# ANTIOXIDANTS – CLINICAL ASPECTS

***(REVIEW)***

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**SUMMARY**

*Interest in nutrition and food consumption in advanced countries has become an important instrument of active care for maintaining the population's health. Basic orientation in food composition and in topical knowledge about the effectiveness of individual nutrients in the human organism form an indispensable part of nutrition and health consciousness and it can significantly facilitate and improve activity in both professionals and non-professionals, or it can correct some subjective opinions and deep-rooted imaginations. At the end of the last century progress in molecular biological research led to better knowledge about previously unknown functions of nutrients in the metabolism and also knowledge about relationships among the individual nutrients. The interest of research institutes was concentrated on selected nutrient groups and also on vitamins. Attention was focused on verification of the role of vitamin E and β carotene in strengthening the antioxidative capacity of the organism and immunity system as prevention against degenerative diseases, atherosclerosis and tumorous diseases. Attention was attracted to the fact that folacin acts preventively against defects of the neural tube and against hyperhomocysteinemia as an independent atherosclerosis risk factor. Also the data about the organism's needs, for example vitamins, are mostly not quite complete, especially in the sphere of detailed knowledge of their preventive effect concerning the so-called civilization diseases, their use in the organism and the reaction of the organism to their insufficient or redundant supply.*

*Key words*: vitamins, reactive oxygen species, civilization diseases, antioxidants

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Many famous theoretical and clinical research institutes have intensively examined the negative role of oxidative stress and the protective effect of vitamins, possibly micronutrients with antioxidative effect. Researchers´ interest in the possible participation of antioxidative stress in the etiopathogenesis of civilization degenerative diseases is still increasing. Free radicals play an important role in the process of cellular senescence, in the pathogenesis of chronic diseases, in carcinogenesis, in the pathogenesis of glaucoma and in the acceleration of atherosclerotic changes of the vascular parries, which is mostly followed by the development of cardiovascular diseases. In the acceleration of the atherosclerosis processes the decisive role is probably played by the oxidation of low-density lipoproteins (LDL).

Free radicals are defined as chemical species that possess one or more unpaired electrons. The term "Reactive Oxygen Species" (ROS) collectively describes free radicals such as $O_2^-$, OH, and other non-radical oxygen derivatives such as hydrogen peroxide ($H_2O_2$). These reactive oxygen intermediates may participate in

reactions that give rise to free radical species. Unstable free radical species attack cellular components, causing damage to lipids, proteins and DNA that can initiate a chain of events, which results in the onset of disease.

Free radicals can originate in various ways: mostly by biochemical redox reactions involving oxygen, which occur as part of the normal metabolism, e.g. $O_2^-$, NO, $H_2O_2$; by phagocytes, as part of a controlled inflammatory reaction, e.g. HOCl, $O_2^-$; occasionally in response to exposure to ionising radiation, UV light, environmental pollution, cigarette smoke, excessive exercise and ischaemia, e.g. $O_2^-$, OH, ROO.

The role of free radicals in disease is very important. Many free radicals and ROS have been implicated in disease development: a highly reactive radical which can attack all biological molecules; a less reactive radical which can travel in the blood and attack a number of biological targets; NO acts on smooth muscle cells in vessel walls causing relaxation. Transition metals are thought to promote free radical reactions, including the Fenton reaction, which results in the formation of hydroxyl radicals.

A number of free radical species fulfil physiologically important roles within the body, for example, superoxide and nitric oxide function as second messengers. However, free radical levels in the body must be carefully controlled as they are highly reactive and can cause tissue destruction. Antioxidants help regulate and control the levels of free radicals at the required physiological concentrations. When the production of free radicals and their removal by the antioxidant system becomes unbalanced, tissue damage and disease can occur.

The body possesses a number of mechanisms both to control the production of ROS and to limit or repair the damage to tissues. The integrated antioxidant system comprises several components to prevent the formation of new ROS, e.g. caeruloplasmin (Cu), metallothionine (Cu), albumin (Cu), transferrin (Fe), ferritin (Fe) and myoglobin (Fe). Scavenging antioxidants remove ROS once formed, thus preventing radical chain reactions: enzymes superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase, metalloenzymes and small molecules ascorbate (vitamin C), tocopherol (vitamin E), carotenoids, glutathione, bilirubin, uric acid, lycopene, flavonoids and other micronutrient elements such as zinc and selenium. Repair enzymes repair or remove ROS-damaged biomolecules. These include DNA repair enzymes and methionine sulphoxide reductase. The antioxidant defence system is a very complex network of interactions, synergisms and specific tasks for each antioxidant. The steady-state balance between prooxidants and antioxidants in the human body may be disturbed by the depletion of antioxidants, whether this occurs endogenously or through a diminished dietary intake. Oxidative stress is defined as the imbalance between oxidants and antioxidants in favour of the oxidants, potentially leading to damage. Oxidative stress is potentially harmful to cells. Normally, antioxidant mechanisms naturally inherent in an organism scavenge ROS; however, under conditions of excessive oxidative stress, cellular antioxidants may be depleted, and ROS can damage cellular components and interfere with cellular activity.

The role of antioxidants and reactive oxygen species (ROS) in chronic degenerative disease has gained recognition since the implication of ROS in the etiology of several degenerative diseases including cardiovascular disease, cancer, neurodegenerative disorders and aging. Current research in this area has led to a new paradigm of human health, with a shift towards emphasis on disease prevention. Oxidative stress is thought to play an important role in atherosclerotic vascular disease. Thus, dietary antioxidants such as ascorbate (vitamin C) and α-tocopherol (the chemically and biologically most active form of vitamin E) can protect against the development and progression of atherosclerosis in experimental models (1, 2).

Numerous observational studies have shown an inverse association between antioxidant intake or body status and the risk of cardiovascular diseases (3, 4, 5). However, several clinical trials, such as the recent GISSI and HOPE trials, have found no benefits of vitamin E supplementation on cardiovascular disease risk (6, 7). A major caveat with observational studies is that they can only show associations and not causal relationships. Clinical trials have a number of limitations, too, such as a relatively short period of antioxidant treatment with a single dose only, and the fact that antioxidant treatment of patients with advance disease (secondary prevention) may not provide information relevant to disease prevention in healthy individuals (primary prevention). For example, both the GISSI and HOPE trials were secondary prevention trials in which > 75% of all participants were treated with aspirin or other antiplatelet agents, and many participants also received β-blockers, lipid-lowering agents, and calcium channel blockers. It is doubtful whether vitamin E can exert beneficial effects above and beyond these standard therapies. In addition, as explained in the current article, vitamin E supplements alone may not be beneficial but may have to be co-administered with vitamin C to effectively lower oxidative stress. (8, 9, 10).

Measuring the individual components of the antioxidant system has provided important information about the defences against free radical attack. However, an overall measurement of total antioxidant capacity (TAC) is desirable owing to the complexity of the antioxidant system. Several methods have been developed to assess the total antioxidant capacity of human serum or plasma because of the difficulty in measuring each antioxidant component separately and the interactions among different antioxidant components in the serum or plasma. Comparisons between results obtained by various methods are not always straightforward (11, 12, 13). The methods based on spectrophotometer measurement of TAC seem to be the most widely applicable and most frequently used.

Whereas many epidemiological and clinical studies use plasma antioxidant concentrations as a measure of antioxidant status, such values require cautious interpretation, as they may not necessarily reflect concentrations in target tissues where oxidative stress is greatest. For example, plasma vitamin E concentrations vary little over a wide range of dietary intakes, whereas concentrations in tissues such as liver, heart and adipose tissue can respond much more markedly. In addition, certain carotenoids can accumulate in specific tissues and therefore their concentration may not reflect those in plasma. Moreover, transient increases in plasma antioxidant concentrations following consumption of antioxidant-rich food and beverages may also give misleading information about the habitual antioxidant status of an individual.

There are many methods in the literature for assessing the antioxidant activity of compounds in vitro and in vivo (14). Most methods (in vitro) assess the ability of compounds to donate H or electron to an oxidizing species introduced into the assay system. Such approaches can give useful information on the attributes required by the compound to function as an antioxidant (e.g. stan-

dard stopped-flow electron spin resonance spectrometry). Most compounds will act as antioxidants if the oxidizing species is strong enough. Results from such in vitro assays should be treated with caution, and certainly not to ascribe them the antioxidant function in the biological system. Many compounds with in vitro antioxidant activity may have little in vivo relevance because they are not bio-available. These chemicals may not be extracted from the food matrix in the gut and may be susceptible to destruction by gut flora. In addition, absorption through the intestinal wall may not take place, or they may be rapidly metabolized by the liver and excreted via the bile. For example, the fruits and vegetables normally consumed in the human diet contain more than forty different types of carotenoids, but only fourteen of these have as yet been identified in tissue and plasma. Similarly, although the process of absorption of all eight tocopherol homologues in our diet is similar, the α-form predominates in blood and tissue, despite some of the other forms being more potent antioxidants in chemical systems. This situation is due to the action of binding proteins, which preferentially select the α-form. These selective mechanisms explain why vitamin E homologues have markedly different antioxidant abilities in the biological system, and illustrate the important distinction between the in vitro antioxidant effectiveness of a substance in the stabilization of, for example, a food product and its in vivo potency as an antioxidant. Thus, the most important approach to assess whether an antioxidant has a role in a biological context is to detect its presence and assess its effects in the organism.

The measured antioxidant capacity of antioxidant compounds of a sample depends on which technology and which free radical generator or oxidant is used in the measurement (15, 16, 17). Therefore, the comparison of different analytical methods constitutes a key factor in helping investigators to choose a method and to understand the results obtained using the method.

The objective of the presented pilot study was to compare level of antioxidants in individuals, antioxidant status and biochemical and anthropometrical parameters.Total antioxidant status, antioxidant vitamins (A, E, C and β-carotene), antioxidant trace element (Se), lipid parameters (total cholesterol, HDL and LDL-cholesterol, triacylglycerols, atherogenic index) and anthropometrical parameters (BMI, waist circumference) were measured in a selected population. Plasma total antioxidant status was measured using a free radicals kit supplied by Sevapharma,a.s. Lipid-soluble antioxidants (vitamin A, E and β-carotene) were measured in hexane extracts of EDTA-anti-coagulated blood plasma. The conditions for HPLC assay with UV/VIS detector were specifically optimized for the separation of each vitamin. Total serum cholesterol, HDL and LDL-cholesterol were determined enzymatically (Bio-La-test, Czech Republic).

The results of the study are still not complete but the details of them were statistically compiled in detail. When assessing the antioxidative capacities of the human organism, it is necessary to pay attention not only to the correlation between the serum concentrations of antioxidants and the level of lipids in serum, but also to the correlation between the age of the examined subjects. The mutual relations of the observed parameters are statistically assessed in a correlative matrix that proves a statistically significant positive correlation of the vitamin E serum levels and BMI, triacylglyceroleamia or waist circumference. A statistically significant negative correlation was proved between ascorbemia and the age, BMI or waist circumference of the examined subjects. A negative correlation was found between the β-carotene level and the BMI or waist circumference, too. A statistically significant positive correlation between the levels of vitamin E and the β-carotene and free radical was observed. When at the same time the serum concentration of vitamin E and β-carotene increases, the level of the free radicals is brought down.

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