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Vitamin C and E consumption and coronary heart disease in men

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1. ABSTRACT

Atherosclerotic cardiovascular diseases (CVD) are a major source of mortality and morbidity in general population. Oxidative modification of low density lipoprotein (LDL) represent 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 which causes CVD. Appropriate nutritional practices are of central importance in managing risk and treatment of CVD; indeed, many current guidelines contain nutritional recommendations to reduce the risk of these diseases. In observational studies vitamin C and E, the most prevalent natural antioxidant vitamins, have suggested that supplemental users have lower rate of coronary events. Despite these data, several large randomized controlled trials (RCTs) have failed to confirm the benefits for vitamin C and E in cardiovascular (CV) prevention. The aim of this review is to examine the studies published in literature which report the effect of supplementation with antioxidant vitamins (C,E) in the primary and secondary prevention of CVD in men due to atherosclerotic process.

2. INTRODUCTION

Cardiovascular disease (CVD) is the single most common cause of death in developed countries and represents an epidemic cause of death and disability responsible for about 30 percent of all deaths worldwide each year (1).

From a diagnostic point of view these pathologies include the ischemic cardiopathy (CHD – coronary heart disease) with its clinical manifestations (acute myocardial infarction [AMI], angina pectoris, and unexpected death), cerebrovascular diseases (ictus cerebri and transitory ischemic attack), and peripheral vasculopaties (2).

Observational studies have demonstrated that acute coronary syndromes (ACS), especially unstable angina (UA) and acute myocardial infarction (AMI) share a common anatomical substrate. These different clinical manifestations derive from a unique basic physio-pathological mechanism that consists in the erosion or in the breach of atherosclerotic plaque; moreover many thrombotic phenomena are associated to these events (3-6).

Even if people at risk from primary and secondary CVD seem less likely to use dietary supplements, despite possible benefits shown in clinical trials, public health organizations need to develop guidelines for the public and for health professionals regarding the uncontrolled use of dietary supplements in the community (7).

Numerous epidemiological studies have identified dietary patterns and food categories associated with a reduced risk of CVD. Although the interpretation of these studies is limited by their observational nature, nevertheless they represent the only feasible approach for drawing conclusions regarding the overall impact on health of dietary patterns and intake of specific food categories (8-10).

Current evidences does not support the indiscriminate use of vitamin C or E to prevent or reduce CVD. In fact, many trials failed to demonstrate any beneficial effect, and some studies also documented an harmful effect (11,12).

3. OXIDATIVE STRESS

Oxidative stress (OS) represents a condition in which cells are exposed to excessive levels of reactive oxygen species (ROS) and the cellular antioxidant defences are inadequate to completely inactivate the ROS generated (13). One of the major consequence of OS is the damage to nucleic acid bases, lipids, proteins, and other cellular structures which can severely compromise cell health and viability or induce a variety of cellular responses through generation of secondary reactive species, ultimately leading to cell death by necrosis or apoptosis. Oxidative damage of any of these biomolecules, if unchecked, can theoretically contribute to human acute and chronic disease development (8,9). Published evidences suggests that ROS participate in the normal aging process as well as in age-related diseases such as atherosclerotic diseases (11,12).

Oxidative stress-induced peroxidation of membrane lipids can be harmful because it leads to alterations in the biological properties of the membrane, such as the degree of fluidity, and can lead to inactivation of membrane-bound receptors or enzymes, which in turn may impair normal cellular function and increase tissue permeability. Moreover, lipid peroxidation may contribute and amplify cellular damage resulting from generation of oxidized products, some of which are chemically reactive and covalently modify critical macromolecules. Cellular DNA damage can be caused by ROS generated under different conditions, and several techniques have been developed to measure the oxidatively modified nucleobases in DNA (14-16).

ROS are highly reactive oxidant molecules generated endogenously through regular metabolic activity, lifestyle activity and diet. They react with cellular components, causing oxidative damage to such critical cellular biomolecules as lipids, proteins and DNA (7,17).

OS induced by ROS is characterized by production of oxidized-LDL (LDL_{ox}), a critical step in the initiation and progression of atherosclerotic lesions (2,15,16). In particular the oxidation of LDL particle ignites a number of events that promote the development of atherosclerotic lesions: foam cells formation by activated macrophages, leukocyte adhesion to endothelium, and release of cytokines from cells within the artery wall which can be directly cytotoxic and causes endothelial dysfunction (18).

Several studies in patients with atherosclerosis or its risk factors have demonstrated that impaired endothelial dysfunction occurs not only in the coronary but also in the peripheral circulation, suggesting that endothelial dysfunction is a generalized, systemic process (19).

4. CLINICAL USE OF ANTIOXIDANTS

The type of diet can influence the pathogenetic development of the atherosclerotic lesions through a direct influence on primary determinants of the atherosclerotic process such as levels of plasmatic lipids (triglycerides, HDL and LDL-cholesterol, phospholipids), structural integrity of the circulating lipids (LDL_{ox}), plasmatic antioxidants (vitamins, trace elements), production of free radicals (FRs), circulating levels of homocysteine, glycaemia, blood pressure, platelet aggregation, and degree of lipidic peroxidation (11,12,20).

The clinical use of antioxidants has gained considerable interest during the last decade. It was suggested from epidemiological studies that diets high in fruits and vegetables might help decrease the risk of CVD. Therefore, supplements of vitamins C and E were applied through protocols aimed to prevent diseases such as atherosclerosis mediated by OS. Despite the biological properties of these vitamins could account for an effective protection, as shown by several clinical and experimental studies, their efficacy remains controversial in the light of some recent clinical trials and meta-analyses (21).

Published data from *in vivo* and *in vitro* studies have shown that peroxidative damage to LDL can promote various degrees of the atherosclerotic process, including damage to the endothelium, the accumulation of foam cells, their proliferation and production. Vitamins with antioxidant effects represent one of the main defence mechanisms of the non-enzymatic body antioxidant system. The ascorbic acid (vitamin C) and the α-tocopherol (the principal form of vitamin E), represent a natural anti-oxidant complexes most studied during the last years. During the last eighty years randomized clinical trials (RCTs) were designed on a wide scale to test the hypothesis of whether dietetic vitamin supplementation with an antioxidant action (alone or in association) was able to reduce the risk of CVD. These studies suggested an epidemiological and experimental positive effect on the development and progression of atherosclerotic plaque with a consequent reduction of cardiovascular events. Recent studies with defined primary and secondary

prevention endpoints have not supported this hypothesis (22).

5. VITAMIN C CONSUMPTION AND CHD

Vitamin C is an important water-soluble vitamin present in two biologically active forms: ascorbic acid and its oxidized derivative, dehydro-ascorbic acid. These forms are inter-convertible, and are collectively termed vitamin C. Most dietary vitamin C is supplied by vegetables and fruit. Both forms of vitamin C are readily absorbed by an active transport and a passive diffusion mechanism. Vitamin C is a reducing agent, as the ascorbate is readily oxidized to dehydro-ascorbate. In this way, vitamin C can act as a hydrogen donor to reverse oxidization thus reacting with FRs and inactivating them before they cause damage to proteins or lipids (23).

5.1. Studies on vitamin C

The First National Health and Nutrition Examination Survey (NHANES) Epidemiological Follow-up Study (11,348 subjects aged 25-74 years) demonstrated that individuals who received the maximum dose of vitamin C (>50 mg/die) showed lower total mortality rates and a reduction of mortality for CVD to 10 years (24). Even Blot *et al.* (25), in a study conducted among a rural population subjects who received a combination of vitamin C (120 mg/die), β-carotene (15 mg/die), selenium (50 μg/die), and vitamin E (30 mg/die), documented a moderate reduction rate of mortality for CVD. Besides, Knekt *et al.* (26) in a cohort study pooling 9 prospective studies that included information on intakes of vitamin C founded a reduced incidence of major CHD events at high supplemental vitamin C intakes (>700 mg/day).

Contrary Wilson *et al.* (27), in a supplementation trial conducted on 578 elderly patients who received 200 mg/day of ascorbate for 6 months, not showed any positive effects on total mortality. Even Sesso *et al.* (28) in a randomized, double-blind, placebo-controlled of vitamin C (500 mg/day for 8 years) involving 14,641 male physician not provided supporting data for the use of this supplement for the prevention of CVD in middle-aged and older men. The same result was found by Kinlays *et al.* (29) in a double-blind RCT with placebo. In this study the supplementation of vitamin C (1000 mg/day) for 6 months in 30 subjects with CAD not improved key mechanism in the biology of atherosclerosis or endothelial dysfunction, or reduced LDL oxidation *in vivo*.

Finally, later reviews and metanalyses about the relationship between anti-oxidants and atherosclerotic cardiac diseases not confirmed this evidence. The analysis of RCT including one hundred and more patients have shown an unclear reduction of the prevalence and mortality of CVD (30).

6. Vitamin E consumption and CHD

Vitamin E is the most important antioxidant vitamin in the body, playing an essential protective role against FRs damage (31,32) and forms part of a group of substances belonging to two closely related families: tocopherols and tocotrienols, with each existing in a number of isomeric forms, α, β, γ, and δ tocotrienol, making a total of eight different group members. Within the vitamin E family of compounds, α-tocopherol is the most biologically active and occurs naturally as one isomer (33). γ-tocopherol is the predominant form of vitamin E found in human diet, while α-tocopherol acetate and synthetic isomers of α-tocopherol are the primary forms of vitamin E supplements (34). The chemical structure of tocopherols and tocotrienols, with an $^{-}$OH group on the ring structure, makes them very effective hydrogen donors. In donating hydrogen vitamin E becomes oxidized itself, whilst preventing the oxidation of more metabolically important components, for example polyunsaturated fatty acids (PUFA) in cell membranes.

Vitamin E function as antioxidant is dependent upon its ability to break radical-propagated chain reactions. As a result, the formation of the tocopheroxyl radical, the odd-electron derivative of vitamin E, is an inherent part of any vitamin E based anti-oxidative reaction. As a lipid-soluble antioxidant α-tocopherol reacts with antioxidant molecules. In turn, α-tocopherol helps protect cell membranes from lipid peroxidation by trapping peroxyl radicals (35). This is important when FRs are present, as these highly reactive substances can attack double bonds, setting up chain reactions, with more FRs being produced. In the case of damage to fatty acids, lipid peroxides are produced and alter the function of the cell membrane and cause possibly irreversible damage to metabolic pathways (36-39).

Vitamin E daily requirements (10 mg) vary with the dietary intake of PUFA. In cellular membranes and circulating lipoproteins vitamin E is the most fat-soluble vitamin compound most represented. For this reason vitamin E is the main antagonist of lipid peroxidation. The protecting action on membranes is important for a good functionality of the endothelial barrier, a consequent reduction of the proliferation stimuli for the muscular cells of the medium tunica, and a reduction of the chemiotactic factors for the monocytes (40).

An ulterior aspect of its protecting ability on the endothelium is supported by a stimulus action on the synthesis of PG_{12} with vasodilatation action and anti-aggregate platelet (41). For its elevated fat solubility vitamin E stretches for a long time on membrane lipoproteins, and therefore its eventual dietetic supplementation does not need to follow a daily re-integration (42).

6.1. Studies on vitamin E

In cellular and molecular studies it has been consistently demonstrated that the oxidant-quenching action of vitamin E provides its beneficial effect by protecting cells from peroxyl radical induced oxidative damage (30,43,44). Both *in vivo* and *in vitro* studies have demonstrated that α-tocopherol inhibits LDL oxidation (45), decreases the release of ROS and the release of pro-inflammatory cytokines, and inhibits monocyte-

endothelial cell-adhesion (44). Studies in animal models have demonstrated a strong inverse relation between the intake of antioxidants and the incidence of atherosclerosis by inhibiting lipid peroxidation and significantly diminish the atherosclerotic process (46,47).

In humans several epidemiological studies have revealed an inverse relationship between vitamin E intake and the progression of atherosclerosis and incidence of CVD. In the Cambridge Heart Antioxidant Study (CHAOS), a randomized secondary prevention study, 2,002 patients with angiographically proven coronary atherosclerosis were enrolled and followed up for a median of 510 days. 1,035 patients were assigned either 800 UI or 400 UI capsules of vitamin E. The remaining 967 patients received identical placebo capsules. The findings from this study revealed that vitamin E supplementation significantly reduced the incidence of death for CVD and non-fatal AMI (48).

Similarly, in a study by Boaz *et al.* (29), the Secondary Prevention with Antioxidants of Cardiovascular disease in Endstage renal disease (SPACE) trial demonstrated a protective effect of vitamin E against CVD endpoints (ischemic stroke, peripheral vascular disease, and unstable angina) and AMI in haemodialysis patients with prevalent CVD. In this study haemodialysis patients (aged 40-75 years) with pre-existing CVD were enrolled and randomized to receive 800 UI/day vitamin E or matching placebo for a median of 519 days. The results showed a significant decrease in CVD endpoints and AMI in patients assigned to receive vitamin E *versus* placebo.

In the Alpha–Tocopherol Beta Carotene Cancer Prevention (ATBC) trial the group of approximately 30,000 patients treated with supplementation of alpha-tocopherol (50 mg/day) or in combination with β-carotene for 5-8 years has shown a lower number of non-fatal AMI, but no reduction of the primary end-point study (total mortality, CV death and AMI) (49).

Blot *et al.* (25) in the Linxian study, conducted in a rural population subjects who received a combination of vitamin C (120 mg/day), β-carotene (15 mg/day), selenium (50 μg/day), and vitamin E (30 mg/day) documented a moderate reduction of mortality for CVD. Particularly, in this study the lower incidence of global mortality has been due to lower incidence of cancer, in particularly gastric cancer.

Finally, in a study by Rimm *et al.* (50), in which 39,000 male health professionals were followed for 4 years, 17% of the men took vitamin E supplements. Men in the upper quintile (median intake 419 UI/day) compared with the men in the lower quintile of vitamin E intake (6 UI day) had a 40% relative risk reduction for non-fatal AMI, death from CHD or coronary revascularization.

In contrast to the studies demonstrating the beneficial effects of vitamin E supplementation against coronary artery disease (CAD), several trials have revealed potential pro-oxidant and pro-atherogenic effects of α-tocopherol when administered in certain population and/or with certain pharmacological agents.

Many papers have shown that in smokers consuming a high polyunsaturated diet, vitamin E may function as a pro-oxidant (51). In this study, 10 subjects who smoked more than one pack of cigarettes per day were fed either a baseline diet with olive oil as the major fat source, a diet with high-linoleic safflower oil as the major fat source or the safflower oil diet plus 800 UI vitamin E per day. LDL oxidation lag time and plasma total F2-isoprostanes were measured as determinants of *in vivo*, oxygen-derived free radical stress. While the safflower di*et al*one demonstrated an increase in plasma isoprostanes relative to the baseline diet, there was no significant change in its effect on the LDL oxidation lag time. In the safflower + vitamin E diet, the lag time increased relative to the baseline diet, and the isoprostanes level increased markedly as well. These data suggest that under the specific conditions that exists in smoker consuming a high polyunsaturated fatty diet, vitamin E can function as a pro-oxidant *in vivo*.

Two important studies (GISSI and HOPE) have reported that vitamin E treatment of patients with CVD had no significant effect on the frequency of primary endpoints, i.e., non-fatal AMI, stroke or death from these disorders.

The Prevention Study of the Italian Group for the Study of the Survival in the Myocardial Infarction (GISSI-Prevenzione) is a randomized secondary prevention trial including 11,000 patients treated for 3.5 years. This study showed that vitamin E supplementation (300 UI/day) isn't associated with a statistically significant reduction of total mortality death for CV events and re-infarctum (52). Even the Hearth Outcome Prevention Evaluation Study (HOPE) has not demonstrated any benefits on CV events with the use of vitamin E (400 UI/day) in 9,541 randomized patients (mean age 55) for 4.5 years (53).

In a study by the Collaborative Group of the Primary Prevention Project (54) investigating the efficacy of anti-platelets and antioxidants in the primary prevention of CV events, it was demonstrated that vitamin E supplementation (300 mg/day) had no effect on any pre-specified endpoint. Similarly, Keith *et al.* (55) showed that vitamin E supplementation in 56 patients with advanced HF failed to affect significantly any marker of oxidative stress and furthermore did not result in any significant improvements in prognostic or functional indexes of HF or in the quality of life of patients.

Finally, an important investigation by the Heart Protection Study Collaborative Group (56) examining 20,536 UK adults with CAD, other occlusive disease or diabetes demonstrated that there was not significant effect on CV primary endpoints in the group randomly allocated vitamin supplementation (600 mg vitamin E, 250 mg vitamin C and 20 mg β-carotene) *versus* the group

receiving matching placebo during five years follow-up period.

7. CONCLUSION

Several naturally occurring constituents have received a considerable attention because of their potential antioxidant activity in the prevention and treatment of atherosclerosis and correlated diseases (57).

The importance of dietary antioxidant intake is suggested by numerous direct and indirect evidences. The possible anti-atherogenic role of antioxidant depends on their ability to neutralize FRs. Indeed, in atherosclerotic disease, the LDL_{ox} have a key role in the activation and facilitation of the atherogenic process; the antioxidants, moreover, are capable of preventing the oxidative catabolism of nitric oxide (NO), a molecule with numerous favorable effects.

While cellular, molecular and animal studies continue to demonstrate the effectiveness of α-tocopherol in reducing the progression of atherosclerosis, large-scale clinical trials showed contradictory results and failed to demonstrated the anticipated beneficial effects in humans. These mixed results, obtained from a variety of clinical trials involving vitamin C and vitamin E, attest the complexity of studying the potential protective role of these vitamins against CAD.

The initial hopeful reports regarding the beneficial role of antioxidant therapies against atherosclerosis derived from purely observational studies, were followed by the negative results of large RCTs. For this reason, at the moment, the treatment with antioxidant vitamins should not be recommended for the prevention or treatment of CAD (58). More studies which investigate the effect of a balanced combination of antioxidants at levels achievable by diet are needed (59,60).

Further studies on the bioactive component of foods may emerge into new treatment concepts and open new horizons to identify other intervention strategies to recover normal homeostasis and prevent vascular endothelial dysfunction (61,62), because the ROS-mediated alteration does not represent the only event in the pathogenesis of those diseases (63). Antioxidants have for a long time been considered as an aid to help against diseases that are presumably aggravated by oxidative stress, such as CVD (64). The outcome of clinical trials undertaken to corroborate this hypothesis, however, remain largely inconclusive.

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Abbreviations: CVD: cardiovascular disease, CHD: cardiac ischemic disease, CAD: coronary artery disease, AMI: acute myocardial infarction, ACS: acute coronary syndromes, UA: unstable angina, OS: oxidative stress,

ROS: reactive oxygen species, LDLox : oxidized-LDL, FRs : free radicals , RCTs: randomized clinical trials, PUFA: polyunsaturated fatty acids, CHAOS: Cambridge Heart Antioxidant Study, SPACE: Secondary Prevention with Antioxidants of Cardiovascular disease in Endstage renal disease, ATBC: Alpha Tocopherol Beta Carotene Cancer Prevention, GISSI: Gruppo Italiano Studio Sopravvivenza dell'Infarto , HOPE: Hearth Outcome Prevention Evaluation Study, HF: heart failure

Key Words: vitamin C, vitamin E, Coronary Heart Disease, Acute Coronary Syndrome, Acute Myocardial Infarction, Cardiac Ischemic Disease, Prevention, men., Review

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