Effects of antioxidant supplementation on the aging process

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Abstract: The free radical theory of aging hypothesizes that oxygen-derived free radicals are responsible for the age-related damage at the cellular and tissue levels. In a normal situation, a balanced-equilibrium exists among oxidants, antioxidants and biomolecules. Excess generation of free radicals may overwhelm natural cellular antioxidant defences leading to oxidation and further contributing to cellular functional impairment. The identification of free radical reactions as promoters of the aging process implies that interventions aimed at limiting or inhibiting them should be able to reduce the rate of formation of aging changes with a consequent reduction of the aging rate and disease pathogenesis. Even if antioxidant supplementation is receiving growing attention and is increasingly adopted in Western countries, supporting evidence is still scarce and equivocal. Major limitations in literature are still needed to be addressed to better evaluate the potential benefits from antioxidant supplementation: 1) an improved understanding of oxidation mechanisms possibly at the basis of the aging process, 2) the determination of reliable markers of oxidative damage and antioxidant status, 3) the identification of a therapeutic window in which an eventual antioxidant supplementation may be beneficial, 4) a deeper knowledge of the antioxidant molecules which in several conditions act as pro-oxidants.In the present paper, after a preliminary introduction to the free radical theory of aging and the rationale of antioxidant supplementation as an anti-aging intervention, we will present an overview of evidence relating antioxidant supplementations with clinical conditions typical of older age (ie, cardiovascular disease, Alzheimer's disease, cancer). We will also discuss studies that have evaluated whether antioxidant supplementation might improve major outcomes of interest in older persons (ie, physical performance, muscle strength, longevity). Given the large amount of data available on the antioxidant supplementation topic, this overview is not intended to be exhaustive. The aim of this paper is to provide the main basis from which future studies should start and indicate which the main limitations that need to be addressed are.

Keywords: aging, anti-aging medicine, antioxidant supplementation, oxidative damage

The free radical theory of aging

More than 300 theories have been proposed to explain the ageing process (Medvedev 1990), but none has yet been generally accepted by gerontologists. However, the initial proposal by Denham Harman that free radicals are causally related to the basic aging process (Harman 1957) is receiving growing acceptance as a possible explanation of the chemical reactions at the basis of ageing (De La Fuente 2002). The free radical theory of aging hypothesizes a single common process, modifiable by genetic and environmental factors, in which oxygen-derived free radicals are responsible (due to their high reactivity) for the age-associated damage at the cellular and tissue levels. In fact, the accumulation of endogenous oxygen radicals generated in cells and the consequent oxidative modification of biological molecules (lipids, proteins and nucleic acid) have been indicated as responsible for the aging and death of all living beings (Finkel and Holbrook 2000; Harman 1957).

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The free radical theory was revised in 1972 (Harman 1972) when mitochondria were identified as responsible for the initiation of most of the free radical reactions occurring in the cells. It was also postulated that the life span is determined by the rate of free radical damage to the mitochondria. In fact, mitochondria, in which there is a continuous generation of free radicals throughout cell life, and especially mitochondrial DNA, are key targets of the free radical attack. Cells which use oxygen, and consequently produce reactive oxygen species, had to evolve complex antioxidant defence systems to neutralize reactive oxygen species and protect themselves against free radical damage. Thus, the increasing oxidative stress in ageing seems to be a consequence of the imbalance between free radical production and antioxidant defences with a higher production of the former (Sastre et al 2000). An ideal "golden triangle" of oxidative balance, in which oxidants, antioxidants and biomolecules are placed at each apex, has been described (Carmeli et al 2002). In a normal situation, a balanced-equilibrium exists among these three elements. Excess generation of free radicals may overwhelm natural cellular antioxidant defences leading to oxidation and further contributing to cellular functional impairment (Bowles et al 1991; Meydani et al 1993).

The identification of free radical reactions as promoters of the aging process implies that interventions aimed at limiting or inhibiting them should be able to reduce the rate of formation of aging changes with a consequent reduction of the aging rate and disease pathogenesis (Harman 2003). In fact, the free radical theory of aging fostered a important body of research investigating the potential role of antioxidant nutrients in therapeutic or preventive strategies (Mayne 2003). However, even if antioxidant supplementation is receiving growing attention and is increasingly adopted in Western countries, supporting evidence is still scarce and equivocal.

Oxidative damage

The simplest free radical is an atom of the element hydrogen, with one proton and a single electron. Free radicals may also be nitrogen- or carbon-centered but O_2-centered radicals are the most important ones in aerobic organisms. Reactive oxygen species are mainly produced in mitochondria, which utilize most of the O_2 consumed for substrate metabolism and ATP production, reducing O_2 to water. Reactive oxygen species, produced under normal aerobic metabolism, are essential for cell signalling and for bacterial defence. In respiring cells, there appears to be a leakage of electrons from the mitochondrial electron transport chain, to eventually yield a variety of such free radicals and active oxygen derivatives that are collectively called reactive oxygen species.

Under normal conditions about 1% of reactive oxygen species daily escapes the control of the endogenous antioxidant defences and contributes to oxidative damage of surrounding tissues, consequently promoting and developing the aging process. Reactive oxygen species can attack any biochemical component of the cell. If the body or cell capacity to neutralise reactive oxygen species is altered, then they will produce acute damage to vital proteins, lipids and DNA. In humans, unbalance between reactive oxygen species production and endogenous antioxidants has been involved in the generation or worsening of more than a hundred pathologic conditions (Gutteridge 1993).

Measuring the free radical activity *in vivo* (ie, increased reactive oxygen production) is confronted with practical and analytical problems: we are left with the surrogate determinations of the end products of oxidation. In fact, the oxidative damage is commonly determined by the quantity of nucleic acid that is damaged with the Comet assay (Hartmann et al 2003), the amount of end products of lipid peroxidation (Cesari et al 2005; Sakamoto et al 2002), or of protein oxidation (Cesari et al 2005).

Physical activity and oxidative damage

The increased production of free radicals during physical exercise has been attributed to several factors, including the increase of catecholamines undergoing auto-oxidation, muscle transient hypoxia and re-oxygenation, lactic acid-induced free iron release from myoglobin, and/or inflammation-related neutrophil function (Ji et al 1998). There is a substantial lack of data regarding the effects of acute or chronic exercise in aging animals or humans. Inconsistent results (partly also due to methodological limitations in the reactive oxygen species measurements) do not allow a clear interpretation of studies regarding exercise-related DNA and protein oxidation, and lipid peroxidation. However, current literature seems to show an increased resistance to oxidative damage with chronic exercise and increased lipid, DNA or protein oxidation after an acute bout of maximal exercise (Polidori et al 2000).

Regular physical activity and exercise are recommended for the maintenance of an optimal health status and the prevention or management of chronic diseases (Department of Health and Human Services, Centers for Disease Control and Prevention, and National Center for Chronic Disease Prevention and Health Promotion 1996; Pate et al 1995). Physical activity (especially if begun in mid-life), quitting cigarette smoking, maintaining normal blood pressure, and avoiding obesity are independently associated with reduced

cardiovascular and overall mortality (Paffenbarger et al 1993). Regular physical activity has shown to reverse age-related body composition modifications in older subjects (ie, by increasing lean mass and reducing adipose tissue) (Fiatarone Singh 1998; Fiatarone et al 1994; Polidori et al 2000), and to confer significant protection against several age-related diseases (eg, non-insulin-dependent diabetes (Hughes et al 1995); cancer (Ji et al 1991); hypertension (Dengel et al 1998); and osteoporosis (Evans 1999)).

Animal (Zerba et al 1990) as well as human (Quindry et al 2003; Tozzi-Ciancarelli, Penco and Di Massimo 2002; Watson et al 2005) models have demonstrated that acute bouts of eccentric exercise produce higher oxidative damage to muscles in aged mice and men compared to young animals or human subjects. However, physical activity may still play an important role in limiting the free radical production and oxidative damage. In fact, even if exercise is associated with an abnormal production of free radicals, physically active older persons benefit from exercise-induced adaptations in the cellular antioxidant defence systems (Fulle et al 2004).

Physical exercise may lead to an increase in antioxidant defences of the organism in younger as well as in older subjects (Lawler and Powers 1998; Leeuwenburgh and Heinecke 2001). Nevertheless, the equilibrium between free radical production and antioxidant defence induction by physical exercise intervention in elders may be more unstable than in younger subjects. This is probably due to the higher rate of oxidative stress occurring in older persons (Ames 1989; Facchini et al 2000; Greco et al 2000; Olinski et al 2003), partly explained by increased number of concurrent clinical conditions and the sedentary lifestyle. Moreover, although age-related modifications occurring in antioxidant defences and repairing systems are not yet fully clarified (Beckman and Ames 1998), a decrease of major antioxidants levels with aging has been suggested (Pinzani et al 1997; Poulsen et al 1996).

An acute bout of exercise increases antioxidant activities in skeletal muscle, heart, and liver with a threshold and magnitude of activation that differs among antioxidant enzymes, tissues, and type of exercise organisms (Ji et al 1998). No significant difference in the antioxidant enzyme response between old and young animals has been suggested (Fiebig et al 1994; Ji 1996; Lawler and Powers 1998). Moreover, endurance training has shown to increase antioxidant enzyme activities even in the senescent muscle (Ji et al 1991).

Antioxidants

Antioxidants are substances, which inhibit or delay oxidation of a substrate while present in minute amounts. Endogenous antioxidant defences are both non-enzymatic (eg, uric acid, glutathione, bilirubin, thiols, albumin, and nutritional factors, including vitamins and phenols) and enzymatic (eg, the superoxide dismutases, the glutathione peroxidases [GSHPx], and catalase). In the normal subject the endogenous antioxidant defences balance the reactive oxygen species production, but for the above-mentioned 1% daily leak. The most important source of antioxidants is provided by nutrition, many belonging to the phenol family.

Nutritional antioxidants act through different mechanisms and in different compartments, but are mainly free radical scavengers: 1) they directly neutralise free radicals, 2) they reduce the peroxide concentrations and repair oxidized membranes, 3) they quench iron to decrease reactive oxygen species production, 4) via lipid metabolism, short-chain free fatty acids and cholesteryl esters neutralise reactive oxygen species (Berger 2005). The body antioxidant defence can be approximated by measuring antioxidant plasma levels (micronutrients, enzymes, other antioxidant), keeping in mind that the circulating compartment only reflects the flow between organs and tissues. The tissue levels of the various antioxidants remains limited to research protocols as tissue biopsies are required.

Vitamin C is the major water-soluble antioxidant and acts as first defence against free radicals in whole blood and plasma. It is a powerful inhibitor of lipid peroxidation and regenerates vitamin E in lipoproteins and membranes. A strong inverse association has been shown between plasma ascorbic acid and isoprostanes (Block et al 2002). Isoprostanes represent a family of prostaglandin isomers which, in contrast to classic prostaglandins formed through an enzymatic action of the prostaglandin-H-synthase from arachidonic acid, result from a free radical-catalyzed mechanism (Morrow et al 1990). For this reason, isoprostanes provide an optimal estimate of oxidative damage to cellular lipids (Morrow 2005) and represent an excellent biomarker of lipid peroxidation for aging studies (Cesari et al 2005).

Bagi et al (Bagi et al 2003) have shown that chronic vitamin C treatment is able to decrease high levels of isoprostanes in animal models. Ascorbic acid combined to α-tocopherol is particularly effective in inhibiting oxidation (Niki et al 1995). Vitamin C reduces α-tocopheroxyl radicals rapidly in membranes and LDL to regenerate α-tocopherol and possibly inhibits α-tocopheroxyl radical-mediated propagation.

Vitamin E is a lipid-soluble vitamin found in cell membranes and circulating lipoproteins. It protects against oxidative damage by acting directly with a variety of oxygen radicals. Its antioxidant function is strongly supported by regeneration

promoted by vitamin C (Maxwell 1995). Vitamin E is thought to have a role in the prevention of atherosclerosis through inhibition of oxidative modifications of LDLs (Steinberg 1997; Witztum 1994). The formation of isoprostanes increases significantly in animals deficient in vitamin E (Morrow and Roberts 1997). Moreover, inhibition of isoprostanes formation by vitamin E supplementation has been shown in humans (Upritchard et al 2003) as well as in animal models (Liu et al 1999). α-tocopherol is quantitatively the major form of vitamin E in humans and has been extensively studied. In contrast, γ-tocopherol, even if representing the most abundant form of vitamin E in the US diet, has received less attention (Jiang et al 2001). Compared with α-tocopherol, γ-tocopherol is a slightly less potent antioxidant with regard to electron-donating propensity, but is superior in detoxifying electrophiles, such as reactive nitrogen oxide species (Jiang et al 2001). The interaction of α-tocopherol with β-carotene is not as evident as the one reported with vitamin C, but it has been shown that α-tocopherol and β-carotene exert a cooperative effect by residing and scavenging radicals at different positions in the lipophilic compartment (Niki et al 1995).

Carotenoids are lipid-soluble antioxidants. Plasma levels of carotenoids are negatively correlated with levels of isoprostanes (Block et al 2002). Carotenoids levels are inversely associated with inflammation (Hu et al 2004), atherosclerosis (Prince et al 1988), cardiovascular disease (Gaziano et al 1995), sarcopenia (Semba et al 2003), and mortality (Hu et al 2004), and positively correlated with physical performance (Cesari et al 2004). Improvements in antioxidant status and reduction of lipid peroxidation have been shown after carotenoids supplementation (Upritchard et al 2003). The most known and studied carotenoid is the β-carotene, a potent antioxidant able to quench singlet oxygen rapidly (Di Mascio et al 1991). β-carotene, α-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin have all been found associated with inflammation (Hu et al 2004; Kritchevsky et al 2000). The association between low serum levels of β-carotene and increased risk for mortality was recently reported (Hu et al 2004).

Vitamin C, vitamin E, and carotenoids have shown to synergistically interact against lipid peroxidation (Niki et al 1995). Higher serum levels of antioxidants are associated with higher strength and physical performance measures (Cesari et al 2004; Semba et al 2003), suggesting that oxidative damage may play an important role for the onset of the disabling process.

Melatonin is a mammalian hormone synthesized from serotonin, mainly in the pineal gland. Besides of its widely documented action regulating the circadian rhythm, it has been reported that melatonin contributes to the reduction of oxidative damage in both the lipid and the aqueous environments of the cell (Aydogan et al 2006). The powerful antioxidant capacity of melatonin (Pieri et al 1995) is exerted by stimulating the expression and activity of glutathione peroxidase, superoxide dismutase, NO synthetase (Nishida 2005). Interestingly, melatonin concentrations are particularly high in mitochondria and the cell nucleus (Aydogan et al 2006), where major oxidation reactions occur.

The balance between oxidant and reducing forces is subtle (Abuja 1998). Trace elements with antioxidant properties such as copper and selenium (Terada et al 1999), may become strongly pro-oxidant both *in vivo* and *in vitro*, as a consequence of their physical properties. This is also the case with vitamins A, C, E, which may become pro-oxidant under defined conditions (Berger 2005).

Vitamin E can also become a pro-oxidant in isolated lipoprotein suspensions (eg, parenteral nutrition solutions in clinical conditions (Neuzil et al 1995). The pro-oxidant effects of selenium have been investigated on cultured vascular cells exposed to parenteral nutrition containing various forms and quantities of selenium (Terada et al 1999). In a recent study, Nakamura and colleagues (Nakamura et al 2006) suggested that Vitamin C may play an important role to prevent the pro-oxidant effect of Vitamin E in LDL oxidation.

Antioxidant supplementation

The last 50 years have been characterized by the understanding of the impact of nutrition and dietary patterns on health (Caballero 2003). An important part of the population is exposed to the risk of trace element and vitamin deficiency for multiple reasons (eg, changes in eating habits in Western Countries, lower food concentration of micronutrients due to intensive agricultural techniques). Children, young women and elders are the most exposed (Caballero 2002; Johnson et al 2002; Ramakrishnan 2002). Efforts to fight nutrient deficiencies have centred on supplemental nutrient administration and on addition of selected nutrients to the food chain in the form of food fortification (Caballero 2003). Supplementation and fortification has also been proposed in healthy individuals with the aim of reducing their risk of future diseases (eg, cardiovascular diseases, diabetes and cancer). Nevertheless, with our increasing understanding of the genetic heterogeneity of human nutrient requirements, it is likely that certain groups or even populations may benefit from higher intakes of certain nutrients. However, the latter

concept is getting closer to the therapeutic modulation of nutrient intake.

Antioxidant supplementation and clinical conditions

Atherosclerosis and cardiovascular disease

In Western Countries, atherosclerotic disease is the major cause of death in the elderly population. Several antioxidants, such as polyphenols and lycopene, have been proposed to delay the progression of this disease. At the beginning of the Nineties, Renaud and de Lorgeril created the so-called "French Paradox", describing how, despite the high intake of saturated fat, the French population presents a low incidence of coronary heart disease events (Renaud and de Lorgeril 1992). Even if their study raised a huge controversy, it has been suggested that beneficial effects from red wine consumption might be related to its high content of antioxidants (Heller et al 1998). Resveratrol, a phytoalexin found in several plants (in particular, red grapes), has shown to be able to up-regulate the nuclear Liver X receptor α and its target genes in macrophages, and to reduce the expression of lipoprotein lipase and scavenger receptor AII (Sevov et al 2006). Through these mechanisms, resveratrol seems to limit cholesterol accumulation in human macrophages. A recent study conducted in cultured human coronary artery endothelial cells has also demonstrated a beneficial effect of polyphenols (ie, catechin and quercetin) on the expression of the plasminogen activator inhibitor-1 gene, potentially providing a further biological explanation to the cardiovascular protective role of these molecules (Pasten et al 2007). However, no definite conclusion can still be drawn given the complex mechanisms in which polyphenols (eg, resveratrol) are involved and which may influence the net results of their supplementation (Iannelli et al 2006).

A number of prospective cohort studies and case-control studies have reported that increased intake of dietary antioxidants including vitamin E, vitamin C, and β-carotene, are associated with reduced risk of atherosclerotic diseases (Kaliora et al 2006). Thus, antioxidants seem to prevent the development and progression of arteriosclerosis (Nakamura et al 2006). From this evidence, a growing interest has been posed into antioxidants as potential inhibitors of the proatherogenic and prothrombotic oxidative events occurring in the artery wall and underlying the atherosclerotic process. In 1999, an American Heart Association Science Advisory recommended that the general population consume a balanced diet with emphasis on antioxidant-rich fruits, vegetables, and whole grains (Krauss et al 2000). Given the absence of data from randomized, controlled clinical trials at the time, no recommendations were made regarding the use of antioxidant supplementation. In a more recent American Heart Association Science Advisory (Kris-Etherton et al 2004), current evidence about the beneficial effects of antioxidant vitamins (such as vitamin E, vitamin C, and β-carotene) on cardiovascular risk has been revised and discussed. Consistently with previous recommendations from the American Heart Association (Mosca et al 2004) and the American College of Cardiology (Gibbons et al 2003), scientific data do not yet justify the use of antioxidant vitamin supplements for cardiovascular risk reduction. However, the controversial results on this topic require further research.

A recent randomized controlled trial, enrolling more than 35,000 healthy women aged 45 years and older, showed no beneficial effect from vitamin E supplementation (600 IU on alternate days for a mean of 10.1 years) for the prevention of major cardiovascular events, cancer, total mortality, and cardiovascular mortality (Lee et al 2005). Similar results were also obtained from the HOPE and the HOPE-TOO trials (The HOPE and HOPE-TOO Trial Investigators 2005), where a possible increased risk of heart failure was also hypothesized in the intervention group (vitamin E 400 IU daily).

Recently, Pham and Plakogiannis (Pham and Plakogiannis 2005) have reviewed evidence on the effects of vitamin E supplementation and cardiovascular and cancer prevention. Their meta-analysis showed that contradicting results regarding the benefits of vitamin E in the prevention of cardiovascular disease and cancer from the considered studies. Authors assured the presence of adequate evidence from large, well-designed studies to discourage the use of vitamin E in the primary prevention of cardiovascular disease. For what concerns secondary prevention, more adequate clinical trials with selected populations are required to examine protective effects of vitamin E in cardiovascular disease.

It is important to underline how positive findings are mostly from observational studies, so that the relationship between vitamin E supplementation (the most promising antioxidant in the prevention of atherosclerotic disease) and lower rates of cardiovascular disease may just reflect an overall healthy lifestyle and dietary intake of supplement users rather than a real protective effect. Nevertheless, a role for

oxidative mechanisms underlying the human atherosclerosis pathogenesis can not be ruled out.

Alzheimer's disease

A number of studies have shown that aging and particularly brain aging are associated with free radicals action. Evidence suggests that reactive oxygen species in brain may play a role in the development of age-related neuronal impairments. The increase in the concentration of the pro-inflammatory cytokines in aged brain tissue may also represent a contributory factor.

The accumulation of oxidative damages to neuronal components with age underlies the molecular basis of brain aging and neurodegeneration (Kolosova et al 2006). Oxidative stress has been implicated in mechanisms leading to neuronal cell injury in various pathological states of the brain (Calabrese et al 2003).

Alzheimer's disease is a progressive disorder with cognitive and memory decline, speech loss, personality changes and synapse loss. The heterogeneity of the etiologic factors of Alzheimer's disease makes it difficult to define the major clinical determinants for the onset and progression of the disease. However, increasing evidence has recently indicated oxidative damage as a potential cause of Alzheimer's disease pathogenesis (Nunomura et al 2006; Onyango and Khan 2006). Moreover, subjects with dementia attributed to Alzheimer's disease have shown an altered balance between oxidant and antioxidant levels (Sinclair et al 1998). Recently, increasing interest has been focused on identifying dietary compounds that can inhibit, retard or reverse the multi-stage pathophysiological events underlying Alzheimer's disease pathology. Alzheimer's disease also involves a chronic inflammatory response associated with both brain injury and beta-amyloid associated pathology.

Animal models have demonstrated that dietary supplementation with antioxidant vitamins can prevent or reverse the age-related changes in antioxidant defences in the central nervous system and decrease oxidative stress (O'Donnell and Lynch 1998). In a recent review, Vina and colleagues (Vina et al 2004) demonstrate that the cognitive function in Alzheimer's disease patients is inversely correlated with systemic oxidative stress. They also confirm the idea that vitamin E may be considered as an effective treatment of Alzheimer's disease. However, the effect of vitamin E on Alzheimer's disease patients shows considerable variations both in its antioxidant function and in its capacity to improve cognitive functions. Therefore, consistently with previous recommendations (Kris-Etherton, and for the Nutrition Committee of the American Heart Association Council on Nutrition Physical Activity and Metabolism 2004), Authors suggest that the determination of the oxidant-antioxidant status of the patient is particularly important to test the effect of antioxidants on given functions.

A major limitation present in most of the intervention studies exploring the effects of antioxidants supplementation (eg, vitamin E) on Alzheimer's disease outcomes is that they have been conducted on subjects who already have been diagnosed with this clinical condition. Therefore, it is difficult to assess the full potential of the specific substances in the prevention of Alzheimer's disease. Moreover, antioxidants are often tested as single agents, while it is becoming clearer that combinations of antioxidants are more effective. As for evidence related to cardiovascular disease, a large part of studies on the topic (mostly from epidemiologic reports) has shown that individuals, who consume higher amounts of fruits and vegetables, as well as vitamin supplement users, have lower rates of Alzheimer's disease. Some reports have suggested that combinations of vitamins with antioxidant properties (in particular, vitamin C and vitamin E) have shown the greatest benefits (Frank and Gupta 2005).

Cancer

While the exact role of free radicals in carcinogenesis and cancer progression is still under investigation, increasing evidence has demonstrated that some antioxidants are associated with a lower incidence of specific types of cancers. Vitamin E, for example, has been shown in some trials to reduce the incidence of breast, lung, and colon cancers, but the most significant results have been obtained with prostate cancer. For example, in the the alpha-tocopherol beta-carotene cancer prevention study (Albanes et al 1995), in which the participants were all male smokers, α-tocopherol supplementation decreased prostate cancer incidence and mortality. Recently, the supplementation en vitamines et mineraux antioxydants (SU.VI.MAX) study, a randomised, double-blind, placebo-controlled primary prevention trial, tested the efficacy of supplementation with a combination of antioxidant vitamins and minerals, at nutritional doses, in reducing the incidence of cancer in a general population not selected for risk factors (Hercberg et al 2004). After a 7.5-year follow-up, antioxidant supplementation was associated with a reduction in cancer incidence in men only. However, Authors discussed that antioxidant supplementation may have beneficial effects on cancer incidence only in healthy subjects, who are not exposed to cancer risk, and with a particularly low baseline antioxidant levels (Hercberg et al 2006). Authors also warned that high dosage antioxi-

dant supplementation 1) may be deleterious in subjects in whom an initial phase of carcinogenesis has already started, and 2) could be ineffective in well-nourished subjects with adequate antioxidant status (Hercberg et al 2006). Consistent with these findings, a trial aimed at evaluating the lung cancer incidence showed that selenium supplementation was beneficial only among individuals with low baseline selenium concentrations (Reid et al 2002).

In the cancer prevention study II nutrition cohort, the authors examined the association between multivitamin supplementation and incidence of colorectal cancer (Jacobs et al 2003). Results were consistent with the hypothesis that past, but not recent, multivitamin use may be associated with a modest lower risk of colorectal cancer.

A recent review of randomised trials comparing antioxidant supplements to placebo/no intervention for the incidence of gastrointestinal cancers has found no evidence that antioxidant supplements prevent gastrointestinal cancers (Bjelakovic et al 2004). On the other hand, antioxidant supplements seem to increase overall mortality. Similarly to what obtained for the cardiovascular disease outcome, Pham and Plakogiannis (Pham and Plakogiannis 2005) found no sufficient evidence that vitamin E is able to reduce the risk of cancer, concluding that vitamin E supplementation for cancer prevention is not recommended.

Physical performance and muscle strength

Whether higher antioxidant intake is beneficial in promoting better physical performance and muscular strength is still controversial. Although findings of some studies have shown improvements (Gao et al 2004; Hauer et al 2003; Takanami et al 2000; Upritchard et al 2003; Wijnen et al 2001), other studies do not support beneficial effects of increased antioxidant intakes on physical performance (Avery et al 2003; Balakrishnan and Anuradha 1998; Barnett and Conlee 2003; Clarkson 1995; Konig et al 2001; Oostenbrug et al 1997; Van der Beek 1991). Nevertheless, it seems reasonable an adequate antioxidant intake is needed to maintain healthy muscular activity (Jackson and Edwards 1990; Maxwell 1995). It has been suggested that controversial results might be explained by the need of better targeting subjects who can really benefit from antioxidant supplementation (Kris-Etherton, and for the Nutrition Committee of the American Heart Association Council on Nutrition Physical Activity and Metabolism 2004). It is likely that only subjects with a low antioxidant status (due to an inadequate antioxidant intake) or those with high levels of oxidative damage should be candidates for an antioxidant supplementation (Patrignani et al 2000). To address this issue, it is needed to better explore the relationship of dietary antioxidants intake with oxidative damage and serum antioxidants levels.

Physical activity

Regarding antioxidants supplementation (eg, vitamin C, vitamin E or glutathione) and their potential protective role against exercise-related oxidative damage, results are again highly inconsistent and/or not adequate, especially in human models (Tiidus and Houston 1995). In fact, the relationship between oxidative stress and physical activity is still poorly understood, particularly in advanced age, since limited data are still available regarding the effects of acute exercise and training in elderly subjects. Moreover, available studies are characterized by low number of subjects, different types and intensities of exercise, and not homogeneous or not completely reliable methods of oxidative damage measurements. Therefore, the paradox of physical activity (which is certainly beneficial at all ages, but simultaneously potentially harmful if not adequately performed due to the free radical excessive production) can not be clarified at the present time.

Since the several current guidelines recommend regular physical activity in the older persons (Department of Health and Human Services, Centers for Disease Control and Prevention, and National Center for Chronic Disease Prevention and Health Promotion 1996; Pate et al 1995), further studies are urgently required to better assess the potential effects of exercise-related free radicals production during exercise.

Longevity

Nutritional supplementation, especially with antioxidants, has been frequently indicated as a potential mean to improve health status and increase longevity (Harman 1962). However, only limited evidence about the protective effects of specific micronutrients is available. Moreover, it is still unclear whether the health benefits from diets at high consumption of fruit and vegetables (van Poppel and van den Berg 1997) can be replicated by antioxidant supplementations (Potter 1997).

The theoretical basis supporting a possible relationship between antioxidant supplementation and longevity are mainly from the evidence showing a relationship of the latter with the rate of mitochondrial oxygen radical generation and the degree of unsaturation of membrane fatty acids (Barja 2002). In fact, these two molecular traits are significantly lower in all the relatively long-lived homeothermic verte-

brates, and may be main causes of the low rate of aging of long-lived animals.

In an animal model, Lipman and colleagues (Lipman et al 1998) showed no effect on age-associated lesions patterns, lesion burden or longevity in ad libitum mice fed with a the diet supplemented with antioxidants (vitamin E and glutathione) and initiated during middle age. Results from the SU.VI.MAX study (Hercberg et al 2004), consistently with findings obtained on incidence of cancer, showed a possible protection for overall mortality in men enrolled in the intervention group. However, no definite conclusion was provided by Authors regarding this possible association, but the recommendation of a lifelong diversified diet including an abundance of foods rich in antioxidant nutrients as previously proposed (Drewnowski et al 1997).

A great interest has been attracted by the potential capacity of melatonin to extend life span (Anisimov 2003). Melatonin is a potent free radical scavenger, especially towards highly toxic hydroxyl radicals. Moreover, melatonin additionally stimulates a number of antioxidative enzymes (Reiter 1998). Unfortunately, current data do not still allow to conclude that melatonin may have a role in extending normal longevity. Moreover, as for many other antioxidants, melatonin can act as a prooxidant under certain conditions (Anisimov 2003; Clapp-Lilly et al 2001; Osseni et al 2000).

Antioxidant supplementation issues

A major limitation of current literature resides in the still limited knowledge of oxidative mechanisms and the lack of valid biochemical markers evaluating the candidate antioxidant compounds. It has also been suggested that antioxidant treatment may need to begin earlier in life to be effective (Kris-Etherton, and for the Nutrition Committee of the American Heart Association Council on Nutrition Physical Activity and metabolism 2004). The discrepancy between the impressive observational data and the clinical trials could also be due to the difference between lifelong exposures to an antioxidant-rich diet versus a limited exposure to antioxidant supplements (ie, the trails follow-up). Moreover, several other potential explanations should be considered when analyzing the lack of agreement between the predicted positive benefits and the results of the clinical trials conducted to date. For example, it may be that only particular antioxidants (possibly in combination with others) might exert protective effects on clinical or biological conditions, or that only specific populations might benefit from an antioxidant supplementation (ie, only subjects with low antioxidant and/or high oxidative stress levels).

An important part of the evidence supporting the beneficial effects of antioxidant supplementation is based on animal data. Results from these studies need to be considered cautiously. In fact, even if these experiments are crucial in the understanding of mechanisms at the basis of biological and clinical hypothesis, the translation of their results to humans may sometimes be problematic and/or misleading.

As for any other medication/intervention, antioxidant supplementation is likely to present its own "therapeutic window". In other words, there might be an optimal early timing after the initial reactive oxygen species production during which supplementation may still have a "preventive effect". Then, it should be also considered that each antioxidant may present a specific and peculiar timing, the combination of antioxidants may modify the "therapeutic window", and/or the length of exposure to supplementation may play a crucial role for the achievement of the effects.

The doses that are required to achieve a therapeutic effect are not definitively determined and represent another issue that needs to be addressed. The belief that if enough of an essential nutrient is good, then more is better is wide spread. Nevertheless, this may not be true. For example, zinc supplements using doses >50 mg/day have been associated with depressed immune response (Chandra and McBean 1994), and chronic exposure to selenium compounds is associated with several adverse health effects (Vinceti et al 2001).

Even if some epidemiological studies shown that antioxidant supplementation may decrease the risk of several clinical conditions, such observations are usually not universal (Butler et al 2002). Even the only capability of reducing oxidative damage through antioxidant supplementation is limited. For example, Mc Call and Frei stated that "except for supplemental vitamin E, and possibly vitamin C, being able to significantly lower lipid oxidative damage in both smokers and non-smokers, the current evidence is insufficient to conclude that antioxidant vitamin supplementation materially reduces oxidative damage in humans" (McCall and Frei 1999). The further step of assessing whether the modification of a biological mechanism (eg, decrease of lipid oxidative damage levels) is able to provide a clinical benefit (eg, reduction of cardiovascular events) is still far to be ascertained.

In conclusion, current evidence does not allow to recommend antioxidant supplementation as a useful mean to prevent age-related pathophysiological modifications and clinical conditions. Several concerns are present not only about their efficacy, but also on their safety. No recommendation will be made until a clearer picture of 1) mechanisms underlying

the aging process, 2) the network existing among the different antioxidant molecules, 3) the relationship between pro-oxidant and antioxidant factor, 4) the pathogenesis of the oxidative damage-related disease, and 5) reliable markers of oxidant and antioxidant levels, will be provided.

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