Original Communication

Carotenoids and Vitamins C and E in the Prevention of Cardiovascular Disease

Graziano Riccioni[1], Nicolantonio D'Orazio[2], Cusenza Salvatore[2], Sara Franceschelli[3], Mirko Pesce[3], and Lorenza Speranza[3]

[1]Cardiology Unit, San Camillo De Lellis Hospital, Manfredonia, Foggia, Italy
[2]Human Nutrition, Department of Biomedical Science, University G. D'Annunzio, Chieti, Italy
[3]Department of Human Movement Sciences, University G. D'Annunzio, Chieti, Italy

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Abstract: Atherosclerotic cardiovascular diseases (CVD) are a major source of mortality and morbidity in the general population. Oxidative modification of low-density lipoprotein cholesterol (LDL-C) represents the most important determinant factor in the development and progression of atherosclerotic lesions. Oxidative damage and the production of free radicals (FRs) in the endothelium are some of the main factors involved in the pathogenesis of the atherosclerotic process that causes CVD. Appropriate nutritional practices are of central importance in managing risk and treatment of CVD; in fact, many current guidelines for a healthy general population contain nutritional recommendations to reduce the risk of these diseases. Observational studies of vitamins C and E, the most prevalent natural antioxidant vitamins, suggest that supplemental use of these vitamins may lower the risk for coronary events. Despite these data, several large, randomized controlled trials have failed to confirm the benefits of vitamin C and E in cardiovascular prevention. The aim of this review is to examine the published studies regarding the effect of vitamins (C and E) and β-carotene supplementation in the prevention of CVD due to atherosclerosis.

Key words: β-carotene**,** vitamin C, vitamin E, cardiovascular disease, antioxidant, prevention

Introduction

Cardiovascular disease (CVD), involving atherosclerosis and hypertension, is one of the leading causes of death in advanced countries [1]. The natural antioxidant system of our bodies consists of a series of antioxidant enzymes [e.g., superoxide dismutase (SOD) and glutathione peroxidase (GSX)], numerous endogenous antioxidant molecules (e. g., glutathione and cysteine) and antioxidant supplements (e. g., vitamins C and E) [2]. Antioxidants can also be obtained from the diet in the form of vegetables, fruits, green tea, and chocolates [3].

Observational and clinical studies have demonstrated that plant-based foods play a role in preventing CVD, since they provide dietary fiber that helps lower blood cholesterol, and antioxidants that help in preventing lipoprotein oxidation; elevated cholesterol levels and lipoprotein oxidation are both strong risk

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factors for the development of atherosclerosis and consequently, CVD [4].

In vitro studies have shown that vitamins C or E and polyphenols inhibit the generation of free radicals (FRs), as well as lipid peroxidation and DNA damage [5]. Observational prospective human cohort studies suggest that higher dietary intake or supplementation of antioxidants (vitamins C and E, folic acid, niacin, β-carotene, selenium, zinc) is associated with a lower risk of CVD and mortality [6]. Administration of moderate doses of vitamins E (400 IU/day) and C (1 g/day) in another study, restored endothelial function, assessed as flow-mediated vasodilation during reactive hyperemia, in children with hyperlipidemia, implicating a protective role of the antioxidants against early atherosclerosis progression [7]. These findings support the notion that antioxidants might be effective in inhibiting the initial stages of human atherosclerosis, even in moderate doses, and less effective in reducing cardiovascular events.

Oxidative stress arises from an imbalance between the production of reactive oxygen and nitrogen species (ROS and RNS) and the capability of biological systems to readily detoxify reactive intermediates [8]. Several studies have reported beneficial effects of therapy with antioxidant agents, including trace elements and other antioxidants, against cardiovascular system dysfunction due to diabetes [9]. Antioxidants act through different mechanisms to prevent oxidant-induced cell damage, acting either directly or indirectly. They can reduce the generation of ROS, scavenge ROS, or interfere with ROS-induced alterations [10]. Increased oxidative stress occurs during an acute myocardial infarction (AMI) and after re-perfusion with a fibrinolytic agent. This increase in oxidative stress may contribute to the pathogenesis of both AMI-related myocardial damage and re-perfusion injury [11]. Numerous clinical trials have evaluated the use of nutritional supplements such as β-carotene, selenium, and vitamins A, C, and E in the prevention of coronary heart disease (CHD) and stroke, yielding conflicting results (positive, neutral, and negative). In many of these clinical trials there are enormous clinical design problems, methodological flaws, varied patient populations, variable dose and types of vitamin use, improper selection of vitamins used, and many other issues that make the studies difficult to interpret [12–13].

Oxidative damage and atherosclerosis

Oxidative stress is involved in the pathogenesis of atherosclerosis [17]. During the past few years, a variety of antioxidants have been used in clinical studies for the prevention and treatment of atherosclerosis [14]. The American Heart Association does not support the use of vitamin E supplements to prevent CVD, but does recommend the consumption of foods rich in antioxidant vitamins and other nutrients [15].

It has been amply demonstrated that both apolipoprotein and lipid peroxidation are the main causes of the conformational changes in both low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C), and that these oxides are a major factor contributing to the formation of atherosclerotic lesions [18–20]. It has also been demonstrated that asymptomatic, non-smoking, non-drinking adults with normal levels of total cholesterol and LDL-C may be at risk of vascular disease if high levels of FRs exists. The amount of the radical becomes crucial for early detection of atherosclerotic risk [21]. The body's ability to defend itself from oxidative damage caused by FRs is then important. It is important, therefore, that the body strikes the right balance between the number of FRs and the defense and repair mechanisms available.

Antioxidants can be divided into two main categories: endogenous antioxidants, which are directly synthesized by the body, including enzymes such as SOD and catalase (CAT), and exogenous antioxidants, taken through food, including vitamins C and E, and β-carotenoids [22, 23]. Previous research on animal and physiological models suggest that antioxidant supplements have beneficial effects that may prolong life. Some observational studies also suggest that antioxidant supplements may prolong life, whereas other observational studies demonstrate neutral or harmful effects [24]. Randomized trials have largely been neutral. However at this time, scientific evidence supports a diet rich in food sources of antioxidants and other heart-protecting nutrients such as fruits, vegetables, whole grains, and nuts instead of antioxidant supplements to reduce risk of CVD [25].

Nutrition and atherosclerotic plaque

Atherosclerosis is a chronic inflammatory process characterized by the formation of fibro-lipid plaques (atheromas) in arterial walls of large/medium size [26].

Over the decades, the gradual growth of the lesion, due to the accumulation of material that is responsive to the proliferation of cellular elements, may cause the protrusion of the same with a progressive reduction of the lumen diameter and of the blood perfusion in tissues located downstream of the affected vessel, with both morphological (atrophy/remodeling) and functional (failure) consequences in the ischemic tissue [27]. The relationship between low levels of HDL-C and the development of CHD can be inferred from epidemiological studies, where even small differences in the level of HDL-C are associated with substantial variations in the risk of major coronary events. This is clear evidence that oxidative stress plays a role in development of atherosclerotic plaque [28].

Numerous studies confirm the relationship between diet and arteriosclerosis, it was found that the saturated fat content in animal tissues mainly increase the level of those fractions of cholesterol heavily involved in the formation of atherosclerotic plaques and are therefore listed among the main risk factors for cardiovascular accidents. Epidemiological data show that half the populations of industrialized countries have levels of circulating LDL that predispose them to atherosclerosis [29, 30].

Antioxidant vitamins

It is now widely accepted that oxidative stress induced by reactive oxygen and nitrogen species is involved in the pathogenesis of various diseases such as atherosclerosis and CVD; consequently, the role of antioxidants in the prevention and treatment of diseases has received much attention from scientists, clinicians, and the general public [30–31]. Antioxidant vitamins E and C, and β-carotene (a form of vitamin A), have potentially health-promoting properties. Although the data are incomplete, about 30 percent of Americans are taking some form of antioxidant supplementation [32].

Vitamins are classified as antioxidants for their ability to scavenge lipid radicals and terminate oxidative chain reactions. Antioxidant vitamins can terminate radical chain reactions by interacting with the lipid peroxyl radical, preventing it from generating a new radical and perpetuating the chain reaction by oxidizing other lipids [33]. The potential benefits of these vitamins are therefore mainly attributed to their antioxidant activity in the oxidation of LDL-C, which is considered an important step in the development and progression of atherosclerosis characteristic of CVD [4].

Carotenoids

Carotenoids have many biological functions, and some are precursors of vitamin A, among which the most important is *β-carotene* [34]. Lutein and zeaxanthin are needed for the healthy function of the macula of the retina. Carotenoids are also lipophilic antioxidants that protect biomolecules from FRs [35]. Carotenoids, in synergy with vitamin E and selenium, prevent the oxidation of cell membranes by inhibiting lipid peroxide radicals. There are interesting studies on the ability of such substances to protect LDL-C from oxidation, since this plays an important role in the etiology and pathogenesis of atherosclerosis [36].

Among the carotenoids, lycopene is an important antioxidant. Recent studies have shown that compared to other carotenoids, lycopenes have antioxidant and anti-radical properties, especially to deactivate singlet oxygen [37]. Lycopene reduces oxidation of lipids and hence the formation of atherosclerotic plaque, responsible for many cardiovascular diseases, where traditional antioxidants, such as vitamin C and vitamin E cannot serve this function [38]. Researchers have reported a drop in blood pressure from a minimum of 4 mmHg to a maximum of 13 mmHg, in hypertensive patients treated for six weeks with an extract of lycopene [39].

Pro

Epidemiological studies suggest that carotenoids exert an important protective role against CVD and certain cancers, although the protective mechanism has not been fully elucidated [40–42]. Other actions, such as their involvement in regulating cell growth and gene expression may also be related to its protective effect [43]. In primary prevention, randomized controlled studies showed that supplementation of 50 mg β-carotene on alternate days for 12 years was not effective in preventing certain cancers, CVD, or deaths from all causes in 22,071 apparently healthy men (40–84 years), including smokers and former smokers [44]. Another study of secondary prevention, involving the same supplementation to 8171 women (minimum age 40 years) with preexisting CVD or at least three risk factors, there was no effect after 9.4 years of follow-up. The results of the β-Carotene And Retinol Efficacy Trial (CARET) study, obtained from 18,314 individuals of both sexes (45–74 years), including smokers, recent ex-smokers, and men exposed to asbestos, were more favorable. These were divided into control and intervention groups, where the second group received

supplementation with 30 mg β-carotene and 25,000 IU of retinyl palmitate (retinol). After four years, there was a 28 % increase in the incidence of lung cancer, 17 % in mortality from all causes, and 26 % in cardiovascular mortality in the supplemented group [45].

Contra

The MRC/BHF Heart Protection Study examined the effects of a cocktail of antioxidant vitamins (600 mg vitamin E, 250 mg vitamin C, and 20 mg β-carotene) or placebo in 20,536 UK adults aged 40–80 years with coronary artery disease (CAD), other occlusive arterial disease, or diabetes mellitus who took supplements for 5 years. Although this supplementation resulted in an increase of antioxidant vitamins in blood, it did not produce a significant reduction in mortality at 5 years [46]. The Alfa Tocopherol β-carotene (ATBC) study demonstrated no effect of β-carotene or vitamin E supplementation on the incidence of large abdominal aortic aneurysm. Supplemental β-carotene actually increased the incidence of cerebral hemorrhage and lead to an increase in deaths from AMI [47].

Vitamin C

Vitamin C is mainly found in fruits and vegetables. Rich fruit sources include cantaloupe, grapefruit, honeydew, kiwi, mango, orange, papaya, strawberries, tangelo, tangerine, and watermelon [48]. Fruit juices containing vitamin C in abundance include grapefruit and orange juices. Several fruit juices are fortified with vitamin C, including apple, cranberry, and grape juices. Rich vegetable sources of vitamin C include asparagus, brussels sprouts, cabbage, cauliflower, kale, mustard greens, pepper (red or green), plantains, potatoes, snow peas, sweet potatoes, and tomatoes and tomato juices. Variables that affect vitamin C content of fruits and vegetables are harvesting season, duration of transport to the marketplace, period of storage, and cooking practices [49].

Ascorbic acid is considered the most important antioxidant in cellular fluids. It can function as a scavenger of several ROS in the aqueous phase, such as superoxide, hydrogen peroxide, hydroxyl radicals and peroxides, and singlet oxygen, and is the first line of antioxidant defense in human plasma against oxidative stress [50]. *In vitro* studies have shown that ascorbate, blocking the FRs in the aqueous phase before they start lipid peroxidation, can protect biomembranes from damage, and this also appears to exert protective action against LDL [51]. Ascorbate protects the membranes and LDL from oxidative modification and may also regenerate the reduced form of β-tocopherol, although this synergistic activity has not been fully demonstrated in biological systems. Ascorbate combined with vitamin E constitutes a powerful system of protection against oxidative damage by FRs [52]. An inadequate daily intake of vitamin C triggers progressive devastating damage caused by FRs on the cell walls; progressive impoverishment and breakdown of collagen in the vascular wall being buffered by derivatives of alpha-lipoproteins leads to the progression of atherosclerosis [53].

Pro

A cross-sectional study of 979 non-smoking women and men aged 20–29 years in the Toronto Nutrigenomics and Health Study was conducted from 2004 to 2008 to determine the prevalence of serum ascorbic acid (vitamin C) deficiency and its association with markers of chronic disease in a population of young Canadian adults. In this cross-sectional study of Canadian men and women aged 20–29 years, 1 of 7 of the young Canadian adults had serum ascorbic acid deficiency, and only 53 % had adequate serum ascorbic acid [54, 55].

An inverse association between serum ascorbic acid and markers of chronic disease was already present in these young adults, suggesting potential adverse health effects [56]. The implications of these findings underscore the importance of obtaining the recommended daily allowance (RDA) for dietary vitamin C in order to decrease the prevalence of serum ascorbic acid deficiency in young Canadians and to potentially decrease the risk of long-term adverse health effects. Vitamin C may increase endothelial nitric oxide (NO) by protecting it from oxidation and increasing its synthesis. Vitamin C and the other antioxidant vitamin, vitamin E, appear to have beneficial effects on vascular endothelial function in healthy subjects and in patients with CVD [57].

Contra

A study led by Sesso *et al.* [58] examined the ability of long-term supplementation with vitamins E and C in reducing the risk of major cardiovascular events among men. The analysis was performed on participants of the Physician's Health Study II, begun in 1997 and completed in mid-2007. Enrolled were 14,641 male

doctors aged 50 and above; 5.1 % (754) of them suffered from CVD. The participants took vitamin E 400 IU on alternate days and 500 mg vitamin C daily. The composite endpoint of major cardiovascular events included: non-fatal myocardial infarction, non-fatal stroke, and death from CVD. During an 8-year period follow-up there were 1,244 major cardiovascular events. Compared with placebo, vitamin E had no effect on the incidence of major cardiovascular events. There was no significant effect of vitamin C on major cardiovascular events either. Neither vitamin C nor vitamin E showed an effect on total mortality, the vitamin E was associated with an increased risk of hemorrhagic stroke (RR 1.74; $p = 0.04$).

The Women's Antioxidant Cardiovascular Study evaluated the effects of ascorbic acid (500 mg/day), vitamin E (600 IU every other day), and β-carotene (50 mg every other day). The composite endpoint was myocardial infarction, stroke, coronary revascularization, or cardiovascular death among 8,171 female health workers at increased risk [60]. The women who took part in the study were aged 40 years and older, with a history of CVD or three or more cardiovascular risk factors, and were followed for a median duration of 9.4 years, from 1995–1996 to 2005. A total of 1,450 women had one or more cardiovascular events. There was no effect of ascorbic acid (RR 1.02), vitamin E (RR 0.94), or β-carotene (RR 1.02), or in the primary composite endpoint individual events (AMI, stroke, coronary revascularization, or cardiovascular death). A marginally significant reduction in the primary endpoint was observed among women with previous CVD who took vitamin E. There were no significant interactions between antioxidant vitamins on the primary endpoint, but the women assigned to ascorbic acid and vitamin E had a significantly lower incidence of stroke. The study data did not reveal any effect of ascorbic acid, vitamin E, or β-carotene on cardiovascular events among women at high risk of CVD [44].

Vitamin E

The term vitamin E is used to describe a group of naturally occurring compounds with antioxidant activity, having eight chemical forms (α-, β-, γ-, δ-tocopherols and α-, β-, γ-, δ-tocotrienols) with different levels of biological activity. The α-tocopherol form is the only form that can meet human needs [61]. Vitamin E is a lipid-soluble antioxidant best known for its ability to inhibit lipid peroxidation and to act as a scavenger of ROS [62]. The role of vitamin E in the human body is not clearly established, but it is known to be an essential compound in some vertebrate species, including humans [63].

CVD is largely attributable to atherosclerosis, and lipid peroxidation plays a central role in the formation of atherosclerotic plaque [64]. This allowed us to hypothesize that antioxidants, including vitamin E, may protect against CVD. To support this view several clinical studies that have suggested a role of vitamin E in the pathophysiology of CVD were performed. Supplementation with vitamins has become popular, and more than half of the adult population in the U. S. is taking nutritional supplements, including 12.7 % that are taking vitamin E [65]. Vegetable oils, nuts (almonds), and green leafy vegetables are the best sources of vitamin E [49].

When taken only from food sources, vitamin E has no documented toxicity. However, in very high doses vitamin E supplements have been shown to have toxic effects [66]. For this the research on vitamin E toxicity is controversial. The recognition of oxidized LDL's participation in the development of atherosclerosis has intensified interest in vitamin E supplementation in preventing CVD [67]. Several meta-analyses of randomized trials were performed to investigate the effects of vitamin E on CVD. The results were largely disappointing, and it was found that there was no overall effect of vitamin E on AMI, stroke, or death due to CVD [68–70]. In fact, study trials conducted on 118,765 participants (59,357 randomized to vitamin E and 59,408 to placebo) demonstrated evidence that vitamin E treatment increased the risk for hemorrhagic stroke by 22 % and reduced the risk of ischemic stroke by 10 %, therefore the results are contrasting [32]. However, it has been suggested that the increased mortality odds ratio in certain trials is not due to the higher dose of vitamin E supplementation. Rather the effect can be explained by a higher proportion of male patients that were included in these trials compared to other trials. The causal relationship of vitamin E supplementation and increased mortality is questionable. Different methodological approaches of meta-analysis yield contradictory results. In particular, high-dose vitamin E supplementation cannot be regarded as conclusively proven to increase mortality [69].

Pro

The evaluation and the association between vitamin E intake and cardiovascular risk have been executed in several observational studies. Among these publications there is the Nurse's Health Study, in which about 90,000 women without CVD, aged 34 to 59

years, were followed for eight years. The results of this study concluded that those who consumed vitamin E supplements (>100 IU) for more than two years had a lower risk of CHD (RR 0.59), after adjusting for age, smoking, other cardiovascular risk factors and nutrients with antioxidant properties [71]. In the same vein, about 40,000 health professional males were followed for four years, and a lower risk of CHD was observed in those who had higher intake of vitamin E (60 IU per day versus 7.5) [72]. Doses of up to 1,000 mg/day in adults appear to be safe, although data are limited and are based on small groups of people who consumed at least 2000 IU per day for a few weeks or months. Finally, some researchers have speculated that antioxidants are more effective in inhibiting the early stages of atherosclerosis, such as fatty-streak formation, than preventing sequelae in the advanced stages experienced by most patients participating in clinical trials [73]. The observed benefits of an antioxidant combination containing vitamin E in the prevention of early stages of cardiac transplant vascular disease is consistent with this hypothesis. The Cambridge Heart Antioxidant Study (CHAOS) was a double-blind, randomized trial evaluating the effect of vitamin E in patients with atherosclerosis. Alpha-tocopherol treatment in patients with angiographically proven symptomatic coronary atherosclerosis substantially reduces the rate of non-fatal AMI, with beneficial effects apparent after one year of treatment [74]. Currently, researchers are in agreement on the antioxidant properties of vitamin E and its involvement in lipid peroxidation, which plays a central role in atherogenesis.

In a recent meta-analysis in individuals with both diabetes mellitus and the haptoglobin (Hp) 2–2 genotype, the antioxidant function of vitamin E supplementation was postulated to provide cardiovascular protection to Hp 2–2 DM [75]. However, it is not known whether the dosages of vitamin E administered in clinical trials are adequate to prevent the peroxidation of lipids in all subjects [32]. In addition to these effects on lipids, vitamin E has important functions in the regulation of membrane enzymes, gene expression, and inflammatory responses. Further studies are needed to explain the essentialness of the vitamin for its ability to scavenge FRs.

Contra

Research has found no negative effects from the consumption of vitamin E in food; however, high doses of α-tocopherol supplements can cause bleeding and stop blood clotting in animals, and *in vitro* tests suggest that high doses inhibit platelet aggregation [76].

Several clinical trials found an increased risk of hemorrhagic stroke in participants receiving α-tocopherol. One was a study comprised of male smokers in Finland who consumed 50 mg/day for an average of 6 years, while another study involved a large group of male doctors in the United States who consumed about 400 IU every other day for 8 years. Therefore, frequent use of α-tocopherol may have adverse effects on health [77].

A meta-analysis performed on 81,788 patients by researchers at the Cleveland Clinic on the evaluation of the effects of antioxidants in CVD showed that when used as secondary prevention, vitamin E did not significantly reduce the risk of cardiovascular events. Furthermore, their results do not support the continued use of vitamin E treatment and discourage the inclusion of vitamin E in future primary and secondary prevention trials in patients at high risk of CVD [68].

Experimental and epidemiological data have shown that supplementation of vitamin E can prevent the onset of cancer and cardiovascular events. Researchers of the Heart Outcomes Prevention Evaluation (HOPE) and Heart Outcomes Prevention Evaluation, and the Ongoing Outcomes (HOPE-TOO) trial, examined whether long-term supplementation with vitamin E was able to reduce the risk of cancer, cancer death, and major cardiovascular events [78]. A cohort of 9,541 patients, mean age 55 years, with a high risk for cardiovascular events, suffering from heart disease or diabetes, were randomized to receive vitamin E (400 IU/day) or placebo for an average of 4.5 years. The results showed that there was no difference between vitamins and placebo in the number of heart attacks, stroke, and death from CHD. Vitamin E had no effect either on secondary targets: unstable angina, congestive heart failure, and complications of diabetes [79].

Conclusion

In the light of what is reported in this review, it can be said that a diet rich in antioxidants may be recommended for all ages, because the prevention of certain diseases is strongly conditioned by a balanced diet which can provide those necessary nutrients and protective substances that participate to improve health and physical well-being, and thus the quality of life of every individual.

Epidemiological studies in recent years suggest that low levels of antioxidants are associated with an increased risk for CVD. Studies of dietary supplementation of antioxidant vitamins to combat vascular damage and reduce the deleterious effects of FRs are rather different. To date, the results are conflicting, and most likely this is due to the fact that not all antioxidants exert equal activity against free radical molecules. Chronic inflammation is known to have a pivotal role in the atherosclerotic process [80].

Recent studies have shown that inflammation leads to an aggravation of oxidative stress, through the interaction and subsequent activation of several transcription factors such as nuclear factor-kappa B (NF-kB), a regulator of several genes involved in atherosclerosis, such as adhesion molecules (ICAM-1 , V-CAM) and cytokines [81].

Some studies have found that supplementation with α-tocopherol results in anti-inflammatory effects *in vivo* and *in vitro* since it leads to a reduction of C-reactive protein (CRP), plasminogen activator, and a number of cytokines and interleukins [82]. Several plausible mechanisms have been suggested. Beta-carotene, in particular, is a poor inhibitor of *in vivo* LDL oxidation [83]. Moreover, cigarette smoke destabilizes the β-carotene molecule, resulting in abnormal signal transduction and the up-regulation of growth factors associated with tumorigenesis [84].

The use of vitamin supplements containing β-carotene and vitamin A, β-carotene's biologically active metabolite, should be actively discouraged because this family of agents is associated with a small but significant excess of all-cause mortality and cardiovascular death [68]. It has been recommended that clinical studies of β-carotene should be discontinued because of its risks. When used in secondary prevention, vitamin E did not reduce the risk of cardiovascular endpoints [85]. Furthermore, given our results and the lack of mechanistic data supporting the efficacy of vitamin E as a potent antioxidant *in vivo*, we do not support the continued use of vitamin E treatment and discourage the inclusion of vitamin E in future primary and secondary prevention trials in patients at high risk of CAD [68].

As for the vitamin supplements that have proven effective *in vitro* antioxidant properties, *in vivo*, for example, the National Health and Nutrition Examination Surveys (II and III) and the Eastern Finland Study both supported a protective role for vitamin C in risk reduction for several endpoints that represent CVD and CAD, in separate models. 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 [86–89]. Researchers from the Cardiovascular Division of Brigham and Women's Hospital of Boston and the Linus Pauling Institute of Oregon State University, Corwallis, conducted a double-blind study of 40 patients who underwent heart transplantation over the past two years and concluded that an additional intake of antioxidant vitamins C and E may delay the progression of early coronary atherosclerosis associated with cardiac transplantation [90, 91].

While we can conclude that diet is a valuable ally in the fight against atherosclerosis in primary prevention, the strategy must be aimed at both the population as a whole, with comprehensive education, change in lifestyle and environmental factors that increase cardiovascular risk, as well as to subjects already at high risk. An apple a day keeps the doctor away, as a well-known proverb promises. There are several studies that have more or less confirmed or questioned the theory that vitamins protect against cardiovascular risk as well as against cancer or other diseases. It is obvious that some of the above studies reach markedly different conclusions. The reasons for these discrepancies are different. Firstly, antioxidants may be useful only for the primary prevention of CVD, i.e. before the disease occurs, while if atherosclerosis has already developed there is no protective action. However, the methodology of these studies, criteria for selection of patients, the uncertain extent of progression of the disease when initiating supplementation, the lack of mechanistic studies containing basic scientific aspects, such as the bioavailability, pharmacokinetic properties, and the nature of the antioxidant sources of vitamins, could account for the inconsistency of the various clinical trials and meta-analyses assessing the efficacy of these vitamins to prevent human diseases.

In addition, it is not always easy to have equivalent dosing regimens in one study and another in order to have comparable results. Finally, there is also a problem with time: randomized trials are often not long enough to observe real benefits. Another possible explanation for inconsistency in observational studies may be that the association between antioxidants and disease exists in addition to social and behavioral factors acting throughout the lifespan.

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Graziano Riccioni, MD, PhD

Via G. De Rogatis, 12
71016 San Severo (FG)
Italia
Tel.: + 39 0882 227022
griccioni@hotmail.com