

Antioxidants and atherosclerotic cardiovascular disease: unresolved issues

Diane L. Tribble

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Department of Molecular and Nuclear Medicine, Lawrence Berkeley National Laboratory, Berkeley, California, USA.

Correspondence and requests for reprints to Diane L. Tribble, PhD, Merck Research Laboratories, Merck & Co., Inc., RY32-557, 126 East Lincoln Avenue, PO Box 2000, Rahway, NJ 07065-0900, USA.
E-mail: diane_tribble@merck.com

Introduction

Interest in the potential cardiovascular benefits of antioxidants has grown in the past several decades on the heels of strong evidence that oxidants are involved in the initiation and progression of atherosclerosis and possibly the precipitation of clinical events, including myocardial infarction and stroke, that are indicative of underlying cardiovascular disease (CVD). Despite these advances in our understanding of disease mechanisms and the inhibitory effects of antioxidants on relevant processes in defined experimental systems, a clear picture of the effects of antioxidants on CVD risk in human populations has remained elusive. Numerous observational epidemiologic studies have shown that greater intakes of antioxidant-rich foods are associated with lower CVD risk. However, large intervention studies in human populations have failed to conclusively demonstrate a beneficial effect of antioxidant supplements on disease outcomes. While such findings suggest that antioxidants are not the panacea some have proposed them to be, careful consideration of the current evidence reveals that it would be premature to dismiss these agents as viable components of a preventive strategy. This review includes a brief summary of the state of evidence regarding the CVD-preventive effects of naturally occurring (dietary) antioxidants and proposes that key issues remain to be resolved before their preventive properties (or lack thereof) may be fully understood.

The involvement of oxidants in cardiovascular disease: a brief synopsis of current evidence

As discussed in several recent reviews [1–3], there is now compelling evidence that oxidants are involved in stimulating multiple processes implicated in the development and expression of atherosclerotic CVD including events as diverse as lipoprotein lipid deposition, endothelial dysfunction, vascular inflammation, vascular cell proliferation, plaque rupture and thrombosis. The prevailing hypothesis links most of these events to oxidatively modified forms of atherogenic lipoproteins [for example, low-density lipoproteins (LDLs)], which serve as sources of lesional lipids and lead to pathophysiologic alterations in the properties of vascular cells through the delivery of biologically active lipid oxidation products. Lipoprotein-independent oxidative or redox-regulated events also may contribute to the disease process by virtue of their role in signal transduction.

The oxidation hypothesis was developed primarily on the basis of results from a large number of studies testing the effects of oxidized LDL on putative proatherogenic events in isolated cell systems. More recently, a large body of evidence in animal models has been assembled, including several seminal studies in genetically altered mice [3–5]. These studies have shown that oxidants/oxidation markers are present in atherosclerotic lesions and that antioxidants and genetic alterations affecting the generation or response to oxidative stress influence atherosclerosis susceptibility. Of particular note are recent studies in hyperlipidemic mice showing that targeted disruption of α -tocopherol transfer protein, which leads to a deficiency in vitamin E, increases atherosclerotic lesion formation [4] and that targeted disruption of 12/15 lipoxygenase, which has been implicated in LDL lipid peroxidation, reduces lesion formation [5].

Evidence from human studies is less extensive. One major limitation has been the lack of non-invasive methods for measuring oxidative processes *in vivo* and especially in the artery wall. As discussed in detail below, observational epidemiologic studies have suggested that dietary antioxidants may reduce risk in human populations but studies directly addressing the preventive properties of antioxidants have as yet been relatively unresponsive. Hence, the pathophysiological importance of oxidative events in the development of CVD and the resulting therapeutic implications, remain unresolved.

A role for antioxidants in disease risk in human populations: postulated but not proven

Observational studies

A host of descriptive, case-control and cohort studies have shown that high antioxidant intakes, either by diets enriched in fruits, vegetables and whole grains or by antioxidant supplementation, are associated with reduced CVD risk [6–15]. In some cases, plasma and/or tissue levels of antioxidants also have been shown to correspond with CVD risk [9]. The most consistent evidence has been obtained for vitamin E. Estimates of relative risk reduction with high vitamin E intake range from approximately 30 to 65% (as compared with low levels of intake) [6–11]. Reduced risk has also been shown for high intakes of β -carotene, but results have been less consistent and the effects have been smaller (ranging from no effect to approximately 50% reduced risk) [9,12–14]. Moreover, in some cases, the benefits have been limited to smokers. A large benefit was

suggested for high vitamin C intake in one major cohort study [15], but the results were not adjusted for vitamin E intake.

While results from these observational studies support the antiatherogenic effects of antioxidants, they do not prove a direct relationship. Indeed, results from observational studies may be confounded by uncontrolled factors including other (unmeasured) dietary components [14]. Antioxidants could, for example, be surrogate markers for other biologically active food constituents that are responsible for the CVD-preventive effects. Foods rich in antioxidants tend to be rich in minerals, flavonoids, indoles and carotenoids other than β -carotene, to contain increased fiber, and to have a lower saturated fat and cholesterol content. Non-dietary lifestyle factors that accompany certain dietary patterns also may confound results from observational studies. For example, in several of the studies cited above, persons using dietary supplements were less likely to smoke and more likely to exercise regularly, both of which have been shown to substantially impact risk. Although attempts are usually made to adjust for these variables, there still could be some residual effects.

Randomized controlled trials

A number of randomized, controlled trials directly addressing the preventive effects of antioxidants have recently been concluded and several are on going. In general, the results have failed to demonstrate a consistent effect of antioxidants on CVD risk in human populations.

The effects of vitamin E have now been examined in four large, randomized, controlled trials. No beneficial effects on CVD endpoints were observed in the Alpha-Tocopherol, Beta-Carotene (ATBC) Study, which involved 5–8 years of daily supplementation with vitamin E (50 mg/day) alone, β -carotene (20 mg/day) alone or vitamin E plus β -carotene (at the same doses) in a population of over 29 000 Finnish male smokers [16]. It has been argued that this study was not specifically designed to address CVD endpoints and that doses of vitamin E were lower than those associated with reduced CVD risk in some observational studies.

In the Cambridge Heart Antioxidant Study (CHAOS), which tested the effects of 400 or 800 i.u. of vitamin E on CVD outcomes in individuals with angiographically proven disease, risk of nonfatal myocardial infarction (MI) was reduced by an astounding 77% relative to placebo [17]. Oddly, there was an insignificant increase in cardiovascular deaths, which effectively reduced the overall benefit of vitamin E.

While results of the CHAOS trial raised hopes that vitamin E might, at the very least, be useful in secondary prevention, the findings have not been confirmed in several recent studies. Neither the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI) Prevention Trial, which examined vitamin E (300 mg/day) alone and in combination with *n*-3 polyunsaturated fatty acids in patients surviving recent MI [18], nor the Heart Outcomes Prevention Evaluation (HOPE) Study, which examined vitamin E (400 mg/day) alone or in combination with ramipril in high-risk patients with a documented history of CVD or with diabetes and additional risk factors [19], showed any beneficial effect of vitamin E on CVD endpoints. Thus, at present, the collective evidence does not support the use of vitamin E in secondary prevention and there is insufficient evidence upon which to make recommendations regarding the use of vitamin E in primary prevention.

Randomized trials addressing the preventive effects of β -carotene have afforded a clearer but more surprising conclusion. Rather than a beneficial effect, results have shown an increase in cancer and CVD risk with β -carotene supplementation, particularly in smokers. In the ATBC Study, there was an 8% increase in total mortality with β -carotene supplementation. An increase in risk of both cancer and cardiovascular endpoints also was observed in the β -Carotene and Retinol Efficacy Trial (CARET), in which the combined effects of β -carotene and retinol were evaluated for <4 years in 18000 men and women with a history of cigarette smoking or occupational exposure to asbestos [20]. In contrast, results of the Physician's Health Study (PHS) did not show any significant effects, either beneficial or deleterious, on cardiovascular endpoints or death from all causes of 12 years of supplementation with 50 mg of β -carotene every other day [21].

Unresolved issues: there is still hope

Do negative results in these antioxidant intervention trials invalidate the oxidation hypothesis? It is generally agreed that the answer to this question is 'no' since the collective data are so compelling. Given this, how can we explain the failure of antioxidant intervention trials to support the rest of the evidence? Perhaps we have not been asking the correct questions or have not been asking the questions in the correct way. To date, most of the human population studies have involved diseased or high-risk populations and some were not designed specifically to address CVD endpoints. However, a number of other issues also may be relevant. It is argued here that the effects of antioxidant supplements on disease risk may be highly dependent on the iden-

tity and dose of the antioxidants tested, the characteristics of the study population, and the nature of the disease outcome criteria.

Antioxidant identity

A number of different oxidants have been implicated in the disease process, but the identification of critical species has remained elusive. Based on studies *in vitro*, a role has been proposed for the superoxide anion, the hydroxyl radical, lipoxygenase-derived lipid hydroperoxides, phagocytic oxidants including reactive nitrogen species and hypochlorous acid and free and/or bound transition metals, to name a few. Since many oxidants are extremely short lived and their effects are fairly non-specific, it has been difficult to confirm their specific involvement *in vivo*. However, some reactive species leave identifiable molecular 'footprints' [22,23]. The development and application of better oxidation markers together with the use of genetically altered mice should soon shed considerably more light on critical oxidation pathways *in vivo*.

Antioxidants differ in their chemical properties and compartmentation and thus ability to intervene in specific oxidation reactions. Without a clear understanding of which oxidants are most important pathophysiologically, it will be difficult to identify, other than through trial and error, which antioxidants may be most effective in countering proatherogenic oxidation events and reducing disease. Much attention has been focused on LDL lipid peroxidation and thus the importance of the major LDL radical-chain-breaking antioxidant, vitamin E. However, vitamin E (and other lipid-soluble antioxidants) will not be effective in protecting LDL against all oxidizing conditions encountered *in vivo* including those that promote direct oxidative modifications of the apolipoprotein B component. Moreover, vitamin E is expected to be relatively ineffective in combating oxidation events outside of the LDL particle, including the accumulation of oxidants in the local milieu.

Antioxidant interdependence

It has long been recognized that antioxidants work together to form a network of protection such that the beneficial effects of one antioxidant may take place only in the context of another (others). Thus, limitations in one component could impact the efficacy of other components and/or the entire system. For example, studies *in vitro* have shown that ubiquinol-10, vitamin C and β -carotene regenerate or spare vitamin E and thereby enhance its antioxidant capacity [24,25]. Thus, selectively elevating one compound (for example, only vitamin E) may not have the maximal expected benefit.

In addition, there are also concerns that selective elevation of one component may compromise the effectiveness of others. For example, α -tocopherol supplementation has been shown to suppress levels of γ -tocopherol [26]. Recent studies have shown that, under some conditions, γ -tocopherol is a more potent antioxidant than α -tocopherol. γ -Tocopherol appears to be particularly effective in trapping electrophilic reactive nitrogen species [27]. Thus, it is conceivable that supplementation with α -tocopherol could reduce antioxidant protection (for example, against reactive nitrogen species) afforded by γ -tocopherol.

The issue of antioxidant interdependence has only recently been considered in the design of antioxidant intervention studies. In one recent trial, the proportion of hypercholesterolemic men exhibiting progression of carotid atherosclerosis was shown to be reduced by 74% following cosupplementation with vitamins E and C, but not with either vitamin alone, relative to placebo [28]. Further evidence of the effects of antioxidant combinations will be available from on-going trials including the Heart Protection Study, which is testing the effects of an antioxidant cocktail.

Antioxidant dose

Extrapolation of results from observational studies usually assumes a linear relationship between antioxidant dose and beneficial effect. However, results from several epidemiologic studies suggest that the relationship between antioxidant intake and disease risk may not be linear. For example, in some studies, carotenoid-related variations in disease outcomes appear to occur largely at lower intakes. In a recent nested case-control study, baseline levels of serum β -carotene were inversely related to subsequent MI, with the increased risk apparent primarily within the lowest two quintiles [9]. These results suggest that there may be a threshold below which risk is increased. This has far different implications for the potential benefits of antioxidants than a linear relationship wherein risk is reduced with increasing intake.

There has also been debate as to the effective doses of vitamin E. Kushi *et al.* [8] reported an inverse association between quintile of vitamin E intake and risk of death from coronary heart disease in postmenopausal women not taking vitamin E supplements. They did not detect a relationship between supplement use and risk. However, in the Nurses' Health Study and the Health Professionals' Follow-up Study, the lowest risk was observed in individuals consuming levels of vitamin E achievable only by supplementation [6,7]. Using data from the Cholesterol Lowering Atherosclerosis Study, Azen *et al.* [10] found that high vitamin E supplement

users exhibited less carotid intimal-media thickness progression than low vitamin E users within the placebo group. These conflicting results obviously lead to entirely different conclusions about the optimal dose range. The former results suggest that supplementation above and beyond dietary levels will have minimal or no impact in individuals that are adequately nourished. The latter results suggest that supplementation will promote the optimal benefit. A greater understanding of dose-response relationships will be necessary for the appropriate design of future studies addressing the preventive effects of antioxidants.

Characteristics of the study populations

Many of the intervention trials completed to date have involved relatively healthy, adequately nourished populations. Perhaps a beneficial effect would be observed in populations with initially low antioxidant nutriture. It is noteworthy that a malnourished population in Linxian, China received benefit from supplemented micronutrients in terms of all-cause mortality and several disease endpoints [29]. In the context of results from other clinical trials, these observations suggest that it might be appropriate to place emphasis on improving micronutrient intake in malnourished individuals/populations rather than promoting intake of supraphysiologic levels in adequately nourished populations.

In addition to baseline nutritional status, whether the population is healthy and/or has pre-existing or advanced disease is undoubtedly important to the outcome. Hypotheses regarding the beneficial effects of antioxidants have focused on their role in preventing the deposition of lipoprotein lipids and the formation of foam cells. These events are associated with the early stages of atherosclerotic plaque formation rather than the expression of advanced disease. As such, the preventive properties of antioxidants may best be addressed by studying the effects of long-term supplementation in individuals that are initially free of disease (that is, primary prevention studies). Studies of this nature are necessarily larger and longer and thus tend to trail behind those involving diseased or high-risk populations.

Nature of disease outcome criteria

A closely related issue may be the nature of the disease outcome criteria. Many of the intervention studies have evaluated the impact of antioxidant supplementation on the end-stage disease events including MI, stroke and cardiovascular deaths. Plaque rupture and thrombosis are probably more important precipitating factors than those affecting the initiation or progression of coronary artery disease. Several recent observational studies have suggested that antioxidants may inhibit lesion

progression (as assessed by ultrasonography) [10,11]. Measures of this type may be more relevant to the pathophysiological events associated with oxidation, and their preferential use in future trials, together with the use of initially healthy populations, could better delineate the role of oxidation and the benefits of antioxidants in disease initiation and progression.

Concluding remarks

While the cardiovascular benefits of antioxidants remain unclear, results obtained over the past decade have moved us closer to an understanding of the possible impact of these dietary components on CVD risk. Clearly, diets emphasizing antioxidant-rich fruits, vegetables and whole grains are an important component in any risk reduction strategy. Despite disappointing results from several recent trials, the successful use of vitamin E as well as vitamin C and other antioxidants as CVD-preventive or therapeutic agents remains an intriguing possibility. In this regard, it appears likely that the cardiovascular benefits of antioxidants may require the right set of circumstances, including optimal levels of a combination of antioxidants. However, based on results to date, even if some antioxidant factor or combination of factors is found to have a beneficial effect on disease outcomes, it will represent only an adjunct to current therapies including lipid-lowering, reduced-fat diets and smoking cessation.

Annotated references

- of special interest
- of outstanding interest

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