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Interventional study with vitamin E in cardiovascular disease and meta-analysis

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ABSTRACT

Cardiovascular disease (CVD) is one of the major causes of morbidity and mortality and atherosclerosis is the common root to most of the CVD. Oxidative stress is one of the most important factors driving atherosclerosis and its complications. Thus, strategies for the prevention and treatment of cardiovascular events had oxidative changes as a potential target. Natural vitamin E consists of a family of eight different compounds, four tocopherols and four tocotrienols. All tocopherols and tocotrienols are potent antioxidants with lipoperoxyl radical-scavenging activities. In addition, α-tocopherol possesses also anti-inflammatory as well as anti-atherothrombotic effects by modulating platelet and clotting system. Experimental and in vitro studies described molecular and cellular signalling pathways regulated by vitamin E antithrombotic and antioxidant properties. While observational studies demonstrated an inverse association between vitamin E serum levels and CVD, interventional trials with vitamin supplements provided negative results.

This review focus on the impact of vitamin E in the atherothrombotic process and describes the results of experimental and clinical studies with the caveats related to the interventional trials with vitamin E to prevent CVD.

1. Introduction

Vitamin E can be considered the most important fat-soluble antioxidant protecting against a variety of oxidative stress-associated pathological processes. The potential role of vitamin E in the prevention of cardiovascular disease was originally proposed by Gey et al. in the "antioxidant hypothesis of atherosclerosis" [1]. This hypothesis considers the suboptimal intake and suboptimal plasma levels of principal antioxidant micronutrients as underrated risk factors for cardiovascular diseases. This theory was complemented with Steinberg's hypothesis which refers to the LDL role and its oxidation in the subendothelium [2], Ross' hypothesis about cellular response to inflammation [3] and Jackson's hypothesis on cytokine response in the arterial inflammatory process [4].

A growing body of evidence suggests that vitamin E is linked to atherosclerosis and thrombotic complications of atherosclerosis. Mechanisms potentially implicated in the anti-atherosclerotic activity of vitamin E can be attributed to its biological functions ranging from its well-established antioxidative properties, as a free radical scavenger, to non-antioxidative properties such as the regulation of signal transduction, cell proliferation, and gene expression. Precise cellular functions of vitamin E independent of its antioxidant or radical scavenging ability were first mentioned in the 1950s, by Hickman and Harris [5]. These functions were later confirmed by Azzi et al. demonstrating that vitamin E inhibits protein kinase C (PKC) activity in concentration-dependent manner [6]. To date, these non-oxidative functions have been characterized and reflect specific interactions of α-tocopherol with enzymes, proteins, lipids, and transcription factors. For example, at the post-translational level, α-tocopherol inhibits 5-lipoxygenase, and phospholipase A2 and activates protein phosphatase 2A and diacylglycerol kinase [7]. At the transcriptional level, α-tocopherol modulated some genes such as scavenger receptors, matrix metalloproteinase-19 and collagenase [7]. Moreover, α-tocopherol inhibits cell proliferation, platelet aggregation and monocyte adhesion [7].

This review will focus on the impact of vitamin E on mechanism of disease regarding the atherosclerotic process addressing its relationship

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List of abbreviations

•OH	hydroxyl radical
8-iso-$PGF_{2\alpha}$	8-iso-prostaglandin $F_{2\alpha}$
ABCA1	ATP-binding cassette transporter
AF	atrial fibrillation
Ang II	angiotensin II
APLT	amino-phospholipid translocase activity
ApoE KO	apolipoprotein E knockout
AT1R	angiotensin-1 receptor
ATBC	alpha-Tocopherol, Beta-Carotene Cancer Prevention
CAD	coronary artery disease
CHAOS	Cambridge Heart Antioxidant Study
CHD	coronary heart disease
CVD	cardiovascular disease
CVE	cardiovascular event
GPx	glutathione peroxidase
H_2O_2	hydrogen peroxide
HAEC	human aortic endothelial cells
HF	heart failure
HOPE	Heart Outcomes Prevention Evaluation
HOPE-TOO	HOPE–The Ongoing Outcomes
Hp	haptoglobin
ICAM-1	intracellular cell adhesion molecule-1
IHD	ischemic heart disease
IL1	Interleukin-1
IMT	intima-media thickness
LDL	low-density lipoprotein
LFLC	low fat/cholesterol
LOX1	lectin-like oxidized low-density lipoprotein (LDL) receptor-1
LXRα	liver X receptors
MCP-1	monocyte chemoattractant protein-1
MetS	metabolic syndrome
MI	myocardial infarction
MLD	minimum lumen diameter
MNC	mononuclear leukocytes
MONICA	Multinational MONitoring of trends and determinants in CArdiovascular disease
MPO	myeloperoxidase
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
Nox2	NADPH oxidase 2
O2	superoxide anion
oxLDL	oxidized LDL
PAD	peripheral artery disease
PAI-1	plasminogen activator inhibitor-1
PKB	protein kinase B
PKC	protein kinase C
PMA	phorbol 12-myristate 13-acetate
PMN	polymorphonuclear leukocytes
PPARγ	peroxisome proliferator-activated receptor γ
PPP	Primary Prevention Project
PS	phosphatidylserine
PT	prothrombin time
RCT	randomized controlled trials
RDA	recommended daily allowance
ROS	reactive oxidant species
RR	relative risk
sNox2 dp	soluble Nox2-derived peptide
SOD	Superoxide dismutase
SPACE	secondary prevention with antioxidants of cardiovascular disease in end-stage renal disease
T2DM	type 2 diabetes mellitus
TF	tissue factor
TNF-α	tumor necrosis factor-α
TT	thrombin time
TxB2	thromboxane B2
VCAM-1	vascular cell adhesion molecule-1
VEAPS	Vitamin E Atherosclerosis Prevention Study
VSMCs	vascular smooth muscle cells
WHS	Women's Health Study

with oxidative stress and clotting pathways. Furthermore, clinical, and interventional studies will be analyzed in the attempts of identifying the caveats of interventional trials for prevention atherosclerotic complications by vitamin E.

2. Oxidative stress and cardiovascular disease

Oxidative stress is defined as an event where a transient or permanent perturbation in the oxidative balance state generates physiological consequences within the cell, depending on the specific target and reactive oxidant species (ROS) concentrations in the cellular medium [8,9]. Thus, the term oxidative stress refers to a change of redox status, where the stimuli for ROS formation overcome the natural defence encompassing natural antioxidants. This change is quite frequent in patients at risk of cardiovascular disease, such as those with hypertension, dyslipidaemia, diabetes, obesity or acute conditions such as sepsis and respiratory failure [10–12]. These clinical settings may be associated with an up-regulation of pro-oxidant enzymatic systems that have been recently recognized as molecular pathways implicated in the progression of the atherosclerotic disease [13]. In particular, NADPH oxidases and myeloperoxidase (MPO) are among the best established enzymatic systems implicated in atherosclerotic progression [14] and are involved in the formation of ROS such as superoxide anion (O2-), hydrogen peroxide (H_2O_2) and hydroxyl radical (• OH) [13].

Atherosclerosis, the common root to most of the cardiovascular diseases (CVD), is a chronic process of progressive hardening and narrowing of arteries that reduces the flow and delivery of blood and oxygen throughout the body, leading to ischemic diseases [13]. It starts from the childhood and progresses along the life with several risk factors favouring its progression. Atherosclerotic plaque progression is caused by molecular changes induced by cytokines, and ROS via interaction between endothelial cells, low-density lipoprotein (LDL) and macrophages. In particular, in the early stages of atherogenesis, LDLs are oxidized by ROS giving formation to oxidized LDL (oxLDLs), which are no longer cleared from sub-endothelial space and start to accumulate in the sub endothelium [13]. oxLDLs are key players in the atherosclerotic process by eliciting an inflammatory response by inducing the production of endothelial adhesion molecules and monocytes, leucocytes and T-cells recruitment (Fig. 1).

The relevance of this effect is documented by the fact that monocytes differentiate into macrophages that internalize LDL and, along with T-cells, further release pro-inflammatory cytokines and ROS to keep oxidizing LDLs [13]. This contributes to the formation of an atherosclerotic plaque by apoptosis and foam cells formation [13] (Fig. 1).

These events lead to formation of the so-called "fatty streak" and ultimately to the atherosclerotic plaque [14]. Plaque rupture or erosion allows platelet to stich to the sub-endothelium and initiate thrombus growth coincidentally with the activation of coagulation cascade activation and eventually acute artery occlusion [14].

Hence, LDL accumulation within artery wall causes is a key step for artery inflammation via migration of inflammatory cells such as monocytes/macrophages, which contribute to perpetuate LDL

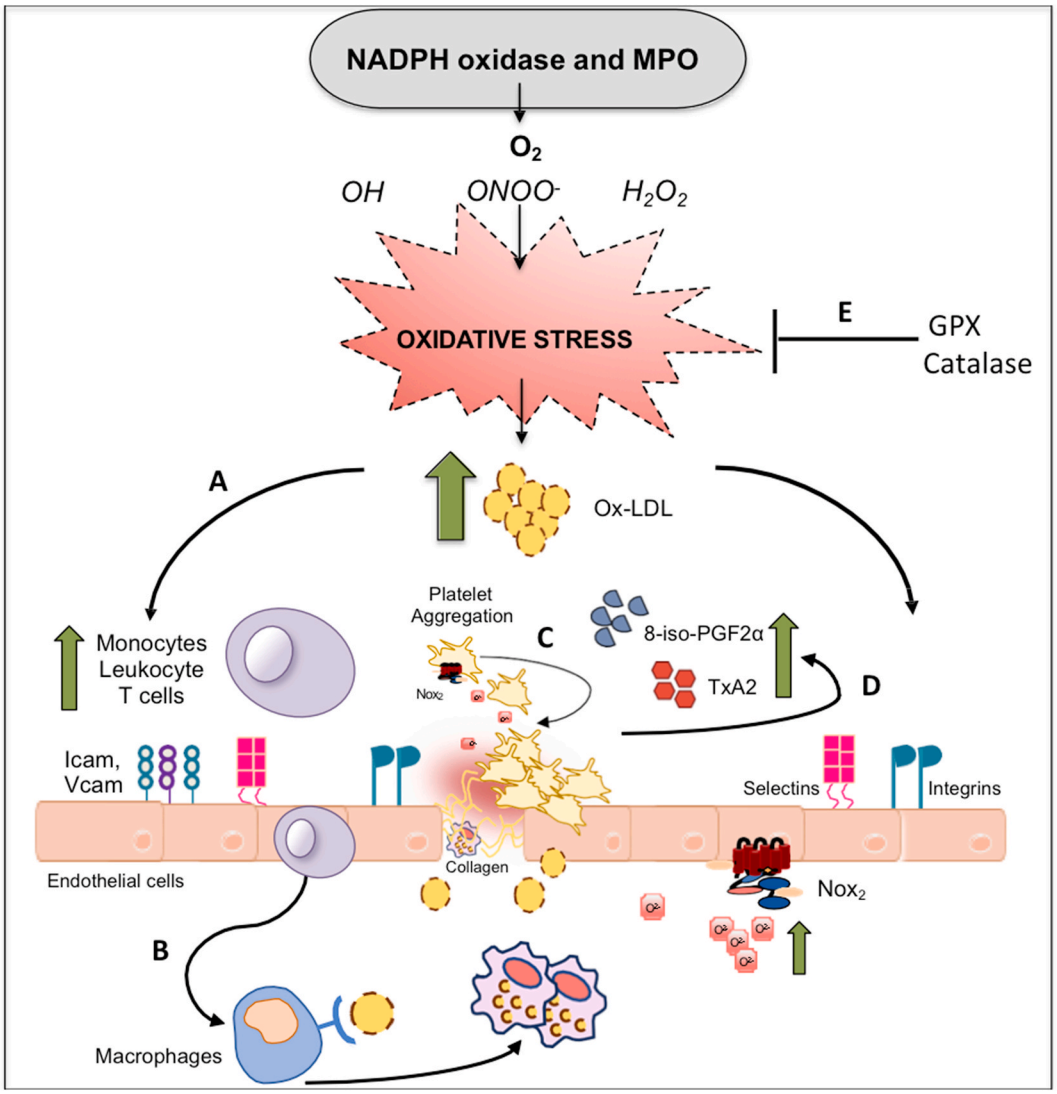

Fig. 1. Schematic representation of oxidative stress role in cardiovascular disease.
(A) NADPH oxidases and myeloperoxidase (MPO) are enzymes involved in the production of ROS such as superoxide anion (O2-), hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH). Oxidative stress contributes to the atherosclerotic plaque progression by molecular changes induced by ROS that oxidize LDLs giving formation to oxidized LDL (ox-LDLs). Ox-LDLs activate the endothelium by inducing the production of adhesion molecules, which recruit monocytes, leucocytes and T-cells. **(B)** Monocytes differentiate into macrophages that internalize LDL and, along with T-cells, release pro-inflammatory cytokines and ROS to keep oxidizing LDLs. **(C)** ox-LDLs behave as a pro-aggregating molecules via interacting with LOX1 and CD36 receptors on platelet surface. **(D)** ox-LDLs induce platelet recruitment by activating the NADPH oxidase pathways with subsequent formation of the pro-aggregating eicosanoids thromboxane A2 and isoprostanes. **(E)** Enzymatic anti-oxidant systems such as GPX and catalase counteract the effect of ROS in the atherosclerotic process.

accumulation via an oxidative stress-mechanism [15]. Concentration of LDL in the circulation is likely to be an important element contributing to LDL permeability and degradation in the artery wall. Bartels et al. [16] studied this issue in cholesterol-fed, LDL receptor-deficient mice treated with an anti-ApoB antisense oligonucleotide versus mismatch control antisense oligonucleotide for 1–4 weeks before injection of iodinated LDL particles. Animals treated with an anti-ApoB antisense oligonucleotide showed approximately 90% reduction of plasma LDL, which was associated with 50% and 85% reduction of aortic permeability and degradation, respectively, of newly entered LDL particles after one week of treatment; conversely, 4 weeks of treatment were necessary to observe reduction of foam cell content, plaque size and aortic LDL pool size. Interestingly, plasma LDL cholesterol lowering was associated with 70% reduction of sub-luminal foam cells and approximately 90% reduction of mRNA expression of inflammatory genes.

Internalization and oxidation of LDL within the vessel wall may be also influenced by intra vessel oxidant status as documented by use of high doses of vitamin E (900 mg/day) in patients undergoing carotid endarterectomy, who were injected with iodinated autologous LDL 24 h before the surgical intervention; the analysis of carotid specimen demonstrated that LDL localized into macrophages and that this phenomenon was prevented by pre-treatment with vitamin E suggesting that LDL is rapidly up-taken and oxidized by foam cells of atherosclerotic plaque [17].

Chronic deposition and accumulation of LDL causes an injury response, which results in recruitment of macrophages, dendritic cells and lymphocytes at site of atherosclerotic lesion. As for non-sterile inflammation, this process may undergo resolution with classical tissue repair or progress to advanced lesion as consequence of defective resolution [18]. Advanced lesion of atherosclerotic plaque is characterized by formation of a necrotic core, which consists prevalently of macrophages but also of vascular smooth muscle cells (VSMC); impaired clearance of necrotic cells, a process called efferocytosis, causes accumulation of inflammatory material that further exacerbates

atherosclerotic lesion eventually leading to its rupture or erosion. Persistent inflammatory stimulus due to continuous accumulation of LDL in the sub-intima coupled with impaired efferocytosis and presence of an inflammatory phenotypes leads to progression of inflammation and atherosclerotic process [19]. In this context, oxidative stress has an important role, as its presence in the plaque is a stimulus for further macrophage accumulation and activation and for the production of oxidant products that perpetuate tissue damage [20].

Human studies in subject affected by genetic modification of ROS producing enzymes such as NADPH oxidase and MPO suggested a potential role for both enzymes in eliciting oxidative stress [21]. Thus, the C242T polymorphism in the gene for the $p22^{phox}$ subunit of NADPH oxidase was evaluated in 237 patients with coronary stenosis during a median follow-up of 7.8 years. The 242T allele was found to be a predictor of lower risk of recurrence of cardiovascular events in high-risk patients and was associated with reduced systemic oxidative stress. Also, patients with genetic deficiency of Nox2, i.e. patients with chronic granulomatous disease, or Nox2 deficiency heterozygosis showed reduced intima-media thickness compared to controls [22]. Similarly, individuals with total or subtotal MPO deficiency or loss-of-function polymorphisms display a reduced rate of CVD [23].

Oxidative stress have been investigated in several settings associated with cardiovascular disease such as type 2 diabetes mellitus (T2DM), hypertension, atrial fibrillation (AF), peripheral artery disease (PAD), obesity, metabolic syndrome (MetS), dyslipidaemia, and smoking/pollution [24]. At this purpose several biomarkers have been studied including analysis of enzyme producing ROS such as Nox2 and MPO or oxidized molecules such as isoprostanes or oxLDL.

2.1. Nox2

Regarding Nox2, we developed an assay to measure its activity in the blood, i.e soluble Nox2-derived peptide (sNox2-dp), which maximally reflects Nox2 activation by leucocytes and platelets and allowed to document an increased activity of the enzyme in several settings associated with enhanced cardiovascular risk. By this assay we found, in fact, over-activation of the enzyme in patients at risk of CVD such as those affected by AF, where Nox2 over-activation was associated directly to increased risk for major adverse cardiac events (MACE) and inversely with Mediterranean diet regimen adherence [25].

In the hypertensive status NADPH oxidase represents the primary source of ROS. Mechanistically, Angiotensin (Ang) II, through angiotensin-1 receptor (AT1R) signalling, mediates the vascular up-regulation and activation of NADPH oxidase. Thus, a study comparing the effect of the angiotensin II inhibitor Irbesartan with diuretic therapy in hypertensive patients demonstrated that Irbesartan-treated subjects presented lower level of O_2^-, a direct product of Nox2 activation [26]. sNox2-dp have been also investigated in patients affected by peripheral PAD, who also showed Nox2 over-activation compared to controls [27].

2.2. Myeloperoxidase (MPO)

MPO is another pro-oxidant enzyme investigated in the setting of cardiovascular disease. Thus, MPO has been found elevated in PAD and coronary artery disease (CAD) patients and independent predictor of vascular outcomes including myocardial infarction and stroke [28–34].

2.3. 8-Iso-prostaglandin F2α

Urinary 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$), which derive from the non-enzymatic oxidation of arachidonic acid, is not only a direct player in the atherosclerotic process but also a validated and accepted reliable biomarker of in vivo oxidative stress, which has been investigated in healthy subjects and patients with cardiovascular diseases [35]. Thus, patients with T2DM have an increased ROS production as documented by enhanced lipid peroxidation and isoprostanes formation [28,29]; of note, change of redox status seems to be influenced by glycaemic profile as urinary 8-iso-$PGF_{2\alpha}$ excretion was lowered after glycemia improvement [30]. Similar data were observed in obese patients who showed increased urinary excretion of 8-iso-$PGF_{2\alpha}$ compared non obese ones and a significant reduction of oxidative stress coincidentally with body-weight reduction [31]. Other clinical settings associated with enhanced urinary excretion of 8-iso-$PGF_{2\alpha}$ include PAD, CAD, MetS and atrial fibrillation (FA) but the intrinsic mechanism accounting for such changes are still unclear [27,36–38].

2.4. oxLDL

Regarding oxLDL, serum levels of this lipoprotein have been found elevated in subjects with or at risk for cardiovascular diseases, such as those with T2DM, obesity, PAD, hypercholesterolemia, MetS and hypertension [29–31]. Almost all these studies reported cross-sectional analysis of ox-LDLs in patients and controls; thereby insufficient data on its predictive value as well as lack of standardized assays limit the use of this biomarker in clinical practice. However, ox-LDLs may have important impact in the thrombogenesis as they behave as pro-aggregating molecules via interacting with specific receptor on platelet surface. Indeed, platelets posses lectin-like oxidized LDL receptor-1 (LOX1) and CD36 that, following the interaction with ox-LDLs induce platelet recruitment by activating the NADPH oxidase pathways with subsequent formation of the pro-aggregating eicosanoids thromboxane A2 and isoprostanes [32]. Noteworthy, once activated, platelets are also able to induce LDL oxidation so inducing a pro-activating loop [32].

2.5. Antioxidants

Finally, some data on antioxidants such as glutathione peroxidase (GPx) corroborated the role of ROS in CVD. Thus, GPx1 activity is either decreased or absent in human carotid atherosclerotic lesions and its absence is associated with lesion severity [33]. Moreover, Freedman et al. found a decreased activity of GPx3 along with an increased platelet reactivity compared to controls [33]. Furthermore, overexpression of GPx4 reduced aortic F2-isoprostane levels, lowered interleukin-1 (IL1)-induced vascular cell adhesion molecule 1 (VCAM-1) expression and delayed lesion progression in experimental animal models [39,40].

The relationship between GPx and cardiovascular events (CVEs) has been investigated in few studies. In a cohort of 636 patients with suspected coronary heart disease (CHD) an inverse relationship between GPx1 activity and risk of CVEs during a follow-up of 4.7 years was reported [41]. Furthermore, a recent prospective cohort study that included 909 AF patients reported a significantly lower value of GPx3 and superoxide dismutase (SOD) in patients experiencing a CVE in a mean follow-up of 43.4 months [42].

3. Vitamin E and thrombosis

Interventional, experimental and in vitro studies showed that vitamin E provides cardiovascular protection acting at several steps of the thrombotic process (Fig. 2). For these studies, the effects of tocopherols, which are the saturated forms of vitamin E, and tocotrienols, the unsaturated form, were tested.

3.1. Vitamin E inhibits the adherence of cells to modified or injured endothelial cells

Several in vitro models consistently showed a positive effect of vitamin E on endothelial cells function. Indeed, the stimulation of human umbilical vein endothelial cells (HUVEC) and human aortic endothelial cells (HAECs) with several agonists including TNF-α, LDL, 25-hydroxycholesterol or ox-LDL increased messenger RNA, surface expression and release of adhesion molecules such as vascular cell

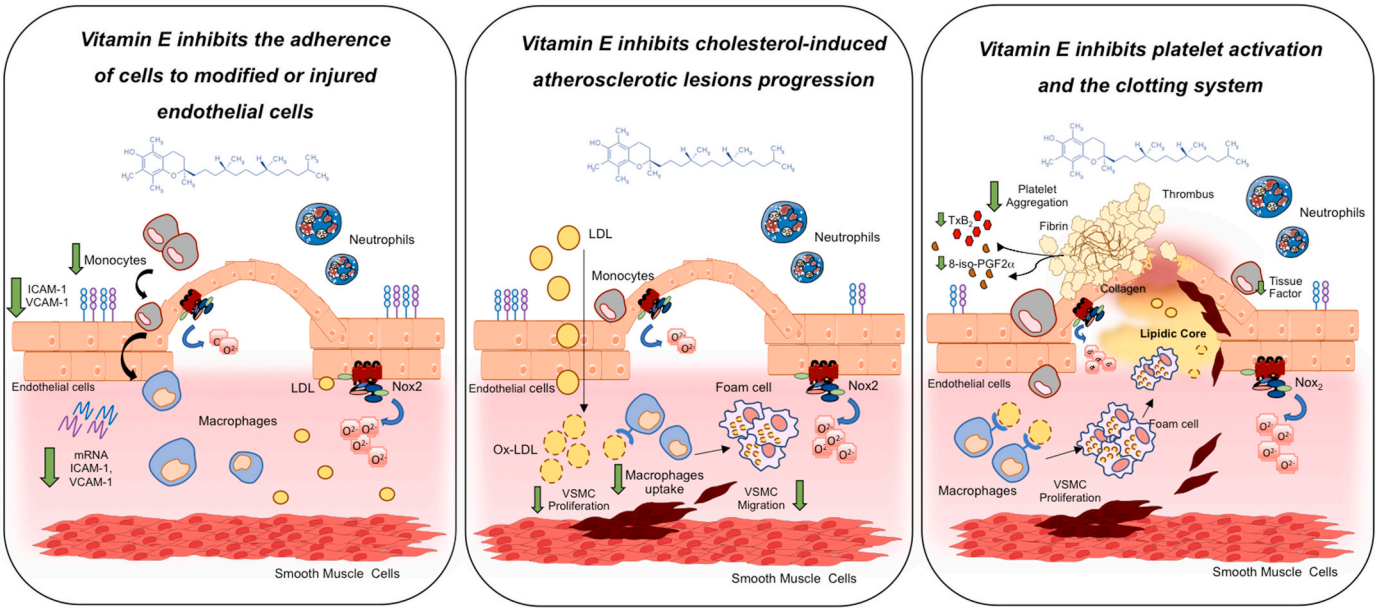

Fig. 2. The antithrombotic effect of Vitamin E.
Vitamin E exerts an antithrombotic effect by acting at different stages of the thrombotic process. **(A)** Vitamin E inhibits the adherence of cells to modified or injured endothelial cells by reducing the expression and the release of adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), intracellular cell adhesion molecule-1 (ICAM-1) and E-selectin, and the adherence of leukocytes to endothelial cells. **(B)** Vitamin E inhibits cholesterol-induced atherosclerotic lesions progression by inhibiting smooth muscle cells (SMCs) proliferation and preventing CD36 induction. **(C)** Vitamin E inhibits agonist-induced aggregation, platelet-leukocytes aggregates and clotting system by reducing monocyte Tissue Factor (TF) and F1+2 prothrombin complex generation.

adhesion molecule-1 (VCAM-1), intracellular cell adhesion molecule-1 (ICAM-1) and E-selectin and increased polymorphonuclear leukocytes (PMN) or mononuclear leukocytes (MNC) cell adherence to endothelium [43–46]. Pre-treatment of endothelial cells with α-tocotrienol (5–25 μM) or α-tocopherol (10–20 μM) yielded a dose-dependent inhibition of leucocyte adhesion on endothelial cells and expression and release of adhesion molecules [43–46]; these inhibitory effects were in part mediated by decreased NF-κB binding activity [43]. In a different in vitro model, vitamin E (0.5–1.0 mM) added to platelet-rich plasma stimulated with thrombin inhibited platelet adhesion to endothelial cells [47].

Together these data indicate that vitamin E impairs endothelium perturbation triggered by inflammatory molecules and lowers its interaction with cells implicated in the atherothrombosis such as monocytes

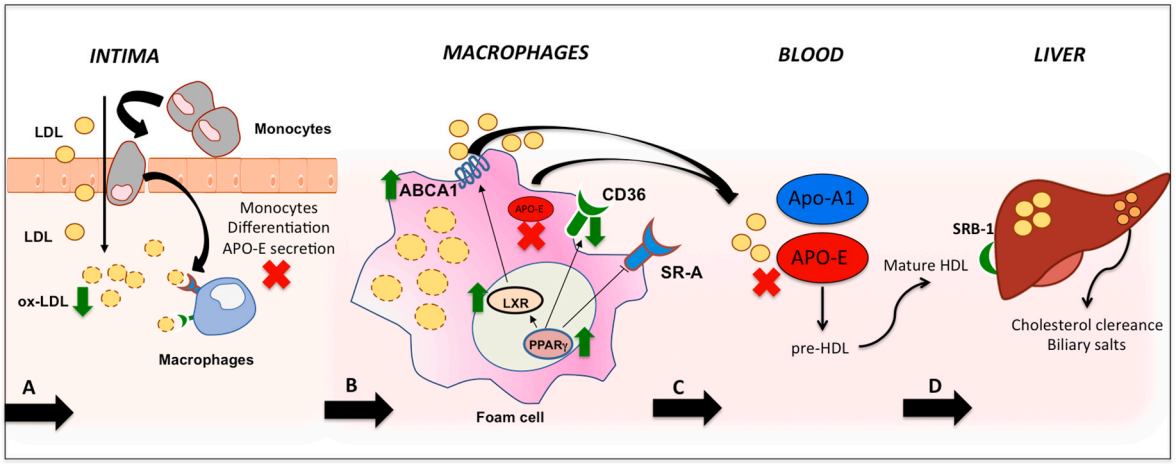

Fig. 3. The Effect of Vitamin E in the reverse cholesterol transport.
(A) In the subendothelial space, LDL is oxidatively modified into oxidized LDL (oxLDL) which causes the attraction of blood monocytes to the subendothelial spaces. The monocytes differentiate into macrophages and subsequently turn into foam cells after taking up oxLDL via scavenger receptors, mainly including scavenger receptor type A (SR-A) and CD36 receptors. Moreover, ApoE is secreted after differentiation of monocytes into macrophages and is associated with the expression of SR-A. (B) A subset of oxidized lipids activates peroxisome proliferator-activated receptors (PPARs), which in turn increase expression of Liver X receptor-α (LXR-α) by macrophages. Free cholesterol is reselase and that is transported out by means of ATP-binding cassette transporter (ABCA1) into pre-HDL particles (C) that contain apoAI or apoE as acceptors. (D) HDL particles are further loaded in the bloodstream with cholesterol to form mature HDL, which in turn delivers its lipid cargo back to the liver through uptake mediated by the scavenger receptor SR-B1. Finally, cholesterol is converted to bile salts in the liver and secreted in this form into the bile. In the absence of apolipoprotein E (apoE) and apoE-containing high-density lipoprotein (apoE-HDL) (X) the cholesterol efflux capacity of HDL is reduced, resulting in cellular lipid accumulation. Vitamin E (↑↓) attenuated this effect reducing oxLDL formation and CD36 expression and stimulating the PPAR–LXR-α–ABCA1 transduction pathway, thereby preventing foam cell formation by the induction of cholesterol efflux from macrophages.

and platelets.

3.2. Vitamin E inhibits cholesterol-induced atherosclerotic lesion progression

Experimental studies consistently showed an anti-atherosclerotic effect on vitamin E. Thus, 50 mg/kg vitamin E added to a 2% cholesterol diet prevented cholesterol-induced atherosclerotic lesions compared to cholesterol-fed rabbits alone [48,49]. Moreover, at cellular levels, vitamin E inhibited PKC activity in smooth muscle cells [48] and prevented CD36 scavenger receptor expression [49]. In Apolipoprotein E knockout (ApoE KO) mice, vitamin E (100 mg/kg) supplementation for 8 weeks significantly reduced the atherosclerotic lesion area by down-regulating mRNA expression of scavenger receptor CD36 and up-regulated mRNA expressions of peroxisome proliferator-activated receptor γ (PPARγ), the liver X receptors (LXRα) and ATP-binding cassette transporter (ABCA1), which are all involved in reverse cholesterol transportation [50] (Fig. 3). In Ldlr (−/−) mice receiving low fat/cholesterol (LFLC) supplemented with vitamin E (500 IU/kg) a lower intima-media thickness (IMT) was detected compared to untreated LFLC mice, coincidentally with the lowest mRNA expression of inflammatory markers such as VCAM-1, MCP-1 and CD36 [51].

In vitro, vitamin E (50-150 μM) inhibited aorta smooth muscle cells (SMCs) proliferation and PKC activity, an effect potentially mediated by down-regulation of CD36 expression and oxLDL uptake and protein kinase B (PKB)/peroxisome proliferator-activated receptor γ (PPARγ) signalling pathway inhibition [52].

3.2.1. Human studies

At variance with experimental and in vitro studies, data in human are equivocal. Thus, in the Vitamin E Atherosclerosis Prevention Study (VEAPS), subjects without clinical signs or symptoms of CVD were randomized to receive DL-α-tocopherol 400 IU/day or placebo every 3 months for an average of 3 years; α-tocopherol supplementation significantly reduced circulating ox-LDLs and LDL oxidation susceptibility. However, vitamin E supplementation did not reduce the progression of intima-media thickness (IMT) over 3-years of follow-up compared to placebo [53]. Same results were obtained in patients with stable coronary artery disease (CAD) included in a randomized, controlled, double-blind trial to assess the effect of RRR-α-tocopherol (1200 IU/d for2 years) supplementation on carotid atherosclerosis, where no significant changes in total carotid IMT were detected [54].

3.3. Vitamin E and experimental thrombosis

In a model of ferric chloride ($FeCl_3$)-induced arterial thrombosis, rats given α- or γ-tocopherol (100 mg/kg/day) for 10 days the time to thrombus was increased with both vitamins (+25% with α-tocopherol and +58% γ-tocopherol) [55]. Moreover, in canine Folts' arterial injury model of stenosis and thrombosis, the intravenous administration (10 mg/kg) of a mixture of α- + γ- + δ-tocotrienols abolished cyclic flow reductions, that is a measure of the acute platelet-mediated thrombus formation [56].

In the above reported studies an increase of endogenous antioxidant activity was also detected, as depicted by decreased arterial superoxide anion generation, lipid peroxidation and LDL oxidation and increased endogenous SOD activity [55].

3.4. Antithrombotic mechanisms of vitamin E

Different studies have been performed to assess if vitamin E exerts an antithrombotic effect by affecting platelet and clotting activation.

Regarding platelet activation, experimental studies provided equivocal results as inhibition of platelet factor 4 and TxB2 or absence of changes of platelet aggregation were reported in animals with genetic hypercholesterolemia given 50 μg α tocotrienol-rich fraction for 6 weeks or animals given an atherogenic diet added with tocotrienol (50 mg/kg) or α-tocopherol (500 mg/kg) for 6 weeks [57–59]. Conversely in vitro studies consistently showed an inhibitory effect of platelet aggregation and TxB2 production with a mechanism involving inhibition of PKC, H_2O_2 and platelet-leucocyte interaction [60].

3.4.1. Human studies

Only few study investigated the antithrombotic effects of Vitamin E supplementation in human. In a double-blinded parallel study, healthy subjects that consumed 100 mg/day of γ-tocopherol displayed reduced platelet aggregation and mean platelet volume [61]. Consistently with a significant effect on platelet reactivity and markers of thrombosis, the supplementation of α-tocopherol (1.200 IU/day) to type 2 diabetic patients significantly lowered plasminogen activator inhibitor-1 (PAI-1) and soluble P-selectin levels [62].

Regarding clotting system Kim et al. showed that vitamin E (0.05 mM–0.1 mM) significantly inhibited phosphatidylserine (PS) externalization and prothrombinase activity on platelet surface; this effect was attributed to increased amino-phospholipid translocase activity (APLT), that transports PS from the outer to the inner membrane leaflet [63]. Moreover, Vitamin E (50 μM) significantly reduced the expression of monocyte tissue factor (TF) antigen and activity as well as F1+2 generation [64]. Conversely, vitamin E (0.25 and 5 mM) had no anticoagulant effect in vitro as evidenced by lack of change of prothrombin time (PT) and thrombin time (TT) [65].

Few human studies evaluated the anticoagulation properties of vitamin E. Vitamin E supplementation (d-α-tocopherol, 670 mg/d) to healthy volunteers for 3 weeks reduced significantly the annexin A5 binding to phosphatidylserine both in non-stimulated and in PMA- and thrombin-stimulated platelets [63]. Ferro et al. explored the effect of vitamin E supplementation on monocyte TF expression and systemic clotting activation in 9 Cirrhotic patients receiving 300 mg vitamin E twice daily + standard treatment or standard treatment alone for 30 days; after vitamin E administration, cirrhotic patients showed significantly reduced monocyte TF antigen and prothrombin fragment F1+2 plasma levels compared to controls [64].

4. Vitamin E and CVD: observational studies

Many population-based cohort studies evaluated the association between systemic and tissue concentrations of vitamin E and the onset of cardiovascular events in general population or in patients with previous cardiovascular diseases (Table 1).

First data supporting the hypothesis that vitamin E was protective against cardiovascular disease (CVD) came from the MONICA (Multinational MONitoring of trends and determinants in CArdiovascular disease) studies. Specifically, in 12 study populations analyzed, cholesterol- and triglycerides-standardized plasma α-tocopherol have a strong inverse association with cardiovascular mortality [66]. A significant inverse association between plasma antioxidant levels and CVD was also found in a population of 6.000 men, where vitamin E/cholesterol molar ratio was lower in patients with angina than in controls [67].

Serum levels of vitamin E were analyzed in 1012 patients with non-valvular AF showing that patients with vitamin E levels below the median values (4.2 μmol/mmol cholesterol) had an increased risk of cardiovascular events after 2 years follow up [68]. Also, Espe et al. demonstrated that in 1046 diabetic patients on haemodialysis the lowest α-tocopherol quartile was associated with a 79% higher risk of stroke and a 31% higher risk of all-cause mortality compared with patients in the highest quartile after 4 years follow up; there was no association between a-tocopherol, MI and sudden death [69].

Conversely, a non-significant association between α-tocopherol and CVD was observed when vitamin E was assessed at tissue level; thus, analysis of vitamin E in adipose-tissue samples from people with acute MI and controls found no relationship between α-tocopherol concentration with the risk of MI [70].

Table 1
Vitamin E and Observational studies.

OBSERVATIONAL STUDIES			
Author/(year)/[reference]	Study type (setting)	Outcome(s)/variable of interest	Main results
MONICA projects (1989) [66]	Comparative study *General population* n=14962 age=middle-aged	IHD mortality	Alpha-tocopherol was inversely associated with the IHD mortality (r2=0.49; p=0.01)
Riemersma et al. (1989) [67]	Comparative study *General population* n= 6000 sex= male age= 35–54 years	CHD incidence	Vitamin E/cholesterol molar ratio was lower in angina than in controls (1.58 ± 0.03 vs. 1.66 ± 0.02, p<0.01)
Cangemi et al. (2013) [68]	Prospective study *Non-valvular AF* n= 1012 sex= 556 male, 455 female age=73.0 ± 8.4	18 fatal MI 14 nonfatal MI 13 fatal ischemic strokes 19 nonfatal ischemic strokes 45 cardiovascular deaths	Lower vitamin E serum levels were found in patients who experienced cardiovascular events compared to those who did not.
Espe et al. (2001) [69]	Prospective study *Diabetic haemodialysis patients* n=1046 sex= 549 male, 497 female age=66 ± 8 years	134 sudden death 172 MI 89 stroke 398 combined cardiovascular events 107 fatal infection 508 and all-cause mortality	Patients in the lowest alpha -tocopherol quartile had a 79% higher risk of stroke and a 31% higher risk of all-cause mortality compared with patients in the highest quartile.
EURAMIC study (1993) [70]	Multicentre case-control study *Patients with acute MI* n=683	MI	Mean alpha-tocopherol concentrations were similar between cases and controls. A low alpha-tocopherol concentration was not associated with risk of myocardial infarction.

Abbreviations: AF= atrial fibrillation; CHD= Coronary heart disease; EURAMIC= European Community Multicenter Study on Antioxidants, Myocardial Infarction, and Cancer; IHD= Ischemic heart disease; MI= myocardial infarction; MONICA= Monitoring trends and determinants in cardiovascular disease.

Observational studies were also conducted to investigate the relationship between CVDs, cardiovascular mortality and the dietary consumption of vitamin E (Table 2).

Dietary vitamin E was reported to be significantly and inversely related to coronary mortality in 5133 men and women aged during a follow-up of 14 years [71]. The Scottish Heart Study found that the risk of undiagnosed coronary heart disease was significantly lower in the highest quintile of dietary vitamin E intake in 10359 men and women [72].

The Nurses' Health Study, a large prospective cohort study including 87245 female nurses, investigated if vitamin E was a protective nutrient against coronary disease. Dietary consumption of several nutrients including vitamin E was obtained by food frequency questionnaires at baseline and updated biennially up to eight years. Women in the highest quintile of vitamin E intake had a reduced relative risk of major coronary disease compared to women in the lowest quintile of the cohort [73]. This protective effect was also found in the Health Professionals Follow-up Study, a prospective investigation of 39.910 male health professionals. Thus, compared with men in the lowest quintile for vitamin E intake, men in the highest quintile had reduced age-adjusted relative risk of coronary disease after 4 years of follow-up [74]. At variance with these findings, the Rotterdam study, which was conducted in 4802 participants free of MI at baseline showed no association between dietary vitamin E intake and MI after 4 years of follow-up [75].

Table 2
Vitamin E and Observational studies: results from dietary and supplement vitamin E intake.

OBSERVATIONAL STUDIES			
Dietary Vitamin E intake			
Author/(year)/[reference]	Study type (setting)	Outcome (s)/variable of interest	Main results
Knekt et al. (1994) [71]	Longitudinal study *General population* n=5133 sex=male and female age=30–39 years	244 fatal CHD	An inverse association was observed between the highest and lowest tertiles of the vitamin E intake and coronary mortality in men (R. R.: 0.68; *p* for trend = 0.01) and women (R. R.: 0.35; *p* for trend = 0.01)
The Scottish Heart Health Study (1992) [72]	Prospective study *General population* n= 10359 sex=male and female age=40–59 years	CHD	The risk of undiagnosed coronary heart disease was significantly lower in the highest quintile of dietary vitamin E intake
Nurses' Health Study (1993) [73]	Prospective cohort study *General population* n=87245 sex=female age=34–59 years	437 non fatal MI 115 coronary diseases death	A statistically significant reduction in the risk of major coronary disease among women with a high intake of vitamin E, as compared with those with a low intake was observed
The Health Professionals Follow-up Study (1993) [74]	Prospective cohort study *General population* n=39910 sex=male age=40–75 years	667 Major coronary disease	The men in the lowest quintile group for vitamin E intake, the men in the highest quintile group had a reduced age-adjusted relative risk
The Rotterdam study (1999) [75]	Prospective Cohort study *General population* n=4802 participants age= 55–95 years	124 fatal or nonfatal MI	No association with risk of MI was observed for vitamin E
Supplement Vitamin E intake			
The Established Populations for Epidemiologic Studies of the Elderly (1996) [77]	Cohort study *General population* n=11178 sex=men and women age=67–105 years	3490 Coronary disease mortality	The use of vitamin E reduced the risk of all-cause mortality and risk of coronary disease mortality
Meyer et al. (1996) [76]	Cohort study *General population* n=2313 sex=men	IHD	Vitamin supplement use was consistently associated with a lower incidence IHD death, MI, angina and first IHD event.

Abbreviations: CHD= coronary heart disease; IHD= Ischemic heart disease; MI= myocardial infarction.

Another group of observational studies investigated if the spontaneous use of supplements affected CVDs (Table 2).

In a cohort study conducted in 2313 men, baseline information on vitamin supplement use and ischemic heart disease (IHD) risk factors was provided. Vitamin E supplement was consistently associated with a

lower incidence of IHD death, MI, angina and IHD event after five years of follow-up [76].

In the Established Populations for Epidemiologic Studies of the Elderly including 11178 persons the use of vitamin E, alone or in combination with Vitamin C, reduced the risk of all-cause mortality and risk of cardiovascular mortality [77].

5. Vitamin E and CVD: interventional studies

Several clinical trials were conducted to assess the association between Vitamin E intake and CVD. The studies have been conducted in the general population (Table 3) or in patients with previous cardiovascular disease (Table 4) and vitamin E was given alone or in association with other antioxidant molecules, including in particular Vitamin C, or n-3 PUFA.

5.1. Primary prevention

The Women's Health Study (WHS) is a randomized, double-blind, placebo-controlled trial including 39815 female health professionals randomized to receive vitamin E, aspirin, both agents, or placebo. The results indicated that vitamin E taken every other day provided no overall benefit for the incidences of MI or stroke in healthy women. However, a significant 24% reduction for cardiovascular death was found [78]. In a subsequent analysis on the same study population, the effect of vitamin E was evaluated on heart failure (HF) risk but no changes were detected [79]. Finally, the WHS trial investigated the effect of vitamin E in a group of diabetic patients at higher risk of CVD such as those with haptoglobin (Hp) genotype [80] showing a protective effect versus total CVD and a non-significant effect in total mortality [81].

Moreover, the Primary Prevention Project (PPP) investigated the efficacy of vitamin E supplementation in people with one or more major cardiovascular risk factors showing no effect of vitamin E supplementation in the cumulative rate of cardiovascular death, nonfatal MI, and non-fatal stroke [82].

The ATBC Study is a randomized, double-blind, placebo-controlled trial undertaken to determine the effect of supplementation in 27271

Table 3
Vitamin E and Interventional studies in primary prevention.

INTERVENTION STUDIES

PRIMARY PREVENTION

Author/(year)/[reference]	Study type (setting)	Type of intervention/doses	Follow-up	Outcome(s)/variable of interest	Main results
The Women's Health Study (2005) [78]	Randomized, double-blind, placebo-controlled trial *General population* n=39815 healthy subjects sex= female age= mean 54.6 years	Vitamin E (600 IU/day).	10.1 years	999 major cardiovascular events	A significant effects was found on the incidences of MI (RR:1.01; 95% CI, 0.82-1.23; p=0.96) or stroke (RR:0.98; 95% CI, 0.82-1.17; p=0.82), as well as ischemic or haemorrhagic stroke. For cardiovascular death, there was a significant 24% reduction (RR: 0.76; 95% CI, 0.59-0.98; p=0.03).
The Women's Health Study (2012) [79]	Randomized, double-blind, placebo-controlled trial *General population* n=39815 healthy subjects sex=female age= mean 54.6 years	Vitamin E (600 IU/day).	10.2 years	220 incident HF events	Vitamin E supplementation did not significantly affect HF risk (HR:0.93; 95% CI, 0.71–1.21; p=0.59).
Levy et al. (2004) [81]	Controlled, randomized, open-label clinical trial *General population* n=721 diabetic individuals with different HP phenotypes	Vitamin E (600 IU/day)	10.1 years	151 total CVD comprising non fatal MI, non fatal stroke, cardiovascular death, PTCA or CABG.	Vitamin E supplementation was associated with 15% reduction in total CVD with a non-significant reduction in total mortality in Hp 2-2 DM individuals (HR: 0.92, 95% CI 0.73-2.6).
The PPP study (2001) [82]	Controlled, randomized, open-label clinical trial *General population* n=4495 subjects with major cardiovascular risk factors. sex= 2583 female, 1912 male age= mean 64.4 years	α-tocopherol (capsules, 300 mg/day)	3.6 years	Cardiovascular death non-fatal MI non-fatal stroke	Vitamin E showed no effect on any endpoint
The ATBC study (1998) [83]	Randomized, double-blind, placebo-controlled trial *General population* n=27271 smokers sex= male age=50–69 years	α-tocopherol (capsules, 50 mg/day) vs α-tocopherol E (50 mg/d) vs α-tocopherol (50 mg/d, plus beta carotene, 20 mg/d) vs beta carotene (20 mg/d) vs or placebo	5–8 years	1204 nonfatal MI 907 fatal CHD	The incidence of primary major coronary events decreased 4% (95% CI, −12%–4%) and the incidence of fatal CHD by 8% (95% CI, −19%–5%) without affecting the incidence of nonfatal myocardial infarction
The Physicians' Health Study II (2008) [84]	Randomized, double-blind, placebo-controlled factorial trial *General population* n= 14.641 physicians sex=male age= 50 years or older	Vitamin E 400 IU every other day and Vitamin C 500 mg daily	8 years	1245 major cardiovascular events	Neither vitamin E nor vitamin C had a significant effect on major cardiovascular events, total mortality total MI, total stroke cardiovascular mortality. Vitamin E was associated with an increased risk of haemorrhagic stroke

Abbreviations: ATBC= The Alpha-Tocopherol, Beta-Carotene Cancer Prevention; CABG= Coronary Artery Bypass Graft surgery; CHD= coronary heart disease DM=diabetes mellitus; HF= heart failure; HP= Haptoglobin; MI= myocardial infarction; PPP= The Primary Prevention Project; PTCA= percutaneous transluminal coronary angioplasty.

Table 4
Vitamin E and Interventional studies in secondary prevention.

INTERVENTIONAL STUDIES

SECONDARY PREVENTION

Author/(year)/ [reference]	Study type (setting)	Type of intervention/doses	Follow-up	Outcome(s)/variable of interest	Main results
CHAOS study (1996) [85]	Double-blind, placebo-controlled study *Patients with coronary atherosclerosis* n=2002 patients	α-tocopherol (capsules, 800 IU/day) vs placebo	1.4 years	Cardiovascular death and non-fatal MI	α–tocopherol treatment significantly reduced the risk of cardiovascular death and non-fatal MI (R.R.: 0.53, Cl 95% 0.34-0.83; p=<0.005).
SPACE study (2000) [86]	Double-blind, placebo-controlled study *Haemodialysis patients with pre-existing CVD* n=196 patients	α-tocopherol (capsules, 800 IU/day) vs placebo	1.4 years	48 primary endpoints (MI, ischaemic stroke, peripheral vascular disease, and UA). 22 MI 11 incidents peripheral vascular disease events 6 cases of UA 11 cases ischaemic stroke	Vitamin E supplementation reduced primary endpoint (R.R.: 0.46, 95% CI 0.27–0.78, p=0·014) and MI (R.R:. 0.3, 95% CI 0.11–0.78, p=0·016) without affecting and cardiovascular mortality.
HOPE Study (2000) [87]	Double-blind, randomized trial *Patients with CVD* n=9541 patients sex= 2545 female and 6996 male age= 55 years or older	α-tocopherol (400 IU/day) vs placebo	4–6 years	1511 MI or stroke or cardiovascular death 670 cardiovascular causes 1056 MI 389 strokes	There were no significant differences in the numbers of deaths from cardiovascular causes (R. R.: 1.05; 95% CI, 0.90 to 1.22), MI (R.R.: 1.02; 95% CI, 0.90 to 1.15), or stroke (R.R.: 1.17; 95% CI, 0.95 to 1.42) among patients taking Vitamin E compared to placebo.
HOPE Study (2002) [109]	Double-blind, randomized trial *Patients with CVD or diabetes with at least one additional coronary risk factor.* n=3654 patients sex= 1358 female, 2296 male age= 55 years or older	α-tocopherol (400 IU/day) vs placebo	4.5 years	The primary outcome: the composite of MI, stroke, or CV death. Secondary outcomes: total mortality, hospitalizations for HF and UA, revascularizations, and overt nephropathy.	Vitamin E had a neutral effect on the primary study outcome (RR: 1.03, 95% CI 0.88–1.21; p=0.70), on each component of the composite primary outcome, and on all predefined secondary outcomes
HOPE-TOO Study (2005) [88]	Double-blind, randomized trial *Patients with CVD* n= 7030 patients sex=1823 female, 5207 male	α-tocopherol (400 IU/day) vs placebo	2.6 years	Composite of MI stroke death from CV causes	There were no significant differences for major cardiovascular events (R.R.: 1.04; 95% CI, 0.96-1.14; p=0.34). Patients in the vitamin E group had a higher risk of heart failure (R.R.: 1.13; 95% CI, 1.01-1.26; p=0.03) and hospitalization for heart failure (R.R.: 1.21; 95% CI, 1.00-1.47; p=0.045).
The GISSI-Prevenzione study (1999) [89]	Randomized trial *Patients with MI* n= 11324 patients sex= 1665 female, 9659 male	α-tocopherol (capsules, 300 mg/day) vs n-3 PUFA vs α-tocopherol + n-3 PUFA vs placebo	3.5 years	Death non-fatal myocardial infarction, stroke	Vitamin E had no statistically significant benefit.
WAVE Trial (2002) [90]	Randomized, double-blind, placebo-controlled trial *Postmenopausal women with coronary stenosis* n=423 patients sex=female age= mean 65 years	Vitamin E (400 IU) + Vitamin C (500 mg) vs placebo vs HRT	2.8 years	Coronary lesions Non-fatal MI Stroke	Vitamin treatment was associated with a non-significant difference in minimum MLD. All-cause mortality was higher in woman assigned to vitamins supplementation compared to placebo (H.R.: 2.8, 95% CI, 1.1-7.2, p=0.047).
The Heart Protection Study (2002) [91]	Randomized, double-blind, placebo-controlled trial *Patients with coronary disease*	Vitamin E (600 mg) + Vitamin C (250) mg + β-carotene (20 mg) daily vs placebo	5 years	Major coronary events Fatal or non-fatal vascular events	No significant differences in non-fatal MI or coronary death (R.R.: 1.02; 95% CI 0.93–1.11), non-fatal or fatal stroke (R.R.: 0.99; 95% CI 0.87–1.12), or coronary or non-coronary revascularisation (R.R.: 0.98; 95% CI 0.90–1.06) were found.

(*continued on next page*)

Table 4 (*continued*)

INTERVENTIONAL STUDIES					
SECONDARY PREVENTION					
Author/(year)/ [reference]	Study type (setting)	Type of intervention/doses	Follow-up	Outcome(s)/variable of interest	Main results
	or other occlusive arterial disease, n= 20536 patients sex= 5082 female, 15454 male age= 40–80 years				

Abbreviations: CHAOS= Cambridge Heart Antioxidant Study; DM=diabetes mellitus; GISSI= Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardio; HF= heart failure; HOPE= The Heart Outcomes Prevention Evaluation; HOPE_TOO= The HOPE-The Ongoing Outcomes; MI= myocardial infarction; HR= hazard ratio. MLD=minimum lumen diameter; RR= relative risk; SPACE= Secondary Prevention with Antioxidants of Cardiovascular Disease in Endstage Renal Disease; UA= Unstable angina; WAVE= The Women's Angiograpic Vitamin and Estrogen.

male smokers with no history of MI assigned to receive daily vitamin E, β-carotene, both agents, or placebo. Among vitamin E recipients the incidence of primary major coronary events decreased by 4% and the incidence of fatal coronary heart disease by 8% without affecting the incidence of nonfatal MI [83].

The Physicians' Health Study II was a double-blind, placebo-controlled trial including 14641 US male physicians at low initial risk of cardiovascular disease, who were randomized to receive supplements of 400 IU of vitamin E every other day or 500 mg of vitamin C daily alone or a combination of both. Compared with placebo, no effect on the incidence of major cardiovascular events was found for vitamin E and C; in particular neither vitamin E or vitamin C had a significant effect on mortality, MI, stroke and cardiovascular mortality; of note, vitamin E was associated with an increased risk of haemorrhagic stroke [84].

5.2. Secondary prevention

The Cambridge Heart Antioxidant Study (CHAOS) tested the hypothesis that the treatment with a high dose of α-tocopherol would reduce the risk of MI in patients with coronary atherosclerosis. In this double-blind, placebo-controlled study, 2002 patients with angiographically proven coronary atherosclerosis were randomized to α–tocopherol or placebo. Results showed that α–tocopherol treatment significantly reduced the risk of cardiovascular death and nonfatal MI [85]. A positive effect of a high dose of α-tocopherol was later confirmed by the secondary prevention with antioxidants of cardiovascular disease in end-stage renal disease (SPACE) study. In this double-blind, placebo-controlled study, 196 haemodialysis patients with pre-existing cardiovascular disease were randomized to receive α–tocopherol or matching placebo. Vitamin E supplementation reduced composite cardiovascular disease endpoints including MI (fatal and non-fatal), ischaemic stroke, peripheral vascular disease and unstable angina without affecting mortality, and cardiovascular-disease mortality [86].

These findings were not supported by the Heart Outcomes Prevention Evaluation (HOPE) Study, which was a double-blind, randomized trial conducted to evaluate the effects of vitamin E in patients at high risk for cardiovascular events versus placebo; the study found no significant differences of cardiovascular death, MI, or stroke among patients taking vitamin E compared to placebo [87]. Similar findings were reported in a sub-group analysis performed in patients with diabetes and in the HOPE–The Ongoing Outcomes (HOPE-TOO) performed in 3994 participants who continued to take daily vitamin E (400 IU) or matching placebo over 7 years [88].

The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI)-Prevenzione trial investigated the effect of vitamin E on morbidity and mortality after MI. 11.324 patients, surviving recent MI, were randomly assigned supplements of n-3 PUFA, vitamin E, both or none. No significant effects were observed after Vitamin E alone or in combination with n-3 PUFA [89].

The Women's Angiographic Vitamin and Estrogen (WAVE) Trial is a double-blind trial including 423 postmenopausal women with coronary stenosis (15%–75%). Participants were randomized to receive vitamin E and vitamin C (400 IU and 500 mg twice daily respectively) or placebo with or without hormone replacement therapies (HRT). Vitamin treatment was associated with a non-significant difference in minimum lumen diameter (MLD) of qualifying coronary lesions. Moreover, all-cause mortality was higher in woman assigned to vitamins supplementation compared to placebo [90].

Any significant benefit was also observed in the Heart Protection Study, a randomized study comparing antioxidant vitamins versus matching placebo in 20536 participants with coronary disease, other occlusive arterial diseases, or diabetes. Participants were randomly allocated to receive antioxidant vitamin supplementation (600 mg vitamin E, 250 mg vitamin C, and 20 mg β-carotene daily) or a matching placebo. No significant differences in the numbers of participants having non-fatal MI or coronary death, non-fatal or fatal stroke, or coronary or non-coronary revascularisation were found between vitamins-vs placebo-allocated participants [91].

6. Vitamin E and CVE: results from metanalyses

Over the past few decades, numerous metanalysis analyzed the relationship between vitamin E and cardiovascular events using the results of interventional randomized controlled trials (RCT) that analyzed the effect of vitamin E on the incidence of cardiovascular death, myocardial infarction, total death and stroke.

To better explore the results of these metanalyses we performed a research on pubmed about on this topic. The last search was run on July 16th, 2021. No language, publication date, or publication status restrictions were imposed. One investigator (L.L.) independently searched in the electronic database combining the following text terms and MeSH terms:

("Vitamin E" [Mesh]AND (("cardiovascular system"[MeSH Terms] OR cardiovascular[Text Word]) AND events[All Fields]) OR "myocardial infarction"[All Fields] OR "stroke"[All Fields]) AND "Vitamin E/therapeutic use". We limited our search to humans and to metanalyses.

One authors (L.L.) independently reviewed titles, abstracts of the metanalyses generated by search. Meta-analyses were excluded if the title and/or abstract showed that the papers did not meet the selection criteria of our meta-analysis. For potentially eligible metanalyses we procured the full text. Meta-analyses not including a control group or that exclusively reported clinical outcomes other than cardiovascular

were excluded.

From seventy-nine articles identified by the initial research, 12 metanalyses [92–103] met the inclusion criteria (Table 5).

6.1. All cause mortality

Seven [92,93,96,98,100–102] out of 12 metanalyses analyzed all cause mortality in patients treated with Vitamin E (Table 5). Some meta-analyses found no beneficial effect of vitamin E [92,93,98,100, 101], others showed increased mortality [96,102]. In particular, Bjelakovic et al. [96] showed that vitamin E singly given versus placebo (20 trials, 58,904 participants) had no significant effect on total mortality (RR 1.02, 95% C.I.: 0.98-1.05) (Table 5); conversely, when vitamin E was given alone or in combination with other antioxidants versus placebo (46 trials, 171,244 participants) or with doses above the recommended daily allowance (RDA, $>$ 15 mg) the mortality significantly increased (Table 5). Miller also found that only high-dosage of vitamin E supplementation (vitamin E $\geq$400 IU/d) increased all-cause mortality [102] (Table 5).

6.2. Cardiovascular death

Six out of 12 metanalyses analyzed the incidence of cardiovascular death in patients treated with vitamin E (Table 2). All of these meta-analyses found no differences between patients treated with vitamin E versus those treated with placebo (Table 5).

6.3. Myocardial infarction

Six [92,93,100,101,103,104] out of 12 metanalyses analyzed the incidence of myocardial infarction in patients treated with vitamin E (Table 5). The first meta-analyses, in chronological order, did not show a reduction in myocardial infarction unlike those published more recently that found some positive results.

Alkhenizan et al. [101] found a significant reduction of non-fatal myocardial infarction in secondary prevention but not in primary prevention; furthermore, no effect in total myocardial infarction or fatal myocardial infarction was observed (Table 5). Also, Myung et al. [104] and Loffredo et al. [94] found a significant reduction of myocardial infarction (Table 5). In particular the metanalysis by Loffredo et al. showed that vitamin E supplementation alone significantly decreased myocardial infarction (random effects R.R.: 0.82; 95% C.I.: 0.70-0.96; p=0.01), an effect driven by reduction of fatal myocardial infarction (random effects R.R.: 0.84; 95% C.I.: 0.73-0.96; p=0.01). This different finding has been suggested to depend on confounding factors related to the combination vitamin E with other antioxidants [94]. Thus, the association of more antioxidants or vitamins derived from the assumption that a mixture of antioxidants could exert synergistically act activity more than a single antioxidant [105]; however, this hypothesis has never been supported by any experimental study [105]. Conversely, an association of more molecules with different antioxidant properties can negatively affect the activity of a single antioxidant and offset its potentially beneficial effect due to deleterious side effects. For example, vitamin C has been shown to exert a pro-oxidant activity when administered “orally” to humans [105,106]; therefore, it cannot be excluded that this “paradoxical” effect has limited the antioxidant property of vitamin E alone. Beta-carotene supplementation is associated with increased mortality, which could counteract, also in this case, the potential beneficial effects of vitamin E [102].

6.4. Stroke

Six [92,95,99–101,103] out of 12 metanalyses analyzed the incidence of stroke in patients treated with vitamin E (Table 5). The first metanalysis [92,99–101,103] did not find any effect of vitamin E supplementation on the incidence of stroke (Table 5). Other two meta-analyses by Schurks [99] by Loh et al. [95] showed a modest decrease of ischemic stroke but also an increase, even if modest, of haemorrhagic stroke, which reinforces the suggestion that vitamin E supplementation has a neutral effect on total stroke (Table 5).

7. Caveats of interventional trials

There is a consistent body of experimental studies suggesting that Vitamin E acts at different stage of atherosclerosis disease including initiation and progression of atherosclerotic plaque where it may prevent endothelial perturbation and inflammation via impairment of ox-LDL accumulation within the arterial wall **(**see Fig. 2**).** Also, studies in vitro and in animals are consistent with an antithrombotic effect via directly quenching ROS or inhibiting PKC or down-regulating TF regulation so lowering thrombin generation and eventually thrombus growth **(**see Fig. 2**).** However, it is still uncertain if these beneficial data can be recapitulated in human beings to suggest a potential usefulness of vitamin E in preventing CVD. RCT and meta-analyses consistently showed an absence of beneficial effects in terms of reduction of MI, stroke or cardiovascular death. It is possible that, as suggested by our group, many studies have been performed with a combination of several antioxidant supplements, which could have overshadowed or counteracted the beneficial effect of vitamin E but this hypothesis must be confirmed by an “ad hoc” study. Another caveat of the RCT regards the absence of any baseline values of vitamin E, which was likely mandatory for vitamin E supplementation. Thus, observational studies have consistently suggested that low circulating levels of vitamin E may be associated with poor vascular outcomes, thereby it could be conceivable that patients needing vitamin E were likely to be better candidates for vitamin E supplementation in primary as well as secondary prevention trials. However, this issue has not been considered so that a potential bias cannot be excluded as supraphysiologic concentrations of vitamin E may be paradoxically pro-oxidant and worsen clinical outcomes as, for example, observed in some cases where high doses of vitamin E were associated with increased mortality. Furthermore, RCT did not take into account data some crucial pharmacodynamic aspects related to vitamin E intake, which are significantly conditioned by the typology of its assumption. Thus, we have shown that plasma concentration of vitamin E is closely dependent on whether it is assumed or not with meals, observing more elevated concentration and, thereby, more antioxidant effect in case of vitamin intake with meals [107]. Finally, it has been suggested that the limited follow-up of RCT with vitamin E did not allow to observe its potential beneficial effects [108] but this hypothesis can be questioned on the light of the fact that other anti-atherosclerotic drugs such as statins provided positive results with similar follow-ups. Given these methodological caveats, we believe that the interventional trials with vitamin E should not be considered negative but, conversely, inconclusive and that further study with a more adequate methodology must be performed to assess the validity of vitamin E supplementation in patients with or at risk of cardiovascular disease.

8. Conclusions

Even if the interventional trials with Vitamin E provided inconclusive results, the data so far reported did not reject the hypothesis that vitamin E or other antioxidants may prevent atherosclerosis and its complications. Among the caveats of the interventional trials above reported, vitamin E dosage and the population who should be candidate for the vitamin E supplementation, i.e. patients with low vitamin levels and, hence, low antioxidant status, are two important issues. We are aware that lack of standardisation for both variables makes difficult an appropriate analysis of this issue but future study should be addressed to solve it. A consequence of this argument regards Vitamin E dosage (low or high doses) to improve the antioxidant status, pharmacokinetic and, in this context, pharmacodynamics studies should be performed to assess the vitamin E quantity necessary to elicit such effect. Until these

Table 5
Vitamin E and metanalyses.

Author/(year)/ [reference]	Patients (n)	n. RCT	Included Studies	Treatment	All-cause mortality R.R. (95% C.I.)	Cardiovascular death R.R. (95% C.I.)	Myocardial infarction R.R. (95% C. I.)	Stroke R.R. (95% C.I.)	Conclusion
Vivekananthan (2003) [92]	81 788	7	ATBC, CHAOS, GISSI, HOPE, HPS, AREDS	Vitamin E vs control	1.02 (0.98–1.06)	1.0 (0.94–1.06)	–	1.02 (0.92–1.12)	No effect of Vitamin E to prevent the events.
Shekelle (2004) [93]	–	8	HATS, ATBC, GISSI HPS, CHAOS, HOPE, SPACE, Haeger, HATS	Vitamin E vs control	0.96 (0.84–1.10)	1.03 (0.81–1.32)	Fatal 0.97 (0.74–1.27) Non fatal: 0.99 (0.89–1.10)	–	No effect of Vitamin E to prevent the events.
Eidelman (2004) [103]	106625	7	Linxian, ATBC, CHAOS, GISSI, HOPE, PPP, HPS	Vitamin E vs control	–	1.00 (0.94-1.05).	Nonfatal myocardial infarction 1.00 (0.92–1.09)	Nonfatal stroke 1.03 (0.93–1.14)	No effect of Vitamin E to prevent cardio-vascular events.
Miller (2005) [102]	135 967	19	ADCS; AREDS; ATBC; CHAOS; DATATOP; GISSI; HOPE; MIN.VIT.AOX; HPS; PPP; PPS; REACT; SPACE; SU.VI.MAX; VECAT; WAVE	Vitamin E vs control	1.1 (0.98–1.04) In 11 studies with high-dosage vitamin E: ***1.04 (1.01 to 1.07) p=0.035***	–	–	–	High-dosage vitamin E supplementation (≥400 IU/d for at least 1 year) increased all-cause mortality
Alkhenizan (2004) [101]	80 645	9	ATBC, HPS, CHAOS, GISSI, HOPE, LI, PPP, SPACE, Steiner	Vitamin E vs control	1.00 (0.94–1.06)	0.97 (0.89–1.06)	Total myocardial infarction: 0.95 (0.86 - 1.06) **Non-fatal myocardial infarction *0.51 (0.38–0.70)***	1.06 (0.94–1.19)	Significant reduction in non-fatal myocardial infarction in patients with pre-existing coronary artery disease.
Kraemer (2004) [100]	83800	19	ADCS, AREDS, ASAP, ATBC, CHAOS, GISSI, HATS, HOPE, HPS, PPP, SPACE, TAA, VEAPS, WAVE	Vitamin E vs control	1.01 (0.899–1.141)	1.03 (0.89–1.19)	0.93 (0.87–1.05)	1.15 (0.94–1.41)	No effect of Vitamin E to prevent the events.
Schürks (2010) [110]	118 765	7	GISSI, HOPE, ATBC, PPP, WHS, WACS, PHS	Vitamin E vs control	–	–	–	Total stroke 0.98 (95% 0.91-1.05). ***Haemorrhagic stroke 1.22 (1.00-1.48), p=0.04, Ischemic stroke:0.90 (0.82-0.99), p=0.02).***	Vitamin E increased the risk for haemorrhagic stroke by 22% and reduced the risk of ischaemic stroke by 10%.
Abner (2011) [98]	246,371	59	*	Vitamin E vs control	1.00 (0.98, 1.02);	–	–		Supplementation with vitamin E appears to have no effect on all-cause mortality
Myung (2013) [97]	–	11	CHAOS, ATBC, HOPE, SPACE, HATS, PPP, HPS, WAVE, WHS, ICARE, PHS2	Vitamin E vs control	–	0.98 (0.92–1.04)	***0.77 (0.65–0.91)***	–	Vitamin E supplementation decreased risk of myocardial infarction.
Bjelakovic (2013) [96]	171,244	46	*	Vitamin E vs control	Vitamin E singly vs placebo:1.02, (0.98-1.05) ***Vitamin E used singly or in combination with other antioxidants versus placebo: 1.03 (1.00-1.05).***	–	–	–	Vitamin E singly or in combination with other antioxidants or with dose above the RDA (>15 mg) significantly increased mortality

(*continued on next page*)

Table 5 (*continued*)

Author/(year)/ [reference]	Patients (n)	n. RCT	Included Studies	Treatment	All-cause mortality R.R. (95% C.I.)	Cardiovascular death R.R. (95% C.I.)	Myocardial infarction R.R. (95% C. I.)	Stroke R.R. (95% C.I.)	Conclusion
					Vitamin E in a dose above the RDA (> 15 mg) versus placebo: 1.03 (1.00-1.05)				
Loffredo [94] (2015)	140491	16	CHAOS, GISSI, Milman, PPP, SPACE, WHS, POPADAD, ATBC, HATS, HOPE, HPS,	Vitamin E vs control	–	–	***Myocardial infarction 0.82 (0.70-0.96) p= 0.01). Fatal myocardial infarction: 0.84 (0.73-0.96); p=0.01.***	–	When supplemented alone, vitamin E reduces myocardial infarction in interventional trials while it appears ineffective when associated with other antioxidants.
Loh [95] (2021)	148 016	18	ATBC, WHS, TACT, VEAPS, HPS, GISSI, WACS, PPP, Linxian, Milman, PHS II, Steiner, Sesso, CHAOS, HOPE, CLIPS, HATS, SPACE, POPADAD.	Vitamin E vs control	–	–	–	Total stroke: 0.98 (0.92–1.04) ***Total ischaemic stroke: 0.92 (0.85–0.99; p=0.04)*** Total haemorrhagic Stroke: 1.17; (0.98–1.39; p=0.08)	Vitamin E may offer some benefits in the prevention of ischaemic stroke

Abbreviations: RDA = Recommended Daily Allowance; ADCS = Alzheimer's Disease Cooperative Study; AREDS = Age-Related Eye Diseases Study; ATBC = Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group; CHAOS = Cambridge Heart Antioxidant Study; DATATOP = Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism; GISSI-Prevenzione = Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarcto Miocardio Prevenzione; CLIPS: Critical Leg Ischaemia Prevention Study; HATS: HDL-Atherosclerosis Treatment Study; HOPE = Heart Outcomes Prevention Evaluation; MIN.VIT.AOX = The Geriatrie/MINéraux, VITamines, et AntiOXydants Network; MRC/BHF HPS = Medical Research Council/British Heart Foundation Heart Protection Study; PPP = Primary Prevention Project; POPADAD: The prevention of progression of arterial disease and diabetes trial; PPS = Polyp Prevention Study; REACT = Roche European American Cataract Trial; SPACE = Secondary Prevention with Antioxidants of Cardiovascular disease in Endstage renal disease; SU.VI.MAX = SUpplementation en VItamines et Minéraux AntioXydants; TACT: Trial to Assess Chelation Therapy (TACT); VEAPS: the vitamin E atherosclerosis prevention study; VECAT = Vitamin E, Cataracts, and Age-Related Maculopathy; WACS: Women's Antioxidant Cardiovascular Study; WAVE = Women's Angiographic Vitamin and Estrogen; WHS: Women's Health Study.
*Not Reported for metanalyses that included more than 20 studies.
Statistically significant results are reported in bold.

studies have been done, supplementation of vitamin E to prevent cardiovascular disease should not be considered.

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