POTENTIAL DANGERS WITH OVERUSE OF ANTIOXIDANT ENZYMES AS NUTRITIONAL SUPPLEMENTS : HOW MUCH HAVE WE REVEALED?

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ABSTRACT

OBJECTIVE: Even though it is known that antioxidants can be obtained from food (mainly fruits and vegetables) the consumption of antioxidant supplements in the general population is broad in extent. The results of epidemiological studies where people were treated with antioxidant supplements are inconclusive and contradictory; however, they are numerous and comprise an intensive field of research. Less is known about the supplementation of antioxidant enzymes because randomized clinical trials are limited, and the potential harmful effects of their overconsumption have only recently started to be investigated. The aim of this brief review is to investigate the potential dangers of antioxidant enzymes overuse (high doses obtained from artificial oral supplements or intravenous infusion) on people's health status.
METHODS: Original articles were searched via the online databases PubMed and Google Scholar published between 1990 and 2012.
RESULTS: Data indicate that high doses of supplementary antioxidant enzymes could act as double-edged swords in cellular redox state as they present health beneficial effects at physiologic doses versus deleterious effects at high doses. Since randomized clinical trials with regard to antioxidant enzymes are scarce there is no official recommended dosage, and if the dose is set too high, safety problems are likely to result. Excessive antioxidant action could adversely affect key physiological processes.
CONCLUSIONS: The use of antioxidant enzymes is not an alternative to regular consumption of fruits and vegetables. Antioxidant compounds within fruits and vegetables may be considered as being more safe and healthy compared to isolated, high doses, such as present in nutritional supplements. Further evidence-based research should focus on the need to answer the questions regarding what effects dose and environment have on pro-oxidant/antioxidant mechanisms before recommending nutritional supplement interventions with antioxidant enzymes.

KEYWORDS : diet, superoxide dismutase , glutathione peroxidase, catalase

Introduction

All aerobic organisms present mechanisms to neutralize the harmful effects of free radicals. Reactive oxygen (ROS) and nitrogen species (RNS) are produced physiologically in low levels due to the normal metabolism of body cells. Apart from the protection offered by the cell's structural construction or the daily consumption of dietary/exogenous antioxidants (as flavonoids in whole natural food or artificial supplements) two main lines of endogenous defense against the detrimental effects of ROS/RNS exist in human serum (Delimaris et al., 2007). The first line of defense consists of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase). The second line of defence mechanisms includes a series of non-enzymatic systems containing lipophilic and hydrophilic antioxidants such as alpha-tocopherol, ascorbic acid, beta-carotene, uric acid, albumin, transferrin, haptoglobin, hemopexin, ceruloplasmin, glutathione, ubiquinol and billirubin (Delimaris, 2008; Delimaris et al., 2008). The two endogenous defense lines, connected together in some cases, result in the regeneration of participating molecules without producing new free radicals.

Despite the fact that antioxidants can be obtained from food (mainly fruits and vegetables) the consumption of antioxidant supplements in the general population is widespread. A growing number of researchers have noticed that there is an industry-driven public obsession with antioxidants, which are equated to safe, health-giving molecules to be swallowed as mega-dose supplements or in fortified foods (Gutteridge, 1999). It is evident that there were always good ideas in biomedical research (sometimes based on reasonable pathobiochemical mechanisms), which, however, were not accompanied by corresponding good results in the “arena” of randomized clinical trials. The clinical studies that indicate the effectiveness of the supplementary administration of antioxidant vitamins and minerals in humans are rather conflicting (Bjelakovic et.al., 2007; Hollman et.al., 2011; Gutteridge &

Halliwell, 2010); however, they are numerous and comprise an intensive field of research. This is not the same for the supplementation of antioxidant enzymes because similar studies at the clinical level (randomized clinical trials) are scarce and there is no official recommended dosage. If the dose is set too high, safety and tolerability problems are likely to result, while selecting too low a dose makes it difficult to establish adequate efficacy (Bretz et.al.,2010). Moreover, in recent years there is an expanding concern regarding the adverse effects of antioxidant vitamins overuse or hypervitaminosis (Herbert, 1994; Bouayed & Bohn, 2010 ; Horrobin, 2001; Schwartz, 1996; Seifried et.al., 2004) but there are only a few studies with respect to the potential harmful effects from the overconsumption of antioxidant enzymes in humans.

The aim of this brief review is to investigate the potential dangers of antioxidant enzymes overuse (high doses obtained from artificial oral supplements or intravenous infusion) on people's health status. Original articles were searched via Google Scholar and PubMed published between 1990 and 2012. All papers identified were English or Greek language full text papers. The reference lists of identified articles for other relevant papers or textbooks were also searched.

The normal metabolism of antioxidant enzymes in humans

Superoxide dismutase

The superoxide dismutase (SOD) catalyze the conversion of the peroxide anion (O_2^-) to hydrogen peroxide (H_2O_2) according to the reaction:

$$M^{(n+1)} + \text{-SOD} + O_2^- \rightarrow M^{n+}\,SOD + O_2 \quad [1]$$

$$M^{n+}\text{-SOD} + O_2^- + 2\,H^+ \rightarrow M^{(n+1)}\,SOD + H_2O_2 \quad [2]$$

where M = Cu (n=1) ; Mn (n=2) ; Fe (n=2)

The H_2O_2 produced can then be removed by catalase or glutathione oxidase. There are three forms of superoxide dismutase in human tissues , each of which has a specific subcellular location and different tissue distribution : a) Superoxide dismutase with copper and zinc (CuZnSOD): located in cytoplasm and organelles of almost all human cells. The molecule has two protein subunits. Each subunit contains a catalytically active atom of copper and zinc (Delimaris, 2008 ; Iakovidis et al., 2011) b) Superoxide dismutase on manganese (MnSOD) is located in mitochondria of almost all cells (Kamiński et.al., 2012). The molecule consists of 4 protein subunits each of which contains a manganese atom. The amino acid sequence of MnSOD is quite different from that of CuZnSOD. Furthermore, the enzyme activity of MnSOD is not inhibited by the presence of cyanide, as in CuZnSO (Delimaris, 2008). The role of serum concentration of Mn-SOD as a potentially useful biomarker for estimating cardiac mitochondrial damage and for diagnosing acute myocardial infarction has been under investigation (Usui et al., 1991) c) Extracellular superoxide dismutase (EC-SOD): it contains copper and zinc, but differs from CuZnSOD. The extracellular SOD is synthesized and secreted only by specific cell types, including fibroblasts and endothelial cells. It is released into the surface of vascular endothelium after administration of injected heparin. The EC-SOD may play a role in regulating vascular tone, because it removes the O_2^- anions that inactivate nitric oxide (Delimaris, 2008). EC-SOD is the major SOD isoenzyme in human plasma and lymph. Oral administration of antioxidant health foods containing SOD originating in plants has the effect of lowering the activity and content of SOD in the blood (Kinoyama et al., 2007).

Catalase

Catalase catalyzes a two-step conversion of hydrogen peroxide to water and molecular oxygen:

$$\text{catalase-Fe (III)} + H_2O_2 \text{ ===> intermediate product I} \quad [3]$$

$$\text{intermediate product I} + H_2O_2 \text{ ===> catalase-Fe (III)} + 2\,H_2O + O_2 \quad [4]$$

Catalase consists of 4 protein subunits, each of which contains a heme group and one molecule of NADPH. It is located within cells in the peroxisomes, which also contain most of the enzymes that produce H_2O_2 levels. However, the actual range of the intracellular concentration of catalase remains unclear, because peroxisomes rupture easily during the laboratory processing of cells. The highest enzyme activity is present in liver and erythrocytes, but smaller amounts of catalase present in all tissues (Delimaris, 2008). The raised human serum catalase activity has been investigated as a diagnostic tool in acute pancreatitis, hemolytic disease and in some liver diseases (Goth et.al., 1991).

Moreover, low levels of catalase could play a role in the greying process of human hair. Low concentration of serum catalase results in an elevation of hydrogen peroxide. This causes the hydrogen peroxide to bleach the hair from the inside out (Wood et.al., 2009).

Glutathione peroxidase

Glutathione peroxidases catalyze the oxidation of the reduced glutathione form (GSH) to the disulfide form (GSSG) and reduce levels of lipid hyperoxides (ROOH) in the reaction:

$$2\ GSH + ROOH \text{ ====> } GSSG + ROH + H_2O \qquad [5]$$

and H_2O_2 in the reaction:

$$2\ GSH + H_2O_2 \text{ ====> } GSSG + 2\ H_2O \qquad [6]$$

There are two major types of glutathione peroxidase. One type (Gpx) is dependent on selenium which catalyzes the degradation of lipid peroxides and H_2O_2. The Gpx consists of 4 protein subunits, each of which contains 1 atom of selenium. Glutathione itself reduces the atom of selenium and the reduced form of enzyme then interacts with the lipid peroxides or H_2O_2. The other type of glutathione peroxidase is the peroxidase of phospholipid peroxides (PhGPx), which is also dependent on selenium. The PhGPx appears to act mainly to peroxides produced by: a) phospholipids which are bound to membranes and b) oxidative damage to DNA . To maintain the balance between GSH and GSSG, the oxidized form of glutathione (GSSG) is reduced to GSH by the glutathione reductase with NADPH as a cofactor. The required amount of NADPH is supplied by the metabolic pathway of pentose phosphate (Delimaris, 2008).

$$GSSG + NADPH + H^+ \text{ ====> } 2\ GSH + NADP^+ \qquad [7]$$

The glutathione peroxidase found in human serum is considered to be synthesized mainly in the kidneys. Although it has a broad distribution in almost all cells (in mitochondria and cytoplasm) its highest levels are found in hepatocytes (Delimaris, 2008).

Potential harmful effects due to overdose of antioxidant enzymes

As a defense against the detrimental effects of ROS/RNS a growing number of individuals use high doses of antioxidant enzymes (artificial supplements), and since there are few human studies, it is difficult to know the proper dosage to take. Selecting a dose too high may result in unacceptable safety problems, while selecting a dose too low may lead to ineffectiveness (Bretz et.al., 2010). Considering the fact that antioxidant enzymes themselves (as oral supplements) would be degraded in the digestive tract, their intravenous infusion is much more effective. The question arises as to whether an overdose of antioxidant enzymes could harm human health. In recent years, not only the poor preventive or therapeutic results, but also the potential dangers from the overconsumption of antioxidant enzymes (and other antioxidants) have been underlined by many researchers (Williams & Fisher, 2005; Shihabi et.al., 2002; Schwartz, 1996; Horrobin, 2001; Bjelakovic et.al., 2007). Widespread use of antioxidant supplements/enzymes has failed to quell the current pandemic of cancer, diabetes, and cardiovascular disease or to stop or reverse the aging process (Howes, 2006). In transgenic mice it was found that overexpression of the major antioxidant enzymes (CuZnSOD, catalase, or combinations of either CuZnSOD and catalase or CuZnSOD and MnSOD) is insufficient to extend lifespan (Poljsak, 2011). In humans, it has been shown that over-consumption of antioxidant enzymes could down-regulate their endogenous production (Gutteridge & Halliwell, 2010). An interesting study indicated that blood superoxide dismutase (SOD) decreased following oral administration of plant SOD to healthy subjects (Kinoyama et al., 2007).

Harms have been reported from pharmacological amounts of antioxidant supplements (Herbert, 1996 ; Gutteridge, 1999). Low dose mixtures, as in multivitamin/multimineral tablets, can sometimes do good, but may be beneficial only for those members of populations whose diet and lifestyle are so bad that they are deficient in certain micronutrients (Gutteridge & Halliwell, 2010). Nevertheless, the results of epidemiological studies where people were treated with antioxidant supplements are inconclusive and contradictory. Recent data indicate that antioxidant enzymes/supplements (although highly recommended by the pharmaceutical industry and taken by many individuals) do not offer adequate protection against oxidative stress, oxidative damage or increase the lifespan. (Poljsak, 2011).

Excessive antioxidant action can adversely affect key physiological processes (Dundar et.al., 2000; Carr, 1999 ; Bjelakovic et.al., 2007). Even if an antioxidant could not act as a pro-oxidant under physiological conditions, it has been suggested that high concentrations of antioxidants may be harmful by destroying the "redox balance/homeostasis" in cells or tissues. Living organisms have developed mechanisms for the advantageous use of free radicals. Important physiological functions that involve free radicals at moderate concentrations or their derivatives include enhancement of signal transduction from various membrane receptors. Flooding the biological system with antioxidants or the overexpression of antioxidative enzymes may be just as detrimental as excessive exposure to free radicals (Droge, 2002). In fact, various ROS-mediated actions protect cells against ROS-induced oxidative stress. Very high doses of antioxidants could produce harmful effects on cellular signaling processes. For example, ROS can induce the release of arachidonic acid and activate tyrosine kinases and mitogen-activated protein kinases, which are critical components of many intracellular signaling cascades, including those required for cell survival and growth. Thus antioxidant supplementation could potentially be harmful to those tissues that are not subjected to substantial oxidative stress (Shihabi et.al., 2002). Moreover, over-consumption of antioxidants (including enzymes) could down-regulate important endogenous antioxidants, depress parts of the immune system, or perhaps increase microbial damage or the normal cellular protective responses to tissue damage (Gutteridge & Halliwell, 2010). Double-edged effects of exogenous antioxidants on cellular responses including oxidative, nitrosative and dicarbonyl metabolisms and other pathways depending potentially on their concentrations: physiologic doses leading to beneficial effects whereas high doses may result in harmful effects (Bouayed & Bohn, 2010).

The effects of antioxidant enzymes/supplements particularly in relation to cancer, should not be overemphasized because the use of those might be harmful for some cancers (Myung et.al., 2010). For example, a significant body of evidence shows that ROS within cells act as secondary messengers in intracellular signalling cascades which induce apoptosis and can therefore function as anti-tumourigenic species (Valko et.al., 2007). Antioxidant overdose may promote the growth of cancer as it is well documented that free radical mechanisms are involved in the elimination of cancer cells. Agents that generate free radicals have repeatedly been shown to kill cancer cells selectively while sparing normal cells. Antioxidants block this cancer-killing effect and accelerate cancer growth both in vitro and in vivo (Horrobin, 2001). For example, a population of smokers is expected to include more cases of subclinical cancer than is a population of nonsmokers, so when such a population is treated with antioxidants, they may accelerate the growth of hitherto undetected cancers (Horrobin, 2001). Nutrients inhibit the continual growth of transformed clones of cells through their prooxidant activity. In contrast, when an antioxidant activity occurs in transformed cells an enhanced growth may result (Schwartz, 1996). It has to be mentioned that oxidation includes distinct biochemical reactions, and it is overly simplistic to lump them into a unitary process that affects all cell types and metabolic pathways adversely (Williams & Fisher, 2005).

Conclusions

Free radicals play an important physiological role in humans. They participate in the metabolism of endogenous and exogenous lipids, in cellular respiration, in the production of prostaglandins and leukotrienes by arachidonic acid, in phagocytosis and in the immune response ((Bouayed & Bohn, 2010). Also, free radicals themselves play a role as scavengers for other free radicals (final reaction of free radical free radical). Perhaps free radicals (at least for most of our lifespan) do not pose a great threat to our wellbeing unless we expose ourselves to an excess of free radical-generating agents such as cigarette smoke or ionising radiation (Gutteridge & Halliwell 2010; Gutteridge 1999). Exogenous antioxidants (antioxidant enzymes, vitamins, minerals) could act as double-edged swords in cellular redox state as they present health beneficial effects at physiologic doses versus deleterious effects at high doses (Bouayed & Bohn, 2010). Since there are few clinical studies, it is difficult to know the proper dosage to take and therefore selecting a dose too high may result in health problems (Bretz et.al., 2010). Guidelines for diet should adhere closely to what has been clinically proved, and by this standard there is no basis to recommend high doses of antioxidant enzyme supplements (Williams & Fisher, 2005). We must realize that the use of antioxidant enzymes is not an alternative to regular consumption of fruits and vegetables. Compounds within fruits and vegetables may be considered as being more safe and healthy compared to isolated, high doses, such as present in nutritional/dietary supplements. Foods rich in antioxidants include all fresh and seasonal fruit and vegetables (peppers, apples, onions, pineapple, dark leafy vegetables, flaxseeds, walnuts, pumpkin seeds, and olives) and olive oil (Poljsak, 2011). Two main factors seem to be predisposing for the beneficial activities of plant

foods: (1) the lower concentration of nutrients and non-nutrients in whole food natural food matrices and (2) the additive or synergistic effects of complex mixtures of phytochemicals and nutrients. Supplementation approaches do generally not take into account both aspects, which could explain the controversial results observed in supplementation studies (Bouayed & Bohn, 2010). It is likely that several antioxidants are still unknown. Moreover, the combination of antioxidants in fruits and vegetables causes their regeneration and enhance their defense against reactive oxygene species (Poljsak, 2011). Consumption of vegetables and plant-derived foods and beverages has a positive impact on the prevention of age-related diseases such as heart disease, cancer and atherosclerosis as well as for longevity (Poljsak, 2011). Multivitamin / multimineral tablets may be beneficial only for those individuals that they are deficient in certain micronutrients (Gutteridge & Halliwell, 2010).

In conclusion, the field of antioxidant research is controversial and confusing to many clinicians because the results of some studies conflict with others, making simple conclusions as to efficacy and safety difficult. An evidence-based approach should focus on the need to answer the myriad questions surrounding pro- and antioxidative mechanisms of action associated with antioxidant use, and what effects dose and environment have on these mechanisms before recommending nutritional or nutritional-pharmacologic interventions (Seifried et.al., 2004). The key to the future success of decreasing oxidative-stress-induced damage should thus be the suppression of oxidative damage without disrupting the wellintegrated antioxidant defense network (Poljsak, 2011).

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