Heart disease and single-vitamin supplementation1–4

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ABSTRACT
Heart disease is the number one cause of death in the United States and has long been recognized to be multifactorial. A growing body of evidence suggests that not only free radical–mediated reactions but also inflammatory responses play major roles in atherogenesis. Vitamin E has both antioxidant and antiinflammatory properties and is the most widely studied vitamin in clinical trials and thus will be the primary example used in this review. Clinical trials of vitamin E efficacy, in hindsight, have been overly optimistic in their expectation that a vitamin could reverse poor dietary habits and a sedentary lifestyle as well as provide benefit beyond that of pharmaceutical agents in treating heart disease. However, it is also apparent that most Americans do not consume dietary amounts adequate to meet established vitamin E requirements. In response to oxidative stressors, vitamin E can decrease biomarkers of lipid peroxidation, is itself killed, and requires optimal vitamin C status to function most effectively. Thus, adequate vitamin E intakes are clearly needed, but what is adequate for what function has yet to be defined. It is noteworthy that in most trials, biomarkers were not used nor were oxidative stress and lipid peroxidation markers used or plasma vitamin E concentrations measured. Am J Clin Nutr 2007;85(suppl):293S–9S.

KEY WORDS Vitamin E, folic acid, biomarkers, oxidative stress, carboxyethyl hydroxy chromans

INTRODUCTION
Heart disease is the number one cause of death in the United States and according to the American Heart Association is also the leading cause of death of women (1). For this presentation, heart diseases include coronary artery disease, hypertensive heart disease, congestive heart failure, peripheral vascular disease, and atherosclerosis, including cerebral artery disease and strokes (2). This review evaluates the use of single-nutrient supplements in the prevention, treatment, or amelioration of heart disease. Vitamin E is the most widely studied with the most clinical trial evidence and thus will be the primary example used.

HEALTHY DIET
Heart disease has long been recognized to be multifactorial; its onset takes decades in most individuals, and diet is well recognized as an important risk factor. Hu and Willett (3) estimated that 74% of coronary events among nonsmokers might have been prevented by eating a healthy diet (nonhydrogenated unsaturated fats, whole grains, abundant fruit and vegetables, and adequate n–3 fatty acids), maintaining a healthy body weight, exercising regularly for ≥30 min/d, and consuming a moderate amount of alcohol (≥5 g/d). Nonetheless, such a diet is apparently extremely difficult to achieve for most Americans. The Women’s Health Initiative Randomized Controlled Dietary Modification Trial (4) aggressively attempted to change women’s diets (reduce total fat intake to 20% of energy, increase intakes of vegetables and fruit to 5 servings/d, and increase grains to ≥6 servings/d), yet the women in the intervention arm only increased fruit and vegetable intake to 1.1 servings/d and had some success in decreasing fat intake. The diet had no significant effect on coronary heart disease but trends toward greater reductions were observed with lower intakes of saturated fat or trans fat or higher intake of vegetables and fruit (4). Given the poor record of individuals making successful changes to their dietary intakes, the possibility of using individual nutrient supplements to stave off the disorder has been a long-standing quest.

CHOLESTEROL AS A BIOMARKER OF HEART DISEASE
Reduction of plasma cholesterol by diet to reduce heart disease risk was unsuccessfully attempted for decades. With the advent of drugs that inhibited cholesterol synthesis (HMGCoA reductase inhibitors, or statins), it was possible to demonstrate that serum cholesterol reduction decreased mortality from coronary heart disease (5), suggesting the use of over-the-counter statins to lower serum cholesterol (6). The data from the statin studies have shown that serum cholesterol is an appropriate biomarker for heart disease risk in that decreasing serum cholesterol also decreases heart disease mortality (5). For example, the Food and Drug Administration has permitted a health claim for phytosterols in lowering heart disease risk given that they lower serum cholesterol (7).

Cholesterol, as an example of a biomarker, gives some guidance as to what might be useful in linking a dietary component, a biomarker, and a disease. Thus, cholesterol as a component of the atherosclerotic lesion is not measurable but cholesterol in the serum or specific lipoproteins can readily be measured. The drug

studies showed that lowering serum cholesterol resulted in decreased mortality from heart disease. Since then it has only been necessary to show that a dietary component reduces serum cholesterol. Unfortunately, no such biomarkers have been described for antioxidants or many of the vitamins tested.

ANTIOXIDANTS AND HEART DISEASE

The question as to why elevated plasma cholesterol, specifically LDL cholesterol, causes atherosclerosis led Steinberg et al (7) to posit that modification of LDLs made these plasma cholesterol carriers more susceptible to uptake by scavenger cells (eg, macrophages). The proposed modification of the protein resulted from lipid peroxidation of the particle. This hypothesis became known as the oxidative modification theory and led to the corollary that antioxidants would be beneficial in stopping lipoprotein oxidation.

Vitamin E: lipid peroxidation and other functions

Vitamin E functions in vivo as a chain-breaking antioxidant that prevents the propagation of free radical damage in biological membranes (8). Specifically, it is a potent peroxyl radical scavenger and especially protects polyunsaturated fatty acids (PUFAs) in membranes and in plasma lipoproteins (9). When lipid hydroperoxides are oxidized to peroxyl radicals, these react 1000 times faster with vitamin E than with PUFAs (10). Specifically, the phenolic hydroxyl group of tocopherol reacts with the peroxyl radical to form the corresponding lipid hydroperoxide and the tocopheroxy radical. In this way, vitamin E acts as a chain-breaking antioxidant, preventing further lipid peroxidation.

A growing body of evidence suggests that not only free radical–mediated reactions but also inflammatory responses play a major role in atherogenesis (11–13). Additionally, scavenger cells (eg, monocyte-macrophages) are critically involved in atherogenesis and secrete biologically active mediators: proinflammatory, proatherogenic cytokines, such as interleukin 1β (IL-1β) and tumor necrosis factor α, as well as chemokines, such as monocyte chemotactic protein 1. Both IL-1β and tumor necrosis factor α stimulate expression of the adhesion molecules vascular adhesion molecule 1 (VCAM-1), intercellular cell adhesion molecule 1 (ICAM-1), and E-selectin (14).

Importantly, α-tocopherol appears to have potent effects on cellular functions that may modulate heart attack risk (15). α-Tocopherol can modulate the inflammatory response by inhibiting 5-lipoxygenase, which decreases monocyte IL-1β release (16). α-Tocopherol also decreases monocyte–endothelial cell adhesion in vitro, which correlates with decreased endothelial cell E-selectin expression (17) and decreased adhesion molecules (ICAM-1 and VCAM-1) induced by oxidized LDL (18, 19). The decreased ICAM-1 expression is mediated by decreased expression of CD11b and VLA-4, possibly by α-tocopherol inhibiting the activation of nuclear factor κB (20). α-Tocopherol inhibits some of these cellular functions through mechanisms mediated by protein kinase C, as shown in model systems such as smooth muscle cell proliferation (21) and platelet aggregation and adhesion (22, 23). α-Tocopherol supplementation also decreases monocyte superoxide production via inhibition of protein kinase C (20, 24).

Vascular homeostasis is another key function regulated by α-tocopherol. Normal vascular function requires responsiveness to nitric oxide. α-Tocopherol mediates nitric oxide production in endothelial cells (25). In an ex vivo study of rabbit aorta, delivery of α-tocopherol mediated by plasma phospholipid transfer protein resulted in the maintenance of relaxation in response to acetylcholine (26). In human aortic endothelial cells in culture, vitamin E resulted in a net increase in the production of vasodilator prostanoids (27).

Unfortunately, many of the studies described were carried out in tissue culture or are a result of ex vivo treatments with α-tocopherol. Very few measurements were made in supplemented humans, so the health benefits of these observations made in tissue culture studies are lacking.

Vitamin E supplement interventions to ameliorate heart disease

The first of the clinical trials to test the efficacy of vitamin E in heart attack prevention was in an English population and showed that vitamin E prevented second heart attacks (28), but subsequent larger trials largely did not show vitamin E benefit (29, 30). Some examples of the better-known trials are listed in Table 1. Nearly 200 trials using vitamin E supplements were carried out in the 10 y after the first trial, and a recent review and meta-analysis claims that vitamin E has neither benefit nor harm (2). It is noteworthy that in most trials, biomarkers were not used nor were oxidative stress and lipid peroxidation markers or plasma vitamin E concentrations measured.

The literature abounds with basic science showing that vitamin E is an antioxidant and inhibits smooth muscle cell proliferation, platelet adhesion and aggregation, and monocyte endothelial adhesion; it has benefit in animal atherosclerosis models and LDL oxidation, platelet effects, and antiinflammatory effects in humans (as reviewed in reference 37). Thus, it is difficult to argue that the mechanistic aspects of vitamin E’s actions are unknown and need further study. Nonetheless, the actual processes that generate the necessity of vitamin E for the human body are unknown, leading to confusion as to why humans require vitamin E.

Vitamin E absorption, transport, metabolism, and excretion

Dietary components with vitamin E antioxidant activity include α-, β-, and γ-tocopherols and -tocotrienols (38). All these molecules have a chromanol ring with a various number of methyl groups and have either a phenyl tail (tocopherols) or an unsaturated tail (tocotrienols). The naturally occurring form of α-tocopherol is RRR-α-tocopherol; chiral carbons are in the R-conformation at positions 2, 4′, and 8′. Chemical synthesis of α–tocopherol results in an equal mixture of 8 different stereoisomers (RRR, RSR, RRS, RSS, SSR, SRR, SRS, and SSS) called all-rac-α-tocopherol. The 2 position is critical for in vivo α-tocopherol activity; only 2R-α-tocopherol forms meet human vitamin E requirements (38).

The liver preferentially secretes α-tocopherol into plasma, under control of the α–tocopherol transfer protein (α-TTP) as shown in vitamin E–deficient humans with genetic α-TTP defects (39, 40) and in α-TTP knockout mice (41, 42). α-Tocopherol is excreted into bile via MDR2 (ABCBC4) (43), an ATP-binding cassette transporter that facilitates biliary phospholipid excretion.

Unlike other fat-soluble vitamins, vitamin E is not accumulated in the liver (or any other tissue) much beyond a 2- to 3-fold
increase above concentrations seen in unsupplemented subjects (44), which suggests that excretion and metabolism are important in preventing hypervitaminosis E. The vitamin E metabolites α-CEHC (2,5,7,8-trimethyl-2-[2’-carboxyethyl]-6-hydroxychroman) and γ-CEHC (2,7,8-trimethyl-2-[2’-carboxyethyl]-6-hydroxychroman) are derived from α- and γ-tocopherols, respectively (45). Vitamin Es are metabolized similarly to xenobiotics in that they are metabolized by cytochrome P450s (CYPs), conjugated, and excreted in urine (45) or bile (46). After ω-oxygenation by CYPs, β-oxidation takes place, and CEHCs can then be sulfated or glucuronidated (47–49).

Vitamin E metabolism seems to be a key event in preventing the accumulation of forms of vitamin E other than α-tocopherol. After administration of an equimolar dose of differentially deuterium-labeled α- and γ-tocopherols to humans, the disappearance rates for γ-tocopherol and α-CEHC were virtually identical and much faster than for α-tocopherol (50). These data emphasize that the body preferentially retains α-tocopherol and that γ-tocopherol is rapidly metabolized and removed from the circulation.

Why CEHC production from γ-tocopherol is so much greater than that from α-tocopherol is unknown, but studies in mice suggest that hepatic vitamin E regulation depends on α-tocopherol concentrations. Hepatic Cyp3a protein is correlated with hepatic α-tocopherol concentrations, even in mice fed diets high in γ-tocopherol (51), and hepatic Cyp3a mRNA is also up-regulated by dietary α-tocopherol but not by γ-tocotrienol (52). CYP3A4 inhibition in rats by sesame lignans (sesamin and sesaminol) elevates plasma γ-tocopherol concentrations and decreases γ-CEHC concentrations; similar effects were seen in rats fed ketoconazole, a known inhibitor of CYP3A (53). These data suggest that α-tocopherol stimulates the metabolism and excretion of non-α-tocopherol forms by increasing CYP3A levels and that inhibition of CYP3A decreases the metabolism of γ-tocopherol, as discussed (51). Moreover, these data emphasize that vitamin E concentrations are limited to prevent excess accumulation.

CYP3A is regulated by a xenobiotic nuclear receptor, the pregnane X receptor (PXR) (54). PXR regulates a constellation of genes involved in xenobiotic detoxification (54–56). PXR is a broad-specificity nuclear receptor that binds lipophilic xenobiotics, including various vitamin E forms (57, 58). On ligand binding, PXR, as a heterodimer with the retinoid X receptor, interacts with the promoter region of genes and induces some cytochrome P450 oxidation systems (phase I), conjugation systems (phase II), and transporters (phase III) that effectively clear

### TABLE 1

Examples of intervention trials with vitamin E

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Dose</th>
<th>Duration</th>
<th>Relative risk (95% CI)</th>
<th>Endpoint</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHAOS</td>
<td>2002</td>
<td>400 or 800 IU RRR-[α]-tocopherol</td>
<td>1.4</td>
<td>0.23 (0.11, 0.47)</td>
<td>Nonfatal myocardial infarction</td>
<td>(28)</td>
</tr>
<tr>
<td>GISSI</td>
<td>11 324</td>
<td>300 mg dl-[α]-tocopherol</td>
<td>3.5</td>
<td>0.98 (0.87, 1.10)</td>
<td>Cardiovascular death, nonfatal myocardial infarction, nonfatal stroke</td>
<td>(30)</td>
</tr>
<tr>
<td>HOPE</td>
<td>9541</td>
<td>400 mg RRR-[α]-tocopherol</td>
<td>4.5</td>
<td>1.05 (0.95, 1.16)</td>
<td>Myocardial infarction, stroke, or cardiovascular death</td>
<td>(29)</td>
</tr>
<tr>
<td>SPACE</td>
<td>196</td>
<td>800 IU RRR-[α]-tocopherol</td>
<td>2</td>
<td>0.46 (0.27, 0.78)</td>
<td>Fatal and nonfatal myocardial infarction, fatal and nonfatal ischemic stroke, unstable angina, peripheral vascular disease, excluding sudden death</td>
<td>(31)</td>
</tr>
<tr>
<td>PPP</td>
<td>4495</td>
<td>300 mg dl-[α]-tocopherol</td>
<td>3.6</td>
<td>1.07 (0.74, 1.56)</td>
<td>Cardiovascular death, nonfatal myocardial infarction, nonfatal stroke</td>
<td>(32)</td>
</tr>
<tr>
<td>MICRO-HOPE</td>
<td>3654</td>
<td>400 IU RRR-[α]-tocopheryl acetate</td>
<td>4.5</td>
<td>1.03 (0.88, 1.21)</td>
<td>Myocardial infarction, stroke, or cardiovascular death</td>
<td>(33)</td>
</tr>
<tr>
<td>VEAPS</td>
<td>353</td>
<td>400 IU dl-[α]-tocopherol</td>
<td>3</td>
<td>$P = 0.81$ for cardiovascular events (14 placebo and 11 vitamin E)</td>
<td>Fatal, nonfatal, or non-Q-wave myocardial infarction, coronary artery bypass graft surgery, percutaneous transluminal coronary angioplasty, unstable angina, transient ischemic attack, cerebrovascular accident</td>
<td>(34)</td>
</tr>
<tr>
<td>HOPE-TOO</td>
<td>3994</td>
<td>400 IU RRR-[α]-tocopheryl acetate</td>
<td>7</td>
<td>1.04 (0.96, 1.14)</td>
<td>Myocardial infarction, stroke, or cardiovascular death</td>
<td>(35)</td>
</tr>
<tr>
<td>Women’s Health Study</td>
<td>39 876</td>
<td>600 IU RRR-[α]-tocopherol</td>
<td>10.1</td>
<td>0.93 (0.82, 1.05)</td>
<td>Nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death</td>
<td>(36)</td>
</tr>
</tbody>
</table>

1 CHAOS, Cambridge Heart Antioxidant Study; GISSI, Gruppo Italiano per lo Studio della Sopravvivenza nell’ Infarto Miocardio; HOPE, Heart Outcomes Prevention Evaluation; SPACE, secondary prevention with antioxidants of cardiovascular disease in endstage renal disease; PPP, Primary Prevention Project; VEAPS, Vitamin E Atherosclerosis Prevention Study.
Vitamin E as an antioxidant in humans

Vitamin E is depleted in humans during oxidative stress. In cigarette smokers, as a result of their increased oxidative stress (assessed by plasma F2-isoprostane concentrations), plasma vitamin E is more rapidly depleted than in nonsmokers (65). Remarkably, smokers with the lowest plasma vitamin C concentrations had the highest vitamin E disappearance, presumably because vitamin C regenerates vitamin E (10). To test this hypothesis, smokers and nonsmokers were supplemented for 2 wk with placebo or vitamin C (1000 mg/d), then their plasma vitamin E disappearance rates were measured (66). Using a crossover design, the same subjects were tested again on the opposite supplement. Marginal vitamin C status in smokers was associated with increased rates of vitamin E disappearance from plasma [as previously observed (65)], and these rates were normalized by prior vitamin C supplementation (66). Importantly, both α- and γ-tocopherol were similarly affected by vitamin C status, which suggests that oxidation of the tocopherols is the mechanism for the faster vitamin E disappearance (66).

The studies of the smokers also made it apparent that additional biomarkers of oxidative and nitrosative damage would be useful. One biomarker of nitration reactions is 5-nitro-γ-tocopherol (67). It is higher in smokers than in nonsmokers (68), coronary heart disease patients (69), and Alzheimer patients (70). Other biomarkers currently under investigation include ascorbyl-4-hydroxy nonenal (71, 72). In addition to F2-isoprostanes (73), β-oxidation metabolites of F2-isoprostanes may be a useful marker of whole-body oxidative stress when measured in 24-h urine collections (74). F2-isoprostanes are free radical–mediated breakdown products of arachidonic acid; longer-chain PUFAs that lead to other breakdown products, such as the neuroprostanes (73) or F2-isoprostanes (75), may also be useful biomarkers of oxidative stress.

In contrast with smokers and others experiencing chronic oxidative stress, persons who exercise experience bursts of increased reactive oxygen species. For example, when oxidative stress was evaluated in endurance runners during a 50-km race compared with a rest day, both F2-isoprostane concentrations and vitamin E disappearance rates were elevated during the race (76). Daily supplementation with vitamin E (400 IU) and vitamin C (1000 mg) for 6 wk before the race prevented the increase in F2-isoprostanes but not in markers of inflammation (77). Taken together, these data strongly support the concept that vitamin E is required for its antioxidant properties, specifically as a lipidsoluble antioxidant preventing the propagation of lipid peroxidation, and that inadequate levels would lead to greater oxidative stress. Supplementation with vitamin E would then be predicted to be beneficial for the prevention of oxidative stress and related disorders.

The longest trial to test whether vitamin E supplements would prevent heart disease was the Women’s Health Study (36). In that study, 40 000 women aged ≥45 y were randomly assigned to receive vitamin E (600 IU) or placebo and aspirin or placebo every other day. The study lasted 10 y. A significant 24% reduction in cardiovascular death was largely attributed to fewer sudden deaths in the vitamin E group (38 compared with 51 in the placebo group). Women aged ≥65 y (10% of study participants) assigned to vitamin E had a significant 26% reduction in major cardiovascular events, a 34% reduction in myocardial infarction, and a 49% reduction in cardiovascular deaths and no reduction in stroke rate. There was no significant effect of vitamin E on total mortality. The only significant adverse effect was an increase in the risk of epistaxis (nosebleeds) (36). It is noteworthy that biomarkers were not used to evaluate vitamin E efficacy in this trial. The authors of the study reported that vitamin E provided no overall benefit and do not support recommending vitamin E supplementation for cardiovascular disease prevention among healthy women (36).

Dietary intakes of vitamin E

The current Recommended Dietary Allowance (RDA) for vitamin E is 15 mg α-tocopherol/d for women and men (38). The Estimated Average Requirement is 12 mg α-tocopherol and is not met by 96% of women and 90% of men (84). Vitamin E is fat soluble so it is found in limited amounts in fruit and vegetables; α-tocopherol is found in seeds, nuts, and some vegetable oils (eg, almond, sunflower seed, and olive oils) (78, 79). On the basis of vitamin E kinetic studies done with deuterium-labeled vitamin E, the RDA values appear correct (80), and cardiovascular disease risk is lower with higher intakes (81). Thus, we are left with increased risk of heart disease with low vitamin E intakes, a population that consumes insufficient amounts of vitamin E to meet its needs, and conflicting data concerning the use of supplements (82, 83). Clearly, additional studies are needed to evaluate the required amount of vitamin E to provide optimal protection against oxidative stress.
OTHER DIETARY COMPONENTS

Fish oil is one of the possible foods that could be used as a dietary supplement to decrease the risk of heart disease. A recent meta-analysis suggested a role for fish oil (eicosapentaenoic acid, docosahexaenoic acid) or fish in secondary prevention because significant reductions in total mortality, coronary heart disease death, and sudden death were reported (84). However, as noted for the vitamin E studies, no biomarkers or intermediate clinical endpoints exist that could be used for evaluating other nutrients. Additionally, because fish oils are highly polyunsaturated, they require adequate protection from oxidation, reinforcing the concept that adequate vitamin E amounts be consumed.

Hyperhomocysteinemia is associated with increased risk of heart disease. It is unclear as to whether supplementation with folic acid will decrease heart disease risk in addition to decreasing homocysteine concentrations. The B-Vitamin Treatment Trials’ Collaboration just reviewed the design and statistical power of 12 randomized trials assessing the effects of lowering homocysteine with B-vitamin supplements on risk of cardiovascular disease (85). They concluded that the individual trials may not have involved a sufficient number of vascular events or have lasted long enough to have a good chance on their own to detect reliably plausible effects of homocysteine lowering on cardiovascular disease risk, but the combined analysis of these trials should have adequate power to determine whether lowering homocysteine reduces the risk of cardiovascular events within a few years. Findings from both the HOPE2 trial (86) and NORVIT (87) showed that the combination of vitamin B-6, vitamin B-12, and folic acid decreased plasma homocysteine but had no demonstrable benefit for heart disease-related outcomes.

CONCLUSION

Several studies reported that vitamin E is associated with decreased chronic disease risk. The Women’s Health Study, a 10-y prevention trial in normal, healthy women, found that 600 IU vitamin E decreased overall cardiovascular mortality by 24% and in women over 65 y decreased it by 49% (36). Antioxidant treatment with vitamins E and C slowed atherosclerotic progression in intimal thickness of coronary and carotid arteries in patients with hypercholesterolemia (88) and heart-transplant patients (89). The Cache County Study reported that antioxidant use (vitamin E > 400 IU and vitamin C > 500 mg) was associated with reduced Alzheimer disease prevalence and incidence in the elderly (90). Regular vitamin E supplement use for ≥10 y was associated with a lower risk of dying from amyotrophic lateral sclerosis (Lou Gehrig disease), a neurodegenerative disease (91). It is therefore not surprising that vitamin E supplements are taken daily by >35 million persons in the United States (92).

Heart disease is such a complex disorder that it is also not surprising that no single remedy has been found to ameliorate it. Dietary and lifestyle changes must be dramatic to achieve heart-healthy goals for most adult Americans. Research on how to achieve and maintain these changes is urgently needed. Biomarkers to assess progress toward these goals are also needed. Although it appears overly optimistic to assume that 1 or 2 nutrients might have benefit, if such nutrients were discovered, it would be important to have a clear idea of adverse effects and the intakes at which these adverse effects become problematic.

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