
Micronutrient Vitamin Deficiencies and Cardiovascular Disease Risk: Advancing Current Understanding

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Abstract: Cardiovascular diseases are responsible for one-third of all deaths in the world. Several factors contribute to the current trend including micronutrient vitamin deficiencies (MVDs), however, opinions regarding the role of MVDs in CVDs are inconsistent; this could impact on micronutrient vitamins intake. The aim of this review was to provide a brief overview of published evidence on the associations between MVDs and CVDs, assess the interactions between micronutrients and cardiovascular endpoints, and identify current related research needs. Literature search conducted on studies published between 1963 and 2016 indicates that MVDs are common and are related to adverse cardiovascular endpoints through various mechanisms, including impaired antioxidant and immune response mechanisms; and anti-inflammatory activities. Some micronutrients directly impact on CVDs; others act as critical cofactors in several biochemical processes. Several methodologic flaws, environmental and individual susceptibility factors, lack of determination of baseline serum levels of complementary micronutrients, absence of uniformly accepted cut-off values, and interactions between micronutrients may partly account for discordant results across studies. Although MVD is a significant risk factor for CVDs, supplementation with single or paired micronutrients for primary prevention of CVDs in healthy adults with no special nutritional needs is discouraged; there is insufficient evidence to determine the benefit/harm of such supplementation.

Keywords: β -Carotene, Ascorbic Acid, Folate, Calciferol, Tocopherol, Deficiency, Heart Disease

1. Introduction

Micronutrient vitamin deficiencies (MVDs) are important public health issues due to the contributions to the pathogenesis, progression and mortality and morbidity burdens in many chronic diseases, including cardiovascular diseases (CVDs). CVDs are the major cause of morbidity and mortality globally, accounting for >40% of total mortality [1] with increasing trends in developed and developing nations. It is posited that > 23 million people are expected to be affected by CVDs by 2030 [2, 3]. Although the use of drug treatment for conventional cardiovascular risk factors has been very effective, recent studies suggest failure to reach the optimal levels of treatment only in some patient groups [4]. This scenario underscores the need for improved prevention and treatment strategies for CVDs as the key action for achieving the best results. Health providers and nutritionists are actively seeking complementary methods, such as the use of diet (fruits and

vegetables) rich in micronutrients and antioxidants to prevent and supplement treatment for CVDs [5]. However, it remains debatable whether MVDs cause CVDs, or whether micronutrient supplementation can postpone the onset of CVDs in those at risk or can mitigate progression and associated complications in those already affected [6]. Granted, there is much research interest on the relationship between MVDs and CVDs. Many studies [7, 8, 9, 10, 11, 12] have been conducted, and a number of trials are currently ongoing. New research findings regarding the function of some micronutrients in the body are being added to existing ones [13], which could provide additional data to identify the potential role of micronutrient supplementation in the primary and secondary prevention of CVDs [14].

Previous clinical trials of some micronutrients have shown significant correlations between deficiency states and cardiovascular risks/diseases [15, 16, 17], yet others showed the converse [18]. For instance, the Heart Outcome

Prevention Evaluation trial, the Cambridge Heart Antioxidant study, the Norwegian Vitamin Trial, and the Western Norway B Vitamin Intervention trial [19, 20, 21] showed no clear evidence of benefits from B vitamin supplementation in terms of CVD risk reduction. These controversies have created confusion among the general public and may have impacted micronutrient intake, leading to fluctuating trends in micronutrient intake across the globe [22, 23, 24]. Nevertheless, the conflicting research findings could be resolved when one considers the effects of inadequately adjusted covariates, such as disparities in study design and methodologic flaws, including unreliable analytical methods [25, 26, 27]; variable doses [15]; the use of serum micronutrient measurements that may not adequately reflect long-term micronutrient intake [27, 28, 29, 30]; individual susceptibility factors; type and source of micronutrients; baseline micronutrient level [16], (e.g., very high or very low micronutrient at baseline); environmental factors; micronutrient interactions; inadequate pairing of micronutrients; and many other issues [31]. These have been shown to produce effects similar in magnitude to the observed health effect [14].

This review provides available evidence in favor of, and/or against the role of micronutrient vitamin deficiency (MVD) on cardiovascular endpoints, and extends the discussion on the pathophysiology and therapeutic implications of MVD in CVD.

2. Methods

A search using Medline, Scopus, and EMBASE databases was conducted to identify published articles within the period 1963–2016 using related terms such as micronutrients, essential nutrients, cardiovascular disease, vitamins, minerals, antioxidants, and anti-inflammatory and immune-modulatory micronutrients. For the purpose of this review, micronutrients were defined as vitamins, minerals, and trace elements essential for life. For each micronutrient, we considered evidence for or against its cardioprotective effects, its pharmacodynamics and pharmacokinetics, and current research needs.

All articles evaluating or reporting on the role of vitamins in cardiovascular disease and published within the study period, and met the inclusion criteria were included.

Articles with obvious methodology flaws (e.g., poor analytical methods) were excluded. If data were duplicated or shared in more than one study, the most recent, well presented and comprehensive study was included. In all, 210 articles (20 short communications, 60 review articles and 130 full research articles) were selected. However, 190 articles met the inclusion criteria and were included in the review.

Associations between vitamins and major cardiovascular events such as coronary artery disease, ischemic heart disease, myocardial infarction, angina and sudden cardiac death were explored.

Each article was evaluated for the type of study (e.g., primary, secondary or tertiary intervention trial), duration,

and experimental dose of the tested vitamin.

Study design (double blind, randomized, controlled trial, placebo controlled trial or open label), methodology quality (high or low) administrated supplement (single or in combination with other micronutrients) and duration of treatment. Only articles published in English were selected and evaluated.

3. Pathophysiology of MVD-induced CVD

Many reports [15-18] indicate that MVD is a significant risk factor for a wide spectrum of CVDs due to the ability to either initiate CVDs or worsen extant impaired cardiovascular function.

The pathophysiological mechanisms underlying MVD-induced CVDs include induction of imbalance in antioxidant defense mechanisms, inflammation and immune system dysfunction.

Central to these pathophysiological pathways is the induction of oxidative stress and subsequent generation of ROS, including peroxide, superoxide, and hydroxyl radicals. These free radicals have been implicated in a wide spectrum of CVDs due to the associated damage to endothelial cells; depletion of nitric oxide (NO), and oxidation of low-density lipoprotein-cholesterol (LDL-C).

Empirical evidences indicate that the micronutrient vitamin system mitigates the aforementioned three MVD-induced pathophysiological processes leading to CVDs in three ways.

These include prevention of oxidant formation, inhibition of already formed oxidant and repairs to oxidant-induced cardiovascular injury, through their antioxidative, anti-inflammatory and immune modulatory activities [32] (Tables 1, 2 and 3).

Table 1. Vitamins with cardio-protective activities.

Vitamins
Vitamin A
Vitamin B ₁
Vitamin B ₃
Vitamin B ₆
Vitamin B ₁₂
Vitamin C
Vitamin D
Vitamin E
Vitamin K
Folic acid

Table 2. Vitamins and cardio-protective mode of actions.

Vitamins	Immune modulators	Anti-inflammatory
Antioxidants		
Vitamin A	Vitamin A	Vitamin A
Vitamin C	Vitamin B ₆	Vitamin B ₆
Vitamin E	Folate (B ₉)	Vitamin B ₁₂
Nicotinamide	Vitamin B ₁₂	Vitamin E
Riboflavin	Vitamin C	Folate (B ₉)
Omega-3 fatty acid	Vitamin E	Vitamin C
-	Vitamin D	Vitamin D

Table 3. Classification of Vitamins based on the strength of their cardio-protective mode of action.

Micronutrient Vitamins	Antioxidants	Immune modulators	Anti-inflammatory
Vitamin A	++	++	++
Vitamin B ₆	++	++	++
Vitamin B ₉ (Folate)	++	++	++
Vitamin B ₁₂	-	++	++
Nicotinamide	++	-	-
Riboflavin (B ₂)	++	-	-
Vitamin C	++	++	++
Vitamin D	+ (indirect)	++	++
Vitamin E	++	++	++

++: Persuasive evidence, typically from clinical trials.

+: Suggestive evidence.

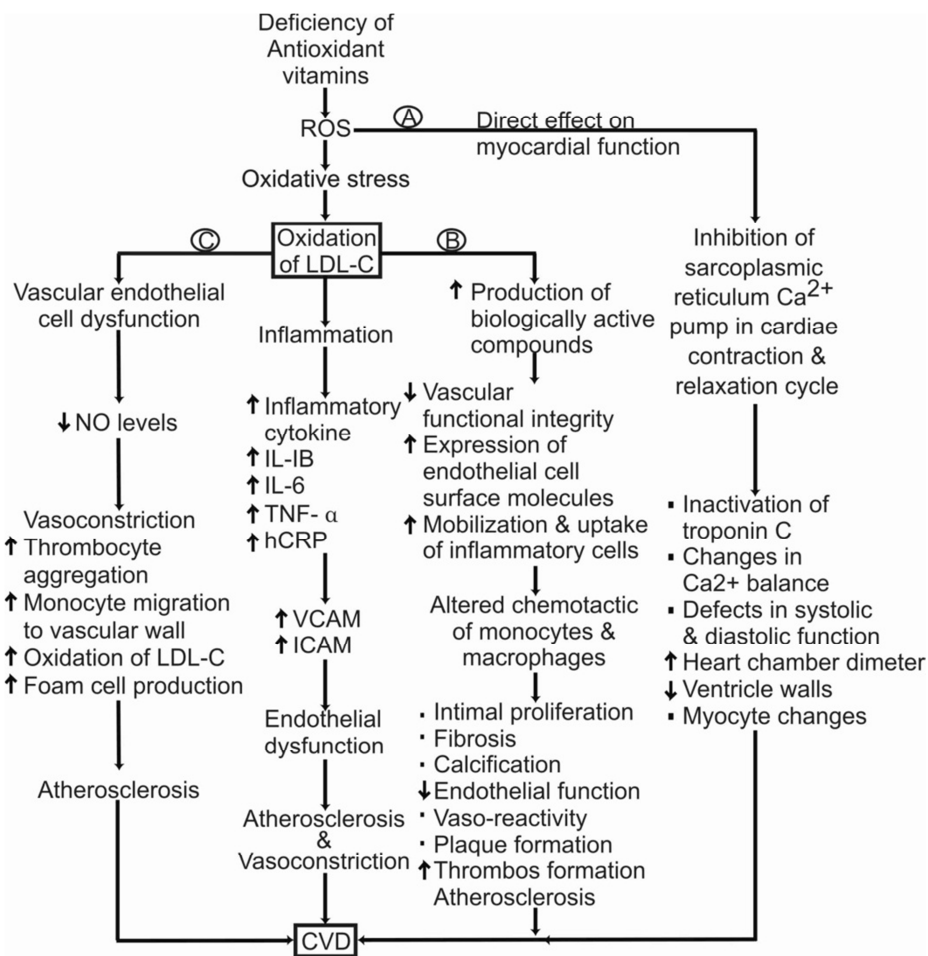
Central to these antioxidant pathways is the inhibition of LDL-C oxidation. Oxidized LDL-C initiates several biochemical and mechanical processes that lead to atherosclerosis. These include increase production of biologically active compounds, which impair the functional integrity of the vascular cells, and increase expression of endothelial cell surface molecules. There is also associated increase mobilization and uptake of circulating inflammatory cells, alteration of chemotactic property of monocytes and

monocyte derived macrophages, increase intimal proliferation, fibrosis, calcification, plaque formation and plaque rupture leading to thrombus formation.

Oxidized LDL-C also inhibits NO synthesis due to its negative impact on vascular endothelial function. Low bioavailability of NO is associated with several processes that lead to atherosclerosis such as vasoconstriction, increase thrombocyte aggregation, monocyte migration to vascular wall, and increase formation of foam cells [33].

Alternatively, the associated inflammatory processes could lead to increase production of inflammatory cytokines such as interleukin (IL)-1B, IL-6, tumor necrosis factor- α (TNF- α), and high sensitivity C-reactive protein and leading to endothelial dysfunction, atherosclerosis, vasoconstriction and CVDs. Furthermore, ROS can directly affect myocardial function through inhibition of the sarcoplasmic reticular calcium-pump in the cardiac cycle.

This action is associated with inactivation of troponin C, changes in calcium balance, defect in systolic and diastolic function, decreased ventricular walls, changes in myocyte morphology (e.g., increase in length and size of myocyte, sarcomeric disorganization and myofibril disarray) [34, 35, 36] and leading to CVDs (Figure 1).

**Figure 1.** Oxidation of LDL and pathogenesis of cardiovascular disease.

Schematic diagram showing three pathophysiologic pathways linking micronutrient deficiencies and cardiovascular diseases, including (A) direct effect on myocardial function, (B) increase production of biological active compounds, and (C) vascular endothelial cell dysfunction .

4. Specific Micronutrient Vitamin Deficiencies and CVD

Although the causes of CVD often overlap, several experimental and clinical studies have yielded a significant amount of data suggesting that deficiencies in vitamins, minerals, and trace elements are major contributors to CVDs and CVD symptoms. To date, available literature suggests that MVDs can cause or worsen the risk factors for CVD [3]. In many of these studies, micronutrient vitamin (MNV) intake over an extended period was associated with decreased risk of cardiovascular events. Although this association has been challenged by some recent studies [37, 38], these studies have important limitations, including the effects of inadequately controlled covariates.

Adequate MNV intake is known to reduce the risk of hypertension and other cardiovascular risk factors. Studies evaluating the relationship between MVD and hypertension suggest MVD is more common in patients with hypertension and other CVDs than in the general population [31]. For instance, the link between deficiencies of vitamins A, C, D, and E and CVD, including hypertension, has long been established. Vitamins A, C, and E constitute the non-enzymatic antioxidant vitamin barrier. Adequate plasma concentrations of these vitamins protects against ROS and, by extension, CVDs. Previous studies indicate lower levels of these vitamins in patients with CVDs [39, 40, 41, 42]. Similarly, deficiencies in several minerals and trace elements, such as calcium, magnesium, and chromium, have been linked to hypertension and other CVDs. A study by Chiplonkar et al. [43], conducted among 109 hypertensive adults and 115 age-sex-socioeconomic status-matched healthy, normotensive adults aged 30–58 years, showed that intake of some micronutrients (potassium, copper, folic acid, and vitamin C) was significantly lower in hypertensive than in normotensive subjects.

Similarly, a study conducted by the hypertension institute in Nashville found significantly lower levels of micronutrients in hypertensive than in normotensive patients. In that study, micronutrient replacement, in addition to other lifestyle modifications, controlled blood pressure in 62% of hypertensive subjects over a period of 6 months, even after discontinuation of antihypertensive drugs; nevertheless, unadjusted lifestyle factors could have confounded the results.

4.1. Vitamin A (β -Carotene) Deficiency and CVD

Vitamin A is a fat soluble vitamin that exists as a pro-vitamin (β -carotene) in plants (roots, leaves, shoots, seed, fruits, and flowers). It is metabolized to retinoic acid. It also exists as a polyisoprenoid compound (retinol) containing a cyclohexenyl ring in animals (eggs, poultry, and fish) (Figure 2A).

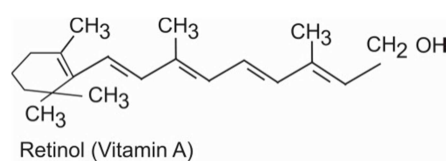


Figure 2A. Chemical structure of retinol (Vitamin A) containing a cyclohexenyl ring.

The double-bond in the carbon chain of the carotinoids may exist in different configurations, including trans and cis configurations (Figure 2B), giving rise to the different isomers of β -carotene.

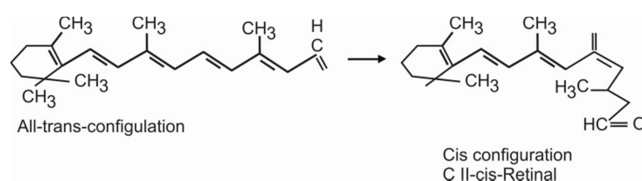


Figure 2B. Chemical structure of carotenoid compounds showing the trans and cis configurations.

Common isomers of β -carotene found in human plasma and foods include all-trans, 13-cis, and 15-cis isomers [45]. The form of β -carotene present in food is important because of the pharmacodynamic and pharmacokinetic bearings. The most common form in humans is the all-trans isomer [46] that is more stable than the cis form. Retinol is stored in the liver as retinol esters. It has two derivatives: retinal and retinoic acid. However, retinol is more potent and they are collectively known as retinoids. Retinoids and β -carotene function as anti-oxidants, anti-inflammatories, and immune modulators; hence, they protect the human body against various diseases, including CVDs. Over 500 plant-derived compounds constitute the carotinoids, however, the major ones include α -carotene (collard greens, pumpkin, corn, yellow pepper, cloudberry), β -carotene (apricots, mangoes, red pepper, kale, spinach, broccoli, carrots), β -cryptoxanthin (orange, persimmon, avocado, pepper, passion fruit), lycopene (guava, watermelon, rose hip, tomato and tomato products, pink grape fruit), and lutein and zeaxanthin (corn, peas, spinach, collard greens, lettuce) [1, 47].

Data from most observational studies [48, 49, 50] found inverse associations between dietary intake or supplementation of vitamin A or β -carotene and CVD risk, including death from ischemic heart disease (IHD) and myocardial infarction (MI). However, several other studies found no association or increased risk of CVD. These studies include the large-scale, randomized double-blind, placebo-controlled trial of β -carotene (50 mg on alternate days) by Hennekens et al. [51], and the prospective, nested case-control study of male physicians without a prior history of CVD by Hak et al. [52]. Likewise, a study by Omenn et al. [53] in a high risk population of 18,314 smokers and former smokers found no association between β -carotene intake CVD risk.

Several factors have been postulated to account for these

conflicting research results across studies, including individual susceptibility factors, treatment doses and duration, sources of β -carotene (dietary or supplementation), baseline plasma carotene level, and the presence of other nutrients in β -carotene-containing foods (including other carotenoid compounds) contributing to the observed cardioprotective effects. There is now considerable evidence that the experimental dose of β -carotene may impact its cardioprotective potential. Csepanyi et al. [54] in their animal study found that at a lower dose (30 mg/kg/day) and short reperfusion times, β -carotene exhibited a significant cardioprotective effect, but that this protection was lost at higher doses (150 mg/kg/day) and higher reperfusion time. Lack of controlling for known physiologic and lifestyle factors, such as smoking, has also been implicated. Streat et al., [55], found that carotene deficiency was associated with a higher risk of subsequent MI in smokers but not in non-smokers. Similarly, Hak et al. [52] showed no protective effect of different carotenoids against incident MI among smokers, but not among non-smokers. Some studies found cardioprotective effects only with dietary β -carotene but not with supplementation [51, 53], suggesting a complementary effect of other nutrients in β -carotene rich foods, including other carotenoids with cardioprotective potential, such as lutein [56]. Besides the carotenoids, other micronutrients present in plants can provide a polyvalent cardioprotective effect through different mechanisms. Hennekens et al. [51] reported a lack of improvement in patients with CVD and cancer treated with all-trans β -carotene, probably due to the absence of the complementary cardioprotective actions of other carotenoids. In a population study of 73,286 female nurses to assess the potential role of other dietary carotenoids in the prevention of coronary artery disease (CAD), Osganian et al. [47] found a moderate but significant inverse association between higher quintiles of intake of β -carotene and α -carotene and the risk of CAD but not with other dietary carotenoids, suggesting that the type of dietary carotenoid affects its cardio-protective potential.

It has also been postulated that supplementation of vitamin A or β -carotene may not provide the desired cardio-protective effect in populations with relatively adequate baseline plasma levels, such as those with large intake of fruits and vegetables. Similarly, healthy individuals on a short-term carotene-deplete diet showed a significant improvement in biochemical markers of lipid peroxidation following supplementation with a β -carotene rich diet [57, 58]. This observation suggests that supplementation of vitamin A or β -carotene to individuals with adequate plasma levels at baseline may not produce any effect or may even be toxic to cells. Accordingly, the United States Preventive Services Task Force does not recommend β -carotene supplementation in healthy populations without known nutritional deficiencies as a means to reduce the risk of CVDs or cancer [59].

The underlying cardio-protective effect of vitamin A or β -carotene is hypothesized to include attenuation of lipid oxidation, in particular, the oxidation of LDL-C in arteries; quenching of singlet oxygen [60]; and scavenging of free

radicals. Furthermore, vitamin A (retinoic acid) exhibits potent anti-inflammatory and immune modulatory effects in atherosclerosis by inducing the formation regulatory T-cells (Tregs), CD4 T-cells that express fork head box protein 3 (FOXP³). Tregs modulate immune and anti-inflammatory responses by suppressing T-cell proliferation and the release anti-inflammatory cytokines including IL-10 and transforming growth factor (TGF)-1. These cytokines can inhibit inflammation and enhance atherosclerotic plaque-stabilization. Results of a study by Mottaghi et al., [61] evaluating the effect of vitamin A supplementation on FOXP³ and TGF gene expression in atherosclerotic patients, showed that vitamin A supplementation caused increased FOXP³ and TGF expression in atherosclerotic patients, leading to increased anti-inflammatory and atherosclerotic plaque-stabilizing effects of TGF. Conversely, deficiency of vitamin A was associated with a significant increase in serum hs-CRP [62], an independent predictor of adverse cardiovascular events [63]. CRP has been shown to worsen pre-existing tissue damage in a complement-dependent pattern [64]. This is in line with a study demonstrating that CRP enhances inflammation in ischemic myocardium by inducing local complement activation [65]; similar results were obtained by several other investigators [66, 67, 68]. In view of the obvious lack of evidence for benefit, supplementary use of β -carotene or vitamin A for primary prevention of CVD in healthy adults without special nutritional deficiency is not recommended.

4.2. B-Vitamin (B-6, B-12 and Folate) Deficiencies and CVD

B-vitamins (B₆, B₉ and B₁₂) function as cofactors in the metabolism of homocysteine. Homocysteine is a sulfur-containing amino acid. It is an intermediate compound in the pathway of methionine metabolism to cysteine, and is normally recycled to maintain serum level at a physiologic limit (Figure 3A).

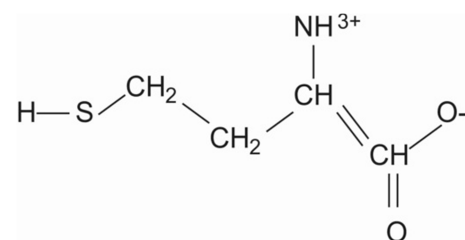


Figure 3A. Chemical structure of homocysteine.

Cysteine is an amino acid used for synthesis of glutathione, an important antioxidant. Normal serum levels of homocysteine are 5–15 μ mol/L [69]. Serum levels of homocysteine 15–29.99 μ mol/L, 30–99.9 μ mol/L, and \geq 100 μ mol/L are regarded as mild, moderate, and severe hyperhomocysteinemia, respectively [70, 71]. Two pathways (remethylation and transsulfuration) are involved in the metabolism of homocysteine to methionine and cysteine, respectively. Vitamins B₆, B₉, and B₁₂ function as cofactors in these pathways (Figure 3B).

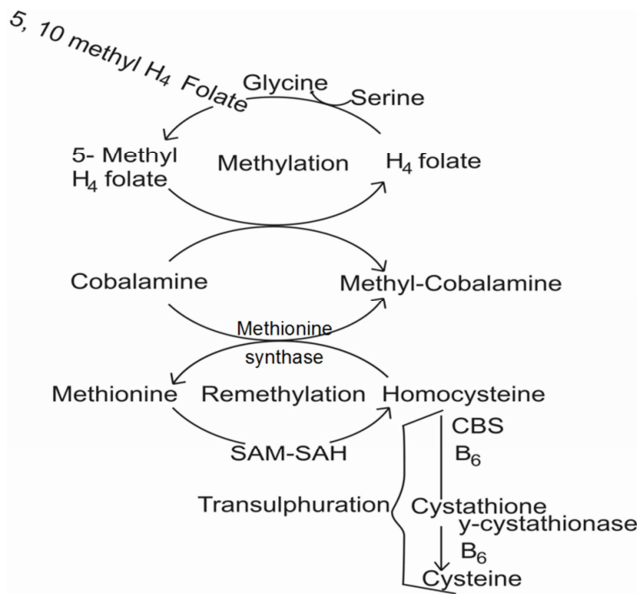


Figure 3B. Schematic illustration of the role of B-vitamins in the biosynthesis and metabolism of cysteine and homocysteine.

Deficiencies of these vitamins are associated with defective homocysteine metabolism leading to elevation in serum homocysteine levels (hyperhomocysteinemia). Numerous observational studies have investigated the relationship between B-vitamins (B₆, B₁₂, and folate) and serum homocysteine level, and CVD risk. Data from these studies have shown that serum homocysteine level is inversely related to serum B-vitamins levels. B-vitamin insufficiencies have been shown to be associated with hyperhomocysteinemia in both sexes [72], and homocysteine levels have been shown to be directly related to CVD risk.

The pathophysiologic mechanisms underlying hyperhomocysteinemia-induced CVD have been postulated to include induction of endothelial damage and dysfunction, enhanced oxidation of LDL-C, smooth muscle proliferation, endothelial-leukocyte interactions, innate autoimmune disorders, inflammation, and oxidative stress [73]. There is enhanced atherosclerosis, cerebrovascular and peripheral disease, and other CVD events, including alteration of platelet function and coagulation factors leading to enhanced thrombogenesis [73, 74, 75, 76] (Figure 3C).

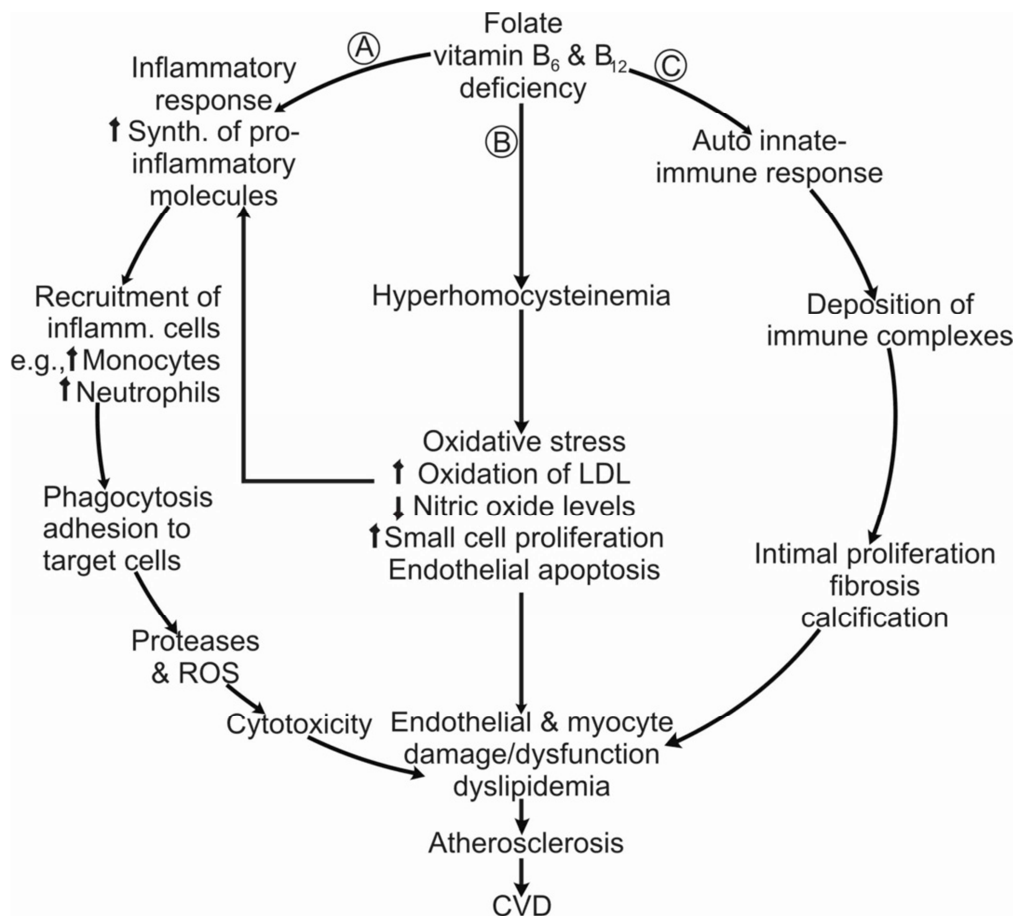


Figure 3C. Schematic illustration of the links between deficiency of the B-vitamins, hyperhomocysteinemia, and cardiovascular diseases.

A: Deficiency of B-vitamins can induce inflammatory responses leading to recruitment of inflammatory cells, phagocytosis, proteases, ROS, cytotoxicity, and endothelial damage.

B: Hyperhomocysteinemia promotes oxidative stress and causes oxidation of LDL-C by free radicals leading to vascular dysfunction including reduction in nitrous oxide levels, increased small cell proliferation, and endothelial apoptosis (Soyding et al. 2007).

C: B-vitamin deficiency can induce autoimmune dysfunction leading to deposition of immune complexes, dyslipidemia, atherosclerosis, and cardiovascular disease.

Decreasing the plasma total homocysteine level by providing the cofactors for its metabolism (folic acid, vitamins B₆ and B₁₂) has been shown to reduce CVD risk [77, 78]. In fact, it is proposed that for every 3 $\mu\text{mol/L}$ decrease in serum homocysteine, there is a mean 16% reduction in CVD risk [79].

High levels of homocysteine are correlated with significant increase risk of CAD, MI [80], peripheral occlusive disease (deep vein thrombosis) [81, 82], and intermittent claudication [83]. According to Nygard et al. [84], a serum homocysteine level of 12% greater than the upper limit of normal is associated with a threefold increased risk of acute MI. Adequate plasma levels of folate and vitamins B₆ and B₁₂ significantly decreased mean serum levels of triglyceride and total cholesterol and simultaneously increased mean serum phospholipid and high density lipoprotein cholesterol (HDL-C) levels [85], and vice versa.

In the Vitamin Intervention for Stroke Prevention study, Tool et al. [15] used high doses of B-vitamins (25 mg vitamin B, 400 μg B-12, and 2500 μg folic acid) to lower homocysteine levels in subjects with a previous history of stroke in order to prevent recurrent stroke, MI, and death. The high dose B-vitamins achieved a better cardioprotective effect than low dose or placebo. In a related study, Spence et al. [16] demonstrated a 21% reduction in the risk of stroke, CVD, or death in a high dose B-vitamin group. In the latter study, the exclusion criteria included very low or very high serum B-vitamin levels at baseline, and those already being treated with vitamin B₁₂. In the nurse health study of 80,082 women, an inverse association between the intake of vitamin B₆ and folate and the risk of MI and cardiovascular death was reported [86]. Merchant et al. [87], in their study of 46,036 individuals aged 40–75 years, reported a 21% lower rate of peripheral arterial disease for each 400 $\mu\text{mol/L}$ increase in intake of B-vitamins. Cui et al. [88] made a similar observation in their study of 58,730 patients, assessing the effect of intake of folate, B₆ and B₁₂ on CVD endpoints.

However, several other studies found no association between vitamin B₆, B₁₂, and folate and homocysteine level and CVDs, leading to the suggestion that the homocysteine-lowering therapies of folic acid, vitamin B₆, and vitamin B₁₂ do not lower the risk of CVD; [69, 89, 90, 91, 92, 93]. For instance, in the Women's Antioxidant and Folic Acid Cardiovascular Study, daily supplementation with 2.5 mg folate, 50 mg vitamin B₆, and 1 mg of vitamin B₁₂ for a mean of 7.3 years produced no effect on cardiovascular endpoints [94].

A study of 12,064 male survivors of MI treated with supplemental folic acid (2 mg) and vitamin B₁₂ (1 mg) for a mean period of 7 years demonstrated no major differences in the outcomes of MI, cardiovascular death, and stroke [95]. The Western Norway B-Vitamin Intervention Trial also produced lack of beneficial effect. Similar negative effect of B-vitamin supplementation was observed in an animal study by Omid et al. [96] that evaluated the impact of folate and vitamin B₁₂ on cardiac function and morphology.

Given the mixed results reported in the aforementioned studies and in view of several methodologic flaws, caution should be exercised in drawing conclusions on the effect of these vitamins on CVD. A study on the role of vitamin B₆ deficiency and serum homocysteine level and CVD indicated that besides impairing cysteine production and, hence, glutathione synthesis, hyperhomocysteinemia chelates copper and impairs copper-dependent enzyme activities, including superoxide dismutase (SOD), leading to increased lipid peroxidation [97]. Therefore, copper deficiency usually co-occurs with vitamin B₂ or B₆ or folate deficiency. This understanding led to the suggestion that copper deficiency plays a significant role in the pathogenesis of hyperhomocysteinemia-induced CVDs. Therefore, lowering serum level of homocysteine alone will not overcome the effect of coexisting copper deficiency on overall cardiovascular health. For instance, supplementation with B vitamins alone to ameliorate hyperhomocysteinemia-induced CVD will fail unless serum levels of copper remain within physiologic limits. This understanding may help to explain why supplementation of B-vitamins alone may fail to reverse hyperhomocysteinemia-induced CVD in some studies [89, 91], as these studies either failed to replace the associated copper deficiency or to ascertain the baseline serum level of copper. More studies are needed to determine at what baseline homocysteine level treatment will produce beneficial effects, in view of the negative results observed in individuals with normal homocysteine levels. In addition, the confounding effect of low level of serum copper should be adequately excluded.

Folate deficiency and CVD

An association between folate deficiency and coronary heart disease (CHD) has been established [79]. Available evidence suggests that 10–25% of deaths from CHD and strokes worldwide may be related to folate deficiency [98]. High intake of folate (0.8 mg) has been found to reduce the risk of IHD by 16%, deep vein thrombosis by 25%, and stroke by 24% [79]. However, the association between folate and CVD has mostly been explored through its effect on homocysteine. Folate is involved in the methylation of homocysteine to methionine. Folate deficiency is associated with increased blood levels of homocysteine, impaired synthesis/activity of endothelial NO, enhanced inflammatory processes that lead to vasoconstriction, atherosclerosis, and by extension CAD [99]. Folate supplementation has been found to decrease serum levels of homocysteine and its sequelae [100]. A high serum homocysteine level is associated with a higher risk of hypertension and stroke [101], however, other prospective studies have questioned the independent role of homocysteine as a risk factor for CVD [102]. Folate is thought to act as a cofactor for endothelial NO synthesis, inhibiting intracellular superoxide generation and stimulating endogenous tetrahydrobiopterin (an enzymatic cofactor of NO synthase), thereby leading to vasodilatation and decreasing the risk of CVD [99].

4.3. Vitamin C deficiency and CVD

Vitamin C is a water soluble antioxidant vitamin with potential to directly scavenge free radicals (singlet oxygen, superoxide and hydroxyl radicals) *in vitro* (Figure 4).

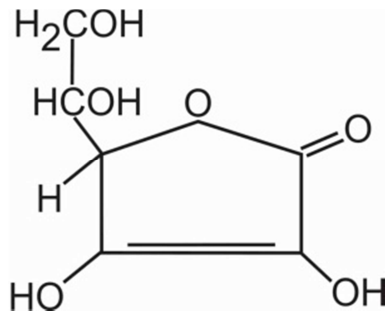


Figure 4. Chemical structure of vitamin C.

Sources of vitamin C include vegetables (e.g., broccoli, green and red pepper, and brussels), citrus, paya and strawberries. The hypothesis that increased intake of vitamin C is protective against CVD and associated morbidity and mortality is validated in many well-conducted population-based studies and clinical trials. Current data support the beneficial effect of vitamin C itself in primary prevention of CVD [47]. However, results of other studies have been less supportive, showing either negative [103, 104, 105, 106, 107] or inconclusive data [108, 109, 110, 111, 112]. Many of these studies may have been confounded by several inappropriately adjusted for covariates, such as poor determination of serum vitamin C level; individual susceptibility factors, including lifestyle variables such as smoking and alcohol intake; the health status of the studied population; and dietary vitamin C intake. It is suggested that a modest intake of vitamin C (100 mg/day) is optimal for maximum reduction of CHD risk among non-smoking men and women [113].

In the Nurses' Health Study, supplementation with vitamin C resulted in a 28% CHD risk reduction. The first National Health and Nutritional Examination Study, an epidemiologic follow-up study, found a 45% and 25% reduction in CHD risk in men and women respectively, who consumed >50 mg/day of vitamin C from dietary sources and took regular supplementation equivalent to 300 mg/day [109].

Osagnian et al., [47] in a report, showed consistent results with a 25% reduction in CHD among women who consumed vitamin C supplements, but not when obtained from the diet. Also, a prospective population based study of men from Eastern Finland showed inverse association between vitamin C deficiency and acute MI [114]. According to a study by Riemersma et al. [104], low plasma vitamin C was not associated with an increased risk of acute MI, irrespective of the smoking status of their study subjects. However, this study was limited by inability to measure plasma vitamin C levels in some patients, underestimation of the confounding effect of

smoking, the differential response rate which was higher in patients than controls, and the likelihood of reporting bias. Another study used a semi-quantitative food frequency questionnaire which is not sensitive enough to detect small differences in CHD risk and hence, may not have detected an effect [47]. In contrast, a study that used a seven-day diet diary demonstrated a strong inverse association between CHD-related mortality and plasma vitamin C level [115].

Therefore, studies that detected significant associations with only vitamin C supplements [47] may have done so because it is easier to assess supplemental intake than dietary intake. Subjects can remember more easily the dose and frequency of supplement intake than exact dietary recall for a certain period of time. The use of a food frequency questionnaire is less precise and does not account for losses of vitamin C during storage and preparation. Some studies [116] associating vitamin C intake with adverse health effects (including increase risk of MI and death) were characterized by small sample sizes and a small number of deaths which could have been a chance finding, or may have been due to confounding. Dose-dose variation could confound results and produce discrepant data across studies. There is an optimum saturation dose at which cells and tissues are fully saturated and cardioprotective effects are enhanced [117]. Relatively high doses of vitamin C are required to inhibit the rapid reaction between superoxide and NO radicals *in vivo* [118].

According to Frei, [119], difficulties in accurately assessing vitamin C in epidemiologic studies could partly account for inconsistent results across studies. Some studies report reduced CHD risk within the dietary vitamin C intake range of 100 mg/day, but not with supplementation [113, 115], whereas available pharmacokinetic studies document optimum cardioprotective effects at a full saturation daily dose of 400 mg of vitamin C [117]. The cardioprotective potential of vitamin C lies in its antioxidant property. Oxidative stress generates ROS that destroys NO, an endothelial vasodilator, leading impaired endothelium mediated vasomotion. Vitamin C directly inactivates ROS and protects NO from being destroyed by free radicals. Vitamin C also enhances endothelial NO production by preventing the inactivation of tetrahydrobiopterin which acts as a co-factor to endothelial NO synthase [120].

4.4. Vitamin D deficiency and CVD

Vitamin D is a steroid-structured, fat-soluble vitamin produced in the skin as a result of exposure to sunlight [3], or from dietary sources such as oily fish [120], eggs, yolk, butter, liver, and fortified foods. Regardless of the source, vitamin D and its metabolites circulate bound to vitamin D binding protein. The peripheral circulating form of vitamin D is 25-hydroxy vitamin D (25(OH)D), while the most potent form is the 1, 25 dihydroxy vitamin D (1,25(OH)D) [121, 122] (Figure 5A).

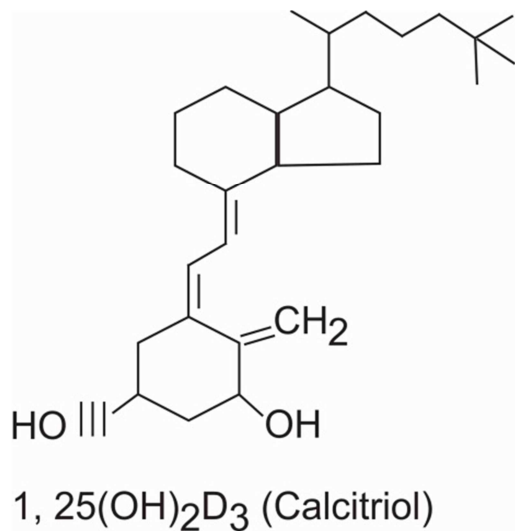


Figure 5A. Chemical structure of 1, 25(OH)₂D₃ (Calcitriol).

Recent studies indicate that vitamin D deficiency (hypovitaminosis D) is very common in the general population [3, 120, 123, 124]. Hypovitaminosis D is widespread and is seen even in areas with high levels of ultraviolet radiation and in developed countries where diets are fortified with vitamin D [125]. It often remains unrecognized and untreated [122]. It is presumed that about 1 billion individuals worldwide may be deficient or have insufficient levels of vitamin D [3]. Links between vitamin D deficiency and cardiovascular pathologies have been established in several experimental clinical trials, cross-sectional, prospective, and epidemiologic as well as preventive trials; available data indicate that vitamin D is potentially an important biomarker for CVD [126]. Vitamin D deficiency has been found to directly or indirectly impact several cardiovascular endpoints including hypertension, IHD, MI [122], endothelial dysfunction, slow coronary flow, subclinical atherosclerosis, and death due to CHD.

In a 10-year Health Professional Follow-Up Study of normal subjects at baseline, Giovannucci et al. found that subjects who subsequently developed low level of serum vitamin D (< 15 ng/mL) had twice the odds for MI, independent of other covariates [153]. Within the same period, a study by Pilz et al. [127] showed that subjects who were deficient in vitamin D had a 50% increased odds for fatal stroke [127]. Their study findings confirmed a previous 10-year follow-up study by Marniemi et al. [128] that also associated vitamin D (25(OH)D) deficiency with MI and stroke in the elderly population [128].

An inverse association between plasma level of vitamin D and hypertension has been reported in human and animal models of vitamin D deficiency [125]. Moreover, plasma 25(OH)D deficiencies and vitamin D receptor polymorphism are correlated with hypertension. One animal study showed that disruption of the vitamin D receptor gene resulted in a rise in blood pressure, renin production, and cardiac hypertrophy [129]. Furthermore, subjects with low plasma active vitamin D (25(OH)D) levels (< 15 ng/mL) had higher odds for current onset of hypertension within 4 years than

those with normal vitamin D levels (> 30 ng/mL).

In the National Health and Nutrition Examination Survey, low plasma levels of vitamin D strongly predicted angina pectoris, MI, stroke [130], atrial fibrillation, and ventricular hypertrophy. In a prospective study of 1471 normal post-menopausal women, Bolland et al. [131] showed that women with a lower vitamin D level at baseline were more likely to have a higher fat mass, be physically inactive, positive history of IHD risks, dyslipidemia, and higher calculated cardiovascular risk at baseline than women with serum levels of 25(OH)D > 50 nmol/L [131]. The consistency of the association between vitamin D deficiency and CVD has led to the suggestion that vitamin D should be considered a cardiovascular marker [121].

Although vitamin D supplementation has shown impressive cardio-protective potential in several studies, its cardio-protective effect has been challenged by studies showing negative correlations between vitamin D supplementation and cardio-protective effects. For instance, in the Women's Health Initiative study [132] and a randomized, double blind, controlled trial in Europe [133], vitamin D deficiency or supplementation produced no effect on cardiovascular endpoints. These discrepancies have been explained on the basis of inconsistencies/inadequacy in experimental doses, study population, and the effect of other coexisting nutrients [134].

Interestingly, despite the significant role of vitamin D in CVD, there is no consensus among researchers regarding what cut-off value should be used to define insufficient or deficient levels of vitamin D. However, an optimum level for cardio-protective effects has been proposed. According to Zittermann et al., [135], serum vitamin D level of 30–35 ng/L provides optimal cardio-protective effects [135].

Based on the Institute of Medicine, a daily intake of 400–600 IU of vitamin D is recommended for an adult [136]. Others are of the opinion that a daily intake close to 1000 IU of vitamin D is more appropriate to maintain health [137]. In most other studies vitamin D deficiency is defined as calcitriol level of < 20 ng/mL, a level of 21–29 ng/mL is defined as insufficient, 30 ng/mL is regarded as sufficient, and > 150 ng/mL is defined as a toxic [138]. Currently, age-specific daily allowances have been suggested, with 200 IU recommended for adults aged 20–50 years, 400 IU for adults 51–69 years of age, and 600 IU for adults ≥ 70 years. For the average older adult, 800 IU is the recommended daily allowance. However, dark-skinned older adults with limited sun exposure may need ≥ 2000 IU per day [134, 139].

Several risk factors for vitamin D deficiency have been identified, including obesity [131, 140], smoking [141], physical inactivity [131], skin color, reduced exposure to sunlight, diabetes mellitus, and variation in distance from the equator. Others include low HDL-C level, malabsorption, renal disease, and medications (such as anticonvulsants and corticosteroids).

The plausible mechanisms underlying the cardio-protective effects of vitamin D are complex and may include down-regulation of the renin-angiotensin-aldosterone system [121] (by decreasing renin-angiotensin activity or down-regulating renin production by the kidneys (Figure 5B).

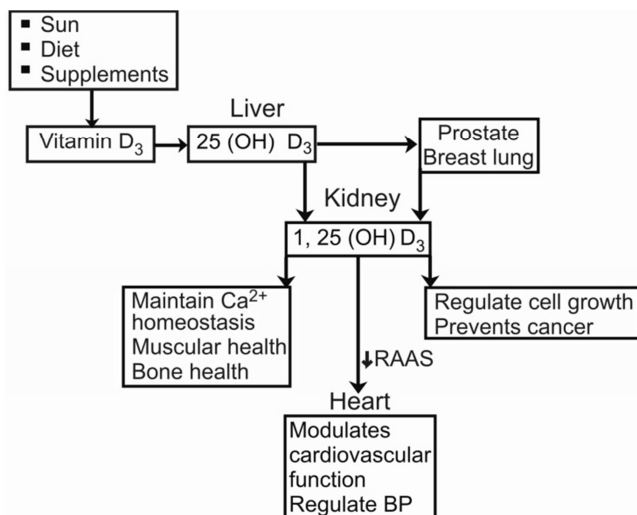


Figure 5B. Schematic representation of the pathways of vitamin D biosynthesis and cardiovascular effects.

This inhibitory action of vitamin D on RAAS may provide indirect antioxidant effect due to the associated improvement in endothelial function as reported previously [142]. Angiotensin II mediated increased production of superoxide is the speculated pathophysiologic mechanism leading to endothelial damage.

Vitamin D inhibits this process and by extension preserves endothelial health [143]. This action significantly contributes to the cardio-protective effect of vitamin D in hypertension and congestive cardiac failure (CCF) as observed previously [129].

One study showed that vitamin D supplementation reduced the mortality risk of patients with renal failure [144]; suppression of the expression of renin genes and regulation of genes involved in renin production have been suggested as mechanisms. Furthermore, vitamin D is known to exert anti-inflammatory effects via several pathways, including inhibition of prostaglandin and the cyclo-oxygenase pathway and up-regulation of anti-inflammatory cytokines. It is posited that vitamin D's anti-inflammatory activities have potential therapeutic benefits in several autoimmune diseases [134]. Vitamin D is also known to possess anti-atherogenic effects; hence, is cardio-protective. Low vitamin D levels may also indirectly affect cardiovascular endpoints due to its etiopathogenic role in several metabolic aberrations such as obesity, diabetes mellitus, dyslipidemia, and hypertension (and hence CVD) [126].

4.5. Vitamin E Deficiency and CVD

Vitamin E is a major lipid-soluble antioxidant, and is the most effective chain-breaking antioxidant within the cell membrane. Several studies have evaluated the role of vitamin E in CVD and have provided results that corroborate the hypothesis that vitamin E has a protective effect against atherosclerosis, and hence CVDs, independent of the effect of other potential confounders [145, 146, 147]. In the Nurses' Health Study [148], those in group with the highest intake of vitamin E had a 34%

decreased coronary risk compared with those in the lowest intake group. Vitamin E supplementation provided a better reduction (40%) than the dietary vitamin E. The Health Professional Follow-Up Study of 40,000 US male professionals aged 40–75 years provided similar results [110]. Likewise, the Iowa Women's Health Study [149] found an inverse association between vitamin E and CVD. However, in the latter, the association was strongest in the group taking supplements than the diet group. A study of middle-aged French Canadian Men [150] corroborated the aforementioned studies' findings. A cross-cultural study of 16 populations of men and women in the Multinational Monitoring of Trends and Determinants in Cardiovascular Disease study found a significant inverse association between vitamin E intake and IHD-related mortality (RR = 0.49; P = 0.01) [151]. Two other cross-sectional studies [152, 153], among 6000 Scottish men aged 35–54 years found inverse association between plasma vitamin E level and angina. In a case-control study in Tunisia, Feki et al., [145], concluded that adequate intake of vitamin E from the diet or supplementation could prevent CHD.

Nevertheless, some other epidemiologic prospective studies found the converse, including the Multiple Risk Factor Intervention Trial, the Elderly Cohort Studies [154], the Rotterdam Study [155], the European Community Multicenter Study on Antioxidants, and the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico prevention trial [156]. The lack of observed association in these studies may be attributable to uncontrolled confounding from unknown or unmeasured confounders such as exercise, diet, concurrent vitamin C depletion or deficiency, and background CVD risk of different study populations. These may produce effects similar in magnitude to the observed health effect. For instance, dietary antioxidants can produce effects similar to experimental antioxidants. Such effects could be additive or synergistic making it difficult to ascertain the specific benefit of the tested antioxidant vitamin [14]. Also, inaccuracies in determination of intake (e.g., dietary intake, vitamin supplementation, lipid standardized, or absolute vitamin E concentrations), changes in dietary habits during the follow-up period or a decrease in vitamin E concentration with time [157; 158], and differences in the selection criteria of cases (e.g., questionnaires, medical examination, and vitamin E status determination) [145] may have impacted the results and weakened the association.

Previous studies [159, 160], mostly clinical trials, that showed lack of benefit of vitamin E supplementation in CVD were characterized by several methodologic flaws, including short period of treatment [160], low dose used [159], small number of events [160], advanced age and high baseline risk of CHD of participants [161], as well as the use of clinical events as endpoints [159, 160, 161].

Vitamin E protects membrane fatty acids from lipid peroxidation by preventing the lipid peroxidation chain reaction in the LDL-C particle [162]. Vitamin E supplementation has been shown to decrease lipid

peroxidation by as much as 40% [162]. Oxidized LDLs are atherogenic due to their immunogenicity, cytotoxicity, and chemotactic properties [163]. Therefore, the anti-atherosclerotic effect of vitamin E is strongest at the point of initiation (primary prevention) than in an already established atherosclerotic lesion (secondary prevention) [145, 164]. This hypothesis is in agreement with results obtained by Klein et al. in a study to assess the effect of two different combined treatments with vitamin E acetate and vitamin C on infarct size and recovery of regional myocardial function [165]. Initiation of treatment before the onset of ischemia resulted in a greater reduction in infarct size and better recovery of regional myocardial function than initiation of treatment during ischemia.

Vitamin E also has the potential to prevent other deleterious processes leading to atherosclerosis. It decreases the secretion of IL-1, reduces platelet adhesion and aggregation [166], decreases monocyte endothelial cell adhesion, and inhibits thrombin formation [165]. Thus, vitamin E intake may have a greater protective effect against the onset of primary atherosclerotic lesions (identified using imaging criteria as endpoints) in younger individuals with a CHD-free background and a prolonged period of intake (>10 years) than in older individuals with pre-existing atherosclerotic lesions, a high CHD risk background, and with short duration of therapy (<5 years) using clinical event endpoints [145], as characteristic of most clinical trials and secondary prevention studies. However, most observational studies support the protective effect of vitamin E against CHD [151].

The mechanism underlying the cardio-protective effects of vitamin E include stabilization of plaque, reduction of platelet adhesion and aggregation, expression of adhesion molecule on the arterial wall, and reduction of vasodilatation [162]. Thus, vitamin E protects against atherosclerosis and, by extension, MI and thrombotic stroke [162]. Adequate levels of vitamin C are required for the reduction of oxidized vitamin E. Reduced vitamin E is very important because it can be re-utilized by cells for free radical scavenging in lipophilic sites [119]. However, recent evaluation of the supplementary use of vitamin E for prevention of CVD by the US Preventive Services Task Force found moderate evidence against supplementation with vitamin E for the prevention of CVD or cancer [59].

5. Micronutrient Combination/Interaction and Effect on CVD

There is compelling evidence that multiple micronutrient deficiencies (MNDs) may be associated with a higher risk of CVD than single MNDs. Likewise, multiple micronutrient supplementation in pharmacologic doses may provide better relief from multiple MND-induced CVD than micronutrient monotherapy. Obviously, paired micronutrient supplementation is more likely to address the pleiotropic

etiology of adverse cardiovascular endpoints than micronutrient monotherapy. The pathophysiologic mechanisms underlying MND-induced vascular abnormalities (e.g., endothelial damage, smooth muscle and cardiac muscle dysfunction, vascular disease, and atherosclerosis) leading to CVD, are etiologically related to oxidative stress, inflammation, and autoimmune dysfunction and are also closely interrelated. Therefore, supplementation of paired micronutrients with antioxidant, anti-inflammatory, and immune boosting potential would provide a synergistic/additive cardio-protective effect than micronutrient monotherapy. Paired micronutrient supplementation can also precipitate interactions between micronutrients. For instance, high manganese supplementation leads to magnesium deficiency and CVDs, with manganese acting as a potential magnesium antagonist [168]. Several previous studies showed that adequate intake of some micronutrients could enhance the pharmacokinetic and/or the pharmacodynamic potentials of other micronutrients.

For instance, some micronutrients act as cofactors for the biosynthesis, transport, activation, and effect of others, while others enhance the absorption and metabolism of other micronutrients. In addition, some micronutrients gain protection from the auto-oxidation by the antioxidant activities of other micronutrients. Some vitamins affect the requirement for other vitamins or minerals and vice versa. A study showed that although an adequate amount of potassium was administered, the serum potassium level did not improve until magnesium was co-administered [169]. Adequate serum magnesium enhances both the absorption and effects of potassium. Similarly, studies have suggested the confounding effect of magnesium and vitamin D, or possible interaction between the two. One study observed a lack of clinical and biochemical improvement in rickets patients treated with vitamin D until magnesium was supplemented [170]. Studies by Rude et al. [171] and Fuss et al. [172] showed that high doses of vitamin D alone failed to cause improvement in serum levels of both 25(OH)D and 1,25(OH)₂D in vitamin D deficiency states until magnesium and vitamin D were co-administered. Administration of high doses of magnesium modified the inverse association of serum 25(OH)D and CVD mortality, according to Rosanoff et al. [173], who cited Deng et al. [174]. Earlier studies showed that calcium intake affects magnesium retention, and vice versa [175], and that hypomagnesemia is often present with hypocalcemia [171]. Furthermore, co-administration of vitamin C prevents magnesium deficiency-induced heart failure, probably due to the ability of vitamin C to scavenge ROS [176].

Similarly, the cardio-protective effect of vitamin E is enhanced and sustained when co-administered with vitamin C [154] due to the synergistic antioxidant effect of both vitamins. The antioxidant activity of alpha-tocopherol results in the production of toxic tocopherol radicals, which are reconverted into alpha-tocopherol by vitamin C and are then reutilized (Figure 6).

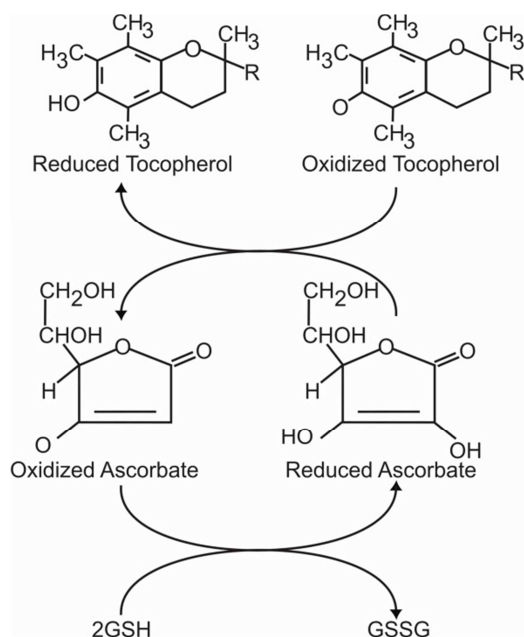


Figure 6. Reduction of oxidized ascorbate by glutathione enhances the reduction of oxidized tocopherol by the reduced ascorbate. This increases the availability of reduced tocopherol for reutilization.

Kennedy and Liebler [177] and Palozza and Krinsky [178] found that tocopherol protects carotenoids from auto-oxidation. Likewise, vitamin C protects tocopherol and β -carotene from oxidative damage [179, 180]. Singh et al. [181] found that vitamins A, C, E, and β -carotene attenuated acute MI a few hours after onset causing a significant decline in total adverse cardiac endpoints [181]. This action was faster and better than the effect of micronutrient monotherapy. Witte et al. [182] used multiple micronutrient supplementation to ameliorate chronic heart failure and IHD and improved quality of life in septuagenarian patients. In a related study, pure selenium deficiency symptoms were found to be rare, but were demonstrated to become obvious when vitamin E deficiency co-occurs with selenium deficiency [182]. Chen et al. [72] reported a higher risk of hyperhomocysteinemia when the deficiency of vitamin B₂ or B₆ co-occurred with folate deficiency.

The aforementioned studies indicate that paired micronutrient supplementation or deficiencies may provide a greater cardioprotective or cardiotoxic effects, respectively, than micronutrient monotherapy, provided treatment with pharmacologic doses is ensured. It should be noted that inappropriate combination doses may result in adverse health outcomes. For instance, supplementation with a high manganese diet exacerbated magnesium deficiency and death in animals fed a high manganese-low magnesium diet compared with animals fed a low manganese-magnesium deficient diet [183]. Furthermore, it is the ratio of copper to zinc in the zinc-copper superoxide dismutase-1 enzyme complex, rather than the absolute amount of copper or zinc, that provides the enzyme complex with higher superoxide scavenging potential and more efficient anti-inflammatory and cardioprotective effects [184]. In support of this view, Reunanen et al., [185], in a study evaluating the association

of serum calcium, magnesium, copper, and zinc concentrations with cardiovascular mortality, reported a higher risk of CHD with the higher risk of CHD-related mortality among those treated with the highest and lowest tertiles of serum copper and zinc.

This may explain, in part, the lack of association between the deficiency of some micronutrients (in some populations) and cardiovascular endpoints as the baseline level of some complementary micronutrients were not determined in most of these studies. Evidently, the effect of one micronutrient may depend on the complementary actions of other micronutrients when present in physiologically relevant levels or at pharmacologic doses.

6. Conclusion

Although opinions are conflicting, there is substantial, significant evidence to suggest that MVD is widespread and is related to adverse cardiovascular endpoints. The lack of association in some studies could partly be due to lack of adequate adjustment for several covariates, including deficiencies in some complementary micronutrient pairings. Supplementation with single or paired micronutrients for prevention of CVD in those with widespread MVDs, pregnant women, children, chronically ill or hospitalized individuals or those known to have nutritional deficiency would be practical and attractive, and is therefore recommended. There is not enough evidence to support supplementation of single or paired micronutrients for prevention of CVD in healthy, well-nourished populations without known nutritional deficiencies. More studies are needed to further assess the usefulness of single or combined micronutrients in healthy populations and in those with adequate micronutrient levels at baseline. Pairing of complementary micronutrients in pharmacologic doses may improve potency, metabolism, and outcomes and may minimize resistance and toxicity.

Abbreviations

CAD, coronary artery disease; CHD, coronary heart disease; CRP, C-reactive protein; CVD, cardiovascular disease; DNA, deoxyribonucleic acid; hs-CRP, high sensitivity C-reactive protein; HDL-C, high-density lipoprotein cholesterol; IL-1B, interleukin-1B; IL-6, interleukin-6; IHD, ischemic heart disease; MNV, micronutrient vitamin; MVD, micronutrient vitamin deficiency; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; NHANES, National Health and Nutrition Examination Study; NO, nitrous oxide; ROS, reactive oxygen species; TNF- α , tumor necrosis factor alpha.

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