Evidence Report

Chapter 1. Introduction

Purpose

The use of nonstandard therapies to prevent or treat disease, in addition to or instead of standard medical treatments, has come to be called complementary and alternative medicine (CAM). The use of CAM for chronic diseases has attracted growing interest. Proponents of CAM consider cardiovascular disease to be particularly well suited for prevention and/or treatment with one class of CAM therapies, that is, those with antioxidant activity, because the pathogenesis of atherosclerosis involves oxidative damage. Such antioxidant therapies include the use of dietary supplements that contain vitamin C, vitamin E, and coenzyme Q10, among others. The purpose of this study was to conduct a systematic review of the scientific literature to assess the evidence for the efficacy of supplements of these three antioxidants for the prevention and treatment of cardiovascular disease.

Specific Aims

The National Center for Complementary and Alternative Medicine (NCCAM) and the Agency for Healthcare Research and Quality (AHRQ) established the following specific aims for this study:

1. To identify controlled clinical trial reports on the efficacy of the antioxidant supplements vitamins C and E and coenzyme Q-10 for preventing and treating cardiovascular disease (CVD) or for modification of a risk factor for CVD,
2. To determine if sufficient evidence exists to recommend further study of these therapies, and
3. To suggest future research.

Cardiovascular Disease

Cardiovascular disease (CVD), defined as coronary artery disease, hypertensive heart disease, congestive heart failure, peripheral vascular disease, and atherosclerosis including cerebral artery disease and strokes, is the leading cause of death in the United States. In 1999, one in five Americans (n=61,800,000) had CVD and 958,775 died from it that year. This figure represented 40.1 percent of all deaths in the United States that year and was equal to the next seven leading causes of death. Cardiovascular death rates in the United States are almost twice the rate of death from cancer.[1] Globally, CVD accounts for an estimated 31 percent of the worldwide mortality and burden of disease from all noncommunicable diseases.[2] Further, rates of CVD are increasing in developed countries. The Heart Outcomes Prevention Evaluation (HOPE) study investigators predict a 29 percent increase in ischemic heart disease mortality and a 28 percent increase in the rates of mortality from cerebrovascular disease in developed countries from 1990 to 2020.[2] The rates of increase are expected to be three to four times higher in developing countries, as they increasingly adopt the sedentary Western lifestyle and its dietary habits.

Characterization by specific disease type demonstrates the significant contribution made by each of these conditions to the morbidity from CVD. In the United States, an estimated 1,100,000 people will develop new or recurrent myocardial infarctions in 2003 (approximately 650,000 will be new attacks and 450,000 recurrent cases). An estimated 400,000 cases of new stable angina and 150,000 cased of new unstable cases of angina occur each year. Of the 958,775 deaths from CVD in 1999, 55 percent were from coronary heart disease, 6 percent were from heart failure, 5 percent were attributed to hypertension, and 17 percent were from stroke. Stroke would be considered the third leading cause of death if considered separately from the rest of CVD.[1] The lifetime risk of developing heart failure was 20 percent as reported in the Framingham Heart Study, with a median survival of 1.7 years in men and 3.2 years in women after diagnosis.[3] Although less common, peripheral vascular disease (PVD), defined as atherosclerotic disease in the arms or legs, affects eight million adults in the United States and causes significant and often disabling pain and disability.[4] Thus, atherosclerotic cardiovascular disease, including stroke, represents a significant source of morbidity and cost for the American public.

Costs of Cardiovascular Disease

The economic cost of CVD in medical care expenditures, lost productivity, and premature mortality is substantial. CVD diagnoses are the most common of all hospital discharge diagnoses and increased 29 percent from 1977 to 1999. Medicare payments for expenses related to CVD hospital admissions in 1998 were $26.4 billion. Estimated total direct expenditures for heart disease in 2002 are expected to be $115 billion, with an estimated loss in productivity from all causes of CVD of $129.7 billion. Combining all expenses and losses for 2002, the American Heart Association has estimated the total cost of CVD in the United States at $329.2 billion.

Risk Factors for Cardiovascular Disease

The major common risk factors for CVD are diabetes mellitus, hypertension, hypercholesterolemia, and smoking.[5] In an evaluation of five large cohorts of young and middle aged men and women (n=72,144), mortality caused by stroke, myocardial infarction, and cancer was significantly reduced in the low-risk cohort, defined by low cholesterol, low blood pressure, and smaller body mass index (BMI).[6] For women in the Nurses Health Study, 82 percent of the cardiovascular mortality was attributed to the lack of adherence to a low-risk lifestyle that included minimizing BMI, exercising regularly, not smoking, and eating a high-fiber diet rich in fruits and vegetables.[7] Over 100,000,000 Americans are estimated to have a total cholesterol above 200mg/dl. Of these, approximately 41,000,000 are at particularly high risk for heart disease, with cholesterol over 240 mg/dl. A 10 percent decrease in total cholesterol is estimated to result in a 30 percent reduction in risk of coronary heart disease. Those with high low-density lipoprotein levels (LDL>130 mg/dl), now about 48 percent of the American population, are at especially high risk.[1] Persons with two or more risk factors are believed to have a 10 percent to 20 percent increase in risk for developing a significant cardiovascular event in the next ten years.[8]

Antioxidants and Cardiovascular Disease

Antioxidants

The Food and Nutrition Board has defined a dietary antioxidant as a substance in commonly consumed foods that significantly decreases the adverse effects of chemically reactive species, such as reactive oxygen and nitrogen species, on normal physiological functions in humans.[9] These reactive species, also called free radicals, possess one or more single unpaired electrons that make them highly disruptive to biological substances when they are allowed to accumulate. Although short lived, this diverse group of compounds is thought to induce oxidative stress, damaging key molecular constituents of cells, and participating in the genesis of chronic diseases such as coronary heart disease.[10] As part of a natural defense system, antioxidants can mitigate the activity of free radicals and other oxidative species that have been implicated in the development of atherogenesis.[11,12] The epidemiologic and observational literature has suggested a beneficial effect of antioxidant-rich foods, as well as specific antioxidants, on the risk of CVD and stroke.[13-19] Because oxidative functions also contribute positively to the health of the cell by their participation in energy metabolism, biosynthesis, detoxification, and cellular signaling, a balance is clearly required between the pro-oxidants and the antioxidant defense system to maintain health.[20]

A number of components in foods have been found to have antioxidant properties. These components include beta-carotene and the other carotenoids, vitamin C, vitamin E, and selenium. Dietary supplements are available that contain each of the putative antioxidants alone and in various combinations. For this report, the funding agencies—the Agency for Healthcare Research and Quality (AHRQ) and the National Center for Complementary and Alternative Medicine (NCCAM)—directed that we focus our analysis on the roles of supplements containing vitamin C, vitamin E and coenzyme Q10 as dietary antioxidants.

The Use of Antioxidants

The overall rate of dietary supplement use in the National Health and Nutrition Examination Survey (NHANES III), conducted between 1988 and 1994, was 40 percent for the general population, a prevalence and pattern of use that have been stable for the preceding 20 years. The prevalence of use is higher than average in women, older adults, white persons, and persons in a higher socioeconomic class or with a higher level of education.[21] In a more recent telephone survey of 2590 members of the general population, prevalence of use during the prior week was reported to be 26 percent for a multivitamin/mineral supplement, including antioxidants, 10 percent for vitamin E alone, and 9.1 percent for vitamin C alone.[22] The most common reason cited for antioxidant use in this study was maintenance of health, a belief frequently cited by users of all dietary supplements.[23]

As mentioned, the use of antioxidant supplements is common in several subgroups of the population. Almost 80 percent of the elderly subjects in one convenience sample reported regular use of at least one dietary supplement. Vitamin E was the most commonly used supplement, and the predominant reason for use was "to improve health."[24] In addition, 10 percent of a group of elderly Europeans reported taking vitamin C.[25] The Women Physicians' Health Study, a large survey of the rates and patterns of dietary supplement use by female

doctors, found that half of these women used a multivitamin-mineral supplement that typically included vitamins E and C and that those who were at risk for heart disease were higher users of antioxidants. However, the general health habits of women who were regular supplement users were also better than average: these habits include eating more fruits and vegetables, consuming less fat, and complying with preventative care recommendations.[26] Thus, it is important to consider that the associated health behaviors of supplement users may confound the effects of antioxidant use reported in observational studies.

Dietary Intervention and Risk of Cardiovascular Disease

Based on the results of a number of observational studies of dietary antioxidants, the American Heart Association has recommended, among other interventions, a diet that includes five to nine servings of fruits and vegetables per day as a means of lowering the risk of CVD.[27] Fruits and vegetables are considered a rich dietary source of a variety of antioxidants, including vitamins C and E. Results of a number of studies support the recommendation to increase consumption of fruits and vegetables.[28-34] In 1998, a meta-analysis of cohort studies showed that the risk of ischemic heart disease was approximately 15 percent lower among individuals in the 90th centile of fruit and vegetable intake than among those in the 10th centile.[35]

Antioxidants and Cardiovascular Risk

The strength of the dietary evidence regarding the benefits of antioxidants is challenged by the fact that the critical components in fruits and vegetables that confer benefit may not be the antioxidants alone.[14] For example, in the "Zutphen" study, a significant inverse correlation was observed between risk of stroke and intake of one specific category of dietary component, the flavonoids, but not vitamins C or E.[36] However, higher dietary levels of vitamin C and E have generally demonstrated protection against coronary artery disease (CAD in other studies). In a cohort of elderly Asian Indian subjects, an inverse relationship was observed between risk of CAD and plasma levels of vitamins C and E.[37] The adjusted odds ratio for CAD, comparing the lowest to the highest quartiles of vitamin levels, was 2.53 for vitamin E (95% CI: 1.11 to 5.31) and 2.21 for vitamin C (95% CI: 1.12 to 3.15). In a study of Finnish men and women, subjects who developed CVD ate more dairy foods and fewer fruits, vegetables, and foods high in vitamin E. For the three percent of participants in this study who used supplements, a trend towards decreased CVD was seen, but these results were not statistically significant,[38] This trend was also observed for vitamin C intake and was not attributable to other common major risk factors for CAD.[38] A review of the major epidemiological studies confirms the favorable association between high intake of antioxidant-rich foods (and high serum levels of vitamins C and E) with decreased risk of ischemic CVD and stroke.[39]

Studies of the effects of antioxidant vitamin status have even demonstrated a positive association between plasma vitamin C and E levels and the structural integrity of various organs. A British study of elderly men and women found an inverse correlation, in men only, between plasma vitamin C levels and decreases in intimal wall thickness (indicative of stenosis). For vitamin E, the male subjects with the lowest levels of this antioxidant were 2.5 times more likely to have significant carotid artery stenosis.[40] The Artherosclerosis Risk in Communities Study, a prospective cohort study designed to investigate the genesis of atherosclerosis, demonstrated a significant inverse relationship between vitamin C intake and wall thickness in both sexes, even

after adjusting for age and major risk factors.[41] In contrast to the previous study,[40] vitamin E intake was significantly correlated with wall thickness only in female patients, although a positive trend was observed for men.

In contrast to the effects of total dietary antioxidant consumption (both whole foods and supplements), observational studies of the effects of antioxidant supplementation alone have not consistently demonstrated a benefit for CVD or stroke.[18] Analysis of data from the Health Professionals Study showed no decrease in risk of stroke for men who used vitamin C or E supplements.[42] Moreover, a prospective cohort study of almost 35,000 postmenopausal women showed a decrease in stroke and cardiovascular death risk with increased dietary vitamin E but not with use of supplemental vitamin E or general antioxidants.[43] In the same large study, intake of supplemental vitamin C was also not associated with a decreased risk of cardiovascular death.[44] In contrast, in another study, both male and female subjects taking vitamin E supplements showed a decrease in carotid artery intimal thickness and were significantly less likely to have stenosis.[40] Thus, the observational data on the effect of supplemental antioxidants are mixed.

Vitamin C and Cardiovascular Disease

Vitamin C, a potent, water-soluble antioxidant, has been known as an essential micronutrient since the late 1700s, when the British Navy supplemented the diet of their sailors with citrus fruits to prevent scurvy. Also known as ascorbic acid, vitamin C is a six-carbon derivative of the sugar hexose, but it cannot be synthesized by primates (see Figure X).[45] Good dietary sources of vitamin C include fruits—especially currants, citrus, and rose hips—and many vegetables. Because of its asymmetrical ring structure, ascorbic acid may exist in four stereoisomers, but L-ascorbic acid is the biologically active form.[46] It represents the primary antioxidant defense in blood,[47] is able to react with virtually all oxygen species, and can terminate free radical chain reactions.[48] Vitamin C also has crucial interactions with a number of other antioxidants. Glutathione is important in recycling oxidized vitamin C, and vitamin C itself is crucial to the regeneration of lipid-bound vitamin E.[47-49]

An association between risk of death from cardiovascular disease and vitamin C intake was reported as early as 1950.[18] Persons at risk for low serum or plasma levels of vitamin C would include smokers, the elderly, and those who are poorly nourished or suffer from chronic disease.[50] Low levels of vitamin C were associated with higher rates of death from CVD or stroke in the Basel study.[51] Conversely, high serum levels of vitamin C appear protective and have been associated with decreased coronary mortality.[52] Data from the First National Health and Nutrition Examination Survey (NHANES I) showed an inverse relationship between all-cause mortality and vitamin C intake, even after adjusting for age, sex, and potentially confounding variables.[53] The inverse relationship between risk for all causes of death and intake of vitamin C was strong in men and weaker for women.[53] The World Health Organization's MONICA study also showed an inverse relationship between plasma vitamin C levels and mortality from coronary artery disease.[54]

Vitamin C is also thought to modify incidence of and risk factors for CVD. Higher plasma levels of vitamin C have been associated with reduced risk of stroke or coronary heart disease and with a lesser degree of stenosis in carotid arteries.[40] In a review of five population studies,

Trout[46] reported that vitamin C supplementation decreased total cholesterol and increased high-density lipoprotein (HDL), mainly in patients with low pretreatment levels of vitamin C. He also noted an inverse relationship between blood pressure and vitamin C. In another study, individuals who received supplements of vitamin E, vitamin C, and beta-carotene displayed inhibited lipid oxidation (in *ex vivo* tissue samples).[55] Other studies have found a decrease in endothelial dysfunction and in monocyte chemotactive processes with vitamin C supplementation.[56]

Vitamin E and Cardiovascular Disease

Vitamin E, the principal lipid-soluble antioxidant, was first discovered in 1936.[57] Vitamin E includes 8 naturally occurring forms, which can be divided into two families of compounds, the tocopherols and the tocotrienols (collectively known as tocols) (see Figure Y). The four tocopherols consist of a six-chromanol ring or head with a phytyl side chain. Three chyral centers exist in the tail at the 2, 4, and 8 positions; thus, a number of stereoisomers are possible. The tocopherols are designated alpha, beta, gamma, and delta, depending on the methyl substitutions in the chromanol ring. The tocotrienols differ from the tocopherols by the presence of three double bonds in the phytyl chain of the former. Thus, by virtue of a chyral center and the presence of the double bonds, tocotrienol can exist in eight different isomers. The tocotrienols are also designated as alpha, beta, gamma, or delta, based on the methyl substitutions in the head ring.[45]Of the 8 naturally occuring forms of vitamin E, only alpha-tocopherol is carried in human blood and is considered to be the active form.

Vitamin E is an essential micronutrient, that is, it must be obtained from the diet. Dietary vitamin E is absorbed in the small intestine, a process that depends on an intact ability to micellize fat and transport it across intestinal cell walls [where it is packaged into chilomicrons for transport]. Thus, severe pancreatic or biliary dysfunction or fat malabsorption may affect vitamin E absorption.[57] For optimal absorption, it is recommended that vitamin E supplements be taken with a meal.[58] Once in the blood stream, vitamin E is bound to plasma carrier proteins, transported to the liver;incorporated into lipoproteins, especially very low-density lipoproteins (VLDL); and secreted into the bloodstream.

Data from NHANES II showed that dietary intake of vitamin E was generally below recommended levels for both men and women.[59] Significant food sources for vitamin E in this survey included foods fortified with the vitamin, salad and cooking oils, peanuts and tree nuts, mayonnaise and other oil-based dressings, and some vegetables. The largest percentage of dietary vitamin E was derived from fats and oils. Acute deficiencies of vitamin E have been generally attributed to severe malnutrition or severe fat malabsorption. Some congenital deficiency syndromes exist as well, but they are rare. Vitamin E deficiency patterns are species specific, but in humans, they primarily involve hematologic and neurologic sequelae.[57]

A significant body of literature exists that correlates dietary vitamin E levels with cardiovascular disease incidence and mortality.[60-65] A protective effect of vitamin E has been reported in 16 European study populations, in which a strong inverse correlation was observed between vitamin E levels and risk of CVD mortality.[66]

The cardioprotective effects of vitamin E are attributed to its antioxidant properties. Specifically, vitamin E is able to extinguish single oxygen species as well as to terminate free-radical chain reactions.[67] Alpha-tocopherol acts as an antioxidant either by donating a hydrogen radical to remove the free lipid radical, reacting with it to form nonradical products, or simply trapping the lipid radical.[68] It is thought to exert its primary protective effects via the protection of LDL from oxidation. This effect has been demonstrated in laboratory animals *in vivo*,[69] in isolated tissues *ex vivo*, and in human populations.[60] For example, in a population-based study, resistance to LDL oxidation was lower in Lithuanian and Swedish men with the higher levels of alpha tocopherol.[70] In a case-control study of 25,000 blood donors, higher levels of alpha-tocopherol were associated with lower risk of developing a myocardial infarction but only in those patients with high cholesterol.[71]

As noted above, antioxidant vitamins have been shown to interfere with the oxidation of LDL. Of the antioxidant vitamins, vitamin E may be the most potent inhibitor of lipid oxidation because it is fat-soluble and constitutes part of the LDL molecule. Oxidation of LDL particles initiates a plaque-forming cascade, which involves the ingestion of oxidized LDL by macrophages, thereby creating foam cells. These foam cells secrete chemotactic molecules that attract more white cells, which damage local endothelium, increase inflammatory cytokines, and promote procoagulant activity.[60] Vitamin E supplementation has been shown to decrease the oxidation of LDL, measured in prolongation of lag-time before LDL oxidation, often experimentally induced by heavy metals such as copper.[72] This protective activity of vitamin E that occurs in the LDL molecule depends on vitamin C to recycle oxidized vitamin E.[47]

Vitamin E may affect the pathogenesis of atherosclerotic vascular disease beyond its direct effects on lipids. The majority of morbidity and mortality from CVD occurs as a result of thrombosis at the site of an unstable atheromatous plaque in an atherosclerotic artery. Vitamin E could affect CVD morbidity and mortality by reducing platelet adhesion, inhibiting vitamin-K-dependent clotting factors, or stimulating nitric-oxide formation by the endothelial cell.[60] Effects on platelet aggregation and adhesion that may affect clot formation have been demonstrated.[73] Furthermore, the oxidized LDL interferes with the normal production of nitric oxide by the endothelium. Nitric oxide is an essential vasodilator and plays an important role in the inhibition of platelet aggregation and smooth-muscle-cell proliferation.[56]

Coenzyme Q10 and Cardiovascular Disease

Coenzyme Q10, a naturally occurring antioxidant, is so widely distributed throughout the human body that it is also known as ubiquinone. Its chemical name is 2,3 dimethoxy,5-methyl-6-polyisoprene parabenzoquinone.[74] It contains 10 isoprene units of five carbons each (see Figure Z). Coenzyme Q10 is a lipid-soluble provitamin that is structurally similar to vitamin K. It is incorporated into the walls of the mitochondria and functions in electron transport and the production of the high-energy compound adenosine triphosphate (ATP). Concentrations are highest in tissues with high-energy demands, such as heart muscle, liver, and kidney tissues.

Although it is present in a wide variety of foods, coenzyme Q10 is mainly supplied by biosynthesis, a process that involves the enzyme HMG-CoA reductase, which is also responsible for cholesterol synthesis. HMG-CoA reductase inhibitors, a class of lipid-lowering drugs referred to as statins, have been shown to decrease levels of coenzyme Q10.[75-77] Levels of coenzyme Q10

can be normalized with oral supplements taken concurrently with the statin drugs, although the clinical significance of this normalization has not been determined.[78,79]

Coenzyme Q10 is believed to exert its effects via three main mechanisms. First, it participates in oxidative phosphorylation as a coenzyme for three critical mitochondrial enzyme systems, complexes I, II, and III. To a lesser degree, free coenzyme Q10 in the cytosol may also contribute to electron transfer outside of the mitochondria as well. By increasing ATP production, it is thought to improve energy function in tissues with high oxidative demands. Second, coenzyme Q10 has significant antioxidant activity. It exists in the cell in both oxidized and reduced forms and is one of the few substances for which there are enzymes whose sole function is to restore their reduced state. Coenzyme Q10 also serves to restore oxidized alpha-tocopherol and thus is important for the function of this important antioxidant as well. Finally, due to its lipid solubility, it is present in the cell membrane phospholipid layer and may influence membrane stability as well.

A number of diseases have been associated with coenzyme Q10 deficiency. These include diabetes mellitus, periodontal disease, muscular dystrophy, and a variety of cardiac conditions such as mitral valve prolapse, angina, coronary artery disease, congestive heart failure, hypertension, cardiomyopathy, and injury following revascularization procedures.[80] Coenzyme Q10 levels are also reportedly low following cardiac surgery and in patients with heart failure (HF) and myocardial infarction (MI). The decrease in coenzyme Q10 levels has been demonstrated to correlate positively with the severity of HF.[81,82] Animal models have demonstrated improved cardiac function and protection from reperfusion injury with coenzyme Q10 supplementation and increased tissue and blood levels of this antioxidant.[83]

In its reduced form, coenzyme Q10 is present in the LDL particle and is believed to act in conjunction with alpha-tocopherol to prevent LDL oxidation, an initiating event in intimal injury and atherosclerosis.[84] In patients with ischemic heart disease, low coenzyme Q10 levels have been correlated with higher levels of total cholesterol, triglyceride (TG) and LDL, known risk factors for coronary artery disease.[85] A protective benefit of coenzyme Q10 was suggested by the results of an observational study of 94 consecutive hospital patients with a variety of medical conditions, including malignancies and HF. Patients who died within six months of hospitalization had lower coenzyme Q10 levels than those who survived.[86]

A meta-analysis of the efficacy of coenzyme Q10 supplementation for the treatment of HF was conducted by Soja and Mortensen[87] in 1997. It suggested significant positive effects on hemodynamic measures such as ejection fraction, stroke volume, cardiac output, and end diastolic volume index.

Safety of Antioxidant Supplements

In general, supplement forms of the three antioxidants, vitamin C, vitamin E, and coenzyme Q10, are believed to be safe, with low toxicity reported and few significant drug interactions.[45,88] Animal studies of oral vitamin E have not revealed significant toxicity, carcinogenicity, or teratogenicity.[89]

For vitamin E, few adverse events have been reported in clinical trials for doses up to 1000 IU (about 660 mg).[90] The tolerable upper intake level is set at 1000 mg.[9] Because of vitamin E's effects on platelets, interactions with anticoagulants and other platelet drugs are of potential concern. At doses greater than 400 IU, reports of a potential interaction with warfarin have been described.[91] In the Alpha Tocopherol Beta Carotene (Cancer Prevention) study (ATBC, discussed in more detail later), alpha tocopherol supplementation was associated with a 50 percent increase in the risk of subarachnoid hemorrhage (p=0.07) and a 181 percent increase in the risk of fatal subarachnoid hemorrhage (p=0.01).[92] A greater number of adenomas were reported from the ATBC study in the subjects taking alpha tocopherol. (relative risk=1.66) However, this finding is postulated to be due to the increased rate of rectal bleeding in this intervention group leading to greater frequencies of colonoscopy rather than actual promotion of polyp formation.[93] The MRC/BHF study in 20,536 patients concluded that the combination of 600 mg (about 900 IU) of vitamin E and 250 mg of vitamin C taken daily for up to 5 years was safe.[94]

For vitamin C, minor episodes of gastric distress have been reported for daily doses greater than several grams.[95,96] This lack of toxicity seems to be sustained over even long periods of use. Although not a toxic effect, vitamin C can interfere with common lab tests for glucose, uric acid, creatine, and fecal occult blood.[97]

Coenzyme Q10 has been described to decrease the effectiveness of warfarin in a case report and may, through effects on glucose in type II diabetics, exaggerate the hypoglycemic effects of diabetic medications.[95] Mild gastrointestinal intolerance has been reported at higher doses (700 mg or more daily), and a number of drugs such as statins and beta-blockers decrease the levels and/or effectiveness of coenzyme Q10.