Antioxidants in Cardiovascular Health and Disease: Key Lessons from Epidemiologic Studies

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The free radical theory of aging posits that oxidative stress is among the major mechanisms in aging and age-related disease, including cardiovascular disease (CVD). Numerous in vitro and animal studies have supported the role of low-density lipoprotein (LDL) oxidation in atherosclerosis. This has led to the hypothesis that antioxidants could be used as an inexpensive means of prevention and possibly, treatment of coronary artery disease, stroke, peripheral vascular disease, and other CVD-related diseases. Epidemiologic cohort studies with large numbers of men, women, and diverse populations have been largely supportive of this hypothesis. However, interventional trials have been controversial, with some positive findings, many null findings, and some suggestion of harm in certain high-risk populations. Because of the mismatch between the epidemiologic studies and the interventional trials, some researchers have advocated ending antioxidant work. Others have questioned the validity of the LDL oxidative hypothesis itself. Clearly, further research is needed to understand the reasons for the mismatch between the epidemiologic and interventional work. Recent smaller interventional studies with carefully chosen populations, such as those under high levels of oxidative stress, have yielded largely positive results. This suggests that we need more hypothesis-driven and rigorous clinical trial designs. This should help clarify the true potential utility of antioxidants in CVD and may lead to a better understanding of the role of oxidative stress in atherosclerosis. © 2008 Elsevier Inc. All rights reserved. (Am J Cardiol 2008; 101[suppl]:75D– 86D)

An important nutritional finding over the past several decades with widespread public health implications is that high consumption of plant foods provides measurable protection against many chronic diseases, including cardiovascular disease (CVD).1 The underlying mechanisms appear to be, at least partially, because of numerous phytochemical compounds contained in plant foods, many of which are potent antioxidants.

Antioxidants are present in human blood, cells, and tissues. They are derived from intrinsic antioxidant defense systems and also from extrinsic sources, such as diet or pharmacotherapy.2 The principal antioxidants in human blood are uric acid and albumin.3 The remainder of the antioxidant capacity is largely because of vitamin C, bilirubin, vitamin E, flavonoids, carotenoids, and serum proteins, such as transferrin and ceruloplasmin.4 Antioxidants from dietary sources have attracted interest, especially vitamin E, vitamin C, β-carotene, and other carotenoids, including lutein, zeaxanthin, and lycopene, which have among the highest singlet oxygen-quenching properties.5 These antioxidants are thought to protect against oxidative stress, a hypothetical etiologic factor in human aging,6 and age-related disease, such as CVD.7–9

In 1979, Goldstein et al7 originally set forth the hypothesis that native low-density lipoprotein (LDL) undergoes an oxidative modification that makes it a target for scavenger receptors that subsequently incorporate the modified LDL into atherosclerotic plaque. This hypothesis is supported by several lines of evidence.9 In addition, there is evidence that antioxidants might protect against this process. For example, most blood carotenoids are associated with plasma lipoproteins, and a large fraction of carotenoids and tocopherols are transported by LDL.10,11 The LDL oxidation hypothesis—a central tenet of the antioxidant/CVD link—is a natural byproduct of the original free radical hypothesis of Harman.6 Indeed, oxidation of several human biologic components, including lipids, proteins, and DNA, results in oxidative byproducts that are measurable in vitro and in...
vivo. Therefore, it is plausible that antioxidants might play a role in preventing CVD and other chronic age-related diseases.

Given the marked differences in CVD mortality across populations, many of whom differ greatly in dietary intake of antioxidant-rich plant foods, dietary antioxidants could be a major protective factor. Studies, such as the Nippon-Honolulu-San Francisco (Ni-Hon-San) study, the fore-runner of the Honolulu Heart Program, the Seven Countries Study, and the Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) study were founded, in part, on such hypotheses. These studies focused on large cross-national differences in CVD, undertook detailed dietary assessments among other data, and initially published cross-sectional analyses of possible diet–disease connections.

Subsequent work in some cohorts investigated blood levels of antioxidants in relation to CVD end points. For example, the MONICA study demonstrated an inverse association (relative risk [RR], 0.49; \( p = 0.01 \)) between lipid-standardized \( \alpha \)-tocopherol levels from stored plasma and mortality attributable to ischemic heart disease in 16 European populations. As the cohorts were observed over time, early promising findings from cross-sectional studies led to more detailed, long-term prospective studies. Some of this work focused on particular antioxidants, especially dietary intakes of \( \beta \)-carotene and vitamins C and E, while others focused on supplement use. Several strongly positive findings supporting a protective role for antioxidants followed in larger cohort studies (see below).

As the evidence began to mount from animal studies and human epidemiologic studies on potential protective effects of antioxidants, excitement in both the lay and medical communities also began to grow. The idea that natural compounds, if taken in supplement form, may offer a broad and inexpensive means of decreasing the risk for CVD and other age-related diseases was a very attractive hypothesis. In 1991, a panel of experts convened by the US National Heart, Lung, and Blood Institute believed that the evidence was strong enough to justify clinical trials. Enthusiasm has grown to the point that approximately 40% of the US adult population is taking some form of dietary supplement, the long-term consequences of which are not yet known.

However, recent findings from clinical intervention studies are cause for concern. Not only have there been several null findings, but some studies have also found possible harm from intake of large doses of single supplements, or from cocktails of supplements. The negative interventional studies have led some researchers to question whether current supplemental forms of antioxidant(s) might do more harm than good. This has led to reevaluation of the oxidative modification hypothesis itself, despite ample evidence from in vitro and animal studies. Therefore, given the current controversy, particularly the disconnect between the epidemiologic studies and the interventional studies, a way forward is needed. Do we abandon years of work in this field or do we sharpen the research questions, methods, and outcomes? This article explores the current state of evidence from epidemiologic studies on antioxidants and CVD, as well as the limitations of such research. It also offers a brief overview of the clinical trial evidence and suggests some potential further avenues of study for this important research area.

**Observational Epidemiologic Studies**

The antioxidant hypothesis was initially supported by in vitro studies, animal work, and cross-sectional human studies. Thus, prospective cohort studies were the next logical step to assess the validity of the findings. Prospective cohort studies are less subject to selection and recall bias because information on exposures is usually collected before disease develops. Such studies work much more efficiently when assessing common predictors and common outcomes, and because there are large variations in diet between different people, especially cross-nationally, dietary antioxidants are a ripe target for such studies. A number of such studies have been conducted, mainly on carotenoids, vitamin C, and vitamin E.

**Carotenoids and Cardiovascular Disease**

Carotenoids were among the earliest antioxidant classes targeted for prospective epidemiologic study. They occur in a class of 600-plus naturally occurring pigments and are synthesized mainly by plants, algae, and photosynthetic bacteria. Vegetables and fruits are the most common food sources for human populations. \( \alpha \)-Carotene, \( \beta \)-carotene, \( \beta \)-Cryptoxanthin, lutein, zeaxanthin, and lycopene are the most common dietary carotenoids and have been the most extensively studied, particularly \( \beta \)-carotene. \( \alpha \)-Carotene, \( \beta \)-carotene, and \( \beta \)-cryptoxanthin are provitamin A carotenoids, whereas lutein, zeaxanthin, and lycopene cannot be converted to retinol and hence have no vitamin A activity. Evidence that the carotenoids are intimately associated with circulating lipoproteins includes their presence in both LDL and high-density lipoprotein (HDL) particles, which suggests a mechanistic role, possibly as protectors against oxidation. Indeed, smaller, denser LDL that has a lower carotenoid concentration is more easily oxidized.

Several prospective cohort studies have shown an inverse association between \( \beta \)-carotene intake through diet or supplements and CVD (Table 1). Among the earliest and largest studies were the Health Professionals Follow-Up Study (HPFS), a study of approximately 40,000 male health professionals, and the Nurse’s Healthy Study (NHS), a study of approximately 80,000 female nurses. The HPFS compared the highest quintile of dietary carotene intake versus the lowest and showed an inverse association with CVD risk among current
Table 1: Selected cohort studies of \( 
\begin{array}{|c|c|c|c|c|}
\hline
\text{Year} & \text{Study} & N & \text{Age (yr)} & \text{Study Population} & \text{Follow-Up (yr)} & \text{Outcomes} & \text{RRR or RR (95\% CI)} \\
\hline
1993 & Health Professionals Follow-up & 24 & 39–910 & US male health professionals & 4 & CAD & RR, 0.30 (0.11–0.82)* \\
1994 & Longitudinal Population Study (Finland) & 25 & 2,748 men & 30–69 Finnish & 12–16 & CAD & RRR, 29% to 70% \\
 & & & 2,385 women & & & & \\
 & & & & & & & \\
1995 & Massachusetts Health Care Panel Study & 27 & 1,299 & US elderly & 4 & CVDM & RR, 0.54 (0.34–0.86) \\
1999 & Rotterdam Study & 28 & 4,802 & Dutch men and women & 4 & MI & RR, 0.25 (0.09–0.67) \\
 & & & & & & & \\
2000 & NHANES III & 29 & 11,327 & US men and women & 10 & Angina Pectoris & RR, 0.57 (0.38–0.86) ¶ \\
2003 & Nurses’ Health Study & 30 & 73,286 & US nurses & 12 & CVD & RR, 0.74 (0.59–0.93) †† \\
\hline
\end{array}
\end{align*}

**Notes:** RRR = reduction in relative risk.

* Adjusted for age, smoking, body mass index, caloric intake, fiber intake, alcohol use, hypertension, aspirin use, exercise, family history, profession, and vitamin use.
† Adjusted for age, smoking, serum cholesterol level, hypertension, body mass index and energy intake.
‡ Adjusted for age, body mass index, high-density lipoprotein cholesterol level, low-density lipoprotein cholesterol level, systolic blood pressure, aspirin use, diabetes, physical activity, current smoking, alcohol intake, and vitamin use.
¶ Adjusted for age, sex, race or ethnicity, education, smoking status, systolic blood pressure, serum cholesterol, high-density lipoprotein cholesterol, history of diabetes mellitus, body mass index, and physical activity.
†† Adjusted for age, smoking, alcohol use, menopausal status, hormone use, exercise, aspirin use, hypertension, cholesterol level, diabetes, caloric intake, vitamin intake, and circulating antioxidants.

**Definitions:**
- CAD: coronary artery disease
- CVD: cardiovascular disease
- MI: myocardial infarction
- CVDM: cardiovascular disease mortality
- NS: not significant
- RR: relative risk
- RRR: reduction in relative risk

**Notes on Studies:**
- The Health Professionals Follow-up Study observed 24,385 male health professionals aged 39–910 years, with a follow-up of 4 years, finding a RR of 0.30 (95% CI, 0.11–0.82) for CAD.
- The Longitudinal Population Study (Finland) involved 2,748 men and 2,385 women aged 30–69 years, with a follow-up of 12–16 years, finding a reduction in relative risk (RRR) ranging from 29% to 70% for CAD.
- The Massachusetts Health Care Panel Study observed 1,299 elderly individuals for 4 years, finding a RR of 0.54 (95% CI, 0.34–0.86) for CVDM.
- The Rotterdam Study involved 4,802 participants aged 55–90 years, with a follow-up of 4 years, finding a RR of 0.25 (95% CI, 0.09–0.67) for MI.
- The NHANES III study observed 11,327 US men and women aged 35–90 years for 10 years, finding a RR of 0.57 (95% CI, 0.38–0.86) for Angina Pectoris.
- The Nurses’ Health Study followed 73,286 US nurses aged 34–59 years for 12 years, finding a RR of 0.74 (95% CI, 0.59–0.93) for CVD.

**Vitamin C and CVD:**

Vitamin C (ascorbic acid) is a 6-carbon lactone. It is synthesized from hepatic glucose in most mammalian species but not in primates. Vitamin C as an electron donor is a potent water-soluble antioxidant in humans and is among the most abundant antioxidants. The antioxidant effects of vitamin C have been demonstrated in many experiments in vitro.

Because the free radical theory of aging posits that oxidative damage is at the root of many aspects of human aging...
and that free radicals increase the risk for age-related diseases, it is natural that it might be speculated that vitamin C should play a protective role against human CVD. However, the prospective cohort data have not generally been as supportive for vitamin C as for α-carotene.

A selection of major prospective studies on the relation of vitamin C and CVD appears in Table 2.24,25,28,35–42 For example, the National Health and Nutrition Examination Surveys (II and III)38,39 and the Eastern Finland Study37 supported a protective role for vitamin C in risk reduction for several end points that represent CVD and coronary artery disease (CAD), in separate models.37,39 With regard to peripheral arterial disease, data from the Rotterdam study32 revealed that vitamin C had a significant protective effect, but only among women. The ARIC study35 assessed dietary antioxidants25 and carotid IMT in men and women aged 55–64 years. When comparing the highest versus the lowest quintile, they found that vitamin C was significantly and inversely related to carotid IMT thickness. This occurred only in women and only in the older group after adjusting for all covariates.

In contrast, the Health Professionals Follow-up Study, the Nurses Health Study, and the Iowa Women’s Health Study did not find a protective effect for vitamin C against CVD.41 Overall, there was some encouraging but not overwhelming support for vitamin C as a protector against CVD (Table 2).

Vitamin E and Cardiovascular Disease

Vitamin E is among the most important fat-soluble antioxidants.43 It consists of a series of methylated phenols and exists in 8 different forms: 4 tocopherols and 4 tocotrienols. Each possesses a chromanol ring, with a hydroxyl group that can donate a hydrogen atom and reduce free radicals. Each also possesses a hydrophobic side chain that enables vitamin E to penetrate biologic membranes. Each version of vitamin E has a somewhat different biologic activity, but α-tocopherol appears to be the most powerful.44 Therefore, there are several reasons to suspect that vitamin E, particularly α-tocopherol, might be a potentially important protector against CVD and other diseases linked to oxidative damage. This hypothesis was particularly enticing after the promising cross-sectional findings from the MONICA study.

Indeed, the initial findings from large, long-term cohort studies, including both the Nurse’s Health Study and the Health Professionals Follow-up Study, generated much excitement. A selection of cohort studies investigating the relation of vitamin E intake and CVD appears in Table 3.24,25,28,36,40,45–47 Among the first and most exciting findings, the Nurses Health Study45 showed that supplemental vitamin E use for ≥2 years predicted lower CVD risk, but this protection was lost at lower doses and shorter follow-up. Similarly, the Iowa Women’s Health Study found a significant protective effect of vitamin E from food sources on the risk for CVD death.46 In another positive finding, almost 40,000 men (aged 45–75 years) were studied for 4 years in the Health Professionals Follow-up Study.24 Consumption of 60 IU/day of vitamin E was associated with an approximate 40% risk reduction for CVD compared with those consuming <7.5 IU/day in a multivariate model. A Finnish study of men and women aged 30–69 years with 14 years of follow-up also found a protective effect for dietary vitamin E intake in men as well as for women when comparing the top versus the bottom quintile.

The ARIC study35 assessed carotid IMT in men and women aged 55–64 years. After comparing the highest versus the lowest quintile, the p-value for trend across quintiles showed that α-tocopherol was significantly and inversely related to carotid IMT thickness. This occurred only in the older women after adjusting for all covariates.

Less impressive results were seen in the Rotterdam Study,32 which assessed vitamin E intake and peripheral vascular disease in older men and women. Vitamin E was found to be of borderline significance and protected men only.

Overall, the epidemiologic findings were quite favorable for vitamin E from both diet and supplements and supported what had been seen in prior in vitro and animal studies. This suggested that vitamin E would be a good choice for clinical intervention trials.

Limitations of Epidemiologic Methods

Although results from epidemiologic cohort studies, overall, are quite encouraging, especially given the difficulty of accurate measurement of dietary antioxidants (or accurate reporting of supplement intake), there are always limitations. For example, we cannot control for the potential effects of unmeasured confounders. When dealing with small-to-moderate effect sizes, as might be seen with antioxidant intake, large uncontrolled confounding factors may blur or overwhelm the antioxidant effects and can lead to biased results.

This can be particularly problematic with dietary studies because “healthy cohort” effects can be quite large. Study participants with healthier diets may have higher dietary or supplemental intake of antioxidants, but they often practice other healthy behaviors that might bias the results.48

Another limit is that causation cannot be assessed. Prospective cohort studies are good for hypothesis generation but cannot establish causation. Thus, it was with great anticipation that interventional clinical trials, particularly double-blind, placebo-controlled, randomized trials, followed the initial cohort studies.

Disappointing Clinical Trials: A Brief Word

The encouraging findings from the prospective cohort studies suggested that clinical trials would find clear benefits for
## Table 2
### Selected cohort studies of vitamin C and cardiovascular disease (CVD)

<table>
<thead>
<tr>
<th>Year</th>
<th>Study</th>
<th>N</th>
<th>Age (yr)</th>
<th>Study Population</th>
<th>Follow-Up (yr)</th>
<th>Outcomes</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>Health Professionals’ Follow-up</td>
<td>39,910</td>
<td>40–75</td>
<td>US male health professionals</td>
<td>4</td>
<td>MI, CVD</td>
<td>NS</td>
</tr>
<tr>
<td>1994</td>
<td>Longitudinal Population Study (Finland)</td>
<td>5,133</td>
<td>30–69</td>
<td>Finnish men (2,748) and women (2,385)</td>
<td>12–16</td>
<td>CAD</td>
<td>NS, RR: 0.49 (0.34–0.98)</td>
</tr>
<tr>
<td>1995</td>
<td>Atherosclerosis Risk in Communities</td>
<td>11,307</td>
<td>45–67</td>
<td>US 4,989 men and 6,318 women</td>
<td>—</td>
<td>Carotid IMT</td>
<td>NS*</td>
</tr>
<tr>
<td>1996</td>
<td>Iowa Women’s Health Study</td>
<td>34,486</td>
<td>55–69</td>
<td>PM women</td>
<td>7</td>
<td>CAD</td>
<td>NS†</td>
</tr>
<tr>
<td>1997</td>
<td>Eastern Finland Study</td>
<td>1,605</td>
<td>42, 48, 54, 60</td>
<td>Finnish healthy men</td>
<td>8</td>
<td>MI</td>
<td>3.5 (1.8–6.7)†</td>
</tr>
<tr>
<td>1999</td>
<td>Rotterdam Study</td>
<td>4,802</td>
<td>55–95</td>
<td>Dutch men and women</td>
<td>4</td>
<td>MI</td>
<td>NS</td>
</tr>
<tr>
<td>1999</td>
<td>NHANES III</td>
<td>7,658</td>
<td>&gt;30</td>
<td>US subjects</td>
<td>—</td>
<td>CVD</td>
<td>0.48 (0.23–1.03) §</td>
</tr>
<tr>
<td>2001</td>
<td>NHANES II</td>
<td>8,459</td>
<td>&gt;30</td>
<td>US subjects</td>
<td>—</td>
<td>CVD</td>
<td>—</td>
</tr>
<tr>
<td>2002</td>
<td>Physicians Health Study</td>
<td>83,638</td>
<td>44–64</td>
<td>US male physicians</td>
<td>5.5</td>
<td>CVDM</td>
<td>RR: 0.88 (0.61–1.27) §</td>
</tr>
<tr>
<td>2003</td>
<td>Nurses’ Health Study</td>
<td>85,118</td>
<td>38–63</td>
<td>US nurses</td>
<td>16</td>
<td>MI, CVD</td>
<td>NS</td>
</tr>
<tr>
<td>2004</td>
<td>Iowa Women’s Health Study</td>
<td>1,923</td>
<td>55–69</td>
<td>Women with diabetes</td>
<td>15</td>
<td>CVD</td>
<td>1.84 (1.12–3.01)</td>
</tr>
</tbody>
</table>

**CAD = coronary artery disease; CADM = coronary artery disease mortality (fatal MI); CI = confidence interval; CVDM = cardiovascular disease mortality; IMT = intima media thickness; MI = myocardial infarction; NHANES = National Health and Nutrition Examination Survey; NS = not significant; PM = postmenopausal; RR = relative risk; RRR = reduction in relative risk.**

* Adjusted for age, gender, race, education, physical activity, smoking, alcohol use, cholesterol, body mass index, diabetes, hypertension, history of cardiovascular disease, aspirin use and vitamin use.

† Adjusted for age, energy intake, body mass index, waist-hip ratio, smoking status, diabetes, hypertension, physical activity, estrogen use, alcohol use, marital status, education.

‡ Adjusted for age, year of examination, season of year.

§ Among participants who consumed alcohol, association with a decreased prevalence of angina (multivariate odds ratio).

∥ Adjusted for alcohol use, smoking history, aspirin use, and medical conditions.

### Table 3
### Selected cohort studies of vitamin E and cardiovascular disease (CVD)

<table>
<thead>
<tr>
<th>Year</th>
<th>Study</th>
<th>N</th>
<th>Age (yr)</th>
<th>Study Population</th>
<th>Follow-Up (yr)</th>
<th>Outcomes</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>Nurses’ Health Study</td>
<td>87,245</td>
<td>34–59</td>
<td>US female nurses</td>
<td>8</td>
<td>CAD, MI</td>
<td>RR: 0.66 (0.50–0.87)*</td>
</tr>
<tr>
<td>1993</td>
<td>Health Professionals Follow-up</td>
<td>39,910</td>
<td>40–75</td>
<td>US male health professionals</td>
<td>4</td>
<td>CAD</td>
<td>RR: 0.60 (0.44–0.81)</td>
</tr>
<tr>
<td>1994</td>
<td>Longitudinal Population Study (Finland)</td>
<td>5,133</td>
<td>30–69</td>
<td>Finnish men (2,748) and women (2,385)</td>
<td>12–16</td>
<td>CAD</td>
<td>RR: 0.68; p for trend &lt;0.01</td>
</tr>
<tr>
<td>1996</td>
<td>Iowa Women’s Health Study</td>
<td>34,486</td>
<td>55–69</td>
<td>PM women</td>
<td>7</td>
<td>CADM</td>
<td>RR: 0.42; p for trend &lt;0.01</td>
</tr>
<tr>
<td>1996</td>
<td>Established Populations for Epidemiologic Studies of the Elderly</td>
<td>11,178</td>
<td>67–105</td>
<td>US men and women</td>
<td>9</td>
<td>M, CADM</td>
<td>RR: 0.53 (0.34–0.84)†</td>
</tr>
<tr>
<td>1998</td>
<td>Multiple Risk Factor Intervention Trial</td>
<td>734</td>
<td>35–57</td>
<td>US men</td>
<td>20</td>
<td>MI, CVDM</td>
<td>NS</td>
</tr>
<tr>
<td>1999</td>
<td>Rotterdam Study</td>
<td>4,802</td>
<td>55–95</td>
<td>Dutch men and women</td>
<td>4</td>
<td>MI</td>
<td>NS</td>
</tr>
<tr>
<td>2002</td>
<td>Physicians’ Health Study</td>
<td>83,638</td>
<td>44–64</td>
<td>US male physicians</td>
<td>5.5</td>
<td>CVDM</td>
<td>NS</td>
</tr>
</tbody>
</table>

**CAD = coronary artery disease; CADM = coronary artery disease mortality (fatal MI); CI = confidence interval; CVDM = cardiovascular disease mortality; M = all-cause mortality; MI = myocardial infarction; NS = not significant; PM = postmenopausal; RR = relative risk.**

* Adjusted for age and smoking.

† Adjusted for age, sex, alcohol use, smoking history, aspirin use, and medical conditions, cigarette smoking, education, and consumption of citrus fruits and vegetables.
<table>
<thead>
<tr>
<th>Year</th>
<th>Study</th>
<th>N</th>
<th>Age (yr)</th>
<th>Study Population</th>
<th>Dose</th>
<th>Follow-Up (yr)</th>
<th>Outcome</th>
<th>RR/Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>Skin Cancer Prevention Study</td>
<td>1,805</td>
<td>&lt;85</td>
<td>Skin cancer patients (M&amp;F)</td>
<td>50 mg</td>
<td>8.2</td>
<td>No effect on CVDM</td>
<td>1.16 (0.82–1.64)</td>
</tr>
<tr>
<td></td>
<td>Physicians’ Health Study</td>
<td>22,071</td>
<td>40–84</td>
<td>Healthy male physicians</td>
<td>50 mg on alternate days</td>
<td>12</td>
<td>No effect on MI CVD</td>
<td>0.96 (0.84–1.09)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MI CVDM</td>
<td>1.00 (0.91–1.09)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CADM</td>
<td>1.09 (0.93–1.27)</td>
</tr>
<tr>
<td>1998</td>
<td>Alpha-Tocopherol–Beta-Carotene Cancer Prevention Study</td>
<td>27,271</td>
<td>50–69</td>
<td>Male smokers with no history of MI</td>
<td>20 mg</td>
<td>6.1</td>
<td>No effect on CAD MI</td>
<td>1.03 (0.91–1.16)</td>
</tr>
<tr>
<td>1999</td>
<td>Women’s Health Study</td>
<td>39,876</td>
<td>&gt;45</td>
<td>Female health professionals</td>
<td>50 mg on alternate days</td>
<td>2.1</td>
<td>MI, CVDM</td>
<td>NS</td>
</tr>
<tr>
<td>2007</td>
<td>Women’s Antioxidant Cardiovascular Study</td>
<td>8,171</td>
<td>PM ≥ 40</td>
<td>Subjects with CVD history/risk</td>
<td>50 mg on alternate days</td>
<td>9.4</td>
<td>MI, Stroke, CVD, CVDM</td>
<td>1.02 (0.92–1.13)</td>
</tr>
<tr>
<td></td>
<td>Vitamin C</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>Women’s Antioxidant Cardiovascular Study</td>
<td>8,171</td>
<td>PM ≥ 40</td>
<td>Subjects with CVD history/risk</td>
<td>500 mg</td>
<td>9.4</td>
<td>MI, Stroke, CVD, CVDM</td>
<td>1.02 (0.92–1.13)</td>
</tr>
<tr>
<td></td>
<td>Vitamin E</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1996</td>
<td>Alpha-Tocopherol–Beta-Carotene Cancer Prevention Study</td>
<td>22,269</td>
<td>50–69</td>
<td>Male smokers</td>
<td>50 mg</td>
<td>4.7</td>
<td>Angina pectoris</td>
<td>0.91 (0.83–0.99)</td>
</tr>
<tr>
<td>2000</td>
<td>Heart Outcomes Protection Evaluation trial</td>
<td>9,541</td>
<td>≥ 55</td>
<td>High CVD risk subjects (M&amp;F)</td>
<td>400 IU (natural)</td>
<td>4.5</td>
<td>No effect on MI, CVDM, stroke</td>
<td>1.05 (0.95–1.16)</td>
</tr>
<tr>
<td>2001</td>
<td>Primary Prevention Project</td>
<td>4,495</td>
<td>64</td>
<td>Subjects at CVD risk (M&amp;F)</td>
<td>300 mg (synthetic)</td>
<td>3.6</td>
<td>No effect on MI, CVDM, stroke</td>
<td>1.07 (0.74–1.56)</td>
</tr>
<tr>
<td>2002</td>
<td>Vitamin E Atherosclerosis Prevention Study</td>
<td>353</td>
<td>≥ 40</td>
<td>Elevated LDL-C (M&amp;F)</td>
<td>400 IU dl-α-tocopherol</td>
<td>3</td>
<td>No effect on IMT or CVD</td>
<td>p = 0.81 for CVD events</td>
</tr>
<tr>
<td>2007</td>
<td>Women’s Antioxidant Cardiovascular Study</td>
<td>8,171</td>
<td>PM ≥ 40</td>
<td>Subjects with CVD history/risk</td>
<td>600 IU on alternate days</td>
<td>9.4</td>
<td>MI, stroke, CVD, CVDM</td>
<td>0.94 (0.85–1.04)</td>
</tr>
<tr>
<td></td>
<td>Secondary Prevention</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1996</td>
<td>Cambridge Heart Antioxidant Study</td>
<td>2,002</td>
<td>62</td>
<td>Coronary disease (M&amp;F)</td>
<td>400 or 800 IU</td>
<td>1.4</td>
<td>Decreased nonfatal acute MI</td>
<td>0.23 (0.11–0.47)</td>
</tr>
<tr>
<td>1997</td>
<td>Alpha-Tocopherol–Beta-Carotene Cancer Prevention Study</td>
<td>1,862</td>
<td>50–69</td>
<td>Male smokers who had an MI</td>
<td>50 mg</td>
<td>5.3</td>
<td>38% reduction in MI</td>
<td>0.62 (0.41–0.96)</td>
</tr>
<tr>
<td>1999</td>
<td>Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto Miocardico-Prevention Trial</td>
<td>11,324</td>
<td>No age limits</td>
<td>Italian post-MI adults (M&amp;F)</td>
<td>300 mg (synthetic)</td>
<td>3.5</td>
<td>No effect on MI, CVDM, stroke</td>
<td>0.98 (0.87–1.10)</td>
</tr>
<tr>
<td>2000</td>
<td>Secondary Prevention with Antioxidants of Cardiovascular Disease in End-stage Renal Disease</td>
<td>196</td>
<td>40–75</td>
<td>Hemodialysis patients (M&amp;F)</td>
<td>800 IU</td>
<td>2</td>
<td>Decreases CVD</td>
<td>0.46 (0.27–0.78)</td>
</tr>
<tr>
<td>2002</td>
<td>Microalbuminuria Cardiovascular Renal Outcomes/HOPE</td>
<td>3,654</td>
<td>65</td>
<td>Diabetes (M&amp;F)</td>
<td>400 IU (natural)</td>
<td>4.5</td>
<td>No effect on MI, CVDM, stroke</td>
<td>1.03 (0.88–1.21)</td>
</tr>
</tbody>
</table>
## Table 4
(continued)

<table>
<thead>
<tr>
<th>Year</th>
<th>Study</th>
<th>N</th>
<th>Age (yr)</th>
<th>Study Population</th>
<th>Dose</th>
<th>Follow-Up (yr)</th>
<th>Outcome</th>
<th>RR/Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>Linxian Trial&lt;sup&gt;49&lt;/sup&gt;</td>
<td>29,584</td>
<td>40–69</td>
<td>Under-nourished Chinese (M&amp;F)</td>
<td>30 mg vitamin E, 15 mg β-carotene, selenium</td>
<td>5.7</td>
<td>M</td>
<td>0.91 (0.84–0.99)</td>
</tr>
<tr>
<td>1996</td>
<td>Beta-Carotene and Retinol Efficacy Trial&lt;sup&gt;53&lt;/sup&gt;</td>
<td>18,314</td>
<td>45–69</td>
<td>Heavy smokers and asbestos workers</td>
<td>30 mg β-carotene, 25,000 IU retinol</td>
<td>4</td>
<td>M</td>
<td>1.17 (1.03–1.33)</td>
</tr>
<tr>
<td>1998</td>
<td>Alpha-Tocopherol–Beta-Carotene Cancer Prevention Study&lt;sup&gt;52&lt;/sup&gt;</td>
<td>27,271</td>
<td>50–69</td>
<td>Male smokers with no history of MI</td>
<td>50 mg vitamin E and 20 mg β-carotene</td>
<td>6.1</td>
<td>No effect on CAD</td>
<td>0.97 (0.86–1.09)</td>
</tr>
<tr>
<td>2000</td>
<td>Antioxidant Supplementation in Atherosclerosis Prevention Study&lt;sup&gt;78&lt;/sup&gt;</td>
<td>520</td>
<td>45–69</td>
<td>Elevated cholesterol levels (M&amp;F)</td>
<td>182 d-α-tocopherol, 500 mg vitamin C</td>
<td>3</td>
<td>Progression of IMT</td>
<td>0.26 (0.11–0.64)</td>
</tr>
<tr>
<td>2002</td>
<td>Intravascular Ultrasonography Study&lt;sup&gt;66&lt;/sup&gt;</td>
<td>40</td>
<td>≥18</td>
<td>After cardiac transplantation (M&amp;F)</td>
<td>500 mg vitamin C, 400 IU vitamin E</td>
<td>1</td>
<td>Greater IMT increase in placebo group</td>
<td>p = 0.008</td>
</tr>
<tr>
<td>2002</td>
<td>Heart Protection Study&lt;sup&gt;79&lt;/sup&gt;</td>
<td>20,536</td>
<td>40–80</td>
<td>Subjects at high CVD risk</td>
<td>600 mg vitamin E, 250 mg vitamin C, 20 mg β-carotene</td>
<td>—</td>
<td>No effect on CVDM</td>
<td>1.05 (0.95–1.15)</td>
</tr>
<tr>
<td>2002</td>
<td>Women’s Angiographic Vitamin and Estrogen Trial&lt;sup&gt;80&lt;/sup&gt;</td>
<td>423</td>
<td>PM</td>
<td>PM women CVD patients</td>
<td>0.625 equine estrogen/day, 400 IU vitamin E and 500 mg vitamin C bid</td>
<td>5</td>
<td>M, MI, Stroke</td>
<td>NS</td>
</tr>
<tr>
<td>2002</td>
<td>MRC/BHF Heart Protection Study&lt;sup&gt;79&lt;/sup&gt;</td>
<td>20,536</td>
<td>40–80</td>
<td>Subjects at high CVD risk</td>
<td>600 mg vitamin E, 250 mg vitamin C, 20 mg β-carotene</td>
<td>5</td>
<td>Coronary events, fatal or nonfatal CVD</td>
<td>NS</td>
</tr>
</tbody>
</table>

CAD = coronary artery disease; CADM = coronary artery disease mortality (fatal MI); CI = confidence interval; CVDM = cardiovascular disease mortality; HOPE = Heart Outcomes Prevention Evaluation; IMT = intima media thickness; LDL-C = low-density lipoprotein cholesterol; M&F = male and female patients; M = all-cause mortality; MI = myocardial infarction; Medical Research Council/British Heart Foundation; NS = not significant; PM = postmenopausal; RR = relative risk.
antioxidants in primary and/or secondary prevention of CVD. Randomized, blinded placebo-controlled trials are largely free of bias and confounding and should theoretically have confirmed the epidemiologic findings.\textsuperscript{49–52} Unfortunately, this was not the case. The positive findings in both primary and secondary prevention have been few, yielding largely negative results, and there has even been some suggestion of harm.\textsuperscript{20}

Among the first major randomized controlled trials of antioxidant supplementation was the Linxian Trial in China, which was primarily a study of cancer. Approximately 30,000 adults aged 40–69 years received 1 of 4 nutrient combinations; 2 of these combinations were vitamin C/molybdenum and \( \beta \)-carotene/vitamin E/selenium, respectively. These antioxidants were studied in subsequent CVD trials. The Linxian Trial documented significantly lower all-cause mortality (RR, 0.91; 95% CI, 0.84–0.99) in those using the latter antioxidant cocktail.\textsuperscript{49} However, the event rate for CVD was too low to calculate a reliable RR for CVD. The population (generally malnourished) was also not representative of typical populations from developed countries. However, the antioxidant-related mortality reduction helped spur enthusiasm for further work in the field.

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study soon followed. This was a Finnish study of approximately 30,000 male smokers aged 50–69 years. Subjects were randomized to 1 of 4 interventions: \( \beta \)-carotene (20 mg/day), \( \alpha \)-tocopherol (50 mg/day, 75 IU), \( \alpha \)-tocopherol and \( \beta \)-carotene, or placebo. Patients were then observed for up to 8 years for cancer-related outcomes; CVD was assessed as a secondary outcome. Unfortunately, \( \alpha \)-tocopherol did not reduce total mortality or CVD mortality.\textsuperscript{50–52} On the contrary, \( \alpha \)-tocopherol was linked to increased risk for mortality from hemorrhagic stroke. Further subgroup analysis showed that systolic blood pressure was a potential effect modifier of \( \alpha \)-tocopherol, and stratification analysis showed that vitamin E users with hypertension had an increased risk of subarachnoid hemorrhage and a decreased risk of cerebral infarction. The ATBC study, which was powered to assess cancer as the primary outcome, also assessed angina pectoris in men.\textsuperscript{51} Results unexpectedly showed that \( \beta \)-carotene was associated with a borderline significant increase in angina.

A psychological turning point in primary prevention trials of antioxidants was the Beta Carotene and Retinol Efficacy Trial (CARET), which studied a population at high-risk for CVD (heavy smokers) as well as asbestos-exposed workers. Investigators studied a combination of 30 mg/day of \( \beta \)-carotene and 25,000 IU/day of retinol (vitamin A).\textsuperscript{53,54} Negative effects of \( \beta \)-carotene and vitamin A led to early termination. Concerns centered on the fact that this population was not representative of the general population, but there was palpable angst in the antioxidant community.

Secondary prevention trials also appeared to be off to a positive start with the Cambridge Heart Antioxidant Study (CHAOS). Investigators assessed approximately 2,000 participants who had angiographically proven coronary atherosclerosis. Participants were observed for approximately 18 months after an intervention of 800 IU/day vitamin E (\( \alpha \)-tocopherol; 537 mg as free \( 2R,4'R,8'R \)-\( \alpha \)-tocopherol)\textsuperscript{55} versus placebo. The intervention group had a significantly lower risk for CVD death and nonfatal MI. Most of the treatment effect was attributable to a lower risk for MI.

Similarly, positive results were seen with the Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto Miocardico-Prevenzione (GISSI) trial. Middle-aged, high-risk subjects who received vitamin E 400 IU/day had a 40% RR reduction for CVD.

The initial excitement began to fade when other trials could not replicate these positive findings. For example, the Heart Outcomes Prevention Evaluation (HOPE) trial assessed vitamin E and ramipril (an angiotensin-converting enzyme inhibitor) in high-risk CVD subjects (aged ≥55 years) with diabetes mellitus and ≥1 additional CVD risk factors. Participants received 400 IU vitamin E from natural sources (RRR-\( \alpha \)-tocopherol) and/or 10 mg ramipril or placebo and were observed for approximately 5 years.\textsuperscript{56,57} There was no effect on CVD outcomes in the vitamin E intervention group. However, subgroup analysis revealed that ramipril had a significant treatment effect on CVD mortality and morbidity.

Negative results were also seen in the Women’s Angiographic Vitamin and Estrogen Trial and the MRC/BHF Heart Protection Study with various combinations of vitamin E, vitamin C, and \( \beta \)-carotene. Worse still, the ATBC Trial found an increased risk for coronary events in subjects aged 50–69 years receiving vitamin E or \( \beta \)-carotene.

A meta-analysis of 15 clinical trials on CVD outcomes failed to show benefits for antioxidants against CVD outcomes. These studies included 8 \( \beta \)-carotene trials with doses of 15–50 mg/day and 8 studies of vitamin E with doses of 50–800 IU/day). Most trials included >1,000 participants. Lack of efficacy was shown across a range of doses, different types and degrees of CVD, and in various populations of men and women.\textsuperscript{58} A systematic review and meta-analysis of the use of antioxidant supplementation all-cause mortality cast further doubt on the efficacy of several antioxidants as preventive agents.\textsuperscript{20} More concerning, this study suggested increased harm from supplemental vitamin E, vitamin A, and \( \beta \)-carotene.

Why the Mismatch?

Studies on nutritional factors and chronic diseases are complex. Several possible confounding factors might be behind the mismatch between the epidemiologic studies and intervention trials. A review of the carotenoid literature by Kritchevsky\textsuperscript{49} suggested that the fact that a diet rich in carotenoid foods is associated with a reduced risk of CVD could be attributable to carotenoids other than \( \beta \)-carotene or to other, unrecognized phytochemicals that correlate with...
A Way Forward: Overcoming Challenges in Study Design

The failure of the interventional studies to find more positive results is perplexing. Many questions remain, including whether (1) the right antioxidants have been used, (2) the appropriate populations have been selected, (3) the studies have been long enough, and (4) the appropriate outcomes have been chosen for clinical trials.

Interestingly, more focused research questions and study populations have produced some new and intriguing findings. Various recent small-scale clinical trials of antioxidants that have been conducted on selected patients with high levels of oxidative stress have found promising results.

As mentioned, the CHAOS trial was a randomized, controlled trial of vitamin E and the risk of death or nonfatal MI in patients who had documented CAD. Theoretically, these patients should have been under greater oxidative stress than healthy patients without CVD because they had established CVD. In CHAOS, the combined end points of death and nonfatal MI revealed an RR of 0.53 in the treatment arm, and this finding was highly significant. Investigators also studied nonfatal MI separately, and the RR was slightly lower and statistically significant (p = 0.049).

Interestingly, the investigators also studied septic shock as an outcome. The RR for septic shock was 0.34, and in critically ill patients, it was 0.28. Again, it was highly significant in the treatment group.

Another small but interesting trial that was conducted in a high oxidative stress population was the Secondary Prevention with Antioxidants of Cardiovascular Disease in End-Stage Renal Disease (SPACE) trial. The SPACE trial was performed in 196 men and women aged 40–75 years who had end-stage renal disease and prior documented CAD. Hemodialysis induces high levels of oxidative stress, which results in accelerated CVD, a reason that this population was selected for antioxidant intervention. The investigators studied the effect of vitamin E (800 IU) treatment on cardiovascular end points, including fatal and nonfatal MI, fatal and nonfatal ischemic stroke, unstable angina, peripheral vascular disease, and sudden death. The overall RR for CVD end points was 0.46 (0.27–0.78) and was statistically significant. When sudden death was excluded, the RR reduction was slightly lower but still significant. For MI, the RR reduction was statistically significant, especially when sudden death was excluded. None of the other end points were statistically significant on their own.

Another interesting secondary prevention trial in a high oxidative stress population was the Intravascular Ultrasound Study (IVUS). The IVUS trial investigated the effects of a daily cocktail of vitamin E (400 IU) and vitamin C (500 mg) on IMT. In total, 40 adult men and women after cardiac transplantation were assessed over a 1-year period. No increase in IMT was seen in the treatment group versus an 8% increase in the placebo group (p = 0.008).

These small trials suggest that careful selection of study subjects, particularly patients undergoing high levels of oxidative stress, may allow differences to be more easily observed in studies of antioxidants and CVD end points.

Conclusion

The antioxidant story has gone from promising to confusing. In vitro, animal and human epidemiologic studies have been quite supportive, but human interventional trials have been mixed. To understand the conflicting data, many challenges lie ahead. Careful reassessment of the evidence is warranted. Both epidemiologic studies and interventional trials can help to further illuminate the role of antioxidants in CVD prevention and treatment. However, more focused research is needed.

First, it must be recognized that a single study cannot be all things to all people. Secondary outcomes must be recognized for what they are—secondary outcomes. Several of the large epidemiologic cohort studies and some of the interventional trials have focused on primary outcomes other than CVD, such as cancer, with CVD as a secondary

β-carotene in the diet or blood. Equally plausible was that low blood carotenoid levels in higher-risk subjects might be because of increased lipoprotein density or greater levels of inflammation, both of which are etiologic factors in CVD.

Such mechanisms may also explain the inverse correlations observed with high intakes or high blood levels of other antioxidants and CVD, including vitamins C and E. Other possible explanations include: (1) vitamin C has been little studied as a single agent in the prevention of CVD; (2) α-tocopherol, the most frequently studied form of vitamin E, may interfere with other potentially more effective forms, such as γ-tocopherol; and (3) some antioxidants or antioxidant cocktails may blunt endogenous antioxidant systems or interfere with medications, such as the protective HDL2 response to statins. A more in-depth treatment of the paradoxical findings of clinical trials is found in an article by Steinhubl.
outcome. Some of these studies had few CVD cases and could not adequately address CVD-related outcomes, yet they were interpreted as null studies. Limited power is an obvious problem.

Second, and related to the former, for both epidemiologic studies and interventional trials, we must investigate representative populations in order to draw generalized conclusions about populations at high risk for CVD.

Third, patient selection for interventional trials needs more careful consideration. If we are truly testing the oxidative stress hypothesis, then patients undergoing higher levels of oxidative stress are more likely to provide measurable outcomes in short-term studies than populations under low oxidative stress. Such studies should be considered a priority.

Fourth, we need better methods to verify the status of single antioxidants (and total antioxidant status) in vivo. Better exposure assessment and more accurate measurement of overall oxidative stress should lead to cleaner outcomes.

Fifth, careful selection of the antioxidants themselves is warranted. All antioxidants are not the same. Just as vitamins perform different functions in the body, antioxidants vary in mechanisms of action, biologic pathways, and functions. Antioxidants cannot simply be thrown together without a priori, evidence-based hypotheses as to their mechanisms of action and their potential outcomes. For example, do we use natural or synthetic forms (eg, natural vitamin E), are we better off using single agents or cocktails, and should we assess whether some antioxidants interfere with other antioxidants in vitro or in animal studies before human trials?

Sixth, pharmacogenetic issues will, at some point, need to be assessed. As we learn more about how different people respond to particular antioxidants based on differences in their genetic profiles, many of the paradoxical findings may become clear.

Greater attention to these and other issues will go a long way to helping us solve the current paradox of antioxidants and CVD risk. Table 4 (see pages 80D and 81D) provides a summary.

Author Disclosures

The authors who contributed to this article have disclosed the following industry relationships:

Bradley J. Willcox, MD, has no financial arrangement or affiliation with a corporate organization or a manufacturer of a product discussed in this article.

J. David Curb, MD, has no financial arrangement or affiliation with a corporate organization or a manufacturer of a product discussed in this article.

Beatriz L. Rodriguez, MD, PhD, has no financial arrangement or affiliation with a corporate organization or a manufacturer of a product discussed in this article.


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