by incompletely reversible airflow limitation [177]. The oxidative inactivation of antiproteinases, epithelial injury, increase in number of neutrophils in the pulmonary microvasculature, and gene expression of proinflammatory mediators seem to

play a key role in this condition. The antioxidant depletion or deficiency in antioxidants may contribute to oxidative stress. Futhermore, airflow limitation seems to be related to dietary deficiency of antioxidants. The effects of antioxidant polyphenol-rich pomegranate juice (PJ) supplementation for 5 weeks on patients with stable COPD has been investigated, but no benefit has been reported [178]. The association between antioxidant nutrients and markers of oxidative stress with pulmonary function in patients with chronic airflow limitation has been also investigated, showing that serum beta-cryptoxanthin, lutein/zeaxanthin, and retinol and dietary beta-carotene, beta-cryptoxanthin, lutein/zeaxanthin, vitamin C, and lycopene were positively associated with FEV1% (0.05, all associations). In addition, serum vitamins beta-cryptoxanthin, lutein/zeaxanthin, lycopene, adietary beta-cryptoxanthin, beta-carotene, and vitamin C were positively associated with FVC%. Erythrocytic glutathione was negatively associated with FEV1%, while plasma thiobarbituric acid-reactive substances (TBARS) were negatively associated with FVC% [179]. It has been observed that erdosteine treatment in current smokers with mild COPD leads to a significant drop in blood ROS and IL-8 in bronchial secretions (observed after day 4 from the beginning of treatment), while 8-isoprostane drop was significant only after day 10. An e-NO decrease was also reported, but it was not significant [180].

15.11 DIABETES TYPE 1 AND TYPE 2

Diabetes is a blood glucose metabolism impairment subdivided into type 1, which is due to the inability of the body to produce insulin, and type 2, when the body produces the insulin needed but the cells are not able to respond to it, making it ineffectual. Type 2 diabetes has been associated with obesity, and it is a condition that can lead to severe illness and even death. In the peripheral nervous system, diabetes causes a progressive deterioration of primarily sensory nerves, and the damage also extends to motor nerves. Approximately 50% of diabetics experience some degree of neuropathy, which is ultimately the leading cause of lower-extremity amputation [181]. In addition, variations in blood sugar level, namely, hypoglycemia or hyperglycemia can lead to atherosclerosis, neuropathy, retinopathy, and nephropathy. The hyperglycemic state that characterizes the diabetic patient is responsible for the high oxidative stress that accompanies this condition. In particular, this seems to be due to superoxide anion production by the cell glucose metabolism, which, in turn, is able to damage oxidative blood balance, leading to protein glycation and O_2 and H_2O_2 release. It has been shown that high α-tocopherol levels, among patients with renal disease and in those using vitamin supplements are associated with lower coronary artery disease (CAD) risk in type 1 diabetes [182]. A randomized and open study [183] has been performed on a total of 36 patients with type 2 diabetes and hypercholesterolemia. They were randomly assigned to a probucol group (500 mg/day, $n = 18$) or an atorvastatin group (10 mg/day, $n = 18$). Over 3 months, total and LDL-cholesterol decreased significantly in both groups. LDL-cholesterol was significantly lower in the atorvastatin group than in the probucol group. HDL-C decreased significantly in the probucol group and did not change in the atorvastatin group. Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) decreased significantly in both groups after 3 months. Urinary 8-OHdG was significantly lower in the probucol group than in the atorvastatin group after the second and third months of administration.

A study involving 12 patients who were treated with oral ALA, 600 mg twice daily, over a period of 4 weeks has shown that short-term oral ALA treatment increases peripheral insulin sensitivity in patients with type 2 diabetes mellitus [184]. Cilostazol (100 mg) was administered to hypertensive type 2 diabetic patients twice daily. At 1 month follow-up the Cilostazol group (26 patients) showed significant decrease in hsCRP, erythrocyte sedimentation rate (38.7%), total leukocyte count, plasma MDA, HbA1c, and an increase in serum albumin and blood reduced glutathione from baseline. The cardiovascular risk assessment in terms of 10-year risk of cardiovascular heart disease (%), calculated using the United Kingdom Prospective Diabetes Study (UKPDS) guidelines, decreased by 6%. Therefore inflammatory and oxidative stress is high in hypertensive type 2 diabetic patients, and Cilostazol reduces those factors as well as coronary heart disease risk in diabetes mellitus [185].

A study involving 36 type 1 diabetic patients showed that neither normalization of glycemia nor vitamin C treatment alone was able to normalize endothelial dysfunction or oxidative stress; when insulin and vitamin C were combined, endothelial dysfunction and decreased oxidative stress normalized to normal levels. Telmisartan significantly improved basal endothelial function and decreased nitrotyrosine plasma level. In patients treated with telmisartan, a near-normalization of both flow-mediated vasodilation and oxidative stress was achieved when glycemia was normalized. Instead, adding vitamin C infusion did not show further effect on endothelial function or nitrotyrosine plasma levels [186]. A double-blind placebo-controlled clinical trial, conducted for 2 months and involving 35 patients with type 2 diabetes mellitus, has shown that lycopene, probably by increasing TAC and inhibiting MDA-LDL formation, may attenuate T cell-dependent adaptive (proatherogenic) immune

response. With enhancement of innate immunity and hence prevention of ox-LDL uptake by macrophage and foam cell formation, lycopene should be effective in prevention of long-term diabetic complications, notably cardiovascular disease [187]. One thousand two hundred and two people seen in dermatology clinics who did not have type 2 diabetes at baseline were studied in a randomized, double-blind, placebo-controlled trial to receive oral administration of Se (200 μg/d) or placebo. The average follow-up was 7.7 years. According to this study, Se supplementation does not seem to prevent type 2 diabetes, and it may increase the risk for the disease [188]. Vitamin C has a dose-dependent effect on the cellular contents of antioxidants and on vitamin E content of LDL in elderly patients with type 2 diabetes mellitus, but these changes are not sufficient to decrease the LDL susceptibility to peroxidation [189]. DEX (dexlipotam: R-lipoic acid) therapy appears to reduce endothelial dysfunction in type 2 diabetes mellitus, especially in men with long history of type 2 diabetes mellitus and having poor glucose control (study involving 114 diabetic recipients). DEX is safe and well tolerated, and dyspepsia appears to be the most relevant side effect of DEX treatment [190]. Oxidative stress plays a key role in long-term b-cell dysfunction in type 2 diabetes. It has been shown that treatment with ALA improves functional outcomes, like insulin sensitivity in type 2 diabetic subjects [191]. Supplementation with vitamin E appears to lower plasma glucose in type 2 diabetic subjects [192], but 900 mg vitamin E/day lowers plasma glucose but may not improve pancreatic response to glucose. In humans no relationship between vitamin E intake and severity of retinopathy in type 2 diabetics has been observed [193, 194]. It has also been observed that there is no association between risk of retinopathy and intake of vitamins C and E from foods and supplements [195]. Plasma vitamin E-to-lipid ratio is lower in diabetic subjects than control subjects, and this effect is even more pronounced in diabetic subjects with neuropathy [196]. The same study has also shown that plasma vitamin E-to-lipid ratio is also inversely related to an assessed score of neuropathy, suggesting that diabetic subjects with neuropathy have higher levels of oxidative stress than those without this complication.

15.12 LIVER DISEASES

The liver is the largest organ in the body, weighing approximately 1.36 kg, and exerts several vital functions. It metabolizes substances present in blood, preparing them for excretion, synthesizes many essential proteins, produces bile, and regulates some key nutrients such as glucose, cholesterol, and amino acids. An increase in liver free radicals can lead to inflammation. Normally the liver uses internally generated antioxidants to neutralize the toxin-derived free radicals, but when the liver antioxidants are low because of alcohol or chronic drug use damage from free radicals increases, resulting in inflammation and formation of scar tissue (fibrosis). Therefore it is important to maintain a constant supply of antioxidants and a healthy lifestyle (abstaining from all alcohol and avoiding environmental toxins) to reduce the strain on the liver. Alcohol lowers liver antioxidant levels, including vitamin E and *S*-adenosyl-L-methionine, making the liver vulnerable. In addition, alcohol lowers glutathione, an important internal antioxidant. Because heavy drinkers consume a substantial number of calories as alcohol, they consume less vitamin- and mineral-rich food, exacerbating alcohol-induced nutritional deficiencies that include low levels of vitamin C, riboflavin, zinc, pyridoxine (vitamin B6), and vitamins. A randomized clinical trial has compared antioxidant and corticosteroid treatments, showing that corticosteroids, in the form of prednisolone 30 mg daily, are superior to a broad antioxidant cocktail in treatment of severe alcoholic hepatitis [197]. A combination of seven different antioxidants administered orally (glycyrrhiza capsules, 500 mg, bid; schizandrae capsules, 500 mg, tid; ascorbate capsules, 2000 mg, tid; L-glutathione capsules, 150 mg, bid; silymarin capsules, 250 mg, tid; lipoic acid capsules, 150 mg, bid; d-α-tocopherol, 800 IU/day) in patients with chronic hepatitis C virus (HCV) infection has been associated with a significant decline in ALT levels in 52% of patients versus 20% of patients who received placebo. Histology activity index (HAI) score at the end of treatment was reduced in 48% of patients versus 26% of patients who received placebo. HCV-RNA levels decreased by 1 log or more in 28% of patients who received antioxidant therapy versus 12% of patients who received placebo (not significant) [198]. Antioxidant therapy, alone or in combination with corticosteroids, does not improve 6-month survival in severe alcoholic hepatitis patients [199]. An analysis of nine randomized clinical trials including 434 patients with alcoholic liver diseases and the supplementation of SAMe has shown no significant effects on all-cause mortality, liver-related mortality, liver transplantation, or complications [200].

15.13 PANCREATITIS

Acute pancreatitis (AP) is an acute inflammatory condition probably due to the activation of enzymes in the pancreatic acinar cells. Chronic pancreatitis (CP) is a progressive inflammatory disorder that is characterized by recurrent episodes of severe abdominal pain. There is evidence that the pathogenesis of both AP and CP can be associated with oxidative stress. In fact, it has been

observed that free radical activity and oxidative stress indices, such as lipid peroxide levels, are higher in AP or CP patients' blood and duodenal juice [201]. Treatment with Se, beta-carotene, L-methionine, vitamins C and E, or placebo for 10 weeks in patients with confirmed CP ($n = 36$) has been associated with significant improvements in quality of life in terms of pain, physical and social functioning, and general health perception [202].

Allopurinol (300 mg), a hydroxyl radical scavenger, has been tested to determine a possible decrease in the rate of PEP, but it does not appear to reduce the overall risk of PEP [203]. Glutamine has been used in AP in combination with standard total parenteral nutrition (TPN; $n = 28$), and a decrease in the duration of TPN therapy and hospitalization without a change in the total cost of parenteral feeding has been demonstrated [204]. Another similar study ($n = 44$) has shown that even though TPN therapy containing glutamine reduces infectious morbidity, it has no significant effect on hospitalization and total mortality [205]. Both studies showed laboratory improvement in AP after administration of glutamine, such as an increase in serum albumin or decrease in CRP. Another study ($n = 14$) has shown that glutamine supplementation does not significantly influence TNF-α or IL-6 release but does reduce median IL-8 release by day 7 in the glutamine group, while it increases in the conventional group [206]. Another nonblinded study examined the administration of glutamine in AP for 10 days starting either on the day of admission or 5 days after admission. Investigators reported an improvement in all clinical findings including hospitalization, infection, and mortality rate [207]. In contrast, another clinical trial ($n = 78$) has shown that CP patients with chronic pain who were admitted to hospital and who received pethidine with or without allopurinol had a reduction in pain and gastric tenderness. Hospitalization also decreased in allopurinol-treated patients [208]. Another clinical study ($n = 13$) has shown that 4-week allopurinol administration does not reduce pain in CP compared with placebo [209]. Allergy, general malaise, and gastrointestinal disturbances were adverse events of allopurinol. It has been shown that 10 g/day vitamin C decreases hospitalization and duration of disease and increases the cure rate in patients ($n = 83$) with AP treated for 5 days. Proinflammatory cytokines and CRP were also diminished by vitamin C administration [210].

A combination of various antioxidants, including Se, beta-carotene, vitamin C, vitamin E, and methionine, administered to CP patients ($n = 28$) has been shown to reduce the pain experienced in this condition [211]. Another study ($n = 36$), using the same antioxidants at the same doses but with greater bioavailability in CP patients, has shown a reduction in pain after 10-week combined antioxidant treatment. Quality of life, physical and social functioning, and health perception were also enhanced [202]. Another clinical trial ($n = 147$) involving the use of the same antioxidant regimen, administered for 6 months, confirmed the previous trials, showing that pain and hospitalization were reduced [212]. The same combination of antioxidants, studied in 12 CP patients, has shown a reduction in pain and hospitalization [213], but some adverse effects, namely, headache, nausea, vomiting, and constipation have been reported. A pilot study involving 20 patients with CP who received 500 mg of curcumin with 5 mg of piperine or placebo for 6 weeks has shown a significant reduction in erythrocyte MDA levels following the curcumin therapy. A significant increase in glutathione (GSH) levels was also observed. There was no corresponding improvement in pain, and no adverse effects were reported [214].

N-acetylcysteine (NAC), a free radical scavenger that stimulates glutathione synthesis, has been tested in a trial involving 106 patients. NAC (600 mg) was given orally 24 h and 12 h before ERCP, and 600 mg was given intravenously twice a day for 2 days after ERCP. The rate of PEP was not significantly reduced. In addition, urine amylase activity, total bilirubin, alanine and aspartate aminotransferases, and white blood cells showed no change [215]. Another double-blind, placebo-controlled trial, involving 256 patients who received intravenous NAC at a loading dose of 70 mg/kg 2 hours before and 35 mg/kg at 4-hour intervals for a total of 24 hours after the procedure, has shown that there were no statistical differences in the incidence or severity of PEP grades between the groups. The mean duration of hospitalization for PEP was not different in the NAC group compared to the placebo group [216]. The results of those studies showed the absence of any beneficial effect of NAC on the incidence and severity of ERCP-induced PEP. A double-blind trial involving 321 patients who were given a single dose of natural beta-carotene 12 hours prior to the procedure has shown that the overall incidence of AP was not significantly different between the beta-carotene group and the placebo groups. The rate of severe PEP was lower in the beta-carotene-treated group [217]. SAMe, a highly bioactive metabolite of methionine and a precursor of glutathione, has been investigated in two clinical trials investigating PEP. SAMe did not enhance the clinical outcomes in either AP [218] or CP [219] patients. However, laboratory indices, such as free radical activity, were better after 10 weeks of SAMe administration in CP patients. Two placebo-controlled clinical trials [220], examining a combined antioxidant therapy on recurrent CP, showed a significant decrease in pain and an elevation in serum antioxidant biomarkers. SAMe has also been examined as an antioxidant, alone or in

combination with Se and beta-carotene, but proved to be ineffective in patients with recurrent PEP.

15.14 RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic progressive autoimmune disorder characterized by symmetric erosive synovitis and destruction of the tissues within the joints leading to consequent physical disability. ROS has been correlated to the etiology of RA in an analysis of 20 randomized clinical trials (RCTs) [11 in inflammatory arthritis and 9 in osteoarthritis (OA); Se for RA ($n = 5$); vitamin E for inflammatory arthritis ($n = 5$); and vitamin E for OA ($n = 7$)]. One study suggests the superiority of vitamin E over placebo, and three RCTs suggest equivalence between vitamin E and diclofenac in the treatment of inflammatory arthritis. In OA, four RCTs compared vitamin E with placebo. Two shorter-term studies have evidenced a positive effect of vitamin E in OA, while two longer-term studies did not. Two further RCTs suggest equivalence between vitamin E and diclofenac in the treatment of OA. An isolated positive result for vitamin C in OA is of doubtful clinical significance [221]. Antioxidants and cardiovascular disease (CVD) risk factors in participants with RA and non-RA control subjects have been studied, observing that plasma levels of antioxidants alpha-carotene, beta-cryptoxanthin, lutein/zeaxanthin, and lycopene were significantly lower in RA subjects compared with non-RA subjects. Compared with non-RA participants, RA subjects were more likely to have increased CRP levels [222].

15.15 KIDNEY DISEASES

Activated macrophages, vascular cells, and various glomerular cells are considered important ROS sources in kidneys. In particular, various diseases affecting the kidneys, namely, glomerulonephritis, tubulointerstitial nephritis, chronic renal failure, and IR injury, have been associated to ROS. Serum sulfite, sulfate, cysteine, homocysteine, cysteine sulfinic acid, and γ-glutamylcysteine are elevated in patients on hemodialysis, suggesting an accelerated catabolism of sulfur-containing amino acids, a reduced elimination of sulfite/sulfate, or both. It has been shown that dietary supplementation with vitamin E (400 mg/day for 6 months) in IgA nephropathy patients, a particular group of patients characterized by low vitamin E level and high oxidative stress, is able to reduce oxidative stress [223]. A study investigating the role of lovastatin or of hypolipemic diet on oxidative stress in hemodialyzed patients has shown that the level of 8-OHdG decreased considerably only in the lovastatin-treated group and that the level of total antioxidant status (TAS) increased significantly in the lovastatin-treated group, decreased in the diet-treated group, and remained unchanged in the untreated group. Therefore lovastatin, but not hypolipemic diet alone, has an antioxidant effect in hemodialyzed patients, although the determinants of the antioxidant effect of statins in patients with chronic renal failure are unclear [224]. NAC has been studied in 30 uremic patients on hemodialysis (HD), suggesting that it could improve the ED by preventing the reduction of FMD in patients on HD [225]. Al-Awadi et al. [226] conducted an RCT to determine whether extracorporeal shock wave lithotripsy (ESWL) produces ischemia and reperfusion injury in kidneys and whether oral administration of antioxidants (2 capsules of Nature Made® antioxidants [each Nature Made® antioxidant capsule (Pharmavite Corporation, Mission Hills, CA, USA) contains high levels of Vitamin A (as beta-carotene) 10,000 IU, vitamin C 250 mg, and vitamin E 200 IU, and mineral supplements like zinc 7.5 mcg and Se 15 mcg] could protect from these complications. At 24 hours after ESWL, the patients who received antioxidants had significantly reduced mean MDA serum concentration, higher levels of serum ascorbic acid and serum albumin, lower α-tocopherol-to-cholesterol ratio, and lower urinary albumin and β_2-microglobulin levels compared with patients who did not receive any antioxidants. These findings point out that treatment with ESWL generates free radicals through an ischemic-reperfusion injury mechanism and that oral administration of antioxidant may protect these patients from short-term renal injury caused by ESWL.

15.16 CONCLUDING REMARKS

This overview on antioxidant clinical use should be updated monthly because of the great number of contributions in this field. They produce evidence-based data on antioxidant benefit to prevent or counteract the ROS-induced damage in acute and chronic diseases as well as in surgical ischemia-reperfusion, surgical trauma, and so on. The choice of the right compound that will be effective to treat a specific disease is a very hard challenge and often a pool of natural or synthetic antioxidant molecules, contemporaneously administered, gives a benefit that cannot be achieved by a single molecule. From this perspective, antioxidant therapy must be considered a relevant support to restore physiological redox balance in several pathological conditions such as neurodegenerative diseases and in cancer prevention. Further insights into the multimodal pathogenesis of uncurable illnesses will help, in the future, to

better define optimal protocols, in terms of scheduling, timing, and synergy with the emerging drugs (especially biologicals), in order to reduce their side effects and enhance their ability to heal.

15.17 CONFLICT OF INTEREST STATEMENT

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in this chapter.

15.18 STATEMENT OF AUTHORSHIP

The authors hereby certify that all work contained in this review is original work of Tommaso Iannitti and Beniamino Palmieri. The authors claim full responsibility for the contents of the article.

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REFERENCES

1. Iannitti T, Palmieri B. Antioxidant therapy effectiveness: an up to date. *Eur Rev Med Pharmacol Sci* 2009; 13(4): 245–278.
2. Jiménez R, Delgado MA, Muguerza B, et al. Physical activity and antioxidant vitamins for the elderly: beneficial effects of a long-term dietary treatment with antioxidant vitamins on the prooxidant effects of physical activity in a large-scale study in aged healthy people. *Free Radic Biol Med* 2004; 36(Suppl 1): 16.
3. Palmieri B, Iannitti T, Sblendorio V. Oxidative stress: how to detect it, cope with and treat basing on evidence? *The Internet Journal of Aesthetic and Antiaging Medicine* 2009; 2(1).
4. Jackson MJ. An overview of methods for assessment of free radical activity in biology. *Proc Nutr Soc* 1999; 58(4): 1001–1006.
5. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. *Cochrane Database Syst Rev* 2008; (2): CD007176.
6. Ghibu S, Richard C, Delemasure S, Vergely C, Mogosan C, Muresan A. An endogenous dithiol with antioxidant properties: alpha-lipoic acid, potential uses in cardiovascular diseases. *Ann Cardiol Angéiol (Paris)* 2008; 57(3): 161–165.
7. Ghibu S, Richard C, Vergely C, Zeller M, Cottin Y, Rochette L. Antioxidant properties of an endogenous thiol: alpha-lipoic acid, useful in the prevention of cardiovascular diseases. *J Cardiovasc Pharmacol* 2009; 54(5): 391–398.
8. Di Geronimo G, Caccese AF, Caruso L, Soldati A, Passaretti U. Treatment of carpal tunnel syndrome with alpha-lipoic acid. *Eur Rev Med Pharmacol Sci* 2009; 13 (2): 133–139.
9. Iannitti T, Palmieri B. An update on the therapeutic role of alkylglycerols. *Marine Drugs* 2010; 8(8): 2267–2300.
10. Lewkowicz P, Banasik M, Glowacka E, Lewkowicz N, Tchorzeweski H. Effect of high doses of shark liver oil supplementation on T cell polarization and peripheral blood polymorphonuclear cell function. *Pol Merkur Lekarski* 2005; 18(108): 686–692.
11. Iannitti T, Palmieri B. The obese patient: clinical effectiveness of a high-protein low-calorie diet and its usefulness in the field of surgery. *Minerva Gastroenterol Dietol* 2010; 56(2 Suppl 1): 1–65.
12. Epstein J, Sanderson IR, Macdonald TT. Curcumin as a therapeutic agent: the evidence from in vitro, animal and human studies. *Br J Nutr* 2010; 103(11): 1545–1557.
13. Kobelt G. Health economic issues in rheumatoid arthritis. *Scand J Rheumatol* 2006; 35: 415–425.
14. Deodhar SD, Sethi R, Srimal RC. Preliminary study on antirheumatic activity of curcumin (diferuloyl methane). *Indian J Med Res* 1980; 71: 632–634.
15. Heng MC, Song MK, Harker J, et al. Drug-induced suppression of phosphorylase kinase activity correlates with resolution of psoriasis as assessed by clinical, histological and immunohistochemical parameters. *Br J Dermatol* 2000; 143: 937–949.
16. Satoskar RR, Shah SJ, Shenoy SG. Evaluation of anti-inflammatory property of curcumin (diferuloyl methane) in patients with postoperative inflammation. *Int J Clin Pharmacol Therapy Toxicol* 1986; 24: 651–654.
17. Lal B, Kapoor AK, Agrawal PK, et al. Role of curcumin in idiopathic inflammatory orbital pseudotumours. *Phytother Res* 2000; 14: 443–447.
18. Lal B, Kapoor AK, Asthana OP, et al. Efficacy of curcumin in the management of chronic anterior uveitis. *Phytother Res* 1999; 13: 318–322.
19. Bundy R, Walker AF, Middleton RW, et al. Turmeric extract may improve irritable bowel syndrome symptomology in otherwise healthy adults: a pilot study. *J Altern Complement Med* 2004; 10: 1015–1018.
20. Durgaprasad S, Pai CG, Vasanthkumar. A pilot study of the antioxidant effect of curcumin in tropical pancreatitis. *Indian J Med Res* 2005; 122: 315–318.
21. Niederau C, Gopfert E. The effect of chelidoniumand turmeric root extract on upper abdominal pain due to functional disorders of the biliary system. Results from a placebo-controlled double-blind study. *Med Klin* 1999; 94: 425–430.
22. Rasyid A, Lelo A. The effect of curcumin and placebo on human gall-bladder function: an ultrasound study. *Aliment Pharmacol Therapeut* 1999; 13: 245–249.

23. Rasyid A, Rahman AR, Jaalam K, et al. Effect of different curcumin dosages on human gall bladder. *Asia Pacific J Clin Nutr* 2002; 11: 314–318.
24. Prucksunand C, Indrasukhsri B, Leethochawalit M, et al. Phase II clinical trial on effect of the long turmeric (*Curcuma longa* Linn) on healing of peptic ulcer. *Southeast Asian J Trop Med Publ Health* 2001; 32: 208–215.
25. Cruz-Correa M, Shoskes DA, Sanchez P, et al. Combination treatment with curcumin and quercetin of adenomas in familial adenomatous polyposis. *Clin Gastroenterol Hepatol* 2006; 4(8): 1035–1038.
26. Usharani P, Mateen AA, Naidu MU, et al. Effect of NCB-02, atorvastatin and placebo on endothelial function, oxidative stress and inflammatory markers in patients with type 2 diabetes mellitus: a randomized, parallel-group, placebo-controlled, 8-week study. *Drugs R&D* 2008; 9: 243–250.
27. Soni KB, Kuttan R. Effect of oral curcumin administration on serum peroxides and cholesterol levels in human volunteers. *Indian J Physiol Pharmacol* 1992; 36: 273–275.
28. Holt PR, Katz S, Kirshoff R. Curcumin therapy in inflammatory bowel disease: a pilot study. *Dig Dis Sci* 2005; 50: 2191–2193.
29. Hanai H, Iida T, Takeuchi K, et al. Curcumin maintenance therapy for ulcerative colitis: randomized, multicenter, double-blind, placebo-controlled trial. *Clin Gastroenterol Hepatol* 2006; 4: 1502–1506.
30. Cheng AL, Hsu CH, Lin JK, et al. Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high risk or pre-malignant lesions. *Anticancer Res* 2001; 21: 2895–2900.
31. Sharma RA, McLelland HR, Hill KA, et al. Pharmacodynamic and pharmacokinetic study of oral *Curcuma* extract in patients with colorectal cancer. *Clin Cancer Res* 2001; 7: 1894–1900.
32. Kuttan R, Sudheeran PC, Josph CD. Turmeric and curcumin as topical agents in cancer therapy. *Tumori* 1987; 73: 29–31.
33. Dhillon N, Aggarwal BB, Newman RA, et al. Phase II trial of curcumin in patients with advanced pancreatic cancer. *Clin Cancer Res* 2008; 14: 4491–4499.
34. Sikora E, Bielak-Zmijewska A, Mosieniak G, Piwocka K. The promise of slow down ageing may come from curcumin. *Curr Pharmaceut Des* 2010; 16(7): 884–892.
35. Rao AV, Snyder DM. Raspberries and human health: a review. *J Agric Food Chem* 2010; 58(7): 3871–3883.
36. Kresty LA, Frankel WL, Hammond CD, Baird ME, Mele JM, Stoner GD, Fromkes JJ. Transitioning from preclinical to clinical chemopreventive assessments of lyophilized black raspberries: interim results show berries modulate markers of oxidative stress in Barrett's esophagus patients. *Nutr Cancer* 2006; 54: 148–156.
37. Mallery, SR, Zwick JC, Pei P, Tong M, Larsen PE, Shumway BS, Lu B, Fields HW,Mumper RJ, Stoner GD. Topical application of a bioadhesive black raspberry gel modulates gene expression and reduces cyclooxygenase 2 protein in human premalignant oral lesions. *Cancer Res* 2008; 68: 4945–4957.
38. Weisel T, Baum M, Eisenbrand G, Dietrich H, Will F, Stockis JP, Kulling S, Rufer C, Johannes C, Janzowski C. An anthocyanin/polyphenolic-rich fruit juice reduces oxidative DNA damage and increases glutathione level in healthy probands. *J Biotechnol* 2006; 1: 388–397.
39. Carmen Ramirez-Tortosa M, Garcia-Alonso J, Luisa Vidal-Guevara M, Quiles JL, Jesus Periago M, Linde J, Dolores Mesa M, Ros G, Abellan P, Gil A. Oxidative stress status in an institutionalised elderly group after the intake of a phenolic-rich dessert. *Br J Nutr* 2004; 91: 943–950.
40. Morillas-Ruiz J, Zafrilla P, Almar M, Cuevas MJ, Lopez FJ, Abellan P, Villegas JA, Gonzalez-Gallego J. The effects of an antioxidant-supplemented beverage on exercise-induced oxidative stress: results from a placebo-controlled double-blind study in cyclists. *Eur J Appl Physiol* 2005; 95: 543–549.
41. Larmo P, Alin J, Salminen E, Kallio H, Tahvonen R. Effects of sea buckthorn berries on infections and inflammation: a doubleblind, randomized, placebo-controlled trial. *Eur J Clin Nutr* 2008; 62: 1123–1130.
42. Roede JR, Jones DP. Reactive species and mitochondrial dysfunction: mechanistic significance of 4-hydroxynonenal. *Environ Mol Mutagen* 2010; 51(5): 380–390.
43. Paradies G, Petrosillo G, Paradies V, Reiter RJ, Ruggiero FM. Melatonin, cardiolipin and mitochondrial bioenergetics in health and disease. *J Pineal Res* 2010; 48(4): 297–310.
44. Leon J, Acuna-Castroviejo D, Sainz RM, et al. Melatonin and mitochondrial function. *Life Sci* 2004; 7: 765–790.
45. Leon J, Acuna-Castroviejo D, Escames G, Tan DX, Reiter RJ. Melatonin mitigates mitochondrial malfunction. *J Pineal Res* 2005; 1: 1–9.
46. Acuna-Castroviejo D, Escames G, Rodriguez MI, Lopez LC. Melatonin role in the mitochondrial function. *Front Biosci* 2007; 12: 947–963.
47. Paradies G, Petrosillo G, Paradies V, Reiter RJ, Ruggiero FM. Melatonin, cardiolipin and mitochondrial bioenergetics in health and disease. *J Pineal Res* 2010; 48(4): 297–310.
48. Reiter RJ, Richardson BA, Johnson LY. Pineal melatonin rhythm: reduction in aging Syrian hamsters. *Science* 1980; 210: 1372–1373.
49. Reiter RJ, Craft CM, Johnson JE. Age-associated reduction in nocturnal pineal melatonin levels in female rats. *Endocrinology* 1981; 109: 1295–1297.
50. Benot S, Goberna R, Reiter RJ, Garcia-Mauriño S, Osuna C, Guerrero JM. Physiological levels of melatonin contribute to the antioxidant capacity of human serum. *J Pineal Res* 1999; 27(1): 59–64.
51. Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol* 1956; 11(3): 298–300.
52. Imhof A, Kövari E, von Gunten A, Gold G, Rivara CB, Herrmann FR, Hof PR, Bouras C, Giannakopoulos P.

Morphological substrates of cognitive decline in nonagenarians and centenarians: a new paradigm? *J Neurol Sci* 2007; 257(1–2): 72–79.

53. Barbieri M, Rizzo MR, Manzella D, Grella R, Ragno E, Carbonella M, Abbatecola AM, Paolisso G. Glucose regulation and oxidative stress in healthy centenarians. *Exp Gerontol* 2003; 38(1–2): 137–143.
54. Shi XM, Yin ZX, Qian HZ, Zhai Y, Liu YZ, Xu JW, Zeng Y. A study on chronic diseases and other related health indicators of centenarians in longevity areas in China. *Zhonghua Yu Fang Yi Xue Za Zhi* 2010; 44(2): 101–107.
55. Willcox DC, Willcox BJ, Todoriki H, Suzuki M. The Okinawan diet: health implications of a low-calorie, nutrient-dense, antioxidant-rich dietary pattern low in glycemic load. *J Am Coll Nutr* 2009; 28 (Suppl): 500S–516S.
56. Mackay J, Mensah G . *Atlas of Heart Disease and Stroke*. World Health Organization, 2004.
57. Arts IC, Jacobs DR, Harnack LJ, Gross M, Folsom AR. Dietary catechins in relation to coronary heart disease death among postmenopausal women. *Epidemiology* 2001; 12: 668–675.
58. Buijsse B, Feskens EJ, Kok FJ, Kromhout D. Cocoa intake, blood pressure, and cardiovascular mortality: the Zutphen Elderly Study. *Arch Intern Med* 2006; 166: 411–417.
59. Geleijnse JM, Launer LJ, Hofman A, Pols HA, Witteman JC. Tea flavonoids may protect against atherosclerosis: the Rotterdam Study. *Arch Intern Med* 1999; 159: 2170–2174.
60. Hertog MG, Kromhout D, Aravanis C, et al. Flavonoid intake and long-term risk of coronary heart disease and cancer in the Seven Countries Study. *Arch Intern Med* 1995; 155: 381–386.
61. Mennen LI, Sapinho D, de Bree A, et al. Consumption of foods rich in flavonoids is related to a decreased cardiovascular risk in apparently healthy French women. *J Nutr* 2004; 134: 923–926.
62. Behrendt D, Beltrame J, Hikiti H, Wainstein M, Kinlay S, Selwyn AP, Ganz P, Fang JC. Impact of coronary endothelial function on the progression of cardiac transplant-associated arteriosclerosis: effect of anti-oxidant vitamins C and E. *J Heart Lung Transplant* 2006; 25: 426–433.
63. Hatzigeorgiou C, Taylor AJ, Feuerstein IM, Bautista L, O'Malley PG. Antioxidant vitamin intake and subclinical coronary atherosclerosis. *Prevent Cardiol* 2006; 9: 75–81.
64. Inatomi Y, Takita T, Yonehara T, Fujioka S, Hashimoto Y, Hirano T, Uchino M. Efficacy of edaravone in cardioembolic stroke. *Intern Med* 2006; 45: 253–257.
65. Ullegaddi R, Powers HJ, Gariballa SE. Antioxidant supplementation with or without B-group vitamins after acute ischemic stroke: a randomized controlled trial. *J Parenteral Enteral Nutr* 2006; 30: 108–114.
66. Yesilbursa D, Serdar A, Senturk T, Serdar Z, Sağ S, Cordan J. Effect of *N*-acetylcysteine on oxidative stress and ventricular function in patients with myocardial infarction. *Heart Vessels* 2006; 21: 33–37.
67. Hozawa A, Jacobs DR Jr, Steffes MW, Gross MD, Steffen LM, Lee DH. Relationships of circulating carotenoid concentrations with several markers of inflammation, oxidative stress, and endothelial dysfunction: the Coronary Artery Risk Development in Young Adults (CARDIA)/Young Adult Longitudinal Trends in Antioxidants (YALTA) study. *Clin Chem* 2007; 53: 447–455.
68. Maçao LB, Wilhelm Filho D, Pedrosa RC, Pereira A, Backes P, Torres MA, Fröde TS. Antioxidant therapy attenuates oxidative stress in chronic cardiopathy associated with Chagas' disease. *Int J Cardiol* 2007; 123: 43–49.
69. Plantinga Y, Ghiadoni L, Magagna A, Giannarelli C, Franzoni F, Taddei S, Salvetti A. Supplementation with vitamins C and E improves arterial stiffness and endothelial function in essential hypertensive patients. *Am J Hypertens* 2007; 20: 392–397.
70. Shinke T, Shite J, Takaoka H, Hata K, Inoue N, Yoshikawa R, Matsumoto H, Masai H, Watanabe S, Ozawa T, Otake H, Matsumoto D, Hirata K, Yokoyama M Vitamin C restores the contractile response to dobutamine and improves myocardial efficiency in patients with heart failure after anterior myocardial infarction. *Am Heart J* 2007; 154: 645 e1–e8.
71. Singh I, Turner AH, Sinclair AJ, Li D, Hawley JA. Effects of gamma-tocopherol supplementation on thrombotic risk factors. *Asia Pacific J Clin Nutr* 2007; 16: 422–428.
72. Suda S, Igarashi H, Arai Y, Andou J, Chishiki T, Katayama Y. Effect of edaravone, a free radical scavenger, on ischemic cerebral edema assessed by magnetic resonance imaging. *Neurol Med Chir* 2007; 47: 197–201; discussion 201–202.
73. Vincent HK, Bourguignon CM, Vincent KR, Taylor AG. Effects of alpha-lipoic acid supplementation in peripheral arterial disease: a pilot study. *J Alternative Complementary Med* 2007; 13: 577–584.
74. Milman U, Blum S, Shapira C, Aronson D, Miller-Lotan R, Anbinder Y, Alshiek J, Bennett L, Kostenko M, Landau M, Keidar S, Levy Y, Khemlin A, Radan A, Levy AP. Vitamin E supplementation reduces cardiovascular events in a subgroup of middle-aged individuals with both type 2 diabetes mellitus and the haptoglobin 2–2 genotype: a prospective double-blinded clinical trial. *Arterioscler Thromb Vasc Biol* 2008; 28: 341–347.
75. Pleiner J, Schaller G, Mittermayer F, Marsik C, MacAllister RJ, Kapiotis S, Ziegler S, Ferlitsch A, Wolzt M. Intra-arterial vitamin C prevents endothelial dysfunction caused by ischemia-reperfusion. *Atherosclerosis* 2008; 197: 383–391.
76. Rodrigo R, Prat H, Passalacqua W, Araya J, Bächler JP. Decrease in oxidative stress through supplementation of vitamins C and E is associated with a reduction in blood pressure in patients with essential hypertension. *Clin Sci* 2008; 114: 625–634.

77. Tardif JC, Grégoire J, L'Allier PL, Ibrahim R, Anderson TJ, Reeves F, Title LM, Schampaert E, LeMay M, Lespérance J, Scott R, Guertin MC, Brennan ML, Hazen SL, Bertrand OF, CART-2 Investigators. Effects of the antioxidant succinobucol (AGI-1067) on human atherosclerosis in a randomized clinical trial. *Atherosclerosis* 2008; 197: 480–486.
78. Brunner EJ, Rees K, Ward K, Burke M, Thorogood M. Dietary advice for reducing cardiovascular risk. *Cochrane Database Syst Rev* 2007; (4): CD002128.
79. Lagneux C, Joyeux M, Demenge P, et al. Protective effects of melatonin against ischemia-reperfusion injury in the isolated rat heart. *Life Sci* 2000; 66: 503–509.
80. Salie R, Harper I, Cillie C, et al. Melatonin protects against ischaemic-reperfusion myocardial damage. *J Mol Cell Cardiol* 2001; 33: 343–357.
81. Reiter RJ, Tan DX. Melatonin: a novel protective agent against oxidative injury of the ischemic/reperfused heart. *Cardiovasc Res* 2003; 58: 10–19.
82. Petrosillo G, Ruggiero FM, Di Venosa N, Paradies G. Decreased complex III activity in mitochondria isolated from rat heart subjected to ischemia and reperfusion: role of reactive oxygen species and cardiolipin. *FASEB J* 2003; 17: 714–716.
83. Herrera E, Jiménez R, Aruoma OI, Hercberg S, Sánchez-García I, Fraga C. Aspects of antioxidant foods and supplements in health and disease. *Nutr Rev* 2009; 67 (Suppl 1): S140–S144.
84. Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr Neuropharmacol* 2009; 7(1): 65–74.
85. Chen J, Berry MJ. Selenium and selenoproteins in the brain and brain diseases. *J Neurochem* 2003; 86: 1–12. Schweizer U, Schomburg L, Savaskan NE. The neurobiology of selenium: lessons from transgenic mice. *J Nutr* 2004; 134: 707–710.
86. Tang BL. Neuronal protein trafficking associated with Alzheimer disease: from APP and BACE1 to glutamate receptors. *Cell Adhesion Migration* 2009; 3(1): 118–128.
87. Zhu X, Raina AK, Lee HG, Casadesus G, Smith MA, Perry G. Oxidative stress signalling in Alzheimer's disease. *Brain Res* 2004; 1000(1–2):332–39.
88. Lovell MA, Ehmann WD, Butler SM, et al. Elevated thiobarbituric acid-reactive substances and antioxidant enzyme activity in the brain in Alzheimer's disease. *Neurology* 1995; 45: 1594–1601.
89. Subburao KV, Richardson JS, Ang L. Autopsy samples of Alzheimer's cortex show increased peroxidation in vivo. *J Neurochem* 1990; 55: 342–345.
90. Markesbery WR, Carney JM. Oxidative alterations in Alzheimer's disease. *Brain Pathol* 1999; 9: 133–146.
91. Munch G, Thorne J, Foley P, et al. Advanced glycation end products in aging and Alzheimer's disease. *Brain Res Rev* 1997; 23: 134–143.
92. Praticò D. Evidence of oxidative stress in Alzheimer's disease brain and antioxidant therapy: lights and shadows. *Ann NY Acad Sci* 2008; 1147: 70–78.
93. Perkins AJ, Hendrie HC, Callahan CM, et al. Association of antioxidants with memory in a multiethnic elderly sample using the Third National Health and Nutrition Examination Survey. *Am J Epidemiol* 1999; 150: 37–44.
94. Masaki KH, Losonczy KG, Izmirlian G, et al. Association of vitamin E and C supplement use with cognitive function and dementia in elderly men. *Neurology* 2000; 54: 1265–1272.
95. Morris MC, Evans DA, Bienias JL, et al. Vitamin E and cognitive decline in older persons. *Arch Neurol* 2002; 59: 1125–1132.
96. Grodstein F, Chen J, Willet WC. High-dose antioxidant supplements and cognitive function in community-dwelling elderly women. *Am J Clin Nutr* 2003; 77: 975–984.
97. Engelhart MJ, Geerlings MI, Ruitenberg A, et al. Dietary intake of antioxidants and risk of Alzheimer disease. *JAMA* 2002; 287: 3223–3229.
98. Morris MC, Evans DA, Bienias JL, et al Dietary intake of antioxidant nutrients and the risk of incident Alzheimer disease in a biracial community study. *JAMA* 2002; 287: 3230–3237.
99. Luchsinger JA, Tang MX, Shea S, et al Antioxidant vitamin intake and risk of Alzheimer disease. *Arch Neurol* 2003; 60: 203–208.
100. Zandi PP, Anthony JC, Khachaturian AS, et al Reduced risk of Alzheimer disease in users of antioxidants vitamin supplements: the Cache County Study. *Arch Neurol* 2004; 61: 82–88.
101. Laurin D, Masaki KH, Foley DJ, et al Midlife dietary intake of antioxidants and risk of late-life incident dementia: the Honolulu-Asia Aging Study. *Am J Epidemiol* 2004; 159: 959–967.
102. Sano M, Ernesto C, Thomas RG, et al A controlled trial of seligiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. *N Engl J Med* 1997; 336: 1216–1222.
103. Petersen RC, Thomas RG, Grundman M, et al Vitamin E and donezepil for the treatment of mild cognitive impairment. *N Engl J Med* 2005; 352: 2379–2388.
104. Jack CR, Petersen RC, Grundman M, et al Longitudinal MRI findings from the vitamin E and donezepil treatment study for MCI. *Neurobiol Aging* 2007; 29: 1285–1295.
105. Zhang S, Rocourt C, Cheng WH. Selenoproteins and the aging brain. *Mech Ageing Dev* 2010; 131(4): 253–260.
106. Rayman MP, Rayman MP. The argument for increasing selenium intake. *Proc Nutr Soc* 2002; 61: 203–215.
107. Cornett CR, Markesbery WR, Ehmann WD. Imbalances of trace elements related to oxidative damage in Alzheimer's disease brain. *Neurotoxicology* 1998; 19: 339–345.
108. Wenstrup D, Ehmann WD, Markesbery WR. Trace element imbalances in isolated subcellular fractions of Alzheimer's disease brains. *Brain Res* 1990; 533: 125–131.

109. Zhang S, Rocourt C, Cheng WH. Selenoproteins and the aging brain. *Mech Ageing Dev* 2010; 131(4): 253–260.
110. Ceballos-Picot I, Merad-Boudia M, Nicole A, Thevenin M, Hellier G, Legrain S, Berr C. Peripheral antioxidant enzyme activities and selenium in elderly subjects and in dementia of Alzheimer's type-place of the extracellular glutathione peroxidase. *Free Radic Biol Med* 1996; 20: 579–587.
111. Lovell MA, Xie C, Gabbita SP, Markesbery WR. Decreased thioredoxin and increased thioredoxin reductase levels in Alzheimer's disease brain. *Free Radic Biol Med* 2000; 28: 418–427.
112. Gerhardsson L, Lundh T, Minthon L, Londos E. Metal concentrations in plasma and cerebrospinal fluid in patients with Alzheimer's disease. *Dementia Geriatr Cogn Disord* 2008; 25: 508–515.
113. Thomas P, O'Callaghan NJ, Fenech M. Telomere length in white blood cells, buccal cells and brain tissue and its variation with ageing and Alzheimer's disease. *Mech Ageing Dev* 2008; 129: 183–190.
114. Tanaka Y, Moritoh Y, Miwa N. Age-dependent telomere-shortening is repressed by phosphorylated alpha-tocopherol together with cellular longevity and intracellular oxidative-stress reduction in human brain microvascular endotheliocytes. *J Cell Biochem* 2007; 102: 689–703.
115. Liu Q, Wang H, Hu D, Ding C, Xu H, Tao D. Effects of trace elements on the telomere lengths of hepatocytes L-02 and hepatoma cells SMMC-7721. *Biol Trace Element Res* 2004; 100: 215–227.
116. Savitt JM, Dawson VL, Dawson TM. Diagnosis and treatment of Parkinson disease: molecules to medicine. *J Clin Invest* 2006; 116(7): 1744–1754.
117. Aruoma OI, Sun B, Fujii H, et al. Low molecular proanthocyanidin dietary biofactor Oligonol: its modulation of oxidative stress, bioefficacy, neuroprotection, food application and chemoprevention potentials. *Biofactors* 2006; 27: 245–265.
118. Aruoma OI, Bahorun T, Jen L-S. Neuroprotection by bioactive components in medicinal and food plant extracts. *Mutat Res* 2003; 544: 203–215.
119. Schon EA, Manfredi G. Neuronal degeneration and mitochondrial dysfunction. *J Clin Invest* 2003; 111: 303–312.
120. Schapira AH, Cooper JM, Dexter D, et al. Mitochondrial complex I deficiency in Parkinson's disease. *J Neurochem* 1990; 54: 823–827.
121. Jansen SL, Forbes DA, Duncan V, Morgan DG. Melatonin for cognitive impairment. *Cochrane Database Syst Rev* 2006; (1): CD003802.
122. Navarro A, Boveris A. The mitochondrial energy transduction system and the aging process. *Am J Physiol Cell Physiol* 2007; 292: C670–C686.
123. Bondy SC, Sharman EH. Melatonin and the aging brain. *Neurochem Int* 2007; 50: 571–580.
124. Caballero B, Vega-Naredo I, Sierra V et al. Favourable effects of a prolonged treatment with melatonin on the level of oxidative damage and neurodegeneration in senescenceaccelerated mice. *J Pineal Res* 2008; 45: 302–311.
125. Dowling GA, Burr RL, Van Someren EJ, Hubbard EM, Luxenberg JS, Mastick J, Cooper BA. Melatonin and bright-light treatment for rest-activity disruption in institutionalized patients with Alzheimer's disease. *J Am Geriatr Soc* 2008; 56: 239–246.
126. Gray SL, Anderson ML, Crane PK, Breitner JC, McCormick W, Bowen JD, Teri L, Larson E. Antioxidant vitamin supplement use and risk of dementia or Alzheimer's disease in older adults. *J Am Geriatr Soc* 2008; 56: 291–295.
127. Weber CA, Ernst ME. Antioxidants, supplements, and Parkinson's disease. *Ann Pharmacother* 2006; 40: 935–938.
128. Medeiros CA, Carvalhedo de Bruin PF, Lopes LA, Magalhães MC, de Lourdes Seabra M, de Bruin VM. Effect of exogenous melatonin on sleep and motor dysfunction in Parkinson's disease. A randomized, double blind, placebo-controlled study. *J Neurol* 2007; 254: 459–464.
129. Sharma A, Agarwal S, Broatch J, Raja SN. A web-based crosssectional epidemiological survey of complex regional pain syndrome. *Regional Anesth Pain Med* 2009; 34: 110–115.
130. Stanton-Hicks M, Janig W, Hassenbusch S, Haddox JD, Boas R, Wilson P. Reflex sympathetic dystrophy: changing concepts and taxonomy. *Pain* 1995; 63: 127–133.
131. Tran de QH, Duong S, Bertini P, Finlayson RJ. Treatment of complex regional pain syndrome: a review of the evidence. *Can J Anaesth* 2010; 57(2): 149–166.
132. Smith HS. The role of genomic oxidative-reductive balance as predictor of complex regional pain syndrome development: a novel theory. *Pain Physician* 2010; 13(1): 79–90.
133. Starr AM, Wessely MA, Albastaki U, Pierre-Jerome C, Kettner NW. Bone marrow edema: pathophysiology, differential diagnosis, and imaging. *Acta Radiol* 2008; 49(7): 771–786.
134. Xanthos D, Francis L, Bennett G, Coderre T. Animal models of chronic pain: chronic post-ischemia pain: a novel animal model of complex regional pain syndrome type I produced by prolonged hindpaw ischemia and reperfusion in the rat. *J Pain* 2004; 5(3 Suppl 2): S1.
135. Zuurmond WW, Langendijk PN, Bezemer PD, Brink HE, de Lange JJ, van Ioenen AC. Treatment of acute reflex sympathetic dystrophy with DMSO 50% in a fatty cream. *Acta Anaethesiol Scand* 1996; 40: 364–367.
136. Zollinger PD, Tuinebreijer WE, Breederveld RS, Kreis RW. Can vitamin C prevent complex regional pain syndrome in patients with wrist fractures? A randomized, controlled, multicenter dose-response study. *Am J Bone Joint Surg* 2007; 89: 1424–1431.
137. Kumar R, Aggarwal A, Faridi MM. Complex regional pain syndrome type 1 and scurvy. *Indian Pediatr* 2009; 46: 529–531.

138. Zollinger PE, Ellis ML, Unal H, Tuinebreijer WE. Clinical outcome of cementless semi-constrained trapeziometacarpal arthroplasty, and possible effect of vitamin C on the occurrence of complex regional pain syndrome. *Acta Orthopaed Belg* 2008; 74: 317–322.
139. Besse JL, Gadeyne S, Galand-Desmé S, Lerat JL, Moyen B. Effect of vitamin C on prevention of complex regional pain syndrome type I in foot and ankle surgery. *Foot Ankle Surg* 2009; 15: 179–182.
140. Eisenberg E, Shtahl S, Geller R, Reznick AZ, Sharf O, Ravbinovich M, Erenreich A, Nagler RM. Serum and salivary oxidative analysis in Complex Regional Pain Syndrome. *Pain* 2008; 138: 226–232.
141. Diamandopoulos GT. Cancer: an historical perspective. *Anticancer Res* 1996; 16(4A): 1595–1602; Weiss L. Early concepts of cancer. *Cancer Metastasis Rev* 2000; 19: 205–217.
142. *General and Systematic Pathology* 4th Ed. Edited by JCE. Underwood. Churchill and Livingstone. 2009.
143. Halliwell B. Oxidative stress and cancer: have we moved forward? *J Biochem* 2007; 401(1): 1–11.
144. Lawless MW, O'Byrne KJ, Gray SG. Targeting oxidative stress in cancer. *Expert Opin Therapeut Targets* 2010; 14 (11): 1225–1245.
145. Almushatat AS, Talwar D, McArdle PA, Williamson C, Sattar N, O'Reilly DS, Underwood MA, McMillan DC. Vitamin antioxidants, lipid peroxidation and the systemic inflammatory response in patients with prostate cancer. *Int J Cancer* 2006; 118: 1051–1053.
146. Kirsh VA, Hayes RB, Mayne ST, Chatterjee N, Subar AF, Dixon LB, Albanes D, Andriole GL, Urban DA, Peters U, PLCO Trial. Supplemental and dietary vitamin E, beta-carotene, and vitamin C intakes and prostate cancer risk. *J Natl Cancer Inst* 2006; 98(4): 245–254.
147. Reid ME, Duffield-Lillico AJ, Sunga A, Fakih M, Alberts DS, Marshall JR. Selenium supplementation and colorectal adenomas: an analysis of the nutritional prevention of cancer trial. *Int J Cancer* 2006; 118: 1777–1781.
148. Hercberg S, Ezzedine K, Guinot C, Preziosi P, Galan P, Bertrais S, Estaquio C, Briançon S, Favier A, Latreille J, Malvy D. Antioxidant supplementation increases the risk of skin cancers in women but not in men. *J Nutr* 2007; 137: 2098–2105.
149. Weinstein SJ, Wright ME, Lawson KA, Snyder K, Männistö S, Taylor PR, Virtamo J, Albanes D. Serum and dietary vitamin E in relation to prostate cancer risk. *Cancer Epidemiol Biomarkers Prev* 2007; 16: 1253–1259.
150. Bardia A, Tleyjeh IM, Cerhan JR, Sood AK, Limburg PJ, Erwin PJ, Montori VM. Efficacy of antioxidant supplementation in reducing primary cancer incidence and mortality: systematic review and meta-analysis. *Mayo Clin Proc* 2008; 83: 23–34.
151. Dennert G, Horneber M. Selenium for alleviating the side effects of chemotherapy, radiotherapy and surgery in cancer patients. *Cochrane Database Syst Rev* 2006; 3: CD005037.
152. Chitturi S, Farrell GC. Etiopathogenesis of nonalcoholic steatohepatitis. *Semin Liver Dis* 2001; 21(1): 27–41.
153. Videla LA, Rodrigo R, Araya J, Poniachik J. Oxidative stress and depletion of hepatic long-chain polyunsaturated fatty acids may contribute to nonalcoholic fatty liver disease. *Free Radic Biol Med* 2004; 37(9): 1499–1507.
154. Freedman RR. Biochemical, metabolic, and vascular mechanisms in menopausal hot flashes. *Fertil Steril* 1998; 70(2): 332–337.
155. Redmon JB, Carey P, Pryor JL. Varicocele—the most common cause of male factor infertility? *Hum Reprod Update* 2002; 8(1): 53–58.
156. Semercioz A, Onur R, Ogras S, Orhan I. Effects of melatonin on testicular tissue nitric oxide level and antioxidant enzyme activities in experimentally induced left varicocele. *Neuroendocrinol Lett* 2003; 24(1–2): 86–90.
157. Krishna MT, Chauhan AJ, Frew AJ, Holgate ST. Toxicological mechanisms underlying oxidant pollutant-induced airway injury. *Rev Environ Health* 1998; 13(1–2): 59–71.
158. Stehbens WE. Oxidative stress in viral hepatitis and AIDS. *Exp Mol Pathol* 2004; 77(2): 121–132.
159. Mapp PI, Grootveld MC, Blake DR. Hypoxia, oxidative stress and rheumatoid arthritis. *Br Med Bull* 1995; 51(2): 419–436.
160. Nathens AB, Neff MJ, Jurkovich GJ, Klotz P, Farver K, Ruzinski JT, Radella F, Garcia I, Maier RV. Randomized, prospective trial of antioxidant supplementation in critically ill surgical patients. *Ann Surg* 2002; 236(6): 814–822.
161. Pradhan AD, Manson JE, Rossouw JE, Siscovick DS, Mouton CP, Rifai N, Wallace RB, Jackson RD, Pettinger MB, Ridker P. Inflammatory biomarkers, hormone replacement therapy, and incident coronary heart disease: prospective analysis from the Women's Health Initiative observational study. *JAMA* 2002; 288(8): 980–987.
162. Watanabe T, Tomita S, Kudo M, Kurokawa M, Orino A, Todo A, Chiba T. Detection of Helicobacter pylori gene by means of immunomagnetic separation-based polymerase chain reaction in feces. *Scand J Gastroenterol* 1998; 33(11): 1140–1143.
163. Mobley HL. Helicobacter pylori factors associated with disease development. *Gastroenterology* 1997; 113(6 Suppl): S21–S28.
164. Davies GR, Simmonds NJ, Stevens TR, Sheaff MT, Banatvala N, Laurenson IF, Blake DR, Rampton DS. Helicobacter pylori stimulates antral mucosal reactive oxygen metabolite production in vivo. *Gut* 1994; 35(2): 179–185.
165. Nielsen H, Andersen LP. Activation of human phagocyte oxidative metabolism by *Helicobacter pylori*. *Gastroenterology* 1992; 103(6): 1747–1753.
166. Zhang QB, Nakashabendi IM, Mokhashi MS, Dawodu JB, Gemmell CG, Russell RI. Association of cytotoxin production and neutrophil activation by strains of *Helicobacter pylori* isolated from patients with peptic ulceration and chronic gastritis. *Gut* 1996; 38(6): 841–845.

167. Soghier LM, Brion LP. Cysteine, cystine or *N*-acetylcysteine supplementation in parenterally fed neonates. *Cochrane Database Syst Rev* 2006; (4): CD004869.

168. Orrell RW, Lane RJ, Ross M. Antioxidant treatment for amyotrophic lateral sclerosis/motor neuron disease. *Cochrane Database Syst Rev* 2007; (1): CD002829.

169. Farinotti M, Simi S, Di Pietrantonj C, McDowell N, Brait L, Lupo D, Filippini G. Dietary interventions for multiple sclerosis. *Cochrane Database Syst Rev* 2007; (1): CD004192.

170. Mehendale S, Kilari A, Dangat K, Taralekar V, Mahadik S, Joshi S. Fatty acids, antioxidants, and oxidative stress in pre-eclampsia. *Int J Gynecol Obstet* 2008; 100: 234–238.

171. Rumbold A, Duley L, Crowther CA, Haslam RR. Antioxidants for preventing pre-eclampsia. *Cochrane Database Syst Rev* 2008; (1): CD004227.

172. Poston L, Briley AL, Seed PT, Kelly FJ, Shennan AH, and Vitamins in Pre-eclampsia (VIP) Trial Consortium. Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia (VIP trial): randomized placebo-controlled trial. *Lancet* 2006; 367: 1145–1154.

173. Spinnato JA 2nd, Freire S, Pinto E Silva JL, Cunha Rudge MV, Martins-Costa S, Koch MA, Goco N, Santos Cde B, Cecatti JG, Costa R, Ramos JG, Moss N, Sibai BM. Antioxidant therapy to prevent preeclampsia: a randomized controlled trial. *Obstet Gynecol* 2007; 110: 1311–1318.

174. Henricks PA, Nijkamp FP. Reactive oxygen species as mediators in asthma. *Pulm Pharmacol Therapeut* 2001;14(6): 409–420.

175. Patel BD, Welch AA, Bingham SA, Luben RN, Day NE, Khaw KT, Lomas DA, Wareham NJ. Dietary antioxidants and asthma in adults. *Thorax* 2006; 61: 388–393.

176. Wood LG, Garg ML, Powell H, Gibson PG. Lycopene-rich treatments modify noneosinophilic airway inflammation in asthma: proof of concept. *Free Radic Res* 2008; 42: 94–102.

177. Orvoen-Frija E, Benoit M, Catto M, Chambouleyron M, Duguet A, Emeriau JP, Ferry M, Hayot M, Jeandel C, Morize V, Nassih K, Ouksel H, Piette F, Prefaut C, Roche N, de Wazieres B, Zureik M. Chronic obstructive pulmonary disease (COPD) in the elderly. *Rev Mal Respir* 2010; 27(8): 855–873.

178. Cerdá B, Soto C, Albaladejo MD, Martínez P, Sánchez-Gascón F, Tomás-Barberán F, Espín JC. Pomegranate juice supplementation in chronic obstructive pulmonary disease: a 5-week randomized, double-blind, placebo-controlled trial. *Eur J Clin Nutr* 2006; 60: 245–253.

179. Ochs-Balcom HM, Grant BJ, Muti P, Sempos CT, Freudenheim JL, Browne RW, Trevisan M, Iacoviello L, Cassano PA, Schünemann HJ. Antioxidants, oxidative stress, and pulmonary function in individuals diagnosed with asthma or COPD. *Eur J Clin Nutr* 2006; 60: 991–999.

180. Dal Negro RW, Visconti M, Micheletto C, Tognella S. Changes in blood ROS, e-NO, and some pro-inflammatory mediators in bronchial secretions following erdosteine or placebo: a controlled study in current smokers with mild COPD. *Pulm Pharmacol Ther* 2008; 21: 304–308 Dal Negro RW. Erdosteine: Antitussive and anti-inflammatory effects. *Lung* 2008; 186(Suppl 1): S70–S73.

181. Obrosova IG. Diabetes and the peripheral nerve. *Biochim Biophys Acta* 2009 Oct; 1792(10): 931–40.

182. Costacou T, Zgibor JC, Evans RW, Tyurina YY, Kagan VE, Orchard TJ. Antioxidants and coronary artery disease among individuals with type 1 diabetes: findings from the Pittsburgh Epidemiology of Diabetes Complications Study. *J Diabetes Complications* 2006; 20: 387–394.

183. Endo K, Miyashita Y, Sasaki H, Ebisuno M, Ohira M, Saiki A, Koide N, Oyama T, Takeyoshi M, Shirai K. Probucol and atorvastatin decrease urinary 8-hydroxy-2′-deoxyguanosine in patients with diabetes and hypercholesterolemia. *J Atheroscler Thromb* 2006; 13: 68–75.

184. Kamenova P. Improvement of insulin sensitivity in patients with type 2 diabetes mellitus after oral administration of alpha-lipoic acid. *Hormones (Athens)* 2006; 5: 251–258.

185. Agrawal NK, Maiti R, Dash D, Pandey BL. Cilostazol reduces inflammatory burden and oxidative stress in hypertensive type 2 diabetes mellitus patients. *Pharmacol Res* 2007; 56: 118–123.

186. Ceriello A, Piconi L, Esposito K, Giugliano D. Telmisartan shows an equivalent effect of vitamin C in further improving endothelial dysfunction after glycemia normalization in type 1 diabetes. *Diabetes Care* 2007; 30: 1694–1698.

187. Neyestani TR, Shariatzadeh N, Gharavi A, Kalayi A, Khalaji N. Physiological dose of lycopene suppressed oxidative stress and enhanced serum levels of immunoglobulin M in patients with Type 2 diabetes mellitus: a possible role in the prevention of long-term complications. *J Endocrinol Invest* 2007; 30: 833–838.

188. Neyestani TR, Shariatzadeh N, Gharavi A, Kalayi A, Khalaji N. Physiological dose of lycopene suppressed oxidative stress and enhanced serum levels of immunoglobulin M in patients with Type 2 diabetes mellitus: a possible role in the prevention of long-term complications. *J Endocrinol Invest* 2007; 30: 833–838.

189. Tessier DM, Khalil A, Trottier L, Fülöp T. Effects of vitamin C supplementation on antioxidants and lipid peroxidation markers in elderly subjects with type 2 diabetes. *Arch Gerontol Geriatr* 2009 Jan–Feb; 48(1): 67–72.

190. Vossler S, Füllert S, Schneider F, Haak E, Haak T, Samigullin R, Tritschler H, Tooke JE, Konrad T. Pharmacodynamic effects of orally administered dexlipotam on endothelial function in type 2-diabetic patients. *Intl J Clin Pharmacol Therapeut* 2007; 45: 385–393.

191. Jacob S, Ruus P, Hermann R, Tritschler HJ, Maerker E, Renn W, Augustin HJ, Dietze GJ, Rett K. Oral administration of RAC-alpha-lipoic acid modulates insulin sensitivity in patients with type-2 diabetes mellitus: a placebocontrolled pilot trial. *Free Radic Biol Med* 1999; 27: 309–314.

192. Paolisso G, D'Amore A, Galzerano D, Balbi V, Giugliano D, Varricchio M, D'Onofrio F, Daily vitamin E supplements improve metabolic control but not insulin secretion in elderly type II diabetic patients. *Diabetes Care* 1993; 16: 1433–1437; Gokkusu C, Palanduz S, Ademoglu E, Tamer S. Oxidant and antioxidant systems in niddm patients: influence of vitamin E supplementation. *Endocr Res* 2001; 27: 377–386.

193. Mayer-Davis EJ, Bell RA, Reboussin BA, Rushing J, Marshall JA, Hamman RF. Antioxidant nutrient intake and diabetic retinopathy: the San Luis Valley Diabetes Study. *Ophthalmology* 1998; 105: 2264–2270.

194. Millen AE, Klein R, Folsom AR, Stevens J, Palta M, Mares JA. Relation between intake of vitamins C and E and risk of diabetic retinopathy in the Atherosclerosis Risk in Communities Study. *Am J Clin Nutr* 2004; 79: 865–873.

195. Millen AE, Klein R, Folsom AR, Stevens J, Palta M, Mares JA. Relation between intake of vitamins C and E and risk of diabetic retinopathy in the Atherosclerosis Risk in Communities Study. *Am J Clin Nutr* 2004; 79: 865–873.

196. Ziegler D, Sohr CG, Nourooz-Zadeh J. Oxidative stress and antioxidant defense in relation to the severity of diabetic polyneuropathy and cardiovascular autonomic neuropathy. *Diabetes Care* 2004; 27: 2178–2183.

197. Phillips M, Curtis H, Portmann B, Donaldson N, Bomford A, O'Grady J. Antioxidants versus corticosteroids in the treatment of severe alcoholic hepatitis—a randomized clinical trial. *J Hepatol* 2006; 44: 784–790.

198. Gabbay E, Zigmond E, Pappo O, Hemed N, Rowe M, Zabrecky G, Cohen R, Ilan Y. Antioxidant therapy for chronic hepatitis C after failure of interferon: results of phase II randomized, double-blind placebo controlled clinical trial. *World J Gastroenterol* 2007; 13: 5317–5323.

199. Stewart S, Prince M, Bassendine M, Hudson M, James O, Jones D, Record C, Day CP. A randomized trial of antioxidant therapy alone or with corticosteroids in acute alcoholic hepatitis. *J Hepatol* 2007; 47: 277–283.

200. Rambaldi A, Gluud C. s-adenosyl-L-methionine for alcoholic liver diseases. *Cochrane Database Syst Rev* 2006; (2): CD002235.

201. Mohseni Salehi Monfared SS, Vahidi H, Abdolghaffari AH, Nikfar S, Abdollahi M. Antioxidant therapy in the management of acute, chronic and post-ERCP pancreatitis: a systematic review. *World J Gastroenterol* 2009; 15 (36): 4481–4490.

202. Kirk GR, White JS, McKie L, Stevenson M, Young I, Clements WD, Rowlands BJ. Combined antioxidant therapy reduces pain and improves quality of life in chronic pancreatitis. *J Gastrointest Surg* 2006; 10: 499–503.

203. Romagnuolo J, Hilsden R, Sandha GS, Cole M, Bass S, May G, Love J, Bain VG, McKaigney J, Fedorak RN. Allopurinol to prevent pancreatitis after endoscopic retrograde cholangiopancreatography: a randomized placebo-controlled trial. *Clin Gastroenterol Hepatol* 2008; 6: 465–71.

204. Ockenga J, Borchert K, Rifai K, Manns MP, Bischoff SC. Effect of glutamine-enriched total parenteral nutrition in patients with acute pancreatitis. *Clin Nutr* 2002; 21: 409–416.

205. Fuentes-Orozco C, Cervantes-Guevara G, Muciño-Hernández I, López-Ortega A, Ambriz-González G, Gutiérrez-de-la-Rosa JL, Gómez-Herrera E, Hermosillo-Sandoval JM, González- Ojeda A. L-alanyl-L-glutamine-supplemented parenteral nutrition decreases infectious morbidity rate in patients with severe acute pancreatitis. *J Parenteral Enteral Nutr* 2008; 32: 403–411.

206. de Beaux AC, O'Riordain MG, Ross JA, Jodozi L, Carter DC, Fearon KC. Glutamine-supplemented total parenteral nutrition reduces blood mononuclear cell interleukin-8 release in severe acute pancreatitis. *Nutrition* 1998; 14: 261–265.

207. Xue P, Deng LH, Xia Q, Zhang ZD, Hu WM, Yang XN, Song B, Huang ZW. Impact of alanyl-glutamine dipeptide on severe acute pancreatitis in early stage. *World J Gastroenterol* 2008; 14: 474–478. No adverse effects were reported in these trials.

208. Salim AS. Role of oxygen-derived free radical scavengers in the treatment of recurrent pain produced by chronic pancreatitis. A new approach. *Arch Surg* 1991; 126: 1109–1114.

209. Banks PA, Hughes M, Ferrante M, Noordhoek EC, Ramagopal V, Slivka A. Does allopurinol reduce pain of chronic pancreatitis? *Int J Pancreatol* 1997; 22: 171–176.

210. Du WD, Yuan ZR, Sun J, Tang JX, Cheng AQ, Shen DM, Huang CJ, Song XH, Yu XF, Zheng SB. Therapeutic efficacy of high-dose vitamin C on acute pancreatitis and its potential mechanisms. *World J Gastroenterol* 2003; 9: 2565–2569.

211. Uden S, Schofield D, Miller PF, Day JP, Bottiglier T, Braganza JM. Antioxidant therapy for recurrent pancreatitis: biochemical profiles in a placebo-controlled trial. *Aliment Pharmacol Therapeut* 1992; 6: 229–240.

212. Bhardwaj P, Garg PK, Maulik SK, Saraya A, Tandon RK, Acharya SK. A randomized controlled trial of antioxidant supplementation for pain relief in patients with chronic pancreatitis. *Gastroenterology* 2009; 136: 149.e217–159.e217.

213. De las Heras Castaño G, García de la Paz A, Fernández MD, Fernández Forcelledo JL. Use of antioxidants to treat pain in chronic pancreatitis. *Rev Española Enferm Dig* 2000; 92: 375–385.

214. Durgaprasad S, Pai CG, Vasanthkumar, Alvres JF, Namitha S. A pilot study of the antioxidant effect of curcumin in tropical pancreatitis. *Indian J Med Res* 2005; 122: 315–318.

215. Milewski J, Rydzewska G, Degowska M, Kierzkiewicz M, Rydzewski A. N-acetylcysteine does not prevent post-endoscopic retrograde cholangiopancreatography hyperamylasemia and acute pancreatitis. *World J Gastroenterol* 2006; 12: 3751–3755.

216. Katsinelos P, Kountouras J, Paroutoglou G, Beltsis A, Mimidis K, Zavos C. Intravenous N-acetylcysteine does not prevent post-ERCP pancreatitis. *Gastrointest Endosc* 2005; 62: 105–111.

217. Lavy A, Karban A, Suissa A, Yassin K, Hermesh I, Ben-Amotz A. Natural beta-carotene for the prevention of post-ERCP pancreatitis. *Pancreas* 2004; 29: e45–e50.

218. Braganza JM. Clinical trial of 24 hours' treatment with glutathione precursors in acute pancreatitis. *Clin Drug Invest* 1995; 10: 147–157.

219. Bilton D, Schofield D, Mei G, Kay PM, Bottiglieri T, Braganza JM. Placebo-controlled trials of antioxidant therapy including S-adenosulmethionine in patients with recurrent non-gallstone pancreatitis. *Drug Invest* 1994; 8: 10–20.

220. Uden S, Schofield D, Miller PF, Day JP, Bottiglier T, Braganza JM. Antioxidant therapy for recurrent pancreatitis: biochemical profiles in a placebo-controlled trial. *Aliment Pharmacol Therapeut* 1992; 6: 229–240; Uden S, Bilton D, Nathan L, Hunt LP, Main C, Braganza JM. Antioxidant therapy for recurrent pancreatitis: placebo-controlled trial. *Aliment Pharmacol Therapeut* 1990; 4: 357–371.

221. Canter PH, Wider B, Ernst E. The antioxidant vitamins A, C, E and selenium in the treatment of arthritis: a systematic review of randomized clinical trials. *Rheumatology* 2007; 46: 1223–1233.

222. de Pablo P, Dietrich T, Karlson EW. Antioxidants and other novel cardiovascular risk factors in subjects with rheumatoid arthritis in a large population sample. *Arthritis Rheumatism* 2007; 57: 953–962.

223. Ong-ajyooth L, Ong-ajyooth S, Parichatikanond P. The effect of alpha-tocopherol on the oxidative stress and antioxidants in idiopathic IgA nephropathy. *J Med Assoc Thailand* 2006; 89 (Suppl 5): S164–S170.

224. Mastalerz-Migas A, Reksa D, Pokorski M, Steciwko A, Muszyńska A, Bunio A, Drobnik J, Pokorna-Kalwak D. Comparison of a statin vs. hypolipemic diet on the oxidant status in hemodialyzed patients with chronic renal failure. *J Physiol Pharmacol* 2007; 58 (Suppl 5) (Pt 1): 363–370.

225. Sahin G, Yalcin AU, Akcar N. Effect of *N*-acetylcysteine on endothelial dysfunction in dialysis patients. *Blood Purif* 2007; 25: 309–315.

226. Al-Awadi KA, Kehinde EO, Loutfi I, Mojiminiyi OA, Al-Hunayan A, Abdul-Halim H, Al-Sarraf A, Memon A, Abraham MP. Treatment of renal calculi by lithotripsy: minimizing short-term shock wave induced renal damage by using antioxidants. *Urol Res* 2008; 36: 51–60.

227. Niki E, Noguchi N, Tsuchihashi H, et al. Interaction among vitamin C, vitamin E, and beta-carotene. *Am J Clin Nutr* 1995; 62(Suppl 6): 1322S–1326S.

228. Bagi Z, Cseko C, Toth E, Koller A. Oxidative stress-induced dysregulation of arteriosal wall shear stress and blood pressure in hyperhomocysteinemia is prevented by chronic vitamin C treatment. *Am J Physiol Heart Circ Physiol* 2003; 285: H2277–H2283.

229. Cyrus T, D Praticó. 2006. Vitamin E and Atherosclerosis. In: *The Encyclopedia of Vitamins*. EVR Preedy, RR Watson, Eds. 623–632. Wallingford, UK.