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Exogenous antioxidants—Double-edged swords in cellular redox state

Health beneficial effects at physiologic doses versus deleterious effects at high doses

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The balance between oxidation and antioxidation is believed to be critical in maintaining healthy biological systems. Under physiological conditions, the human antioxidative defense system including e.g., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH) and others, allows the elimination of excess reactive oxygen species (ROS) including, among others superoxide anions ($O_2^{\bullet -}$), hydroxyl radicals ($OH^{\bullet}$), alkoxyl radicals ($RO^{\bullet}$) and peroxyradicals ($ROO^{\bullet}$). However, our endogenous antioxidant defense systems are incomplete without exogenous originating reducing compounds such as vitamin C, vitamin E, carotenoids and polyphenols, playing an essential role in many antioxidant mechanisms in living organisms. Therefore, there is continuous demand for exogenous antioxidants in order to prevent oxidative stress, representing a disequilibrium redox state in favor of oxidation. However, high doses of isolated compounds may be toxic, owing to prooxidative effects at high concentrations or their potential to react with beneficial concentrations of ROS normally present at physiological conditions that are required for optimal cellular functioning. This review aims to examine the double-edged effects of dietary originating antioxidants with a focus on the most abundant compounds, especially polyphenols, vitamin C, vitamin E and carotenoids. Different approaches to enrich our body with exogenous antioxidants such as via synthetic antioxidants, diets rich in fruits and vegetables and taking supplements will be reviewed and experimental and epidemiological evidences discussed, highlighting that antioxidants at physiological doses are generally safe, exhibiting interesting health beneficial effects.

Introduction

Humans live in the presence of various ubiquitous environmental stressors including UV radiation, microbes, allergens and various pollutants such as increased ozone, cigarette smoke and polycyclic aromatic hydrocarbons, which can amplify the generation of reactive oxygen species (ROS) in the body.[1-5] ROS can be defined as intermediate oxygen carrying metabolites with or without an unpaired electron, comprising oxyradicals (i.e., oxygen-centered free radicals) such as superoxide anions ($O_2^{\bullet -}$), hydroxyl radicals ($OH^{\bullet}$), alkoxyl radicals ($RO^{\bullet}$) and peroxyradicals ($ROO^{\bullet}$) and non-radicals such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and singlet oxygen ($^1O_2$), able to oxidize other components and turning them into free radicals, often causing a chain reaction leading to the formation of numerous new radicals.[6-9] Prominent radicals that may be formed in vivo include both relatively stable radicals such as the urate radical ($UrH^{\bullet -}$), the ascorbyl radical ($Asc^{\bullet -}$), the vitamin E radical ($VE^{\bullet}$) and phenoxyl radicals ($Phl^{\bullet}$), and reactive radicals encompassing carbon-centered free radicals [e.g., lipid radicals ($L^{\bullet}$)] and sulphur-centered radicals [e.g., glutathiyl radicals ($GS^{\bullet}$)], which, in aerobic medium, can result in species with higher oxidative potential [such as lipid peroxyl radicals ($LOO^{\bullet}$), lipid alkoxyl radicals ($LO^{\bullet}$) and thiyl radicals ($GSOO^{\bullet}$, $GSO^{\bullet}$ and $GSO_2OO^{\bullet}$)].[7,10] Radical chain reaction typically continues until the system becomes anaerobic or the substrate [e.g., membrane fatty acids (LH)] is depleted; however the chain reaction can be stopped when two radicals form non-radical products or by the presence of chain-breaking antioxidants (e.g., vitamin E and polyphenols).[7,10,11]

Physical stressors such as acute aerobic, anaerobic and intense exhaustive exercise can result in excessive reactive oxygen production.[12-14] In this regard, the superoxide radical ($O_2^{\bullet -}$), resulting from monoelectronic reduction of oxygen, is considered to be the precursor of ROS including $OH^{\bullet}$, $RO^{\bullet}$, $ROO^{\bullet}$ and H_2O_2.[9] For instance, the superoxide radical ($O_2^{\bullet -}$) can react with nitric oxide ($^{\bullet}NO$), a nitrogen-centered radical, generating a highly reactive molecule, the peroxynitrite anion ($ONOO^-$), also termed a reactive oxygen and nitrogen species (RONS), able to cause DNA fragmentation and lipid oxidation.[7,8,10] Animal experiments have shown that stressful situations such as immobilization stress and sleep deprivation stimulate excessive production of such toxic oxygen metabolites.[15,16] Emotional stress and depressed mood are also associated with a massive formation of oxygen free

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REVIEW

Table 1. Human antioxidant defense systems include endogenous (enzymatic and non-enzymatic) and exogenous antioxidants, with the diet being the main exogenous source

Antioxidant defense system	
Endogenous antioxidants	**Exogenous antioxidants**
Enzymatic antioxidants	**Prinicipal dietary antioxidants from fruits, vegetables and grains**
• Superoxide dismutase (SOD): enzyme detoxifying superoxide radical ($O_2^{\cdot -}$)	• **Vitamins**: vitamin C, vitamin E
• Catalase (CAT) and glutathione peroxidase (GPx): enzymes involved in the detoxification of peroxides (CAT against H_2O_2, and GPx against both H_2O_2 and ROOH)	• **Trace elements**: zinc, selenium
• Glutathione reductase: enzyme involved in the regeneration of glutathione	• **Carotenoids**: β-carotene, lycopene, lutein, zeaxanthin
• Thioredoxin reductase: enzyme involved in the protection against protein oxidation	• **Phenolic acids**: chlorogenic acids, gallic acid, cafeic acid, etc.,
• Glucose-6-phosphate dehydrogenase: enzyme involved in the regeneration of NADPH	• **Flavonols**: quercetin*, kaempferol*, myricetin*
Non-enzymatic antioxidants (principal intracellular reducing agents)	• **Flavanols:** proanthocyanidins and catechins
Glutathione (GSH), uric acid, lipoic acid, NADPH, coenzyme Q, albumin, bilirubin	• **Anthocyanidins**: cyanidin* and pelagonidin*
	• **Isoflavones**: genistein*, daidzein* and glycitein*
	• **Flavanones**: naringenin*, eriodictyol* and hesperetin*
	• **Flavones**: luteolin* and apigenin*

*and their glucosides.

radicals.[9,17-21] Overproduction of oxygen-derived radical species can further result from diets excessive in fat and carbohydrates and are relatively deficient in antioxidant vitamins.[7,22,23] Other conditions or pathways which may amplify ROS formation favouring oxidative stress include metabolism of alcohol or pharmaceutical agents, therapeutic (x-ray) radiation, hyperthermia, inflammation and iron overload.[3,24,25] Therefore, our antioxidant system has to be efficient against these stressful conditions, some of which can routinely occur in our daily lives, avoiding the onset of oxidative stress, constituting a causative or associated risk factor for a number of human diseases including chronic complications such as cardiovascular diseases (CVD),[7,26] cancer,[7,27] and neurodegenerative diseases,[7,28] with probably over 100 associated diseases in total.[29] Furthermore, our antioxidant system, which is incomplete without exogenous reducing compounds such as vitamin C, vitamin E, carotenoids and polyphenols, has the role to quench excess oxygen-derived reactive species generated during normal cellular metabolism utilizing molecular oxygen such as during mitochondrial respiration (in which 85% of inhaled oxygen is metabolized) and processes catalyzed by NAD(P)H oxidase and xanthine oxidase.[7,9]

The following review focuses on the double-edged effects of natural antioxidants. We report several studies showing controversial results of exogenous antioxidants, discussing that the type, dosage and matrix of exogenous antioxidants may be determining factors impacting the balance between beneficial or deleterious effects of these natural compounds.

Evidence of Double-Edged Effects of Exogenous Antioxidants

Definition, necessity and sources. Owing to the fundamental role of antioxidants in human life and health, and their general popularity due to increased media attention, the demand for these compounds by the general public has been recently increasing. Antioxidants have been defined as substances that, when present at low concentrations compared to an oxidizable compound (e.g., DNA, proteins, lipids or carbohydrates), delay or prevent oxidative damage caused by the presence of ROS.[30,31] ROS at high doses become deleterious, exhibiting pathophysiological actions, whereas, at low doses they may be beneficial for normal physiological actions (reviewed in ref. 7, 26, 32 and 33). Exogenous antioxidants play a key role in this delicate equilibrium between oxidation and antioxidation in living systems.[7,9,34,35]

Our antioxidant defense system includes endogenous (enzymatic and non-enzymatic) antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione (GSH), among others and exogenous antioxidants such as vitamin C, vitamin E, carotenoids and polyphenols, with the diet being the main source (**Table 1**).[9,34,36,37] Endogenous and exogenous antioxidants act interactively (e.g., synergistically) to maintain or re-establish redox homeostasis, such as during the regeneration of vitamin E by glutathione (GSH) or vitamin C to prevent lipid peroxidation processes,[7] which can affect membrane fluidity and damage membrane proteins by e.g., inactivating receptors, enzymes and ion channels, even disrupting membrane integrity resulting eventually in cell death.[17] Catechins might prevent the consumption of vitamin E by scavenging hydrophilic radicals near membrane surfaces, whereas vitamin E scavenges lipid peroxyl radicals ($LOO^{\bullet}$) as hydrogen donor to stop free radical chain reactions (chain-breaking antioxidant).[11] The intake of complete foods rich in naturally-occurring antioxidants, including nutrients (e.g., vitamins) and phytochemicals (e.g., polyphenols) has been widely recommended by many health organizations, such as within the "five a day campaign."[38] Indeed, humans are not capable of synthesizing these antioxidant compounds de novo; and plant food (e.g., apples, plums, bananas, tomatoes, potatoes, onions, broccolis, etc.,) constitutes the natural source of these antioxidants.[9,34,36,37,39] In addition to their natural occurrence in foods, fortification, supplementation with isolated components and intake of synthetic antioxidant additives such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT),

tert-butyl hydroquinone (TBHQ) and propyl, octyl and dodecyl gallates (used initially to protect and to preserve the nutritional quality and to increase shelf-life of processed foods)[40,41] constitute further sources of antioxidants.

In vitro evidence. In vitro studies have highlighted the cytoprotective activity of plant food constituents such as polyphenols and mixtures and their preventive effects against oxidative stress-induced cell death.[42-46] Thus, although the antioxidant activity of phytochemicals is well recognized,[7,9,34,35] they can also display prooxidant activities under certain conditions, such as at high doses or in the presence of metal ions.[47-50] The prooxidant or antioxidant activity intimately depends on their concentration. In this regard, recent studies employing cell models have highlighted the prooxidative activity of several polyphenols already known as antioxidants such as quercetin, catechins including epicatechin and epigallocatechin-3-gallate (EGCG) and gallic acid.[50-55] For example, at high doses, it has been demonstrated that quercetin (50 μM) can potentiate superoxide radical ($O_2^{\bullet-}$) generation within isolated mitochondria and cultured cells.[51] In another study, the antioxidant activity of quercetin was observed only at low doses (0.1–20 μM) while higher concentrations (>50 μM) decreased cell survival and viability, thiol content, total antioxidant capacity and activities of SOD, CAT and glutathione S-transferase.[52] It has also been demonstrated that flavonoids (quercetin and fisetin) at low concentrations (10–25 μM) protect rat H4IIE cells against H_2O_2-induced cytotoxicity, DNA strand breaks and apoptosis, whereas high concentrations (50–250 μM) caused cytotoxicity, DNA damage and apoptosis.[50] It was also shown that flavonoids at high concentrations can generate ROS by autoxidation (e.g., myricetin and quercetagetin) and redox-cycling (e.g., quercetin).[56-59]

In addition to the concentration of antioxidants, the presence of metal ions has been reported to play an important role. It was revealed that EGCG in the presence of transition metals causes oxidative damage to isolated and cellular DNA.[53] Dietary antioxidants such as phenolics can display prooxidant activities in the presence of metal ions owing to their reducing capacity and forming chelates, such as with the transition metals iron and copper, which are important properties of these compounds in plants.[3,47,48,53] The mechanism of the antioxidative action of natural compounds is considered as primary when antioxidants act directly on free radicals (-R•) by a scavenging process characterized by the donation of hydrogen atoms (resulting in the formation of -RH) or electrons (resulting in the formation of -R⁻).[60-62] It is secondary, when the antioxidants absorb UV radiation or intervene in anti-oxidation processes as chelators of transition metal ion catalysts, act as deactivators of singlet oxygen ($^1O_2$) or convert hydroperoxides (ROOH) to non-radical species.[60-63] However, the strong reducing power of antioxidants may also affect metal ions, especially Fe^{3+} and Cu^{2+}, increasing their ability to form highly reactive hydroxyl radical concentrations, potentially harmful radicals, originating from peroxides via the Fenton (2) reaction.[7,10]

$$\text{(1)} \qquad \text{Antioxidant (AH)} + Fe^{3+}\,(\text{or } Cu^{2+}) \rightarrow A^{\bullet} + Fe^{2+}\,(\text{or } Cu^{+}) + H^{+}$$

$$\text{(2)} \qquad H_2O_2 + Fe^{2+}\ (\text{or } Cu^{+}) \rightarrow {}^{\bullet}OH + {}^{-}OH + Fe^{3+}\ (\text{or } Cu^{2+})$$

Such conditions could be problematic in organisms overloaded by iron as in the case of hemochromatosis,[7] a disease characterized by increased iron absorption and storage from the diet. As a consequence, the metal chelating activity of several phenolics may result in the reduction of the prooxidant capacity of metal ions, however, phenolics may also act as prooxidants by chelating metals in a manner that maintains or increases their catalytic activity.[48] In vitro, it has been shown that the pH influences oxidoreductions of phenolic compounds, suggesting that the pH of biological tissues could impact antioxidant/prooxidant activities of phenolics and their chelating activity. For example, a decrease in pH causes a reduced chelating effect of phenolics toward iron, possibly due to increased solubility of the complexes.[48,64] The effect of pH could however be different for various phenolics. While at pH 7.4 certain phenolics have displayed prooxidant activities which at lower pH (5.8) were reported to possess antioxidant properties (e.g., γ-resorcyclic acid), others have exhibited antioxidant activities (e.g., hydrobenzoic acid).[48,64]

Antioxidant phenolics, when scavenging free radicals, can form less reactive phenoxyl radicals, which are stabilized by delocalization of unpaired electrons around the aromatic ring.[11] However, even though these radicals are relatively stable, they can also display prooxidant activities inducing cellular damage (reviewed in ref. 53). It is well established that one of the chemopreventive mechanisms of polyphenols (or fruits and vegetables rich in antioxidants) against cancer development is the inhibition of initiation, the first step of carcinogenesis occurring following oxidative DNA damage leading to mutagenesis.[27,65,66] In a recent review, the prooxidant activity of individual dietary polyphenols and their ability to induce mitochondrial dysfunction and consequently apoptosis has been suggested as a possible anticancer mechanisms.[53]

It is worth noting that beneficial or harmful effects of natural compounds may also occur independently from their (anti-) oxidative properties e.g., as a result of the activation of particular cellular pathways including inflammatory processes, nitrogen and dicarbonyl metabolisms for which a close relation exists (**Fig. 1**).[7,67-69] Indeed, inflammation, nitrosative stress (resulting from excessive production of reactive nitrogen species) and carbonyl stress (resulting from excessive accumulation of reactive dicarbonyl compounds) may exacerbate or provoke oxidative stress and vice versa.[3,7,10,17,67,70] Besides the biphasic effects of antioxidants on oxidative metabolism, it has also been reported that natural compounds can display double-edged effects on inflammatory reactions. For example, β-carotene at low doses exhibited antioxidant[71] and anti-inflammatory[72] properties in human HL-60 cells, whereas, at high doses prooxidant activity[71] and pro-inflammatory effects[72] [an increase in the production of pro-inflammatory mediators tumor necrosis factor-α (TNFα) and interleukin-8 (IL-8)] have been reported. To the best of our knowledge, there is still no work reporting that antioxidants, in certain cases as described above, can provoke nitrosative stress or carbonyl stress despite that these stresses may result from oxidative stress disturbances; however, their protective potentials on theses stresses have been demonstrated.[73,74]

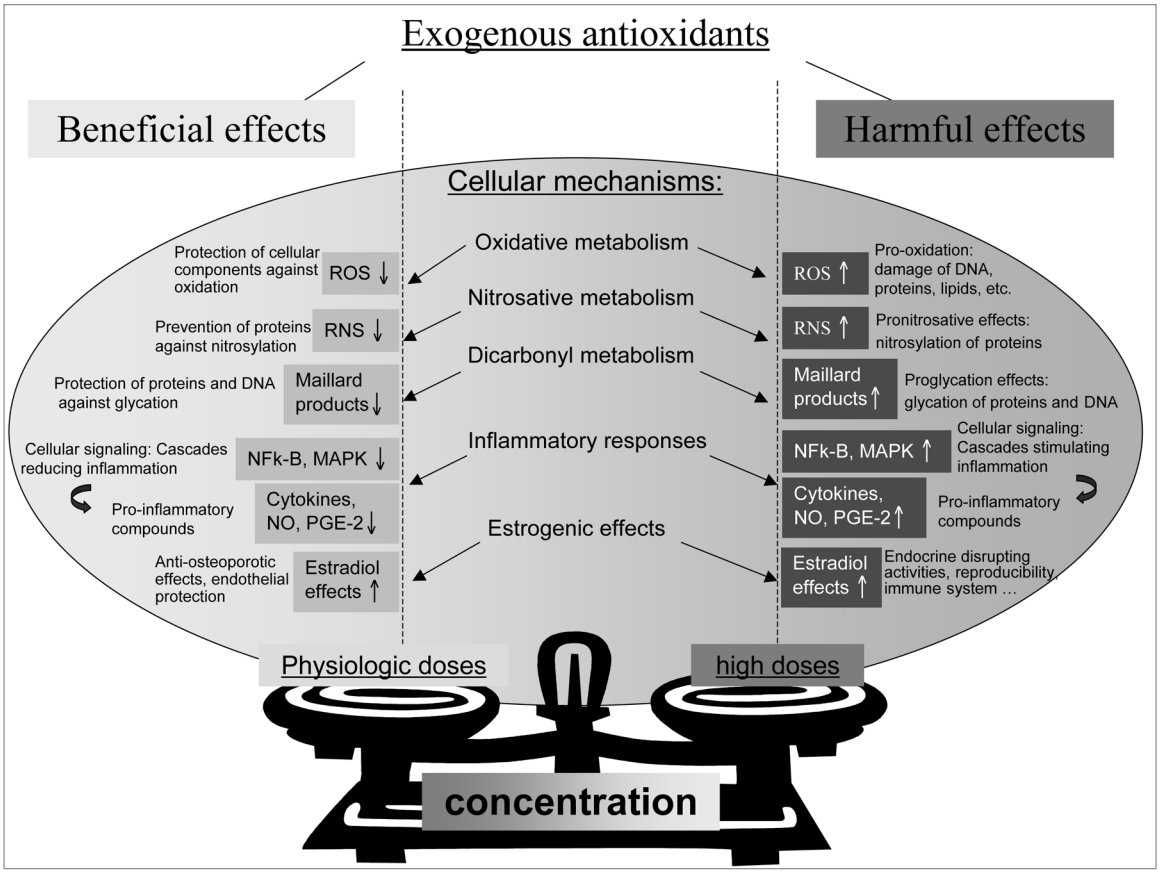

Figure 1. Double-edged effects of exogenous antioxidants on cellular responses including oxidative, nitrosative and dicarbonyl metabolisms and other pathways such as inflammatory processes depending potentially on their concentrations: physiologic doses leading to beneficial effects whereas high doses may result in harmful effects.

Other activities of natural compounds might result in beneficial as well as harmful effects such as due to the estrogen-like activity of isoflavones, as they are able to bind to ß-estradiol receptors (**Fig. 1**).[75,76] Independently of their antioxidant activity, polyphenols are also able to exert modulatory effects in cells, resulting in outcomes depending on the activated pathways among others, e.g., by interacting with intracellular signaling cascades (e.g., the nuclear factor kappa B (NFκB) and the mitogen-activated protein kinase (MAPK)) or by binding to the ATP-binding sites of a large number of proteins, including mitochondrial ATPase, calcium plasma membrane ATPase, protein kinase A, protein kinase C and topoisomerase (reviewed in ref. 69). It also has been revealed that some antioxidants (e.g., quercetin and naringenin) are able to inhibit certain cytochrome P450 enzymes (CYP1A1 and CYP3A4, respectively) involved in the bioactivation of chemical carcinogens,[77] constituting another proposed chemopreventive mechanism of polyphenols against cancer development including lung cancer.[78] In addition, it has been demonstrated that polyphenols (e.g., chlorogenic acid, EGCG and rutin) at pharmacological (non-nutritional) doses could interact with $GABA_A$ receptors or modulate neurotransmitters (e.g., serotonin and noradrenaline), resulting in interesting pharmacologic properties on the central nervous system including anti-anxiety and antidepressant activities.[9,79]

In vivo evidence: animal experiments, epidemiological data and human intervention trials. In animal experiments, it has been demonstrated that long-term intake of some natural food items such as apple,[80] olive oil[81] and honey[82] reversed several side effects associated with aging including brain oxidative stress,[80,81] cognitive deterioration[82] and anxiety.[80-82] Transgenic mice having vitamin E deficiency in the brain suffered from oxidative stress in this vital organ, developing anxious behavior without abnormalities in the locomotor performance.[83] It was also demonstrated that isolated polyphenols (e.g., quercetin, rutin and epigallocatechin-3-gallate) are able to reverse oxidative stress toxicity induced by certain conditions (e.g., pharmacological treatment, ischemia-reperfusion) in rat models.[84,85] Individual antioxidants from plant foods have also displayed cytoprotective activities and interesting pharmacological properties in rodents such as antidepressant and anxiolytic effects, among others.[9,79,86]

The potential adverse effects of exogenous antioxidants on consumer health has first concerned synthetic antioxidants including BHA and BHT, following their carcinogenity and toxicity at higher doses in rodents[87,88] and monkeys,[88] possibly resulting from prooxidative properties at higher concentrations. Surprisingly, it has also been reported that BHA displayed anticarcinogenic activity against various carcinogens

Table 2. Compilation of data from epidemiological (retrospective and prospective) investigations and human intervention trials highlighting the role of the diet (fruits and vegetable, supplements) on human diseases or biomarkers of health

Epidemiological and dietary intervention studies employing plant foods	References	Supplementation	References
Retrospective epidemiological data:		Human intervention trials with supplements:	
• apple,[78,98,99] onion[86,99] and white grapefruit[78] possessing preventive properties against developing coronary diseases[99] and lung cancer[78,98] as measured by cancer incidence[78,98] and CYP1A1[78]	Knekt et al.[98,99] & Le Marchand[78]	• no negative effects with respect to β-carotene supplementation (50 mg every other day) for 12 yrs in healthy subjects	Reviewed by Goralczyk,[138]
• reduced risk of lung cancer with higher dietary intake of β-carotene	Reviewed by Peto et al.[136]	• negative impact of supplementation by a combined treatment with vitamin A (retinyl palmitate) at 25,000 IU and β-carotene (30 mg) for 4 yrs on incidence of human lung cancer and cardiovascular diseases (CARET# study)	#Beta-Carotene and Retinol Efficacy Trial[111]
• plant-based diet may prevent against several types of cancer including breast, prostate and colon cancers, based on comparisons between traditional Eastern diets (e.g., Korea) rich in plant foods versus Western diets (e.g., U.S, high intake of calories and fats and limited intake of plant foods). Changes in Korean dietary habits due to "Westernization" of diet have increased incidence of cancer	Reviewed by Lee et al.[27]	• negative impact of supplementation of β-carotene (20 mg/d) for 5–8 yrs on incidence of lung cancer (ATBC study)*	*The Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group[110]
Prospective cohort epidemiological trials:		• vitamin E (50 mg/d) and β-carotene (20 mg/d) supplements for 6 yrs (median) failed to show beneficial effects on total stroke incidence or mortality in male smokers participating in the ATBC study ("Finish smoking study"). Increased risk of fatal subarachnoid hemorrhage and intracerebral hemorrhage by vitamin E and beta-carotene supplements, respectively	Leppälä et al.[139]
• protective effects of fruits and vegetables against coronary heart disease when consumed >4 servings/d for >8 years	Joshipura et al.[104]		
• Meta-analysis of eight independent cohort prospective studies: Consumption of >5 servings/d of fruits and vegetables caused a stronger reduction in strokes (ischaemic and haemorrhagic stroke) compared to 3–5 servings, the latter consumption reducing stroke incidence significantly compared to <3 servings/d	He et al.[105]		
Human dietary intervention trials:		• vitamin E (100 mg twice/d) and vitamin C (250 mg twice/d) supplements during two months failed to reduce oxidative DNA damage in smokers	Prieme et al.[117]
• decreased levels of oxidative DNA damage in healthy human volunteers consuming fruits and vegetables	Reviewed by Halliwell[65]	• supplementation with vitamin C at 500 mg/kg over 6 weeks increased oxidative lymphocyte DNA damage of 30 healthy volunteers	Podmore et al.[118]
• Adoption of traditional Mediterranean diets (moderate energy intake, limited animal fat and diversity and high intake of plant-based foods such as olive oil, cereals, legumes, nuts and vegetables), reduced several CVD risk factors in subjects at risk (primary prevention) and/or cardiovascular events/mortality in patients following a first cardiac event (secondary prevention)	Reviewed by Lairon[137]	• positive effects of long term supplementation of various minerals (Zn, Se), and β-carotene (15 mg), vitamin E (30 mg) on incidence on cancer in general (Linxian trial)	Blot et al.[114]

in animal models.[89-91] This disagreement could be explained by the doses administered and, perhaps, the duration of the treatment. In certain parts of the world, humans have consumed daily doses of BHA and BHT of ca. 0.1 mg/kg.[88] An LD_{50} of ca. 2,000 mg/kg of these synthetic antioxidants has been reported for most animals,[88] raising the question on the toxicity of these additives on human health at chronic exposure. Interestingly, high concentrations of antioxidants including BHT and BHA in food items, can also increase spoilage of food items, rather then result in prolonged shelf-life due to pro-oxidant activities.[92] As a consequence, tendencies emerged to replace synthetic antioxidants in foods and pharmaceutical preparations by natural antioxidants, due to presumably increased safety and higher acceptance by the consumer.[41]

With respect to humans, many of the health beneficial functions of dietary ingredients, including antimutagenicity, anticarcinogenity and anti-aging, among others, have been discussed in relation to their antioxidant properties.[27,35,66] Epidemiological investigations have played a key role in investigating the preventive action of diets rich in naturally occurring antioxidants on disease development and progression.[78,93-99] Indeed, regular consumption of fruits and vegetables has been shown to be inversely associated with lower mortality, presumably due to the protection offered by plant foods against the development of chronic human diseases related to oxidative stress such as cancer or CVD.[78,93-100] Even though more recent, prospective studies such as results of the EPIC study indicate that these retrospectively obtained results, at least with respect to cancer, might have been somewhat overestimated, still significant reduction of consumption of fruits and vegetables on e.g., colorectal cancer was found.[101] However, it has been hypothesized that specific food items e.g., apples and onions confer protection against lung cancer and coronary heart disease (**Table 2**).[78,98,99] Epidemiologists have postulated that the health beneficial effects of apple and onion against lung cancer may be attributed to few or even individual components, such as quercetin, owing to its potent chemopreventive activity against carcinogens in vitro[77] and in in vivo animal studies.[102,103]

It is interesting to note that in a prospective cohort study monitoring human volunteers for several years (8–14 years),

Table 3. Examples of antioxidant concentration in fruits, vegetables and in supplement preparations available on the market

Dietary antioxidants	Rich dietary sources	Concentration in foods (mg/100 g)	Concentration in supplements*** (mg/capsule)
Vitamin C	bell pepper, citrus fruits[140,141]	10–170	100–1000
Quercetin	apples, onions[140]	4–46	100–800
Carotenoids	leafy vegetables, plums, tomatoes, watermelon, carrots[140,141]	0.2–10	5–15
EGCG	green tea[142]	5–450#	25–360
Selenium*	fish (dairy producs, potato, rice)[140,141]	1–150*	0.07–0.20
Vitamin E	fish, meat, leafy vegetables[140,141]	0.2–10	400 IU**
Isoflavonoids	soy, beans, peanuts[140,143]	0.1–155	50–150

*µg/100 g; #mg/cup (ca. 225 mL of tea beverage); **1 IU alpha tocopherol = 0.667 mg; ***Internet data.

a reduction (albeit being non-significant) of the risk to develop coronary heart disease by intake of fruit and vegetables has been noticed for persons consuming more than 4 servings/d, and that this protection was more pronounced (and significant) in persons with a high consumption of fruits and vegetables (≥8 servings/d).[104] In addition, the pooled meta-analysis of eight prospective studies showed a negative relation between higher consumption of fruits and vegetables, and stroke risk (ischaemic and haemorrhagic stroke).[105] Based on these results, it was concluded that consumption of more than 5 servings/d of fruits and vegetables causes a more pronounced reduction in strokes than with 3–5 portions/d,[105] a recommended portion being somewhat vaguely defined as 80–100 g. However, the average fruit and vegetable intake in most developed countries is only about 3 servings/d.[105]

Nevertheless, there is increasing evidence that the observed associated health advantageous effects of plant food consumption may not be attributable to a specific compound, but rather to the whole fruit and vegetable, following additive or synergist actions of complex mixtures of phytochemicals and nutrients.[27,106] While earlier epidemiological and observational studies have suggested that increased carotenoid intake can go along with decreased risk of developing certain types of cancer, such as digestive tract cancer,[107] or lung cancer[108,109] and decreased risk of markers of CVD,[100] many individual supplementation trials in humans failed to result in observed health beneficial effects or even suggested that antioxidant compounds can be toxic under certain conditions such as at high doses or when synergistic compounds are lacking. For example, supplementing β-carotene alone (20 mg/day),[110] β-carotene and retinol (30 mg/d β-carotene and 25,000 IU retinyl palmitate)[111] over several years increased the lung cancer incidence in smokers. The same was observed in asbestos workers.[112]

However, it has also to be stated that some supplementation trials employing β-carotene, especially in healthy subjects, did not find increased mortality due to cancer,[113] or found even decreased overall mortality due to decreased incidence of cancer[114] (**Table 2**). Long-term supplementation studies with natural sources of antioxidants are virtually non-existing; however, short-term supplementation studies employing natural sources of antioxidants such as carotenoids demonstrated decreased oxidative stress markers and improved blood lipids.[115,116]

Nevertheless, the absence of beneficial activities of individual antioxidants, and even toxic effects[8,27,73,106,117,118] may be explained by the dose-dependent behavior these components exhibit outside their natural matrix, highlighting the important properties of complex mixtures such as of whole foods containing essential elements (vitamins, minerals), dietary fiber and non-nutrient phytochemicals including flavonoids, phenolic acids, several carotenoids, and many more. Several studies have shown that supplementation with isolated forms of vitamin C, vitamin E or β-carotene had no beneficial effects.[8,73,106] For example, supplementing diets of 30 healthy individuals with high doses of vitamin C (500 mg/d) caused an increase of oxidative damage in the DNA from lymphocytes, suggesting prooxidative effects at elevated doses.[118] In another study, supplementation with vitamin E and vitamin C failed to reduce oxidative DNA damage in smokers.[117] In contrast, some studies on healthy human volunteers consuming fruits and vegetables rich in vitamin C decreased levels of oxidative DNA damage[65] (**Table 2**). It has been suggested that at physiological conditions, the antioxidative properties of vitamin C outweigh its possible prooxidant activity.[3,118] Human trials and in vitro studies showed that oxidative stress causes a rapid depletion of vitamin C and vitamin E.[3,119] Vice versa, deficiency of vitamin E has also shown to provoke oxidative stress disturbances in transgenic rats.[83] Synergistic actions between vitamin C and vitamin E therefore appear important in their preventive activity against lipid peroxidation.[7]

Another example showing the importance of dosing on health concerns EGCG, a dietary antioxidant existing in green tea, marketed also in other preparations owing to its proposed preventive activity against oxidative stress. It has been demonstrated that EGCG at pharmacological doses (30 and 60 mg/kg) abolishes anxiety in mice;[120] at 150 mg/kg however this tea polyphenol caused death to mice (100% mortality) in less than 24 h, presumably due to its high hepatotoxicity noticed above 100 mg/kg.[54] Among green tea catechins, it has been revealed that, at higher doses, the most cytotoxic was EGCG, which is also the most abundant tea catechin.[54] Interestingly, despite green tea being viewed as a healthy drink with chemopreventive potential against cancer development,[66] tea, when consumed very frequently (>1 l/d), has been associated with increased incidence of esophageal cancer in some countries such as northern

Iran or India, even though this has been discussed to be due to consumption of hot tea,[121,122] further more, green tea has been shown to be able to produce H_2O_2 in the mouth cavity.[123]

In general, antioxidants when delivered as dietary supplements contain isolated (synthetic or concentrated) compounds in concentrated form. For example, a typical vegetarian diet contains 20 times less quercetin than a single dose of many supplements of this antioxidant available on the market.[53] High, isolated concentrations of carotenoids, EGCG and vitamin C are also common (**Table 3**). Carotenoid supplements for example mostly contain β-carotene, lycopene or lutein and xeaxanthin, and contain often the manifold of a typical daily intake. Unfortunately, for many dietary antioxidants, no upper tolerable intake level (UL) has been established, with exception for some vitamins.[124,125] While carotenoids have been taken also for its vitamin A activity and against macular degeneration,[36,126,127] especially lycopene has been marketed as an antioxidant.[128] Negative effects of taking high amounts of lycopene, also from diets, have been hypothesized to cause skin alterations and contribute to adverse effects such as abdominal problems (French Food Safety Agency AFFSA, www.afssa.fr/Documents/NUT2004sa0336.pdf).

Impact of Antioxidants on the Double-Edged Effect of ROS

In addition to the different effects antioxidants could exhibit in vivo depending on their present concentration, the double-edged effects of oxygen metabolites is also recognized and well documented.[7,33] The redox state of a cell and its oscillation determines its cellular functioning (reviewed in ref. 7 and 33). At low doses, ROS possess a crucial role in many physiological functions such as cellular signaling, gene expression, the regulation of immune responses and fostering antioxidative defense mechanisms.[7,10,32,33] For example, it was demonstrated that at least 40 various genes can be activated by H_2O_2 in mammalian cells.[32] The balance between oxidant production and antioxidant protection is believed to be critical in maintaining healthy biological systems. Therefore, antioxidants at high doses could, despite acting as prooxidants, also disrupt the redox balance following their potential to interact with ROS present at physiological concentrations required for optimal cellular functioning, leading to cellular dysfunction.[33] This assumption was reinforced by findings showing that transgenic animals overexpressing antioxidant enzyme systems (e.g., SOD and GPx) display abnormalities in function, including overexpression of certain genes such as immediate early genes (IEGs)[129] and certain proteins.[25] GPx overexpression in transgenic mice for example resulted in their development into a thermosensitive phenotype, suggesting a dysfunction in thermoregulation.[25]

At high concentrations, ROS are toxic compounds leading to lipid peroxidation and the oxidation of other sensitive biomolecules such as proteins and DNA.[7,17,32] When this situation occurs, cells enter an oxidative stress state, characterized by the disequilibrium between oxidant production and antioxidant protection in favor of the former.[7,17] Oxidative stress can cause cellular dysfunction by e.g., inducing changes in gene expression, protein expression, cellular signaling, membrane fluidity, potentially resulting in cell death.[7,17] Dietary antioxidants play a key role in reinforcing our antioxidant system to eliminate the excess of oxygen metabolites. An interactive and often synergistic action occurs between endogenous and exogenous antioxidants to maintain a balance between oxidation and antioxidation.[7] It has been estimated that concentrations of antioxidant micronutrients such as vitamin C, vitamin E and carotenoids range between high micromolar and low millimolar levels in human plasma and organs, while polyphenol concentrations are in the high nanomolar to low micromolar range.[130] However, polyphenols have been reported to be more efficient than vitamin C against oxidative stress at tissue levels.[130] In this respect, it has been suggested that phenolics are among the most active substances from natural sources, displaying a variety of health-promoting properties such as cytoprotective, antibacterial, antiviral, anti-aging, antiinflammatory, antiallergenic, antimutagenic, vasodilatory, anxiolytic, antidepressant and cognitive enhancing effects.[9,35] Polyphenols including phenolic acids and flavonoids are the most abundant class of antioxidant phytochemicals, existing in fruits and vegetables in concentrations around up to several 100 mg/100 g,[131] and thereby constituting the major class of antioxidants derived from the diet, with estimated intakes in westernized countries around 0.4–1.0 g/d and capita.[132,133] It is noteworthy however that the total amounts of antioxidative constituents present in the food matrix may not be completely extractable by the gastrointestinal (GI) tract, depending on several parameters, such as complexation by the food matrix or the presence of potential inhibitors of absorption. For carotenoids, for example, these factors have been summarized in the mnemonic term SLAMENGHI,[36,134] (comprising factors species, molecular linkage, amount compounds, matrix effects, effectors of absorption and bioconversion, nutrient status of host, genetic factors, host-related factors, interaction of all factors). In the GI tract, once nutrients and phytochemicals are present in soluble and bioaccessible form, they may be taken up by the epithelium and exert their antioxidant activity.[135] However, to be bioactive in other organs, additional factors of bioavailability such as absorption by the gut mucosa, transport to their place of action, formation of phase I and phase II metabolites and excretion do play a role.

Conclusion

The balance between oxidation and antioxidation (redox balance) is critical in maintaining a healthy biological system.[7,9,17] In cellular redox state, the double-edged effect does not only concern ROS, but also antioxidants. Physiologic doses of exogenous antioxidants are required to maintain or re-establish redox homeostasis.[7,34] However, high doses of exogenous antioxidants may disrupt redox balance. Considering epidemiological studies and trials on humans taking antioxidant compounds, it is evident that the health benefits of phytochemicals and nutrients were observed predominantly when being consumed within their natural food matrices (fruits, vegetables, grain, etc.).

Compounds within plant foods may therefore be considered as being more safe and healthy compared to isolated, high doses, such as present in supplements. Two main factors seem to be predisposing for the beneficial activities of plant foods: (1) the general low concentration of nutrients and non-nutrients in these natural food matrices and (2) the additive or synergistic actions of complex mixture profiles of phytochemicals and nutrients. Supplementation approaches do generally not take into account both aspects, which could explain the controversial results observed in supplementation studies.

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