Association between oxidative stress and nutritional status in the elderly

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Summary

Ageing is a dynamic and progressive process that is characterized by the occurrence of morphological, biochemical, functional and psychological changes in the organism. The aim of the present article is to provide updated concepts on oxidative stress, covering its importance in aging, as well as nutritional status and supplementation with antioxidants (substances that prevent or attenuate oxidation of oxidizable substrates, such as lipids, proteins, carbohydrates and deoxyribonucleic acid) in the geriatric population. Evidence suggests that there is an inverse relationship between oxidative stress and nutritional status in elderly individuals. Although an increase in oxidative stress in chronic diseases associated with aging has been proven, such as Parkinson’s disease and Alzheimer’s disease, up to now there has been no consistent clinical evidence proving the efficiency of supplementation with antioxidants against oxidative stress. In this context, supplementation is not recommended. On the other hand, the elderly should be encouraged to eat antioxidant foods, such as fruits and vegetables. Maintaining a normal weight (body mass index between 23 and 28 Kg/m²) should also be stimulated.

Uniterms: aging; oxidative stress; nutritional status; antioxidants; dietary supplements.

Oxidative stress

The role of oxidative stress in aging was originally studied in 1956 when Harman proposed that the process is partially associated to the accumulation of oxidative damage in biomolecules such as lipids, proteins, carbohydrates and deoxyribonucleic acid (DNA).⁷

Oxidative stress is classically defined as an event resulting from an imbalance between the amount of pro-oxidant and antioxidant substances.⁸ Both substances are generated in a redox setting, in which oxidation implies a gain in electrons, and reduction in a loss. As the production and action of pro-oxidant and antioxidant substances depends on this redox system, many authors have been using the term imbalance of the redox system to refer to oxidative stress.⁹,¹⁰ Pro-oxidant substances include those relating to oxygen (ROS) and nitrogen (RNS), which correspond to highly reactive molecules that constantly attack...
the human body through biochemical reactions that are a normal part of cellular metabolism, or exposure to environmental factors.\textsuperscript{5,11}

Popularly known as free radicals, reactive substances correspond to any atom or molecules bearing unpaired electrons (odd number of electrons) in its outermost orbit. This lack of pairing is responsible for their high reactivity.\textsuperscript{12,13} The main reactive substances include: hydroxyl radical (OH), superoxide radical (\(O_2^-\)), peroxynitrite (ONOO\textsuperscript-), hydrogen peroxide (H\(_2\)O\(_2\)), singlet oxygen (\(^1\)O\(_2\)), nitric oxide (NO\textsuperscript-), hypochlorite (ClO\textsuperscript-), hydroperoxyl radical (HO\(_2\)), and alkoxyl (LO\textsuperscript- ) and peroxyl (LOO\textsuperscript- ) radicals. The term free radical is not always appropriate because the reactive substance is not necessarily a free radical. For example, \(H_2O_2\) does not have a solitary electronic in its outermost orbit, but is a highly oxidant substance. The same occurs with peroxynitrite, hypochlorite, singlet oxygen and hydroperoxide (ROOH).\textsuperscript{14} Therefore, it is more appropriate to replace the term free radical with reactive substance.

The nitric oxide synthases (NOS) enzyme system is an important source of reactive substances in aging. Physiologically, the NOS converts L-arginine into nitric oxide, resulting in control of the smooth muscle of the vessels. There is evidence that the expression of NOS enzyme and bioavailability of L-arginine decrease during aging, which leads to the accumulation of superoxide radicals.\textsuperscript{14} An excess of this radical stimulates decoupling of the NOS enzyme, resulting in contraction of the vessel. These situations limit the blood supply to the heart, alter the consumption of O\(_2\) in the myocardium and increase apoptosis of endothelial cells. The uncoupled NOS (eNOS) has been demonstrated in diseases such as arteriosclerosis, diabetes mellitus (DM) and high blood pressure (HBP).\textsuperscript{15}

Although reactive substances are essential for a variety of cellular defense mechanisms (bactericide activity, mitochondrial respiration\textsuperscript{16}, regulation of relaxation/contraction of the smooth musculature of the vessels\textsuperscript{17}), they may cause oxidative damage in biomolecules (lipids, proteins, carbohydrates and DNA) when present in numbers above their neutralization, carried out by the Antioxidant Defense System.\textsuperscript{18} The oxidation of biomolecules may be associated with aging and various diseases.\textsuperscript{19,20}

As stated, the reactive substances may attack all of the main classes of biomolecule, being unsaturated lipids the most susceptible.\textsuperscript{21} Oxidative damage occurring in lipids is denominated lipid peroxidation and involves initiation, propagation and termination stages.\textsuperscript{22}

The final products of lipid peroxidation include aldehydes and gaseous hydrocarbons. The product that is most frequently measured is malondialdehyde (MDA), which reacts with proteins and amino acids.\textsuperscript{23} It has been reported that the MDA may be higher in elderly individuals, particularly in the following comparisons: elderly compared with adults\textsuperscript{24}, institutionalized with community residents\textsuperscript{25}, and those of advanced age (> 80) relative to the ones of less advanced age (approximately 60 years old).\textsuperscript{24}

The clinical importance of the reaction between MDA and proteins is stronger in atherogenesis. The MDA-LDL (low density lipoprotein) complex can be used to measure pro-inflammatory and pro-atherogenic processes, which inevitably cause generation of foam cells.\textsuperscript{26}

In addition to the damage to lipids induced by reactive substances, we can also point out the oxidation of proteins. This oxidation may occur via nitration and carbonylation.

Carbonylation of proteins occurs as a result of the action of advanced lipid peroxidation products (MDA, glyoxal, acrolein, 4-hydroxynonenal, isoketals) and advanced glycation (glyoxal and methylglyoxal) on the nucleophilic sites in proteins, peptides (cysteine, histidine and lysine), aminophospholipids and DNA. This aggression generates irreversible carbonylation, resulting in dysfunction of molecules, cells, tissues and organs.\textsuperscript{27} These products have been identified in patients with Alzheimer’s disease (MDA, 4-hydroxynonenal, methylglyoxal and isoketal)\textsuperscript{28} and Parkinson’s disease (MDA, 4-hydroxynenal and methylglyxol)\textsuperscript{29} and patients with atherosclerosis (isoketal).\textsuperscript{30}

The nitration of protein is measured by peroxynitrite, a powerful oxidant that may damage protein and DNA in addition to lipids. Nitration of tyrosine is of particular importance in the aging process. Tyrosine is a determinant amino acid in the synthesis of neurotransmitters (dopamine) and norepinephrine and epinephrine. Individuals with Parkinson’s disease show reduced production of dopamine.\textsuperscript{31} The nitration of protein is important, particularly in amyotrophic lateral sclerosis and atherosclerosis, but has also been detected in cerebrospinal fluid of patients with Alzheimer’s and Parkinson’s.\textsuperscript{32} Studies have found an increase in the formation of peroxynitrite, protein nitration and superoxide dismutase (SOD) (antioxidant enzyme) and the presence of 4-hydroxynonenal during aging.\textsuperscript{15}

**Antioxidant Defense System**

The function of the antioxidant system is of fundamental importance to neutralize the action of reactive substances. Antioxidants are defined as any substance that prevents or significantly attenuates the oxidation of an oxidizable sub-
strate, such as lipids, proteins, carbohydrates and deoxyribonucleic acid – DNA. The defense system is constituted of various enzymatic and non-enzymatic components. The enzymatic includes: glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rd), superoxide dismutase (SOD) and catalase. Non-enzymatic substances include endogenous antioxidants – reduced glutathione (GSH), ubiquinone (Coenzyme Q10), uric acid, alpha lipoic acid, metallothionein, albumin, transferrin and ceruloplasmin – and exogenous antioxidants, i.e., those ingested from foods – vitamin E (especially α-tocopherol), carotenoids (β-carotene, α-carotene, lycopene, lutein, zeaxanthin, astaxanthin, canthaxanthin), vitamin C, flavonoids, mannitol, aminoguanidine and pyridoxine. The antioxidants that attack peroxynitrite are GSH and vitamins C and E (all sources of electrons that can reduce the peroxynitrite). Diet is a source of nutrients that function as antioxidant cofactors even in the “endogenous” category. Classic examples are the constituents of red bell pepper (cysteine), Brazil nut (selenium) and oyster (zinc), which are necessary for the function of the GSH, GSH-Px and Zn-SOD, respectively.

In addition to the classification that differentiates enzymatic and non-enzymatic antioxidants, there is also a classification that emphasizes hydrophilic and lipophilic compartments. In the hydrophilic compartment (aqueous) ascorbate, flavonoids, albumin, bilirubin, GSH, GSH-Px, SOD, catalase and other substances are present. On the other hand, in the lipophilic compartment, carotenoids and α-tocopherol are present.

The antioxidant defense system may be verified individually or globally. Those that are verified individually (catalase, SOD, glutathione system, vitamin C, carotenoids, tocopherols, etc.) generally use high performance liquid chromatography. On the other hand, the methods that verify the defense system globally use fluorescence equipment, which can measure the hydrophilic compartment alone, or both compartments (hydrophilic and lipophilic).

It is important to underline that the beneficial action of a determined antioxidant is the result of a fine balance between antioxidants (present in the hydrophilic and lipophilic compartments) and the magnitude of the generation of ROS and RNS. If this interaction is not respected, the undesirable phenomenon known as pro-oxidant may occur. It may occur, for example, in the presence of an isolated supplementation with a single antioxidant. Given this fact, it would be understandable to imagine that the supplementation with a mixture of antioxidants would avoid the undesirable pro-oxidant action. However, unfortunately, the optimal combination and the required dose of each antioxidant to the mix are still unknown, despite great efforts in the area.

Evidence suggests that cellular oxidation may occur in the aging process, participating in the genesis of many non-transmittable chronic diseases that affect elderly individuals. Oxidative stress is considered as an independent risk factor for some diseases, such as osteoporosis. However, the ROS may be cause or an effect of diseases associated with oxidative stress, such as Alzheimer’s disease, Parkinson’s disease, strokes, multiple sclerosis and respiratory disease syndrome in adults. This increase in cellular oxidation leads to a greater incidence of cardiovascular diseases, cancer and neurodegenerative diseases. The accumulation of oxidative damage with age may occur via an increase in the generation of oxidized substances, reduction in antioxidant capacity, reduction of repairs to oxidative damage, decrease in the generation of oxidized macromolecules or a combination of these mechanisms.

Figure 1 presents the sources generating reactive substances, as well as the cellular response to the conditions that could result in oxidative stress.

A study conducted on two groups of elderly people (with and without osteoporosis) found lower plasmatic
levels of antioxidants (GSH-Px and total antioxidant capacity) in those with osteoporosis in comparison with those without this condition. Recently, antioxidant activity and the levels of lipid peroxidation have been examined in the elderly and adults resident in both rural and urban areas. The authors verified that the elderly population resident in the urban area showed lipid peroxidation – thiobarbituric acid reactive substances (TBARS) – and total antioxidant status (TAS) higher than the same population in the rural area, as well as higher SOD enzyme activity and lower GSH-Px enzyme activity. The same group of researchers examined the relationship between the level of oxidative stress and cognitive decline in elderly individuals in urban and rural areas. They confirmed previous results showing that in comparison with elderly individuals in rural areas there was greater lipid peroxidation in individuals in urban areas, together with greater cognitive decline, indicating that residing in urban areas in comparison with rural areas may represent a risk factor for an increase in lipid peroxidation and cognitive decline. The apparently unexpected antioxidant behavior, had already been pointed out by other authors, who suggested that aging should not necessarily be associated with an overall decline in antioxidant capacity as, despite the activity of some antioxidants declining with age, the activity of others remains the same or increases. However, the behavior of the antioxidant defense system in the elderly faced with the increase in oxidation of biomolecules is a thesis still subject to debate and study. In parallel, supplementation with exogenous antioxidants is controversial.

A large, randomized clinical trial evaluated 20,536 adults aged 40 to 80 in the United Kingdom and did not find a reduction in mortality, the incidence of cancer or any type of vascular diseases in 5 years of follow up with daily supplementation of vitamin E (600mg), vitamin C (250mg) and beta-carotene (20mg). Another large, randomized, double blind and controlled study with 29,133 male smokers (50 to 69 years) evaluated the effects of supplementation with alpha-tocopherol and beta-carotene (separately and in conjunction) for 5 to 8 years and did not find a reduction in the incidence of lung cancer. On the contrary, an increase in incidence of this type of cancer was identified in the participants using beta-carotene supplements. A series of studies with vitamin C and E did not show evidence of a reduction in mortality owing to cardiovascular diseases. A trial known as the Rotterdam Study evaluated how 4 years of supplementation with antioxidant vitamins had a protective effect against myocardial infarction, but it did not find evidence of protection with vitamins C and E. In 2003, a review of the data from a committee of the American College of Cardiology and American Heart Association (AHA) concluded that there was no basis for the recommendation of Vitamin C or E supplements for the prevention or treatment of coronary disease. In fact, a meta-analysis of clinical studies with Vitamin E suggested that the use of a high dose of this vitamin (more than 400 IU/day) may in fact increase mortality.

The results of a recent study have indicated that pharmacological doses (250 mg) of alpha-tocopherol may induce cardiotoxicity in healthy rats.

A recent review article also covered the issue of supplementation and oxidative stress directed at metabolic syndrome (MetS), a condition that is frequently present in the elderly. MetS consists of a set of abnormalities, such as an increase in arterial pressure, change in blood sugar, hypertriglyceridemia, low levels of HDL (high density lipoprotein) and abdominal obesity. The pathogenesis of such clinical manifestations is related to oxidative stress. Despite the strong relationship, there is not recommendation for supplementation with antioxidants in MetS, nor any diseases associated with oxidative stress, such as Alzheimer’s disease, Parkinson’s disease, strokes, multiple sclerosis and adult respiratory syndrome.

On the other hand, supplementation with selenium, zinc and vitamins A, C and E has revealed a protective effect against cognitive impairment in a cohort study conducted on elderly residents in a community. A reduction in DNA damage was also identified in elderly women after supplementation with carotenoids (lutein, lycopene and β-carotene), especially when a mixture of the three carotenoids was administered. Recent work has verified a decrease in oxidative stress (hemolysis of erythrocytes and MDA) after supplementation with vitamin E in the Chinese elderly.

Supplementation with agents that attenuate carbonylation have also been described. Experimental studies indicated that carnosine plays an important role as anti-aging molecule. Carnosine also stimulates the enzyme calcium ATPase and the calcium pump. This is important because the transmission of nerve impulses depends on calcium. In fact, neuronal toxicity generated by reactive substances is associated with a change in calcium in Alzheimer’s disease. It was shown that the addition of carnosine increases the activity of the calcium pump and the enzyme ATPase, and reduces MDA in brain cultures. The results suggest that carnosine may increase nerve transmission and reduce lipid peroxidation.
Aminoguanidine is a powerful inhibitor of the final byproducts of advanced glycoxidation, preventing the formation of glyoxal and methylglyoxal. Recently, aminoguanidine was studied in physiological aging. After 3 months of supplementation with aminoguanidine, there was a significant reduction in the concentration of serum, aorta and heart Ne-(carboxymethyl)lysine, improving the accumulation of proteins connected to Ne-(carboxymethyl)lysine and proteins connected to 4-hydroxynonenal in the serum of 344 elderly Fisher rats.

Pyridoxamine and pyridoxal are natural forms of vitamin B6, which are involved in the metabolism of amino acids and glycogen, the synthesis of nucleic acids, hemoglobin, sphingomyelin and the synthesis of other neurotransmitters such as serotonin, dopamine and GABA. Pyridoxine prevents the formation of MDA and methylglyoxal.

**Nutritional Status and Oxidative Stress**

According to the American Dietetic Association, nutritional evaluation relates to a complete approach for determining the nutritional status of the patient, with the medical history (anamnesis), social, nutritional and medication history as well as physical examination, anthropometric measurements and laboratorial data.

Nutrition is one of the greatest determiners of the health of the individual and their well being. As the elderly are particularly vulnerable to poor nutrition, the nutritional evaluation should be part of routine clinical practice, especially in fragile individuals, the sick, the institutionalized and hospitalized.

In the normal course of aging, there are physiological and biological changes that affect or are a consequence of the nutritional status of the elderly individual. One of these changes is the reduction and redistribution of body fat (accumulation in the abdominal region and reduction at the extremities) together with a reduction in muscle mass. Furthermore, there is a reduction in total body water, loss of taste and smell, reduction in the production of pepsin and hydrochloric acid and a consequent reduction in the ingestion of foods. It should be taken into account that poor nutrition, generally characterized by low weight, predisposes the elderly individual to various complications, such as reduced immunity, frailty syndrome (characterized by involuntary weight loss, exhaustion, weakness, reduction in walking speed and balanced and reduced physical activity) and an increase in morbidity and mortality.

There is still a lot of divergence about the cutoff points for the body mass index (BMI) in the elderly population. In 1994, a classification was suggested for the BMI in the elderly with a low weight of < 22 kg/m², and adequate weight between 22 and 27, and an excess weight of > 28. This cutoff point was used in previous studies. In a multicenter study (7 countries in Latin America and the Caribbean) coordinated by the Pan American Health Organization, the city of São Paulo was the Brazilian representative in the research, counting on 1894 elderly participants. The study was a great national contribution in relation to anthropometrics of the elderly. The cutoff point adopted for the BMI in the research was a low weight: ≤ 23 kg/m²; adequate weight of: 23 to 28; and excess weight of ≥ 28. However, many studies have used the WHO references (1997), even though they are not the most appropriate as they refer to adults and not the elderly.

Excess weight is an ever present reality, including among the elderly, as demonstrated in recent studies. When analyzing the prevalence of excess weight (BMI ≥ 27 Kg/m²) in two Brazilian municipalities (Santa Catarina and Bahia), the authors found this condition at 52.8% (Antônio Carlos – SC) and 28.2% (Lafaiete Coutinho – BA) in the elderly population. In another study, the prevalence of excess weight (BMI ≥ 27 Kg/m²) was found at 48% in the elderly individuals evaluated. Using a different cutoff point in the studies cited above, recent work has found a prevalence of excess weight (BMI ≥ 28 kg/m²) at 41.4% in the population aged over 65 years evaluated.

Studies have verified the association between plasmatic levels of oxidative stress markers (8-iso-PGF2α and 8-Oxo-dGuo), indicators of nutritional status (BMI) and the risk of cardiovascular disease (waist-to-hip ratio – WHR). Examining the elderly in communities, previous studies have identified an inverse association (p < 0.01) between BMI values and plasmatic levels of carotenoids. Android obesity (established in the study as a WHR ≥ 0.86 – cutoff point, was adopted by the authors through logistic regression) was associated with greater lipid peroxidation (8-iso-PGF2α in urine) in Adult Italian women (24 to 63 years) with obesity (BMI ≥ 28 Kg/m²). This same biomarker (8-iso-PGF2α) was also associated with obesity (≥ 30 Kg/m²) in the elderly (≥ 70 years) in a study conducted by Japanese researchers. Recognized as one of the complications of poor nutrition, fragility syndrome has been related to oxidation of DNA bases (8-Oxo-dGuo, a marker for oxidation of purine bases).
Examining the products of lipid (hydroperoxides) and protein (protein carbonyl) oxidation, previous research has verified that these plasmatic biomarkers are higher both in elderly individuals as well as the obese (elderly > young; elderly obese > elderly non-obese; elderly obese > young obese).80

When analyzing the nutritional status, oxidative stress and the occurrence of infections in the elderly long-term residents of institutions in the city of Botucatu (SP), the researcher concluded that low weight, identified in 40% of those institutionalized, was associated with low concentrations of α-tocopherol, albumin, and total serum protein.81 Another study identified a positive association between BMI and α-tocopherol in a follow-up study conducted on elderly Italians.82 A recent study found that the elderly (both obese and non-obese) presented lower levels of endogenous antioxidants (GSH-Px, catalase and SOD) when compared to adult individuals.80

The World Health Organization83 establishes a minimum consumption of 400 grams per day of fruit and vegetables for the prevention of chronic, non-transmissible diseases, such as strokes, cancer and cardiac diseases.84 These foods, rich in antioxidant substances, may attenuate oxidative stress and the emergence of diseases.85

A study has shown that greater ingestion of fruits and vegetables is associated with lower lipid peroxidation, as well as a higher plasmatic concentration of lipophilic antioxidants (β-carotene, α-tocopherol and lycopene).86 Recent work has verified the positive associated between consumption of foods rich in antioxidant substances and plasmatic levels of α-tocopherol, vitamin C and total antioxidant activity.87 On the other hand, a positive correlation was not verified between the ingestion of foods that are a source of β-carotene and plasmatic levels of this antioxidant in elderly residents of a community in Ireland.88 Furthermore, it should be reiterated that the absorption of certain nutrients (for example, vitamin B12, calcium, iron and β-carotene) can be harmed by the occurrence of atrophic gastritis (condition in which stomach acidity is reduced).89,90 The absorption of the nutrients above is dependent on an acidic pH.91

An interventional study with an elderly community evaluated the effects of the ingestion of fruits and vegetables (5 daily portions for 3 months) on the plasmatic markers of oxidative stress (β-carotene, α-tocopherol, MDA). The results showed that although plasmatic levels of β-carotene (p<0.05) and α-tocopherol (p>0.05) had suffered an increase, the levels of MDA also increased, albeit slightly. The authors concluded that the surprising behavior of the MDA may be the result of insufficient ingestion of antioxidants from the diet and could be associated to the insignificant (p>0.05) increase in the levels of α-tocopherol.82 This same biomarker (MDA) was analyzed in a study conducted in Spain. The authors identified that the ingestion of cooked vegetables above 154.9 grams and moderate ingestion of wine (150 mL) are protectors against oxidative damage (plasmatic MDA).93

**Conclusion**

There is a relationship between oxidative stress (markers of lipid peroxidation, proteins and DNA, endogenous antioxidants and total antioxidant activity) and nutritional status (poor nutrition, excess weight and exogenous antioxidant) in elderly individuals.

Although an increase in oxidative stress has been proven in chronic diseases associated with aging, up to now there is no consistent clinical evidence that supplementation with antioxidants can reverse or attenuate the damage resulting from oxidative stress. Thus, supplementation with antioxidants is not recommended for the treatment of age related diseases. The indiscriminate use of antioxidant supplements should be discouraged due to the undesirable pro-oxidant phenomenon that has been widely described with the use of such supplements. The beneficial action of a determined antioxidant is the result of a fine synergy between the antioxidants present in the hydrophilic and lipophilic compartments. Therefore, supplementation with a single antioxidant may cause irreversible impairment of the antioxidant defense system. Elderly individuals with chronic diseases or otherwise should be encouraged to consume a varied diet containing foods that are sources of antioxidant substances. Indeed, this consumption should be encouraged in all stages of life. The maintenance of a healthy weight (BMI between 23 and 28 kg/m²) should also be stimulated.

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**Resumo**

Associação entre estresse oxidativo e estado nutricional de idosos.

O envelhecimento é um processo dinâmico e progressivo que se caracteriza pela ocorrência de modificações morfológicas, bioquímicas, funcionais e psicológicas do organismo. O objetivo do presente artigo é fornecer conceitos atualizados sobre estresse oxidativo, abordando sua importância no envelhecimento, assim como os
tado nutricional e suplementação com antioxidantes (substâncias que evitam ou atenuam a oxidação de substâncias oxidáveis, como lipídios, proteínas, carboidratos e o ácido desoxirribonucleico) na população geriátrica. Evidências sugerem que existe uma relação inversa entre estresse oxidativo e estado nutricional em indivíduos idosos. Embora seja comprovado o aumento do estresse oxidativo nas doenças crônicas associadas ao envelhecimento, como doença de Parkinson e doença de Alzheimer, não há, até o momento, evidências clínicas consistentes que comprovem a eficiência da suplementação com antioxidantes contra o estresse oxidativo. Nesse contexto, a suplementação não é recomendada. Por outro lado, idosos devem ser encorajados a ingerir alimentos antioxidantes, como, por exemplo, frutas e vegetais. A manutenção do peso dentro da faixa de normalidade (índice de massa corporal entre 23 e 28 kg/m²) também deve ser estimulada.

**Unitermos:** envelhecimento; estresse oxidativo; estado nutricional; antioxidantes; suplementos dietéticos.

**References**

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