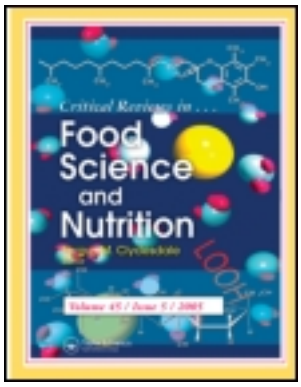

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Antioxidants in Food: Mere Myth or Magic Medicine?

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Antioxidants in Food: Mere Myth or Magic Medicine?

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The powerful action of antioxidants in preventing premature lipid oxidation in food suggests that the same compounds, when consumed with the daily diet, could unfold antioxidative/anti-aging effects in the human body. Therefore, it has been hypothesized that antioxidants are helpful in preventing various diseases. More detailed chemical and physiological examination of antioxidants shows, however, that the extrapolation of in vitro data to in vivo behavior may be misleading. Indeed, such a procedure fails to take into account the mismatch between most in vitro models (e.g., cell cultures) and in vivo systems. For example, the physiological relevance of pro-oxidative and other physiological activities of antioxidants have been largely underestimated. Actually, contrary to the antioxidant hypothesis, clinical trials testing the health benefits of dietary antioxidants have reported rather mixed or negative results. Many clinical studies have not taken into account the nutrikinetic and nutridynamic nature of antioxidants. Further, oxidative stress is not only an inevitable event in a healthy human cell, but responsible for the functioning of vital metabolic processes, such as insulin signaling and erythropoietin production. In the light of recent physiological studies it appears more advisable to maintain the delicate redox balance of the cell than to interfere with the antioxidant homeostasis by a non-physiological, excessive exogenous supply of antioxidants in healthy humans.

Keywords Antioxidant, in vitro study, cell culture, intervention study, reactive oxygen species, cancer

INTRODUCTION

Antioxidants, such as *tert*-butyl-4-hydroxyanisol, 3,5-di-*tert*-butyl-4-hydroxytoluol, members of the vitamin E family, ascorbic acid, and citric acid, are frequently used food additives (Table 1). They protect items which are susceptible to oxidative degradation and prevent them from a premature loss of quality. Their efficiency, even at dosages of 0.1% or lower, has been proven since a long time (Pokorny, 2007; Pokorný, 2007) and explained by either.

- scavenging of free radicals (phenols may donate a hydrogen atom to a fatty acid radical, forming a reconstituted fatty acid and a more stable phenol radical, thus breaking the chain reaction)
- low redox potential (sacrificing themselves in favor of other, less easily oxidized food constituents) or
- complexing of catalytic trace metal ions (which would otherwise accelerate lipid oxidation by facilitating electron transfer reactions).

Supported by advances in high-performance liquid chromatography coupled to mass spectrometric techniques and rapid bioassays, such as ORAC (oxygen radical absorbance capacity), a wealth of compounds with anti-oxidative properties has been detected and identified in foods including fruits, vegetables, oilseeds, nuts, teas, cocoa, coffee, spices, meat, and cereals (Hall, 2001). Starting in the late 1990s food producers have transformed this knowledge into the idea that antioxidants in food could also protect sensitive constituents of human cells from oxidation, thereby obviating severe diseases, such as atherosclerosis, cancer, and cataract (Frei, 2004).

This idea met the spirit of the time: Food was no longer well marketable using the same old nutritional and quality promises; food was no longer regarded as a source of just energy and building blocks but as a carrier of "functional ingredients" (Hahn et al., 2002; Hahn and Ströhle, 2004). To equalize food and a human body in terms of susceptibility to antioxidants appears so convincing and established that a recent review

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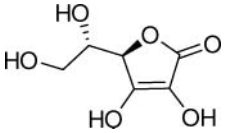
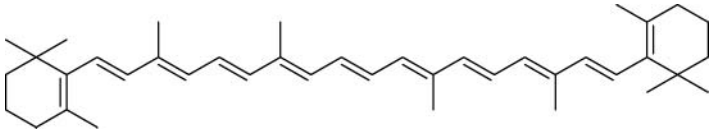
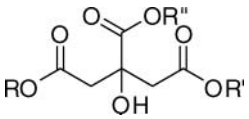
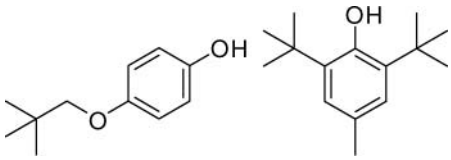
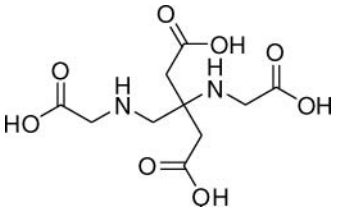
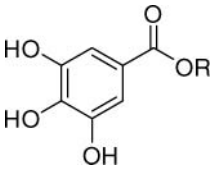
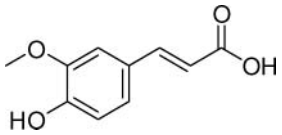
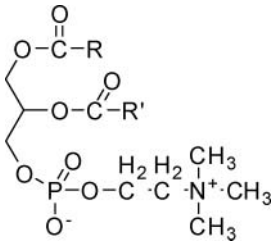
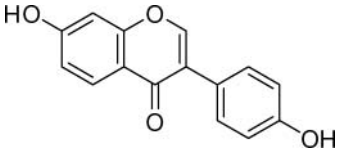
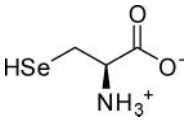
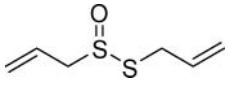
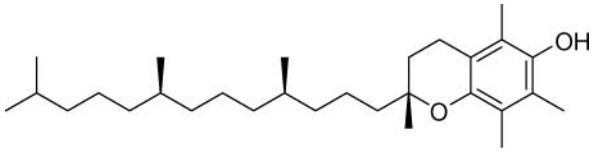
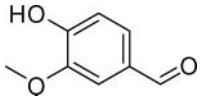
Table 1 Common antioxidants added to or present in food

Antioxidant	Representative/chemical structure
amino acids and certain peptides	
ascorbates, isoascorbic acid and ascorbyl palmitate	
carotenes and carotenoids	
citrates, lactates, fumarates	
t-butyl-4-hydroxyanisol, 3,5-di-*t*-butyl-4-hydroxytoluol	
ethylene diaminotetraacetate	
gallic acid esters and other phenol carboxylic acids	
non-enzymatic browning (*Maillard* reaction) products, ketoenols, melanoidines, pronyl-lysine phenols (simple phenols, phenol carboxylic acids, phenylpropanes, flavanoids, di- and oligomers)	

(Continued on next page)

Table 1 Common antioxidants added to or present in food *(Continued)*

Antioxidant	Representative/chemical structure
phosphates (di-, tri-, oligo-), phosphatidylcholin, phosphatidylethanolamine	
phythoestrogens	
sulfites (mono-, hydrogen-, di)	
selenium (as selenocysteine in glutathione peroxidase and in thyroxine dejodase)	
thiosulfinates, such as allicin, glutamylcysteinyl glycine and other sulfur compounds	
tocopherols and tocotrienols	
vanillin and some other phenolic flavors	

*The term antioxidant is not restrained by any internationally accepted definition (Becker et al., 2004).

concludes that antioxidants are "traditionally recognized to be food components that have anti-aging effects" (Yamashita, 2009). Not only food enriched with antioxidants, but also an impressive number of plant extracts are now in the marketplace, often decorated with mysterious "health" attributes. The present note intends to pour some water into the (highly antioxidative) wine.

THE FREE RADICAL THEORY OF CHRONIC DISEASE

The rapid advances in understanding the patho-biochemical processes mediated by free radicals and other reactive oxygen species in the late 1980s and early 1990s resulted in the so-called "free radical theory" of chronic diseases (Goldstein and Witz, 1990; Halliwell, 1989; Hennig and Chow, 1988; Jürgens et al., 1987; Vuillaume, 1987) and aging (Pacifici and Davies, 1991). Several population-based observational and cross-sectional analyses indicated that a high dietary intake of antioxidants as well as higher plasma concentrations of vitamin E, vitamin C, and β-carotene may prevent cardiovascular disease (Eichholzer et al., 1992; Gey et al., 1987; 1991; 1993; 1993; Knekt et al., 1994; Osganian et al., 2003; 2003; Riemersma et al., 1991; Rimm et al., 1993; Stampfer et al., 1993) and cancer (Eichholzer et al., 1992; Stähelin et al., 1991; 1991; Tamimi et al., 2005; Zhang et al., 1999). Similar studies found that persons who took vitamin E supplements had a lower risk of coronary disease (Rimm et al., 1993; Stampfer et al., 1993).

Figure 1 Endogenous and exogenous sources replenish electrons to control oxidative damage in vivo (PUFA Polyunsaturated Fatty Acid, GSH Glutathione, PPP Pentose Phosphate Pathway).

CHARACTERISTICS OF ANTIOXIDANTS

The general definition of an antioxidant is based on activity rather than on structure or mechanism (Table 1). Halliwell and Guteridge (1995) defined an antioxidant as "any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate"; later, the same author (Halliwell, 2007) defined an antioxidant as "any substance that delays, prevents, or removes oxidative damage to a target molecule." Similarly, Khlebnikov et al. (2007) have defined the term antioxidant "as any substance that directly scavenges ROS or indirectly acts to up-regulate antioxidant defenses or inhibit ROS production."

Some assays detect inhibition of peroxidation (malonaldehyde, carotene bleaching, conjugated diene); others detect electron or radical scavenging (2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, and the ferric reducing antioxidant power (FRAP) assay) (Moon and Shibamoto, 2009). The rapid ORAC assay has gained increasing popularity, although the results often coincide with the total phenols as determined by the Folin-Ciocalteu reagent (Zheng and Wang, 2001). Not only the results of various assays differ for a certain extract or compound (Dudonné et al., 2009; Krings and Berger, 2001), but variants of the same assay may also give deviating results (Alarcón et al., 2008). This is not surprising, as the quantitative in vitro capacity of an antioxidant may depend on pH, solvent, oxidation levels, and other reaction conditions (Frankel and Finley 2008). Phenols are more active in the (non-physiological) alkaline range, and the hydrophilic ascorbic acid will not perform well in a linoleic acid micelle assay (Robbins et al., 2006).

JANUS FACED IN VITRO BEHAVIOR OF ANTIOXIDANTS

Reliable biomarkers of oxidative damage in human tissues, such as 8-oxo-2′-deoxyguanosine (DNA oxidation), malondialdehyde, and isoprostanes (Basu, 2004) (lipid oxidation), certain carbonyls (protein oxidation), and carboxyl methyl lysine (reaction between carbohydrates and free amino groups) (Valko et al., 2007) are usually elevated in smokers and in patients with Alzheimer's disease, cancer, autoimmune diseases, hepatitis, metabolic syndrome, neurodegenerative diseases, and arthritis (Frei, 2004). If there was a causal correlation between the functional integrity of some of the cellular constituents and the risk of these diseases, antioxidants should contribute to a risk reduction if they reach their site of action. Nevertheless, it is obvious that oxidative processes not only occur within cells, but in all the compartments of the human body. Oxidation of circulating LDL-particles is one example of such processes and is known to be one etiological factor in atherogenesis. Even within the intestine, non-absorbed as well as secreted antioxidants could show health effects.

The contradicting findings in studies with antioxidants may result from a deeper biochemical ambivalence of antioxidants. Above a certain concentration some antioxidants become pro-oxidants. For example, α-tocopherol is sacrificed during the autoxidation of unsaturated fatty acids to form a hydroxyalkylquinone. Thus, a strong oxidant is accumulating over time, and the intermediate radicals may accumulate as well and initiate, in spite of their relative inertness, new chain reactions on the long-term (Fig. 1). Ascorbic acid turns into a pro-oxidant in the presence of Fe^{3+} ions producing O_2^- ions and OH radicals along the Fenton reaction. Likewise, the pro-oxidative character of carotenoids and flavonoids may result from accumulating reactive intermediates or stabilizing interactions with heavy metal ions. A similar situation was reported for complex extracts from spices (Bonanni et al., 2007).

More specifically, many secondary plant metabolites with antioxidant properties possess other, possibly adverse bioactivities. Not so long ago, polyphenols and others have been classified "antinutrients." An example is sesame lignans, known phytoestrogens, which were shown to complement tocotrienol in the skin in preventing oxidative damage induced by UV-B light (Yamashita 2009). Such compounds and other famous antioxidants, such as quercetin show genotoxic activity in vitro and pro-apoptotic effects in cell systems (Stopper et al., 2005). The same applies to resveratrol, a plant defense phenol with numerous physiological activities (Pinto et al., 2004). The sales of red wine boomed after its supposed activity in protecting against tumor development had been made public. (Intriguingly, the well-proven carcinogenic potential of ethanol has not been brought to the awareness of a broader public.) Likewise, the consumption of cranberries and cinnamon is promoted by reports on the absorption of hydrolyzable proanthocyanidins which are said to protect lipoproteins and reduce inflammatory processes (Beecher, 2004). However, hydrolyzable proanthocyanidins are potentially toxic at least to ruminants (through the formation of pyrogallol), while non-hydrolyzable species are considered to be non-toxic because they are not absorbed (Reed, 1995).

Thermal treatment of food results in the formation of roast flavors, brown pigments (melanoidins), and antioxidants along

the so-called Maillard reaction. The antioxidant capacity of beer, for example, was equally distributed over fractions with different molecular mass and highly correlated to color—the deeper the color, the more pronounced was the antioxidant capacity. Extracts of roasted wheat germ and coffee scavenged free radicals as measured by the DPPH assay (Krings et al., 2006). The radical-scavenging activity assay and a repair enzyme based in vitro DNA protection assay (3D-assay), when applied to fractions of the total extract, did not give concurrent data. Moreover, results strongly depended on chosen concentration of the fraction. Lee and Shibamoto (2002) discussed a correlation of antioxidative and mutagenic properties in food model systems and in actual foods. Similarly, Oikawa (2008) reported that antioxidants may also induce DNA damage in human cultured cell lines; it is concluded that "some antioxidants play paradoxical roles acting as 'double-edged sword.'"

CAN CELL CULTURES MIMIC PHYSIOLOGICAL CONDITIONS?

Cell culture based assays were suggested to overcome the Janus faced in vitro results and to account for the complex bioactivities of some compounds (Liu, 2007). For experimental reasons, mainly cancer cell lines are used (Wolfe et al., 2008). However, the results of cell culture experiments with antioxidants strongly depended on the cell type, the cell culture medium, and the absence or the presence of other antioxidants (Babich et al., 2009; Long et al., 2000; 2007; Long and Halliwell, 2009; Roques et al., 2002; Wee et al., 2003). Therefore, some of the data generated in the cell culture arena showing beneficial or potentially harmful effects of antioxidants may simply represent artifacts (Long and Halliwell 2009; Long et al., 2000).

There are several discrepancies between cell cultures and more complex in vivo systems (Horrobin, 2003):

- "The types and rates of nutrient and oxygen supply, and carbon dioxide and metabolite removal, are different";
- "The endocrine environment is different, both in terms of the amounts and patterns of hormones present and their kinetic change";
- "The antibiotic environment is different: in vivo cells are not normally bathed in penicillin, streptomycin, and other antibiotics";
- "The lipid environment is different. The phospholipid composition of cells in culture is quite different from the phospholipid composition of the parent in vivo cells";
- "Even when appropriate constituents are present in culture fluid, their concentrations may be dramatically different from anything seen in vivo."

There is evidence that results from in vitro studies, testing non-physiological concentrations in cell cultures, cannot be transferred to in vivo conditions (Schmitt et al., 2007). A simple one-to-one extrapolation of in vitro data to in vivo behavior must therefore be misleading: "What happens in a Petri dish or in preclinical assays may not happen in people" (Bjelakovic and Gluud, 2007).

TAKING THE STEP TO IN VIVO

The comparison of epidemiological and in vivo intervention studies shows an apparent contradiction: Diets high in fresh fruits and vegetables decrease the risk of some chronic diseases. The results of in vivo intervention studies and of epidemiological studies with specific antioxidants, however, are rather equivocal. Supplementation of humans with vitamin C and E showed, for example, a significant lowering of in vivo oxidation in some, but not in all interventional trials (Frei, 2004; Huang et al., 2000; McCall and Frei, 1999).

Clinical trials testing the health benefits of dietary antioxidants gave mixed results, but overall failed to confirm the hypothesis. For example, 15 randomized trials with large cohorts of patients (1000 or more) and a follow-up of up to 12 years were analyzed (Vivekananthan et al., 2003). Tocopherol did "not provide benefit in mortality compared with control treatment or significantly decrease risk of cardiovascular death or cerebrovascular accident. β-Carotene led to a small but significant increase in all-cause mortality and with a slight increase in cardiovascular death." Similarly discouraging data were obtained from an extended meta-analysis of vitamin E-supplementation studies (Miller et al., 2005) and from another one conducted on data from 68 randomized trials with 232,606 patients (Bjelakovic et al., 2007). While in the latter study ascorbic acid and selenium had "no significant effect on mortality," "treatments with β-carotene, vitamin A, and vitamin E" were supposed to even "increase mortality."

There has been no lack of attempts to explain the negative outcome of these laborious studies. Some authors have criticized methodical shortcomings (e.g., questionable data combination of studies with heterogeneity in the study samples, selection bias due to the exclusion of such trials where no deaths occurred, etc.) (Bell and Grochoski, 2008; Blatt and Pryor, 2005; DeZee et al., 2005; Hemilä, 2005; Jialal and Devaraj, 2005; Krishnan et al., 2005; Lim et al., 2005; Meydani et al., 2005; Taylor and Dawsey, 2007). It is also possible (although not proven) that antioxidants of plant origin, such as ascorbic acid or β-carotene are useful plasma markers of fruit and vegetable intake, but the marker does not need to be the bioactive constituent. Antioxidants may exert their effects not through an antioxidant mechanism, but by indirectly affecting up-regulation of genes involved in defense or DNA-repair mechanisms, thus promoting the maintenance of metabolic homeostasis or cell integrity. β-Carotene, for instance, has shown no effect in preventing lung cancer when applied in population-based trials, but exerted a significant increase of cancer in heavy smokers. This compound induces cytochrome P450 enzymes, thus enhancing the biotransformation of benzo[*a*]pyrene (B[*a*]P) to the

powerful carcinogen B[a]P-7,8-diol-9,10-epoxid (Paolini et al., 2003; 1999). Also discussed are: patients already diagnozed with a disease may respond less or better (Osawa and Kato, 2005) or differently to an antioxidant; unknown synergistic/antagonistic effects among antioxidants or antioxidants and pharmaceuticals may hide the effect aimed at (Leger, 2006); methods for measuring plasma levels of antioxidant, antioxidant metabolites, and metabolic target may have been insufficient (Frankel and Finley, 2008).

Based on these findings some have exclaimed the end of the antioxidant hypothesis (Heyden, 2003). For example, Greenberg (2005) stated that it may be "past time for the scientific and public health communities to loosen their ties to a theory that lacks predictive ability for human diseases." However, most clinical studies simply have not taken into account the specific nutrikinetic and nutridynamic nature of antioxidants. There are many ways a prevention human interventional trial might fail (Blumberg and Frei, 2007; Frei, 2004; Hatfield and Gladyshev, 2009; Jialal and Devaraj, 2003). Frequent drawbacks were recently summarized: "The intervention dose was too high or low, the planned trial duration was too short, an unexpected side effect ended the trial early, intervention adherence was poor, too many control participants 'dropped in' to the intervention, only subgroups of participants were susceptible to the intervention, or the intervention itself affected end point detection" (Kristal 2008).

EVALUATING THE RESULTS OF INTERVENTION STUDIES

- **Aspects "Dosage" and "Baseline Nutrient Status."** Effects of bioactive constituents are co-determined by the dose applied. The trace element selenium is a good example: A distinct U-shaped dose-response curve exists between the supply of selenium and the risk of cancer. This non-linear relationship implies that more of a potential cancer-preventing nutrient, such as selenium is not necessarily better. A person's baseline nutrient status and the amount of selenium intake determine whether selenium supplementation will cause a net benefit (Waters et al., 2005). In general, the benefit of a micronutrient supplementation is greater in people with an inadequate intake. This can be demonstrated by the results of an intervention trial with 30.000 people carried out in the Chinese province of Linxian. The daily administration of β-carotene, vitamin E, and selenium to people with a sub-optimal intake over a period of several years resulted in a 20% risk reduction for cancer of the stomach, and the total mortality was decreased by about 10% at the same time. Likewise, the results of the French study SU.VI.MAX with more than 13,000 adults indicated that the administration of a physiologically matched combination-supplement can possess a preventive benefit in people with a sub-optimal intake of antioxidants, which is often prevalent in men (Galan et al., 2005). These results have led to the notion "that it is time to move beyond the belief that any particular agent administered at the same dose to all participants will benefit the overall population" (Rayman et al., 2009).
- **Aspect "Synergism" Antioxidants Show Distinct Synergetic Effects.** The vitamins E and C are, for example, integrated into an anti-oxidative network together with other compounds such as ubiquinol or α-lipoic acid. In this network vitamin C is the most important antioxidant in the hydrophilic phase, while vitamin E is effective in the lipophilic cell compartments (Packer et al., 2001). Because most chronic diseases are of multi-causal origin, the supplementation with a single antioxidant seems unreasonable. The negative results of respective interventional trials with single or few antioxidants, as for example in SELECT (Selenium and vitamin E Cancer Prevention Trial; (Lippman et al., 2009)) or PHS-II (Physicans' Health Study II; (Gaziano et al., 2009)) are not surprising. Consequently, it was stated that "single-agent interventions, even in combinations, may be an ineffective approach to primary prevention in average-risk populations" (Gann, 2009).
- **Aspect "Time."** Chronic nutrition-associated diseases may evolve over decades. It is unlikely that intervention trials will detect hard clinical end points within a few months (Ames et al., 2007; Waters et al., 2008). The results of several cohort studies indicated that the protective effect of folic acid-containing multivitamins towards colorectal tumours clearly emerge only after a use of ten years and longer (Fuchs et al., 2002; Giovannucci et al., 1998; Jacobs et al., 2001).
- **Aspect "Chemistry."** The binding status of a chemical (speciation) determines its physiology. For example, L-selenomethionine, which was used in the SELECT trial cited above, is metabolized in a different way than selenite or selenium-enriched baker's yeast. The latter forms have anti-cancerogen efficacy as it was shown in human and animal trials (Hatfield and Gladyshev 2009). The impact of stereochemistry was shown for vitamin E and its role in atherosclerosis. In comparison with *RRR*-α-tocopherol, the synthetic all-rac- α-tocopherol exhibited a different biopotency (Brigelius-Flohé et al., 2002).
- **Aspect "Bioavailability."** In vivo antioxidant activity may start already in the gastrointestinal tract (Halliwell et al., 2000). Absorption after ingestion is an obvious prerequisite for any cellular activity of an antioxidant. There is ample controversy on this issue for both phenols (Fernandez-Panchon et al., 2008; Karakaya, 2004) and carotenoids (Southon and Faulks, 2001). It was concluded from intervention studies that flavonols are absorbable and accumulate in plasma (Crozier et al., 2000), while a more recent study emphasized multiple effects of food processing on their bioavailability (Hackman et al., 2007). The situation is not only complicated by the chemical diversity of natural antioxidants in food, but also by numerous conjugated (glycosylated, esterified, oligomerized) forms which may all differ in gastrointestinal absorption (Miller and Ruiz-Larrea, 2002). After absorption the

antioxidant has to reach the place of action and must not be converted to inactive metabolites. This is at least questionable for flavonoids, as these molecules can undergo metabolism in human tissue and colon bacteria, thereby loosing part or all of their antioxidant capacity (Halliwell et al., 2005). Once absorbed and present in plasma or cells, flavonoids can exert multiple biological functions besides antioxidant activity, such as affecting enzyme activity of cyclooxygenases and lipoxygenases and act as receptor ligands of the estrogen and other receptors (Halliwell et al., 2005; Virgili and Marino, 2008). Taken together, it seems doubtful that what we eat as an antioxidant finally reaches the intracellular sites aimed at.

REVISION OF THE ROLE OF REACTIVE OXYGEN SPECIES (ROS) IN HUMANS

Oxidative stress is defined as an imbalance of pro-oxidative and antioxidative processes in the human body. Many authors have supposed that elevated levels of ROS must cause severe diseases, like elevated levels inevitably in spoil potato chips.

ROS are endogenous species produced in a vitamin B_2 dependent reaction by NADPH oxidase to help, for example, in phagocytosis and cell signaling (Yazdanpanah et al., 2009). In an animal model, increased oxidative stress induced by the deletion of superoxide dismutase genes did not result in accelerated aging (Doonan et al., 2008; Van Raamsdonk and Hekimi, 2009). ROS were a major effector of blood-cell development in *Drosophila* (Owusu-Ansah and Banerjee, 2009), and elevated transcription of genes involved in ROS formation in mice blood cell progenitors indicated that they might play a similar role in mammals (Tothova et al., 2007). In zebrafish H_2O_2 signaled to leucocytes in wound healing (Niethammer et al., 2009). The p53 tumor suppressor was identified as a part of the regulatory means of cells to cope with oxidation under conditions of average stress (Olovnikov et al., 2009). Besides this, ROS were involved in several other biochemical and physiological processes, such as insulin signaling, control of ventilation, and erythropoietin production (Dröge, 2002).

The vital role of a balanced antioxidant status of the cell was emphasized by recent work on metabolic alterations and cancer (Schafer et al., 2009). An oncogene (ERBB2) over-expressing breast epithelial cell line was compared to a normal cell line. In the latter, mitochondrial oxidative stress was attenuated by glucose which bypassed glycolysis and generated NADPH, a powerful antioxidant, through the pentose phosphate pathway. This protected normal cells from oxidative damage. ERBB2 expression in the mobile cancer cells maintained glucose uptake (by activating a cancer inducing pathway), compensating for energy depletion, and thus protected cancer cells from starvation. The same was achieved in normal, glucose-starved cells supplemented with an antioxidant (50 μM trolox), showing that regular NADPH production prevented pathological oxidative stress. This means that an exogenous antioxidant could even contribute to the survival of a detached cell on its way to dedifferentiation and cancer initiation (Fig. 2).

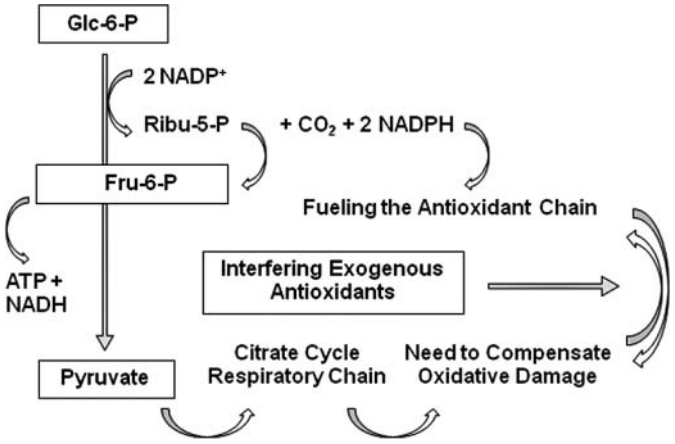


Figure 2 Increasing oxidative stress in mitochondria damages matrix-detached human breast cells. Oncogene over-expression maintains their glucose uptake through activating a cancer-inducing pathway. At the same time, oxidative stress is compensated by the conversion of glucose to NADPH through the pentose pathway. Trolox, a synthetic antioxidant, was able to replace NADPH in normal detached cells. The intriguing question is if exogenous antioxidants may abet the conversion of normal cells to cancer cells through this interference.

CONCLUSION

The gap between the antioxidative effects in vitro compared to the much more complex situation in vivo is obvious. Looking at the profound physiological relevance of antioxidants it seems appropriate to call for more detailed investigations (Davies et al., 2005), particularly on absorption, nutrikinetics, clinical effects, and toxicity of continuous ingestion. Forced by worldwide food laws the toxicology of some synthetic antioxidants is well-known, which applies to many isolated natural antioxidants to a much lesser extent (Pokorný, 2007). Remarkably little work has been done on the structure-activity relationship of antioxidants (Kim and Lee, 2004). There is conflicting evidence concerning the potential benefit of higher intakes of especially single antioxidants due to different reasons. As a consequence, a permanent intake of non-physiological dosages of isolated antioxidants should not be recommended to healthy consumers. This must not be confused with a high intake of fruit and vegetables, which is considered safe and beneficial. The present antioxidant hype promotes the sales of side-steams of food processing, such as grain husks or apricot kernel powder. Two decades ago nobody would have seriously classified such materials as edible. It is high time to readjust the biased views on antioxidants, and to base medical and "health" statements on sound data. A vision for a path forward could be that health effects have to be shown on a broad basis of scientific data—and this must be underlined—by weighing the evidence. Data from human studies are required as well as those explaining mechanisms. This approach is becoming public policy in the European

Community (EC) in order to protect the consumer against misleading claims. Regulation (EC) No 1924/2006 on nutrition and health claims made on foods and the corresponding regulation (EC) No. 353/2008 establishing implementing rules for applications for authorization of health claims emphasize that health claims will only be permitted if based on and substantiated by generally accepted scientific data, especially in human studies of the target group.

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