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*Review Article*

# Vitamins and cardiovascular disease

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CVD is a major cause of mortality and morbidity in the Western world. In recent years its importance has expanded internationally and it is believed that by 2020 it will be the biggest cause of mortality in the world, emphasising the importance to prevent or minimise this increase. A beneficial role for vitamins in CVD has long been explored but the data are still inconsistent. While being supported by observational studies, randomised controlled trials have not yet supported a role for vitamins in primary or secondary prevention of CVD and have in some cases even indicated increased mortality in those with pre-existing late-stage atherosclerosis. The superiority of combination therapy over single supplementation has been suggested but this has not been confirmed in trials. Studies have indicated that β-carotene mediates pro-oxidant effects and it has been suggested that its negative effects may diminish the beneficial effects mediated by the other vitamins in the supplementation cocktail. The trials that used a combination of vitamins that include β-carotene have been disappointing. However, vitamin E and vitamin C have in combination shown long-term anti-atherogenic effects but their combined effect on clinical endpoints has been inconsistent. Studies also suggest that vitamins would be beneficial to individuals who are antioxidant-deficient or exposed to increased levels of oxidative stress, for example, smokers, diabetics and elderly patients, emphasising the importance of subgroup targeting. Through defining the right population group and the optimal vitamin combination we could potentially find a future role for vitamins in CVD.

**Antioxidants: Vitamin E: Vitamin C: β-Carotene: Cardiovascular disease: Heart disease**

CVD is believed to become the biggest cause of morbidity and mortality in men and women in the world in 2020[1], emphasising the great need for retarding the increase in disease incidence. Individuals with a high dietary intake of fruit and vegetables have a clear reduction in the incidence of CHD[2–5], stroke[6–9] and cardiovascular mortality[10,11]. Reactive oxygen species and free radicals have been implicated in the pathophysiology of CVD[12], with vitamins E and C and β-carotene being hypothesised as the fundamental protective components in fruit and vegetables. It has also been hypothesised that flavonoids and fibre are also likely to be potential fundamental protective components in fruit and vegetables.

The body is equipped with antioxidative enzymes, such as glutathione peroxidase and superoxide dismutase, and vitamins including vitamins E and C and β-carotene which cooperate and in some cases act synergistically to provide protection against oxidative stress. Atherosclerosis is the underlying cause of CVD, involving the accumulation of modified LDL in the intima of the arterial wall[13] enabling plaque progression[14] and the occurrence of cardiovascular events[15]. LDL particles contain about 2700 fatty acids of which approximately half are polyunsaturated and are susceptible to oxidation[16].

The identification of the oxidative modification hypothesis of LDL[14] and the strong correlation between the levels of oxidised LDL[17] and the *ex vivo* oxidative susceptibility of LDL[18] to the apparent extent of atherosclerosis provide a rationale for a role of oxidative stress in atherosclerosis. Oxidised LDL acts as a chemokine that stimulates the recruitment of circulating monocytes into the intimal space[19,20] and inhibits the exit of resident macrophages[20], enabling foam cell formation and cell-mediated LDL peroxidation. Oxidised LDL is cytotoxic[21–23] and also reduces NO bioavailability[24–26], which results in endothelial dysfunction. In accordance with the response-to-injury hypothesis of atherogenesis, this results in the progression of the atherosclerotic lesion[14] and consequent cardiovascular events[15]. The role of these vitamins (vitamins E and C and β-carotene) is emphasised by their inhibitory action on the oxidative modification of LDL[27,28] and their improvement of endothelial dysfunction[15,29]. Their therapeutic role has been supported by animal studies[30–33] and has been further supported by changes in lipid peroxide levels[34], *ex vivo* oxidisability of LDL[35,36], and plasma levels of these vitamins[37–41] being potential good predictors of future cardiac events and cardiovascular mortality.

**Abbreviations:** WACS, Women's Antioxidant Cardiovascular Study; WAVE, Women's Angiographic Vitamin and Estrogen.

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Susceptibility to LDL peroxidation is dependent on the levels of these vitamins[41] and only once they are fully depleted is rapid oxidation possible[42]. As a consequence these vitamins are frequently referred to, perhaps simplistically, as antioxidant vitamins. The role of these vitamins in reducing LDL oxidation has been most consistently shown for vitamin E while the data has been mixed for vitamin C and β-carotene. Therefore greater emphasis has been put on vitamin E in exploring a preventive or therapeutic role for these vitamins in CVD.

The role of these vitamins in CVD has long been emphasised mainly on the basis of their hypothesised antioxidant properties and the majority of trials have been initiated on this basis. However, in recent years research has greatly expanded in this area and studies now strongly support that the concept that these vitamins have other fundamental non-antioxidative properties, including actions on different aspects of the inflammatory responses that are involved in the pathogenesis of CVD. Each vitamin may have its own non-antioxidative properties, which will be discussed more in detail below, and as a consequence these vitamins target different aspects of the pathogenesis of CVD and as a result more emphasis can be put on the role of combination therapy. Through these properties a further novel role for these vitamins in CVD may be proposed. In the present review we will focus on putative antioxidant roles of these vitamins, but it should be emphasised that their non-antioxidative properties may be relevant to the modulation of CVD risk.

### *Vitamin E*

Vitamin E is the main chain-breaking lipid-soluble vitamin in plasma and LDL[43], present in a complex of four isomers (α-tocopherol, γ-tocopherol, β-tocopherol and δ-tocopherol), of which α-tocopherol is biologically the most active[44]. Supplementation with pharmacological doses ($\geq$150 IU/d, $\leq$1200 IU/d) of vitamin E has been shown to reduce LDL peroxidation[45,46] (1 mg vitamin E is equivalent to 1·49 IU vitamin E).

Atherosclerosis is now accepted to be a chronic inflammatory disease[27,47] and vitamin E has shown to mediate anti-inflammatory effects beyond its antioxidative properties[48–51]. Through these non-antioxidative properties vitamin E may target aspects of atherosclerosis beyond the oxidation of LDL, therefore extending its potential protective role in CVD. Vitamin E potentially reduces foam cell formation by decreasing monocyte recruitment[49,52], through reducing chemokine secretion[53] and by reducing the expression of scavenger receptors on macrophages (CD36)[54,55]. Vitamin E can also potentially reduce the progression of atherosclerosis by reducing adhesion molecule expression[48,51], inhibiting smooth muscle cell proliferation[56,57] and platelet aggregation[58,59] and by enhancing NO bioavailability[60]. These effects have been shown to be partly mediated *via* non-antioxidant mechanisms causing inhibition of signalling pathways, particularly protein kinase C[61,62], that have potentially been activated by oxidised LDL. Vitamin E has been shown to prevent oxidised LDL-induced NF-κB activation through suppressing protein kinase C[63] and inhibiting IκB degradation[64], further reducing the inflammatory response that is mediated in CVD.

Another anti-atherogenic property of vitamin E is its ability to modulate gene expression, such as up-regulating endothelial NO synthase mRNA expression and consequently NO levels[65], hence protecting the endothelium. Vitamin E has been shown to prevent endothelial dysfunction through protecting the endothelium against reactive oxygen species and oxidised LDL[66] and through stimulating endothelial cell proliferation[67,68] and reducing endothelial apoptosis[69,70]. These effects are mediated by mechanisms beyond that of inhibition of oxidation of LDL, which include inhibition of oxidised LDL-induced protein kinase C stimulation[66], possibly *via* an activation of a phosphatase PP2A[71], modulation of the Bcl-2 family of apoptosis-related proteins[72], by inhibiting caspase-3 activity[69] and by inhibiting the oxidised LDL-induced up-regulation of angiotensin II receptor (AT1R) mRNA and protein. These properties have been further supported by animal studies[73].

These effects of α-tocopherol have only been confirmed by *in vitro* studies and animal studies but not yet *in vivo*. The importance of vitamin E in protecting against atherosclerosis has been further supported by the vitamin E-deficient mouse model, which suffered from increased levels of oxidative stress and atherosclerosis[74].

### *Vitamin C*

The independent role of vitamin C in CVD has not been extensively assessed in clinical trials. However, as LDL oxidation occurs substantially in the sub-endothelial space[75], vitamin C may be most important in maintaining the reduced state of vitamin E. Water-soluble antioxidant vitamins, predominantly vitamin C, work to prevent the consumption of hydrophobic antioxidant vitamins such as vitamin E and β-carotene[76] and ensure their recycling[77], therefore playing an important role in maintaining antioxidative protection. Therefore vitamin C can act synergistically with these other vitamins, enhancing the benefit achieved with supplementation. Like vitamin E, vitamin C has been shown to have additional non-antioxidant properties. Vitamin C has been shown *in vivo* to suppress endothelial apoptosis mediated by inflammatory cytokines and oxidised LDL[78] and it has been shown to promote the proliferation of endothelial cells and the inhibition of vascular smooth muscle growth[67] *via* the extracellular signal-regulated kinase-signalling pathway[79]. It has also been suggested that vitamin C has a role in preventing restenosis postangioplasty[80]. In fact the combination of vitamins C and E exhibited a stronger positive effect than vitamin C or vitamin E did on their own. Vitamin C has the ability to modulate gene expression and through down-regulating intercellular adhesion molecule-1 gene expression it can reduce monocyte adherence to the endothelium[81]. Vitamin C has also been shown to enhance NO synthesis in endothelial cells[82] and *in vivo* it has been shown to have sustained beneficial effects on endothelial-derived NO-dependent flow-mediated dilation[83]. Vitamin C supplementation has also been shown to reduce vascular smooth muscle cell apoptosis and therefore prevent plaque instability in late-stage atherosclerosis[84].

### *β-Carotene*

β-Carotene is a fat-soluble vitamin present together with vitamin E in the lipid core of LDL particles[41]. It is an excellent

trapper of singlet oxygen and potentially a second-line antioxidative defence for LDL particles once vitamin E has been utilised[42]. The role of carotenoids in oxidative protection has been inconsistent, data indicating neutral[10,45,85], anti-[86,87] and pro-oxidant[88,89] properties. The pro-oxidant effects have been proposed to be due to the tendency of β-carotene radicals reacting with oxygen to give rise to peroxyl radicals that mediate lipid peroxidation[86]. Serum carotenoid levels have been inversely associated with atherogenic factors[90], risk of atherosclerosis[91] and cardiovascular mortality[92]; however, these studies looked at the possible effect of a combination of carotenoids and did not assess the independent effect of β-carotene.

High dietary intake of vitamin E[93–97], vitamin C[98,99] and β-carotene[96,100,101] has been inversely associated with the incidence of CHD. High dietary intake of β-carotene has been associated with a reduced CVD mortality[102] and all-cause mortality[103]; however, this was restricted to elderly individuals.

The favourable safety profile of these vitamins[104,105] has allowed several clinical trials to be conducted attempting to confirm their role. At this point the results have been inconsistent, with a few small trials suggesting a protective role while large-scale trials have concluded no benefit with vitamin supplementation in patients at high risk of CVD[106–116], or with pre-existing CVD[117–120].

There have been several explanations for this lack of correlation between observational studies and randomised controlled trials. The lack of benefit in randomised controlled trials could suggest that these vitamins are not the protective components in fruit and vegetables. As the results of observational studies can be as a consequence of confounding factors it is possible that other components of fruit and vegetables are the mediators of cardiovascular protection, such as flavonoids, fibre, etc.

However, the lack of benefit could also be a consequence of the differences in duration, vitamin dosages and target population between observational studies and randomised controlled trials.

Observational studies have been conducted on an average for 11 years while randomised controlled trials have continued for an average of 4 years, which can suggest that supplementation needs to be conducted for a longer period of time to gain benefit. Steinberg[121] hypothesised that antioxidants were targeting early stages of atherosclerosis so that the average 4·5-year duration of the majority of trials was too short to achieve beneficial effects. However, none of the pre-existing trials have indicated any trend towards a protective role and the two trials conducted over more than 10 years[108,113] have further disputed the role of antioxidants in CVD. Therefore before the trial duration is extended other areas should be addressed. The lack of detailed knowledge of the mechanism of oxidative modification has restricted us in defining an optimal antioxidant vitamin. The lack of efficient biomarkers for oxidative stress has not allowed us to assess *in vivo* effectiveness of these vitamins' antioxidant properties and define the optimal vitamin dosage. Whether the dosage of these vitamins plays a role in their beneficial effects is addressed in the present review.

The targeted population is still undefined; however, pre-existing evidence is suggestive of targeting subgroups such as smokers, diabetics and the elderly.

These vitamins have been shown to mediate effects beyond their antioxidative properties; however, at this point these have only been shown *in vitro* and not yet explored in *in vivo* studies. The present review will address the hypotheses that have been put forward to try to explain the lack of benefit with these vitamins in randomised controlled trials, provide further evidence regarding their role in CVD and explore what the future may entail for vitamin therapy in CVD.

## Dosage, oxidative markers and isomers

The optimal vitamin dosage has not yet been defined. Nutritional doses of vitamin E (about 4–8 mg/d)[93–96] and vitamin E supplementation for at least 2 years with >100 IU/d[96,97] with a maximum dose of 1000 mg/d[104] have been beneficial in CHD. However, the majority of observational studies have shown disappointing findings in regards to supplemental intake of vitamin E (≤25 mg/d up to ≥250 mg/d)[93,94]. Randomised clinical trials supplementing with 330–800 IU vitamin E per d have also been disappointing[108,110,115,122–125]. The doses of these vitamins used in trials have been questioned, on the one hand for being suboptimal and on the other for being too high. Studies by Jialal *et al.*[126] and Simons *et al.*[127] and findings from observational studies support the concept that the dosages used in trials are not suboptimal. The use of mega-doses of these vitamins has been disputed due to their potential pro-oxidant[45,128,129] and pro-atherogenic effects[130] and their negative drug interactions[131,132]. Even though adverse effects are uncommon and shown to occur at doses well above those used in trials, it is possible that these override their beneficial effects, giving no net gained benefit.

The Vitamin E Atherosclerosis Prevention Study (VEAPS) trial[110] indicated that a level of oxidative protection is needed to be achieved to gain anti-atherogenic effects, which is suggested by trials to be achieved with 800 IU RRR-α-tocopherol per d[133–135]. Vitamins' antioxidative effectiveness is assessed *ex vivo* or through plasma or urinary levels of oxidised biomarkers and it is not clear whether this accurately estimates arterial wall oxidation. These vitamins have been shown to reduce levels of oxidative stress in plasma but not in plaques[121]. Out of eighteen large-scale trials, only three assessed the effect that vitamin supplementation had on the level of oxidative stress (Table 1)[107,108,110–112,114–116,118–120,122–126,133–136].

Failure to achieve the oxidative threshold could be the underlying reason behind the disappointing findings of trials. Through identifying more accurate oxidative biomarkers we could assess whether these vitamins mediate their predicted antioxidative effects and identify dose–response curves for optimal oxidative and inflammatory protection.

It has been proposed that the vitamin isomer used in trials is relevant in regards to its effects. The trials that have concluded a positive effect with vitamin E all used RRR-α-tocopherol[112,133–135] and five out of nine trials that indicated neutral effects used all-rac α-tocopherol[110,115,119,122,124]. Stereoisomers differ structurally and as a result this can restrict their participation in signalling pathways and in other processes, which can result in them not mediating their non-antioxidative actions including the anti-inflammatory effects discussed previously. It is therefore possible that due to the vitamin E

British Journal of Nutrition

**Table 1.** Trials assessing antioxidant effectiveness*

| Trials | Plasma levels of antioxidants | Level of LDL oxidation |
|---|---|---|
| Supplémentation en Vitamines et Minéraux Antioxydants (SU.VI.MAX)[107] | na | na |
| Atorvastatin Simvastatin Atherosclerosis Progression (ASAP)[112] | Yes | Yes |
| Chinese Linxian study[116] | Yes | na |
| Transplant-associated arteriosclerosis[133] | Yes | na |
| Women's Angiographic Vitamin and Estrogen (WAVE)[118] | Yes | na |
| MRC/BHF Heart Protection Study (HPS)[119] | Yes | na |
| HDL-Atherosclerosis Treatment Study (HATS)[120] | Yes | na |
| Women's Antioxidant Cardiovascular Study (WACS)[136] | Data not yet available | Data not yet available |
| Women's Health Study (WHS)[108] | Yes | Yes |
| Vitamin E Atherosclerosis Prevention Study (VEAPS)[110] | Yes | Yes |
| Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC)[115] | Yes | na |
| Primary Prevention Project (PPP)[122] | na | na |
| Heart Outcomes Prevention Evaluation (HOPE)[125] | na | na |
| Secondary Prevention with Antioxidants of Cardiovascular disease in End-stage renal disease (SPACE)[134] | na | na |
| Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)[124] | na | na |
| Cambridge Heart Antioxidant Study (CHAOS)[135] | Yes | na |
| Carotene and Retinol Efficacy Trial (CARET)[114] | na | na |
| Physicians' Health Study II (PHS)[111] | na | na |

na, Not been assessed in the trial.
* This Table looks at how intervention studies that are assessing the role of antioxidants in CVD have tried to assess the effectiveness of antioxidants, with some of them measuring antioxidant plasma levels and a few measuring the level of LDL oxidation.

isomer that is used for supplementation in trials the non-antioxidative effects of vitamin E are not observed. All-rac α-tocopherol has a lower bioactivity than RRR-α-tocopherol[137,138] and has been shown to lack anti-inflammatory properties[139] at dosages where this is achieved by RRR-α-tocopherol[140].

## The interaction of exogenous and endogenous vitamins: are we using the wrong vitamin?

Traber[141] hypothesised that single supplements may interfere with the uptake, transport, distribution and metabolism of other non-supplemented antioxidant nutrients. The disappointing results of clinical trials could therefore be a result of vitamins' negative interaction with other potentially protective vitamins. Even though studies have emphasised a role for α-tocopherol, γ-tocopherol has been shown to have an anti-atherogenic role[142] and a superior anti-inflammatory effect to that of α-tocopherol[143]. The main constituent of vitamin E supplementation is usually α-tocopherol, which has been implicated in reducing γ-tocopherol levels[46,144,145] through competing for the same intestinal uptake mechanism[145]. Therefore the lack of benefits in trials could potentially be due to the gain from one vitamin causing the loss in protection mediated by another vitamin.

As we still lack knowledge regarding the mechanism of LDL oxidation *in vivo* the optimal vitamin in this context has not been defined. Total carotenoid intake has been associated with a reduced cardiovascular incidence and mortality[5,10] and the lack of benefit with β-carotene is suggestive that this is the wrong carotenoid. Lycopene is a singlet oxygen scavenger which is part of the carotenoid family and has been predicted to be a stronger antioxidant vitamin than β-carotene[146]. High plasma levels of lycopene have been associated with a reduced risk of atherosclerosis[147,148] and CVD[149,150]. The effects of lycopene have not yet been assessed in large-scale trials.

## Is combination therapy superior to single vitamin supplementation? Should we avoid β-carotene?

These vitamins show different efficacy depending on the type of oxidative stress and the body compartment in which it takes place. The lack of knowledge regarding where and how LDL undergoes oxidative modification has restricted us in defining the optimal vitamin type. As these vitamins each possess a specific role in the antioxidant defence system, through the use of a combination of vitamins the overall protection would potentially be broadened.

The potential superiority of combination therapy may be predicted from the following:

(1) Protective effects seen in observational studies with high dietary intake of fruit and vegetables containing several of these vitamins;
(2) Lack of benefit in randomised clinical trials with single compound supplementation;
(3) Pro-oxidant effect of these vitamins in the absence of required cofactors;
(4) Experimental data for the cooperative and synergistic effects of vitamins.

In fruit and vegetables there is a natural interaction between hydrophobic (for example, vitamin E) and hydrophilic (vitamin C) antioxidant vitamins that is lost with single vitamin supplementation and this could account for the lack of benefit. Supplementation with only one of these vitamins could result in an imbalance of endogenous antioxidants which weakens the antioxidant defence system and enables pro-oxidant effects to emerge[151], as with tocopherol-mediated atherosclerosis seen with high doses of vitamin E[152]. Through increasing the dietary intake of fruit and vegetables this results in increased levels of these vitamins in the 'right environment'. Therefore through combination supplementation using doses of vitamins in physiological ratios we can optimise antioxidant status without resulting in an imbalance in the endogenous

antioxidant levels. These vitamins have shown to act synergistically to mediate protection against oxidative stress. Vitamin C has been shown to regenerate vitamin E from its oxidised to its active state[77,153,154], to minimise its pro-oxidant effects[155] and to cause synergistic inhibition of LDL peroxidation[156,157]. β-Carotene has also been shown to act synergistically with vitamin E[158]. It can then be hypothesised that this enhanced protection against oxidative stress should provide a greater anti-atherogenic effect and as a consequence reduce the incidence of clinical endpoints. However, clinical trials using a 'cocktail' of vitamins have not indicated any such positive effects[107,116–119]. Jialal & Grundy[159] and Fuller *et al.* [160] concluded that combinations of these vitamins at doses similar to those used in trials (400–800 IU vitamin E per d, 1 g vitamin C per d and 30 mg β-carotene per d) did not provide further oxidative protection of LDL compared with a high dose of α-tocopherol (800 IU/d) on its own. This is therefore suggestive that the combination of these vitamins does not cause a greater reduction in lipid peroxidation than that attributable to single vitamin supplementation.

The majority of the 'cocktail' supplementations used in trials have included β-carotene[107,111,116,119,120,161] despite the pre-existing data disputing a role for β-carotene as an antioxidant vitamin, and even indicating pro-oxidant effects at the dosages used in trials[88,89,162]. Together with the negative effects seen in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC)[106] and the Beta-Carotene and Retinol Efficacy Trial (CARET)[114] its use in CVD is highly questionable, particularly in smokers. The trials including β-carotene supplementation have overall failed to show a beneficial role in CVD (Table 2) [107,111,116,119]; however, a combination of vitamins excluding β-carotene has indicated a beneficial role in atherosclerosis (Table 3) [112,163]. *In vivo* the carotenoids do not appear alone but in a heterogeneous mixture, possibly acting synergistically[164,165]. Through supplementation with only one carotenoid this can potentially lead to negative effects. Therefore the lack of overall protection with combination therapy could be as a result of a net negative balance between β-carotene pro-oxidant and vitamin E and C antioxidant effects. The beneficial effects in the Transplant-Associated Arteriosclerosis Trial[133] and the Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) trial[112] do, however, support a role for vitamins C and E in combination in preventing the progression of atherosclerosis. However, trials have shown contradictory results with the combined supplementation of vitamin C and vitamin E with regard to clinical endpoints, with one suggesting protective effects[163] and the other indicating neutral effects[117].

## The role of vitamins in the progression and complication of atherosclerosis: should we start these vitamins earlier?

The role of these vitamins in preventing the progression of atherosclerosis and destabilisation of plaques has not been fully confirmed. Oxidised LDL has been shown to stimulate smooth muscle proliferation[166,167] and platelet aggregation[168] and to be an independent marker for the destabilisation of plaques[169]. These vitamins have *in vitro* been shown to reduce platelet aggregation[170,171] and modulate smooth muscle phenotype[71], potentially playing a role in retarding the progression of late-stage atherosclerosis, hence attempts to use them in secondary prevention. However, the neutral effects seen in secondary prevention trials may be indicative of the wrong timing of supplementation. Animal studies and observational studies have indicated a therapeutic role through assessing their effect on early lesions while in trials the primary endpoints have been the incidence of major vascular events. Steinberg & Witztum[172] suggested that antioxidant vitamins are only protective when given before the development of disease, prioritising a role for them in primary prevention.

A meta-analysis of secondary prevention trials concluded that there was a lack of anti-atherogenic effect of vitamin supplementation[173] and individuals with late-stage atherosclerosis and pre-existing CVD actually had increased cardiac and all-cause mortality with vitamin supplementation[119,136,174,175]. This negative effect on fatal and non-fatal CHD is not seen in individuals without pre-existing CHD[176] and the use of vitamins in these individuals has even suggested a 30 % reduction in overall mortality[177]. The negative effects of these vitamins on late-stage atherosclerosis may be due to their limiting effect on ischaemic pre-conditioning[178], negative interaction with drugs commonly taken by these patients such as nitrates, warfarin and diuretics[177] and their pro-oxidant effects[179] that can destabilise the plaque. These findings are suggestive that vitamin supplementation may have an adverse effect on plaque-related complications and, if so, its use should be restricted to those with early stages of disease, excluding individuals with late-stage atherosclerosis. However, this is difficult in practice. In Western populations atherosclerosis begins early in life, implying that such supplementation should be initiated in childhood and continued for decades. At the same time most adults, certainly those with overt CVD, will have late atherosclerotic lesions.

## Directing vitamin use to subgroups

Jialal *et al.* [180] concluded that LDL preparations from different individuals showed different susceptibility or resistance to oxidation. Studies have indicated inter-individual variation in the response seen with antioxidants[181], suggesting that individuals exposed to increased levels of oxidative stress or who were antioxidant deficient would gain more benefit[182]. Vitamin E has been shown to have a variable antioxidant effect that is dependent on the rate of lipid peroxidation[183] and supplementation studies with vitamin E have indicated no significant effect on lipid peroxidation *in vivo* in healthy individuals[184,185]. These results dispute the role of vitamin E supplementation in individuals with normal baseline levels of antioxidants and oxidative stress (who then appear to be 'non-responders'). As the majority of trial participants meet their RDA of these so-called antioxidant vitamins and with none of the large clinical trials assessing baseline levels of oxidative stress it is possible that the inclusion of 'non-responders' dilute the overall beneficial effect that is seen with responders, accounting for the disappointing overall findings. Clinical trials targeting individuals with an abnormal antioxidant status have shown more consistent benefits[109,133,134,161], indicating a role for subgroup targeting.

The Cambridge Heart Antioxidant Study (CHAOS) trial[135] concluded that there was a significant reduction in

**Table 2.** Intervention studies: combination of antioxidants including β-carotene[121,255,292]*†

| Target group | Trial | Study design | Antioxidant type and dosage in treatment group | Results and conclusion |
|---|---|---|---|---|
| Primary prevention | SU.VI.MAX[107] | Participants: approximately 12 800 middle-aged men and women were treated for 7·5 years<br>Primary endpoint: incidence of cancer, ischaemic CVD and all-cause mortality | Vitamin E: 30 mg/d<br>Vitamin C: 120 mg/d<br>β-Carotene: 6 mg/d<br>Se: 100 mg/d<br>Trace elements (Zn): 20 mg/d | No difference in the incidence of cardiovascular events and all-cause mortality between the intervention and placebo group |
| Primary prevention | Physician Health Study II[111] | Participants: 15 000 healthy male physicians aged about 55 years old were treated for 12 years<br>Primary endpoint: incidence of CVD, cancer and eye disease | Firstly randomised to β-carotene or placebo, then further randomised to vitamins E, C and multivitamins<br>Vitamin E: 400 IU<br>β-Carotene: 50 mg (alternate days)<br>Vitamin C: 500 mg/d<br>Multivitamins (daily) | Awaiting CVD results |
| Primary prevention | Chinese study in Linxian Province[116] | Participants: approximately 30 000 men and women at high risk of CVD were treated for 5·2 years<br>Primary endpoint: incidence of cancer, cancer mortality and mortality from other diseases | First factor (nutrient combination):<br>Vitamin E: 30 mg/d<br>β-Carotene: 15 mg/d<br>Se: 50 μg/d<br>Second factor: Vitamin C: 120 mg/d<br>Mo: 30 μg/d | First factor: supplementation caused no reduction in the incidence of CVD<br>Second factor: combination of vitamin C and Mo caused no reduction in incidence of CVD |
| Secondary prevention | MRC/BHF Heart Protection Study[119] | Participants: approximately 20 500 individuals with pre-existing CVD or diabetes were treated for 5 years<br>Primary endpoint: incidence of vascular and non-vascular mortality and major morbidity | Vitamin E: 600 mg/d (all-rac-α-tocopherol)<br>Vitamin C: 250 mg/d<br>β-Carotene: 20 mg/d | No significant difference in all-cause and cardiovascular mortality and incidence of vascular events between the vitamin- and placebo-allocated groups. There was an increase in all-cause mortality in the supplemented group compared with the placebo group |
| Secondary prevention | HATS[120] | Participants: 160 patients with pre-existing CHD, low HDL and normal LDL levels were treated for 3 years<br>Primary endpoint: arteriographic evidence of coronary stenosis change and the first occurrence of cardio vascular event (death, myocardial infarction, stroke or revascularisation) | Vitamin E: 800 IU/d (d-α-tocopherol)<br>Vitamin C: 1000 mg/d<br>β-Carotene: 25 mg/d<br>Se: 100 μg/d<br>Second treatment: Simvastatin and niacin 2 × 2 factorial design | In combination with simvastatin and niacin, antioxidants blunted their effect on the rise in HDL-2 levels (15 % reduction), the reduction in level of stenosis (0·4 % regression *v.* 0·7 % progression) and there was a 12 % reduction in the number of individuals free of events |
| Secondary prevention | The Indian Experiment of Infarct Survival-3[161] | Participants: approximately 130 patients with suspected acute myocardial infarction were treated for 28 d<br>Primary endpoint: effect on cardiac enzymes and complications of acute myocardial infarction | Vitamin E: 400 mg/d<br>Vitamin C: 1000 mg/d<br>β-Carotene: 25 mg/d<br>Vitamin A: 50 000 IU/d | The combined antioxidant treatment caused a significant reduction in infarct size, QRS score, reduced occurrence of angina pectoris and a 30 % reduction in risk of cardiac endpoints compared with the placebo group |

SU.VI.MAX, Supplémentation en Vitamines et Minéraux Antioxydants; HATS, HDL-Atherosclerosis Treatment Study.

*This Table includes intervention studies (double-blinded randomised controlled trials) that assess the effect of a combination of antioxidants (that include β-carotene) on CVD. It provides information regarding the structure of the intervention studies and their outcomes.

†1 mg vitamin E per d is equivalent to 1·49 IU vitamin E per d.

**Table 3.** Intervention studies: combination of antioxidants excluding β-carotene[121,255,292]* †

| Target group | Trial | Study design | Antioxidant type and dosage in treatment group | Results and conclusion |
|---|---|---|---|---|
| Primary prevention | St Francis Heart Study[163] | Participants: approximately 1000 asymptomatic apparently healthy individuals with elevated coronary Ca score were treated for 4·3 years<br>Primary endpoint: Composite of atherosclerotic cardiovascular events | Vitamin E: 1000 IU/d<br>Vitamin C: 1 g/d<br>Atorvastatin: 20 mg/d | Participants with elevated Ca scores had a 42 % significant reduction in composite endpoint with antioxidant therapy compared with placebo. However, this study was underpowered |
| Primary prevention | ASAP[112] | Participants: 520 middle-aged high-risk men and postmenopausal women with hypercholesterolaemia (>5 mmol/l) were treated for 3 years<br>Primary endpoint: level of IMT in common carotid artery | Vitamin E: 272 IU/d (RRR-α-tocopheryl acetate)<br>Vitamin C: 500 mg/d (slow-release ascorbic acid) | 33 (95 % CI 4, 62) % significant reduction ($P=0·024$) and 14 % NS reduction in IMT in supplemented men and women respectively compared with placebo group<br>The lack of benefit in women could be due to a lack of reduction in isoprostane levels in women |
| Secondary prevention | WACS[136] | Participants: approximately 8200 female health professionals with pre-existing CVD or more than three CVD risk factors and >40 years old were treated for 4 years<br>Primary endpoint: incidence of cardiovascular events (non-fatal myocardial infarction, stroke and coronary revascularisation) and total CVD mortality | Vitamin E: 600 IU/d (RRR-α-tocopherol)<br>Vitamin C: 500 mg/d<br>β-Carotene: 50 mg/d<br>Out of 8171, 5442 participants were randomised to receive: folic acid/ vitamin $B_6$/vitamin $B_{12}$ $2\times2\times2$ factorial design | The combination of vitamin C and vitamin E alone had no effect on the primary endpoints compared with the placebo and single supplementation groups<br>There was a significant reduction in the incidence of stroke with the combination of vitamin C and vita min E ($P=0·03$)[114] |
| Secondary prevention | WAVE[118] | Participants: 423 postmenopausal women with at least 15 to 75 % coronary stenosis at baseline were treated for 5 years<br>Primary endpoint: the level of progression of coronary lesions | Vitamin E: 400 IU/d<br>Vitamin C: 500 mg/d<br>Second treatment: Conjugated equine oestrogen: 0·625 mg/d $2\times2$ factorial design | With antioxidant supplementation there was an increase of 0·044 mm/year in the coronary stenosis compared with placebo group<br>It was also associated with an increase in all-cause mortality rates compared with placebo (hazard ratio: 2·8; 95 % CI 1·1, 7·2; $P=0·047$) |
| Secondary prevention | Transplant Associated Arteriosclerosis Trial[133] | Participants: forty cardiac transplant patients (0–2 years after cardiac transplantation) were treated for 1 year<br>Primary endpoint: changes in the intimal index | Vitamin E: 400 IU twice daily (RRR-α-tocopherol)<br>Vitamin C: 500 mg twice daily | After 1 year there was no change in intimal index in the supplemented group while there was an 8 % increase in the placebo group. The magnitude of benefit was larger in cardiac transplant patients with endothelial dysfunction[293] |

ASAP, Atorvastatin Simvastatin Atherosclerosis Progression; IMT, intima-to-media thickness; WACS, Women's Antioxidant Cardiovascular Study; WAVE, Women's Angiographic Vitamin and Estrogen.

* This Table includes intervention studies (double-blinded randomised controlled trials) that assess the effect of a combination of antioxidants (that exclude β-carotene) on CVD. It provides information regarding the structure of the intervention studies and their outcomes.

† 1 mg vitamin E per d is equivalent to 1·49 IU vitamin E per d.

cardiovascular events with α-tocopherol supplementation. Brown[186] concluded that a large number of these patients had a 3·5-fold increase in frequency for a polymorphism in the endothelial NO synthase gene that made them more prone to endothelial dysfunction and of greater need for vitamin E, hence further supporting subgroup targeting. However, it is still hard to accurately identify individuals exposed to increased oxidative stress due to the lack of efficient biomarkers for oxidative stress.

Patients with cardiovascular risk factors are exposed to greater amounts of oxidative stress[187], which contributes to endothelial dysfunction[188,189]. The enhanced level of oxidative stress is partly due to their reduced dietary intake of these so-called antioxidant vitamins[190] and this could possibly be responsible for the increased rate of atherosclerosis seen in these patients. The use of vitamins could retard the development of cardiovascular risk factors and reduce the risk of CVD.

Vitamin supplementation has been shown to improve endothelium-dependent dilatation in smokers[191] and in hypercholesterolaemic[192], hypertensive[193] and diabetic patients[194].

*Subgroup 1: smokers*

Smoking is associated with an increased progression of atherosclerosis[195] and of heart disease[196], possibly mediated by exposure to increased levels of oxidative stress[197–199]. In smokers, plasma ascorbic acid, α-tocopherol and β-carotene levels are significantly depleted[200–202], partly as a consequence of increased utilisation[203,204], reduced regeneration of ascorbic acid[205] and their poorer diet[206]. Smokers have also been shown to have a down-regulated enzymic antioxidant defence system with reduced levels of catalase and glutathione peroxidase[207] making them further prone to oxidative damage.

Supplementation with a combination of vitamins re-establishes a normal antioxidant status[208] and reduces oxidative stress[209–211] in smokers. The Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study[112] concluded a greater anti-atherogenic benefit in smokers than non-smokers. These vitamins have also on the other hand been shown to mediate a pro-oxidant effect with increased levels of oxidative stress[162] and the likelihood of this is enhanced in smokers[45,212]. However, the use of a combination of vitamins excluding β-carotene may prevent the increased likelihood of pro-oxidant effects and the negative findings encountered in the Beta-Carotene and Retinol Efficacy Trial (CARET)[114] and the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study[115].

*Subgroup 2: obese and overweight patients*

In obese and overweight individuals fat-soluble vitamins can potentially become retained in visceral tissue, which can cause reduced serum levels of these vitamins. It has been shown that obese children have significantly reduced circulating levels of vitamin E and β-carotene[213,214] and reduced LDL β-carotene and vitamin E levels[215] compared with normal-weight children. As a result these individuals may be more prone to oxidative stress with an increased likelihood of endothelial dysfunction and LDL oxidation. This can partly account for the increased risk of CVD in obese or overweight individuals and supports a therapeutic role for supplementation with these vitamins in these individuals. Vitamin E supplementation in obese and overweight individuals has been shown to improve the metabolic profile (HbA1c, serum malondialdehyde levels and erythrocyte glutathione peroxidase activity were reduced)[216], increase antioxidant levels and reduce pro-oxidants[216,217]. Randomised controlled trials are required to assess whether this reduction in oxidative stress reduces the development of CVD in obese or overweight individuals.

As obesity is a major risk factor for CVD, a large number of individuals in secondary prevention trials are likely to be overweight or obese. As the majority of trials do not directly assess the antioxidative actions of these vitamins *in vivo* and obese individuals are prone to the retention of vitamins in adipose tissue, it is plausible that these vitamins do not mediate their predicted effect in these individuals, possibly partly accounting for the lack of benefit seen in trials. This further emphasises the importance of identifying accurate biomarkers to assess vitamins' antioxidant effectiveness *in vivo*.

*Subgroup 3: hypercholesterolaemic patients*

Studies have shown that hypercholesterolaemic individuals have increased plasma lipid peroxide levels[218,219] and possess LDL that is more susceptible to oxidation[220,221]. As α-tocopherol activity has been shown to be inversely related to cholesterol content in plaques[222], these individuals are also prone to have a diminished antioxidant status. This suggests that these individuals would benefit from vitamin supplementation.

Vitamin supplementation has been shown to alter lipid profile, mediating a reduction in total cholesterol, TAG and LDL levels[223], and positively correlating with HDL levels in individuals without diagnosed disease[224,225]. The use of vitamins in hypercholesterolaemia can be questioned as they have been shown to blunt the beneficial effect of simvastatin/niacin[120]. However, α-tocopherol has been excluded as a potential cause of this response[226]. Nonetheless vitamin E supplementation in hypercholesterolaemic patients has resulted in a small but significant decrease in HDL-cholesterol levels and therefore caution still needs to be taken in regards to vitamin E supplementation[227].

Statins have been shown to reduce vitamin E, β-carotene and ubiquinol-10 levels[228] and it has therefore been suggested that they may worsen the antioxidant status. This could possibly be due to statins reducing the circulating LDL fraction and therefore the delivery of these vitamins. This fact further emphasises a probably beneficial role of vitamin supplementation in hypercholesterolaemic patients.

In recent studies it has been shown that patients taking 10 mg atorvastatin per d gain an increase in plasma level of vitamin E (+42 %; $P<0{\cdot}01$)[229] and dual therapy with vitamins and statins has appeared to provide greater cardiovascular protection than statins on their own[230]. The lack of negative interaction between these agents further emphasises a beneficial role of supplementation with these vitamins in hypercholesterolaemic patients.

*Subgroup 4: hypertensive patients*

It has also been hypothesised that oxidative stress plays a role in the pathogenesis of hypertension and hypertension-induced damage through reducing NO levels and inducing endothelial dysfunction[231]. Hypertensive patients have been shown to be exposed to increased levels of lipid peroxidation and to have abnormal antioxidant status[232]. Observational trials have shown an inverse correlation between fruit and vegetable intake[233], serum levels of putative antioxidant vitamins[234,235] and the development of high blood pressure. These vitamins have been shown in *in vitro* studies to play a role in the aetiology of hypertension by restoring NO activity and endothelial function[193,236,237]. Vitamin E (400 IU/d) and vitamin C (1000 mg/d) supplementation resulted in beneficial effects on endothelium-dependent vasodilatation and arterial stiffness[193] and in significantly lower systolic, diastolic and mean arterial blood pressure levels compared with the placebo group[238]. Hypertensive patients have been more consistently shown to possess a reduction in ascorbic acid levels than those of any other antioxidant vitamins[239], possibly indicating an advantage of vitamin C supplementation over the other vitamins in these patients. Dietary intake[240] and plasma levels[241] of ascorbic acid have been inversely related to blood pressure in some studies but not all[242,243]; in view of the lack of long-term benefit[244] further research is required.

*Subgroup 5: diabetic patients*

Diabetic patients are exposed to increased levels of lipid peroxidation[245] as a result of LDL glycation[246] and their increased levels of the small dense LDL subfraction[247], contributing to their high risk of macrovascular complications[248]. Vitamin E supplementation with doses that are greater than 800 IU/d in type 1 and 2 diabetic patients have been shown to reduce the oxidisability of LDL[249,250] and improve endothelial function[194,251]. Supplementation with high-dose α-tocopherol has been associated with a reduced incidence of CHD[252] and microvascular complications[253] in diabetic users compared with non-users. Supplementation with 400 IU vitamin E per d also resulted in a significant reduction in cardiovascular events compared with a placebo group[254]. However, its long-term effects have not been confirmed by trials[255,256], possibly due to the use of lower vitamin dosages than those that have indicated short-term benefit in the small supplementation studies. At this point the available data are still too sparse to suggest the recommendation of vitamins to diabetic patients and more emphasis should be placed on targeting other diabetic-associated atherogenic factors.

*Subgroup 6: patients with end-stage renal failure*

IHD remains a leading cause of death in end-stage renal failure patients. Vitamin supplementation has been beneficial to patients with end-stage renal failure[134,257] through reducing their increased levels of oxidative stress[258,259]. The Secondary Prevention with Antioxidants of Cardiovascular disease in End-stage renal disease (SPACE) trial showed a 70 % reduction in myocardial infarct rates in haemodialysis patients with pre-existing CVD when supplemented with high-dose vitamin E[124,257].

*Subgroup 7: cardiac transplant or acute myocardial infarction patients*

Atherosclerosis is a major complication of transplantation that limits the prolonged benefit of the transplant[260].

Vitamin supplementation has been beneficial to cardiac transplant[133] and acute myocardial infarction patients[161,261] in reducing their increased levels of oxidative stress[262,263], making these vitamins a possible novel treatment for improving survival in these patients. The Indian Experiment of Infarct Survival[161] and the Myocardial Infarction and Vitamins (MIVIT) pilot[261] trial both confirmed a role for these vitamins in preventing post-myocardial-infarction complications and cardiac events. They have also been implicated in reducing the rejection of allogenic grafts[264], further emphasising a role in transplant patients.

The increased risk of congestive heart failure in vitamin E-supplemented post-myocardial-infarction patients[125,265] indicates, however, the need of further trials. The authors of these trials hypothesised that these negative findings were due to pro-oxidant generation mediated by vitamin E.

*Subgroup 8: elderly individuals*

Elderly individuals are exposed to increased levels of oxidative stress[185]. Cohort studies[102,103,266] and trials[108] have both indicated benefit with supplementation in the elderly. In a subgroup analysis of the Women's Health study, only individuals above the age of 65 years gained a reduction, of 26 %, in cardiovascular events[108]. The Atherosclerosis Risk in Communities (ARIC) study concluded an age-relationship between dietary intake and carotid atherosclerosis, with supplementation only showing benefit in women above the age of 55 years[267]. This therefore suggests that vitamin supplementation would be of benefit to elderly individuals.

## Discussion

Antioxidant research has so far failed to confirm a role for vitamin E, vitamin C and β-carotene in the primary or secondary prevention of CVD. A total of nine primary and eleven secondary prevention trials, including approximately 150 000 and 60 000 participants respectively, have been disappointing. If there is a role for these vitamins in CVD, why is it that we have not identified it through trials? It has long been known that a high intake of fruit and vegetables is associated with a reduced incidence of CVD and it was initially hypothesised that vitamin E, vitamin C and β-carotene were the fundamental protective components that mediated this effect; as a consequence a range of studies was initiated to confirm their role.

*Observational studies*

It has long been believed that observational studies show that a high dietary intake of these vitamins is associated with a reduced risk of CVD and that there is a discrepancy between these studies and trials. However, even though this is true in

British Journal of Nutrition

regards to vitamin E, this is not the case when it comes to vitamin C and β-carotene. Observational studies have shown an inverse correlation between dietary intake of vitamin E (about 4–8 mg/d) and the incidence of CHD[93–95]. But the majority of studies have indicated no benefit with increased dietary intake of vitamin C[92,94,96,98,102] and β-carotene[93,268,269] and those that have indicated a beneficial role have not adjusted for vitamin E intake[95] and hence its effects. The lack of benefit with a high dietary intake of β-carotene (about 890–5500 μg/d) and vitamin C (about 50–170 mg/d)[93] is suggestive that β-carotene and vitamin C are not the relevant protective components in fruit and vegetables, therefore making one question whether they have a protective role in CVD.

### *Randomised controlled trials*

*Vitamin E.* The positive evidence achieved with vitamin E in observational studies has led to more emphasis being put on vitamin E supplementation in randomised controlled trials. However, these positive findings achieved with vitamin E have not been possible to reproduce in randomised controlled trials. The underlying reason behind this discrepancy is still unclear. Even though observational studies have indicated a protective role for dietary intake of vitamin E, the beneficial role of vitamin E supplementation in CVD has only been supported by three studies[96,97,266]. Trials looking at the effect of vitamin E supplementation using dosages between 330 and 800 IU/d have not supported a protective role for vitamin E in CVD. It has been argued that the dosages of vitamin E used in trials are too high, which causes the loss of beneficial effect. However, the supplementation of vitamin E with lower doses of ≤4·91 IU/d[94] has not indicated any benefits even though this is equivalent to the level of vitamin E that is achieved with dietary intake and that has shown benefits in observational studies. Results from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) trial showed an increased incidence of haemorrhagic stroke in the vitamin E-supplemented group compared with the placebo group[115]; however, a prospective cohort study including participants from this trial concluded that those with higher circulating α-tocopherol within the normal range had a significantly lower total and CVD mortality[106].

*Vitamin C.* With regard to vitamin C, studies have shown that supplementation with >500 mg vitamin C per d is associated with a lower risk of CHD[93,98], suggestive that higher doses of vitamin C are required to mediate protective effects. However, the secondary prevention trial Women's Antioxidant Cardiovascular Study (WACS)[117] concluded no independent benefit of 500 mg vitamin C per d on cardiovascular endpoints, therefore disputing its independent therapeutic role in CVD.

*β-Carotene.* Randomised controlled trials that have assessed the effect of single supplementation with 20–50 mg β-carotene per d have not only been disappointing but have even been shown to increase the risk of CVD[114,115]. The levels of β-carotene used in trials is about 10 000 times greater than the levels used in dietary intake and it is possible that β-carotene mediates pro-oxidant effects at these levels, accounting for the negative results achieved.

*Combinations.* The lack of benefit seen in trials with the independent supplementation of vitamin E, regardless of the dosage used, or β-carotene or vitamin C supplementation is highly suggestive that supplementation with a single vitamin does not provide any reduced risk of CVD. As fruit and vegetables contain a range of vitamins in potential symbiosis this suggests that to gain benefit from vitamin supplementation it is necessary to try to mimic this environment through using a 'cocktail' of vitamins. The majority of large-scale trials have used a 'cocktail' of vitamins that has included β-carotene (Table 2)[107,111,116,119,120,161] and the majority of these[107,116,119] have not shown a beneficial effect and even indicated a negative effect in supplemented patients in comparison with non-supplemented patients[119,120]. It can be hypothesised to be due to the pro-oxidant effects of β-carotene overriding the antioxidative effects mediated by the other vitamins in the supplementation. Trials that have assessed the role of a combination of vitamins excluding β-carotene have indicated a potential beneficial role in atherosclerosis[112,133] and on atherosclerotic cardiovascular events[163] but have not reduced the risk of CVD in large-scale trials[117]. The Women's Angiographic Vitamin and Estrogen (WAVE)[118] and WACS[117] trials showed disappointing findings with the combination of vitamin E and vitamin C in a secondary prevention study. In the WAVE trial there was actually a non-significant increase in coronary stenosis and all-cause mortality in post-menopausal women with 15–75 % coronary stenosis who were supplemented with the combination of vitamins E and C, compared with the placebo group. The reason behind the disappointing findings can be three-fold. First, the target group in the WACS and WAVE trials were women and it has been shown through other studies that they do not benefit significantly from vitamin supplementation with regard to CVD[107,112]. Second, these trials were secondary prevention trials while the two other trials[112,163] that showed positive results were primary prevention trials. The oxidative modification hypothesis and the findings from prospective studies have suggested a beneficial role for supplementation with these vitamins in primary prevention; however, this has not been confirmed by clinical trials in regards to clinical endpoints. A role for these vitamins in secondary prevention has been disputed, with the evidence pointing towards an increase in total mortality in supplemented individuals with late-stage atherosclerosis[161,162,257]. The WACS and WAVE trials could have potentially included individuals who suffered from late-stage atherosclerosis, causing the negative effects mediated by this to blunt the predicted positive effect, giving an overall neutral effect. The neutral outcome or increase in clinical endpoints seen with the combination of vitamins C and E in secondary prevention trials suggests that supplementation is not an effective therapy in pre-existing CVD. However, the benefits seen in primary prevention trials suggest that the combined vitamin C and E supplementation may play a preventive role in those without pre-existing CVD. Third, the results from the WACS and WAVE trials could indicate that these vitamins are not the protective components in fruit and vegetables, further minimising the hope of a protective role for these vitamins in CVD.

*Subgroup targeting.* Let us consider the possibility that these vitamins have an optimal dose beyond which further intake does not mediate additional protection against oxidative

stress (Fig. 1) and hence does not reduce LDL oxidation further. Salonen *et al.* [270] showed that *ex vivo* oxidisability and levels of lipid peroxide products were some of the strongest predictors of a 3-year increase in carotid wall thickness, which further supports a role for lipid peroxidation in atherosclerosis. As women are exposed to fewer cardiovascular risk factors[271] and have higher baseline serum concentrations of vitamins[272] they may be exposed to less oxidative stress compared with men. Therefore a lower intake of these vitamins may be required to achieve the optimal effect for the maximum protection against lipid peroxidation in women compared with men, explaining the benefit achieved with dietary intake in women but not in men[93,94]. Trials using pharmacological doses of vitamin E (about 330–800 IU/d) have shown a trend towards a reduction in the incidence of CHD in men but not in women[107,112] and it is possible that at these dosages men achieve an optimal effect while in women supplementation moves them further along the plateau phase. Therefore supplementation would provide greatest benefit to those furthest away from their optimal level such as smokers, diabetics, and cardiac transplant and elderly patients. While the major large trials assessing the role of these vitamins have shown them to lack a beneficial role in CVD, the smaller trials assessing subgroup targeting have indicated a beneficial role in patients with end-stage renal disease[134], cardiac transplant[133] and acute myocardial infarction[261]. The combination of vitamins C and E in a secondary prevention trial has only shown benefit on clinical endpoints when targeting individuals (cardiac transplant patients) who are exposed to demonstrably increased levels of oxidative stress[133]. Therefore through exploring subgroup targeting further in large-scale trials we could find a therapeutic role for these vitamins in CVD.

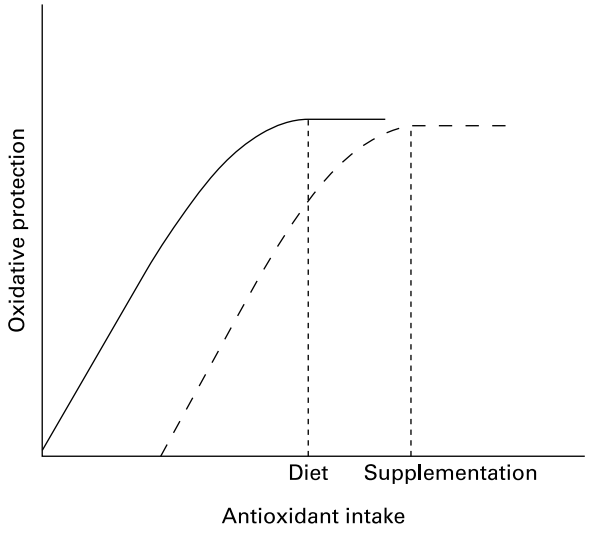


**Fig. 1.** Illustration of a hypothesis for the putative protective mechanism of antioxidants. The hypothesis suggests that antioxidants reach an optimal effect at a specific antioxidant concentration and that in women (—) the optimal antioxidant effect is reached with a lower antioxidant intake, i.e. dietary intake, than in men (- - -) in whom supplementation is needed to reach this optimal effect. It can be hypothesised that this is due to the pre-existing antioxidant levels being lower in men than in women and men being exposed to increased levels of oxidative stress.

To finally come to a conclusion on the role of vitamins in CVD one should probably conduct a primary prevention trial, using a combination of vitamins with 800 IU vitamin E per d and vitamin C $>500$ mg/d exclusive of β-carotene, and targeting subgroups that will potentially gain the most benefit such as diabetics, smokers, etc.

*Flavonoids, fibre and folic acid.* Individuals who consume large amounts of vitamins are less likely to smoke, have higher physical activity, are of higher socio-economic status[273] and more likely to consume other vitamins and to eat less saturated fat[274]. A high intake of these vitamins could therefore act as a marker for other dietary or non-dietary factors, explaining the lack of benefits seen in trials. For example, a high dietary intake of fibre has been associated with a relative risk of 0·77 (95 % CI 0·61, 1·00) for CHD in observational studies[275–279]. Flavonoids have shown an inconsistent role in CHD, with observational studies indicating a reduction in CHD mortality in those with a higher dietary intake of flavonoids compared with those with a lower dietary intake[280,281], while other studies have not indicated any beneficial role in CHD[282–286]. A high dietary intake of isoflavones has been shown to be associated with a reduced incidence of cerebral and myocardial infarction in women[287], possibly through its ability to reduce the progression of atherosclerosis[288]. The role of flavonoids and fibre in CVD has not yet been assessed in randomised controlled trials. A high dietary intake of folic acid has been associated with a reduced incidence of CHD in one study[289]; however, a meta-analysis of randomised controlled trials showed it to have a neutral effect on CVD[290]. There is a great need to assess the role of fibre and flavonoids in large-scale trials to be able more accurately to identify the protective components in fruit and vegetables.

*Non-antioxidative properties.* The causative role of oxidative stress in atherosclerosis has not been confirmed by *in vivo* studies and could therefore be an epiphenomenon[172]. The identification of the 'oxidative stress response to inflammation' hypothesis[291] makes the likelihood of a causative role for oxidative stress in atherosclerosis less plausible, therefore also making the disease-preventing roles for antioxidants less likely. However, as previously emphasised, vitamins such as vitamin E and vitamin C have been shown to mediate additional effects beyond their antioxidative properties including anti-inflammatory effects through altering gene expression and acting on signalling pathways that are activated by oxidised LDL. Therefore if oxidative stress does not play a role in atherosclerosis, it is still acknowledged that atherosclerosis is an inflammatory disease, and through their anti-inflammatory properties these vitamins can potentially still have a major role in atherosclerosis and CVD. Therefore the beneficial effect seen with these vitamins in observational studies could be due to these non-antioxidative properties. The lack of benefit seen with supplementation could be as a result of the lack of cofactors that are potentially present in fruit and vegetables that consequently can result in these properties not being fulfilled in trials. This further emphasises that supplementation should not only be a combination of vitamins E and C but also the relevant minerals and vitamins present in fruit and vegetables. These propositions have not yet been confirmed *in vivo* but through exploring these properties in a clinical context a future novel role in disease prevention for these vitamins can potentially be identified.

## Conclusion

To resolve the discrepancy between observational studies and randomised clinical trials the design of the study has been the main alteration, either by increasing participant size, trial duration or type of supplementation, but this has left us empty handed. Through getting back to basic science and exploring whether oxidative stress has a causative role in atherosclerosis, a role for these vitamins in CVD will be further supported and also we will be enabled to define the optimal vitamin dose and type. The discovery of efficient and standardised oxidative biomarkers will enable the assessment of vitamins' antioxidant efficiency and the identification of individuals who would potentially be in greater need of vitamin supplementation. Future trials should look at the other components in fruit and vegetables, particularly flavonoids and fibre, to hopefully identify a novel preventative and therapeutic agent that can be used to prevent the rise in CVD around the world. The evidence is still insufficient to support a role for routine vitamin supplementation and at this stage more emphasis should be put in recommending a healthy lifestyle.

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British Journal of Nutrition

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British Journal of Nutrition