

Free Radicals: The Pros and Cons of Antioxidants

Efficacy of Dietary Antioxidants to Prevent Oxidative Damage and Inhibit Chronic Disease^{1,2}

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EXPANDED ABSTRACT

KEY WORDS: • vitamin C • vitamin E • β -carotene • oxidative biomarkers • clinical trials

Many in vitro studies have shown that dietary antioxidants, such as vitamin C (ascorbic acid), vitamin E (α -tocopherol), β -carotene, and flavonoids, act as effective antioxidants in biological systems such as plasma, lipoproteins, and cultured cells (1–3). For example, vitamin C effectively inhibits lipid and protein oxidation in human plasma exposed to various (patho)physiologically relevant types of oxidative stress, such as activated polymorphonuclear leukocytes, reagent or myeloperoxidase-derived hypochlorous acid, cigarette smoke, or redox-active iron or copper ions (1,3). Vitamin E is the most abundant lipid-soluble antioxidant in human lipoproteins and tissues and acts as a chain-breaking antioxidant against lipid peroxidation (3). β -carotene, lycopene, lutein, and other carotenoids and oxy-carotenoids are efficient singlet oxygen quenchers and, thus, may be important in protecting the eye and skin against UV-induced oxidative damage (2,5). These small-molecule dietary antioxidants interact with each other in an “antioxidant network” and complement antioxidant enzymes and metal-binding proteins present in cells and extracellular fluids.

There is ample evidence, mainly from case-control studies, that biomarkers of oxidative damage in human plasma, urine, and cells are increased in subjects with certain diseases or associated risk factors. For example, the DNA oxidation markers 8-oxo-2'-deoxyguanosine (8-oxodG) and 8-oxo-guanine are increased in smokers and patients with certain types of cancer, autoimmune diseases, hepatitis, and cystic fibrosis (6,7). F₂-isoprostanes and their metabolites, which are reliable markers of in vivo lipid peroxidation, are elevated in hepatic

cirrhosis and Alzheimer's disease and in subjects with coronary risk factors, such as cigarette smoking, diabetes mellitus, obesity, hypercholesterolemia, or hyperhomocysteinemia (6,8). Furthermore, plasma and tissue levels of protein carbonyls, indicative of protein oxidative damage, are increased in smokers, several neurodegenerative diseases, cataractogenesis, and rheumatoid arthritis (6). Thus, it is possible, although not proven, that these diseases are in part caused by oxidative damage to critical biological macromolecules and that dietary intake of, or supplementation with, antioxidants may lower disease risk or be useful in disease treatment.

Clinical trials of antioxidant supplementation of smokers and nonsmokers with vitamin C, vitamin E, and β -carotene, alone or in combination, generally did not find significant decreases in biomarkers of oxidative DNA damage, usually 8-oxodG (6,7,9). In contrast, observational studies suggest an inverse association between dietary intake or plasma levels of antioxidants and oxidative DNA damage (3,5,6). Furthermore, vitamin C or vitamin E supplementation lowers lipid oxidative damage, measured as F₂-isoprostanes, in some subjects, such as smokers with increased body mass index, patients with alcohol-induced chronic liver disease, hypercholesterolemics, and diabetics, but apparently not in healthy, non-smoking individuals or patients with coronary artery disease (3,6,8). Although human data on protein oxidative damage are sparse, preliminary evidence suggests that vitamin C supplementation lowers protein carbonyl levels in healthy individuals with low baseline ascorbate levels and nitrotyrosine levels (a marker of peroxynitrite-mediated “nitrative stress”) in patients with *Helicobacter pylori* gastritis (3,6). Finally, vitamin E supplementation has been reported to lower protein carbonyl levels in smokers (3). Overall, current evidence suggests that antioxidant supplementation can lower oxidative biomarkers in subjects with increased oxidative stress levels, but not in healthy individuals with “normal” oxidative stress levels.

Despite this evidence, clinical trials of antioxidant supplementation with vitamin E and β -carotene have failed to show benefit with respect to disease outcome and sometimes have found adverse effects (2,10). These clinical trial data are in stark contrast to the compelling evidence from observational studies that dietary intake or plasma levels of antioxidants are

¹ Presented as part of the conference “Free Radicals: The Pros and Cons of Antioxidants,” held June 26–27 in Bethesda, MD. This conference was sponsored by the Division of Cancer Prevention (DCP) and the Division of Cancer Treatment and Diagnosis, National Cancer Institute, NIH, Department of Health and Human Services (DHHS); the National Center for Complementary and Alternative Medicine (NCCAM), NIH, DHHS; the Office of Dietary Supplements (ODS), NIH, DHHS; the American Society for Nutritional Science; and the American Institute for Cancer Research and supported by the DCP, NCCAM, and ODS. Guest editors for the supplement publication were Harold E. Seifried, National Cancer Institute, NIH; Barbara Sorkin, NCCAM, NIH; and Rebecca Costello, ODS, NIH.

² Supported by grants AT00066, AT02034 and HL60866 from the National Institutes of Health.

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correlated with decreased disease risk of, or mortality from, heart disease, stroke, and many types of cancer (4,11–14). For example, dietary intake and plasma levels of β -carotene are associated inversely with lung cancer, yet 2 large trials of β -carotene supplementation found an increased risk of lung cancer in smokers and workers exposed to asbestos occupationally (2,5). These results suggest that β -carotene is a marker of fruit and vegetable intake, which is known to reduce chronic disease risk, but not a main active constituent. Furthermore, observational data suggest an inverse association between vitamin E supplementation and heart disease risk (3,11) but numerous large clinical trials with vitamin E supplements have failed to show any benefit in the secondary prevention of heart disease (10). Finally, observational studies have shown strong inverse associations between vitamin C intake or plasma levels and morbidity or mortality from all causes, cardiovascular diseases (CVD), and cancer (4,12,13); however, clinical trial data are lacking to conclude whether vitamin C is responsible chiefly for the observed health benefits or—similar to β -carotene—merely is a marker of fruit and vegetable intake.

Although clinical trials have been promoted as the “gold standard” for demonstrating health benefits of antioxidant supplements, they suffer from limitations that can hamper their interpretation. In particular, the following issues need to be addressed before definitive conclusions can be drawn:

Primary vs. secondary prevention

Most clinical trials conducted thus far have examined the effect of antioxidant supplements on secondary prevention of disease. In contrast, most observational studies have investigated primary prevention. Thus, while dietary or supplemental antioxidants consumed over a long period of time by healthy individuals may effectively prevent or delay development of chronic diseases, such as CVD, cancer, and neurodegenerative diseases, treating patients diagnosed with these diseases with antioxidant supplements no longer may provide significant benefit. For example, vascular oxidative stress causing oxidation of low-density lipoproteins and endothelial dysfunction is pivotal for the initiation of atherosclerosis but may become progressively less important during the later stages of the disease, when mural thrombosis, smooth muscle-cell proliferation, and deposition of extracellular matrix contribute significantly to plaque growth. Similarly, mutagenic oxidative DNA lesions may initiate carcinogenesis but be less important in tumor promotion and metastasis. Thus, a thorough understanding of disease pathology is crucial for the proper timing and duration of antioxidant supplementation in intervention studies.

Standard therapy

Most clinical trials conducted thus far have examined the effects of antioxidant treatment in conjunction with standard therapy, which may obliterate the potential beneficial effects of the antioxidant studied. For example, many secondary prevention trials of CVD have compared patients on multi-drug therapy, including statins, aspirin, angiotensin-converting enzyme inhibitors, and β -blockers, with patients receiving the same multi-drug therapy plus vitamin E. If some of the benefits of vitamin E are derived from its ability to inhibit platelet aggregation and, thus, mural and occlusive thrombosis, the effects of vitamin E may be obliterated by the simultaneous use of aspirin or other nonsteroidal anti-inflammatory drugs.

Pharmacokinetic behavior of antioxidants

Most clinical trials conducted thus far have not determined antioxidant levels, such as plasma levels of vitamins C and E, at baseline and following supplementation. Without such data, it is impossible to know whether the supplementation had the intended effect of increasing antioxidant levels in the subjects studied. For example, individuals with a relatively moderate daily intake of about 200 mg of vitamin C from the diet and a multivitamin may already have body and tissue saturation, and supplementing such individuals with vitamin C will not further increase their vitamin C body status. Vitamin E and β -carotene are lipid-soluble compounds, the absorption of which from the diet is highly dependent on the food matrix. For example, vitamin E taken on an empty stomach is poorly absorbed or not absorbed at all.

Mechanism of antioxidant action

Equally important to a thorough understanding of the disease pathology (see above) is a thorough understanding of the mechanism of action of the antioxidant to be tested. For example, vitamin E may be the wrong antioxidant for treatment of CVD because myeloperoxidase-derived reactive nitrogen or halogenating species, such as peroxynitrite, nitrogen dioxide, or hypochlorous acid, appear to play a significant role in LDL oxidation and vascular nitrate and oxidative stress. These oxidants can cause oxidative damage to biological target molecules by a mechanism not affected by vitamin E. In addition, a water-soluble antioxidant, such as vitamin C, protects different targets than does a lipid-soluble antioxidant, such as vitamin E. Therefore, failure of one antioxidant to prevent or treat a specific disease does not mean that all other antioxidants will also fail.

Oxidative biomarkers

Virtually no clinical trials conducted thus far have assessed baseline oxidative stress levels in the subjects studied by measuring at least one relevant oxidative biomarker. Without this information, it is impossible to know which subjects are under increased oxidative stress and, thus, may benefit from antioxidant treatment. It also is not known whether the antioxidant supplements had the intended effect of lowering oxidative stress.

Due to these limitations of clinical trials, it remains impossible to conclude whether oxidative stress plays a causal role in CVD, cancer, and other chronic diseases in humans or whether oxidative stress is an epiphenomenon unrelated to the

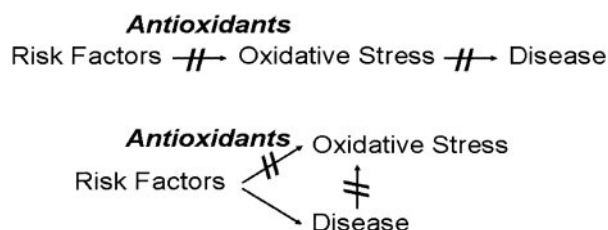


FIGURE 1 Relationships between oxidative stress and disease. In the linear model (*top*), risk factors enhance oxidative stress, which in turn causes disease. Antioxidant intervention lowers oxidative stress and hence inhibits disease. In the branched model (*bottom*), risk factors enhance oxidative stress and cause disease independently of each other. Disease itself also may cause oxidative stress. Antioxidant intervention lowers oxidative stress without affecting disease.

pathogenesis or even a consequence of the disease (Fig. 1). Thus, it is pivotal that ongoing and future clinical trials assess antioxidant levels and oxidative stress before and after antioxidant supplementation, measuring at least 2 established oxidative biomarkers, such as 8-oxodG, F₂-isoprostanes, chlorotyrosine, nitrotyrosine, or protein carbonyls. Without such information, it is impossible to know whether most antioxidant trials conducted thus far have failed to show benefit because supplementation did not lower oxidative stress or because oxidative stress does not play a causal role in the disease under study. Therefore, well-controlled clinical trials are needed that simultaneously assess and compare the effects of antioxidant supplementation in nonantioxidant-saturated populations on body antioxidant status, oxidative biomarkers, and disease outcome. While such trials will help clarify the role of antioxidants in secondary disease prevention, it may never be known with certainty whether antioxidants are effective in primary prevention of disease because these types of trials may be prohibitively expensive and logistically unfeasible.

LITERATURE CITED

1. Frei, B., England, L. & Ames, B. N. (1989) Ascorbate is an outstanding antioxidant in human blood plasma. *Proc. Natl. Acad. Sci. U.S.A.* 86: 6377–6381.
2. Institute of Medicine (2000) National Academy of Sciences, Food and Nutrition Board, Panel on Dietary Antioxidants and Related Compounds. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. National Academy Press, Washington, DC.
3. Packer, L., Traber, M. G., Kraemer, K. & Frei, B., eds. (2002) *The Antioxidant Vitamins C and E*. AOCS Press, Champaign, IL.
4. Carr, A. C. & Frei, B. (1999) Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. *Am. J. Clin. Nutr.* 69: 1086–1107.
5. Mayne, S. T. (2003) Antioxidant nutrients and chronic disease: use of biomarkers of exposure and oxidative stress status in epidemiologic research. *J. Nutr.* 133 (suppl. 3): 933S–940S.
6. McCall, M. R. & Frei, B. (1999) Can antioxidant vitamins materially reduce oxidative damage in humans? *Free Radic. Biol. Med.* 26: 1034–1053.
7. Loft, S. & Poulsen, H. E. (2000) Antioxidant intervention studies related to DNA damage, DNA repair and gene expression. *Free Radic. Res.* 33 (suppl): S67–S83.
8. Roberts, L. J. 2nd & Morrow, J. D. (2002) Products of the isoprostane pathway: unique bioactive compounds and markers of lipid peroxidation. *Cell Mol. Life Sci.* 59: 808–820.
9. Huang, H. Y., Helzlsouer, K. J. & Appel, L. J. (2000) The effects of vitamin C and vitamin E on oxidative DNA damage: results from a randomized controlled trial. *Cancer Epidemiol. Biomarkers Prev.* 9: 647–652.
10. Vivekananthan, D. P., Penn, M. S., Sapp, S. K., Hsu, A. & Topol, E. J. (2003) Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. *Lancet* 361: 2017–2023.
11. Rimm, E. B., Stampfer, M. J., Ascherio, A., Giovannucci, E., Colditz, G. A. & Willett, W. C. (1993) Vitamin E consumption and the risk of coronary heart disease in men. *N. Engl. J. Med.* 328: 1450–1456.
12. Khaw, K. T., Bingham, S., Welch, A., Luben, R., Wareham, N., Oakes, S. & Day, N. (2001) Relation between plasma ascorbic acid and mortality in men and women in EPIC-Norfolk prospective study: a prospective population study. *European Prospective Investigation into Cancer and Nutrition. Lancet* 357: 657–663.
13. Osganian, S. K., Stampfer, M. J., Rimm, E., Spiegelman, D., Hu, F. B., Manson, J. E. & Willett, W. C. (2003) Vitamin C and risk of coronary heart disease in women. *J. Am. Coll. Cardiol.* 42: 246–252.
14. Frei, B. (2003) To C or not to C, that is the question! *J. Am. Coll. Cardiol.* 42: 253–255.