British Journal of Nutrition (2006), **95**, 217–229
DOI: 10.1079/BJN20051616

Review article

Does nutrition have a role in peripheral vascular disease?

Juan J. Carrero[1] and Robert F. Grimble[2]*

[1]*Department of Biochemistry and Molecular Biology, University of Granada, Spain*
[2]*School of Medicine, University of Southampton, UK*

(Received 27 January 2005 – Revised 2 September 2005 – Accepted 2 September 2005)

Peripheral vascular disease (PVD) is a manifestation of systemic atherosclerosis in the lower limbs, and PVD patients have a 3- to 5-fold increased risk of cardiovascular mortality compared with age-matched controls. Nevertheless, recent reports show how PVD patients are undertreated with regard to CVD risk-factor reduction and the use of lipid-lowering or antiplatelet drugs. There is appreciable evidence that demonstrates the beneficial effects of certain nutrients and dietary habits in the prevention of CVD, but there has been little attention paid to the role of nutrients in PVD. The purpose of the present review is to provide an overview of our understanding of how foods could possibly benefit PVD. In the last few decades, several nutrients have arisen as potentially health-promoting in PVD. While nutritional interventions in PVD show positive clinical effects for fish oil, carnitine or vitamin E, others such as olive oil or vitamin C seem to interact only at a biochemical level by decreasing risk factors. Moreover, only epidemiological associations exist for the potential role of fibre, folates or vitamin B_6 in this disease. In all cases, the limited data available provide no clear-cut evidence in favour of the clinical benefit of nutritional interventions aimed at reducing risk factors and ameliorating symptoms in PVD patients. No practical recommendations can be given at this stage, and further studies are clearly needed.

Peripheral vascular disease: Fish oil: Olive oil: Vitamin E: Vitamin C

Atherosclerosis is the common form of arteriosclerosis in which deposits of yellowish plaques (atheromas) containing cholesterol, lipoid material and lipophages are formed within the intima and inner media of large- and medium-sized arteries. Atherosclerosis is also the most common cause of chronic arterial narrowing that reduces blood flow to the lower limbs at rest or during exercise. Atherosclerosis of the lower extremities defines what is known as peripheral vascular disease (PVD). Patients with PVD may be asymptomatic or present with intermittent claudication (IC), ischaemia rest pain, and/or gangrene. It is estimated that PVD, including asymptomatic stages, occurs in approximately 12 % of the adult population, and the incidence of PVD increases with age, such that almost 20 % of individuals over the age of 70 years have this disease (Hiatt *et al.* 1995; Anonymous, 2000; Halperin, 2002).

IC is the most common symptom, present in 15–40 % of patients with PVD (Hirsch *et al.* 2001). It is defined as walking-induced pain in one or both legs that does not go away with continued walking and is relieved only by rest, as is associated with a diminished ability to perform daily activities. In approximately 25 % of patients with IC, there is a progression to critical ischaemia, for example, rest pain and gangrene that may eventually necessitate amputation (Hertzer, 1991). A commonly used non-invasive test for PVD diagnosis is the measurement of systolic blood pressures in the ankles and arms with a Doppler ultrasonic instrument, from which the ankle:brachial index (ABI) is derived. A low ABI is highly predictive not only of the presence of arterial occlusive disease but also of subsequent cardiovascular mortality (Hiatt *et al.* 1995). An ABI greater than 0·90 is considered normal, 0·70 to 0·89 is considered mild disease, 0·5 to 0·69 moderate disease, and less than 0·5 severe disease (Tabet *et al.* 1996). On the other hand, the degree of functional impairment is established according to the distance that the patients can walk without pain or without onset of claudication, that is, pain-free walking distance (PFWD).

PVD is closely associated with high risk for myocardial infarction and stroke (Leng & Fowkes, 1993; Muluk *et al.* 2001), and PVD patients have a 3- to 5-fold increased risk of cardiovascular mortality compared with age-matched controls (Criqui *et al.* 1992; Vogt *et al.* 1993; Leng *et al.* 1996; Henke *et al.* 2004). This increased risk, which appears to be independent of classic risk factors (Leng & Fowkes, 1993;

Abbreviations: ABI, ankle:brachial index; Hcy, homocysteine; HHcy, hyperhomocysteinaemia; IC, intermittent claudication; LC, L-carnitine; PFWD, pain-free walking distance; PLC, propionyl-L-carnitine; PVD, peripheral vascular disease.

* **Corresponding author:** Dr Robert F. Grimble, fax +44 2380 594379, email R.F.Grimble@soton.ac.uk

Brevetti *et al.* 1998*a*), and is only partially explained by the expected association of PVD with coronary and cerebrovascular disease (Leng & Fowkes, 1993; Muluk *et al.* 2001), is strongly related to the severity of PVD itself (Leng & Fowkes, 1993; Brevetti 1998*b*). Because of the systemic nature of atherosclerosis and the high risk of ischaemic events, patients with PVD should be considered candidates for secondary prevention strategies that include antiplatelet drug therapy and aggressive atherosclerotic risk-factor modification (Hiatt, 2001; National Cholesterol Education Programme, 2002). Nevertheless, patients with PVD are undertreated with regard to risk-factor reduction and the use of lipid-lowering or antiplatelet drugs, as compared with patients with CHD (McDermott *et al.* 1997; Tornwall *et al.* 2000). Consensus guidelines for the specific management of PVD patients should be considered, as well as strategies to ensure their implementation (Mukherjee *et al.* 2002; Cassar *et al.* 2003; Henke *et al.* 2004).

Atherosclerosis is an inflammatory disease of the vascular system. An initial impairment in the functional properties of the endothelium of the affected limb vessels could explain the origin of the PVD, which would elicit a series of changes directly related to initiation, progression, and clinical complications of atheromatous plaque. Diet can affect the development of this plaque not only through modulation of serum lipids but also by influencing the immune and inflammatory processes present in the endothelium and known to be associated with the development of this disease (Ross, 1999). There is appreciable epidemiological evidence that demonstrates the beneficial effects of certain nutrients and dietary habits in the prevention of CVD. Based on this evidence, international health societies have produced a number of nutritional recommendations that should be taken into consideration for primary as well as for secondary cardiovascular risk prevention. The latest World Health Organization Study Group (2003) report recommends, for primary CVD prevention, a regular fish consumption to provide about 200–500 mg EPA and DHA/week, a replacement of saturated fat by monounsaturated fat (oleic acid), and an increase in the consumption of fruit and vegetables.

However, there is little attention paid to the role of food components in PVD. It has been shown that malnutrition is common in patients with PVD and is associated with changes in other markers that predict an increase in complication (Spark *et al.* 2002). Although many factors including chronic vascular illness, diabetes, sedentary lifestyle, nutritional deficiencies and ageing itself may contribute to the progression of PVD and the ischaemia-induced muscle weakness, only skeletal muscle disuse and undernutrition (or nutrient intake below desirable amounts) are potentially reversible with targeted interventions. The Edinburgh Artery Study (Donnan *et al.* 1993) is the biggest prospective cross-sectional study that has evaluated the relationships between the ABI and dietary factors. This study, which included 1592 men and women (aged 55–74 years), described a positive association between a higher consumption of fibre-containing foods with greater mean ABI in PVD males (less disease severity). Higher consumption of meat was also associated with low mean ABI in PVD males and females, together with positive associations with cereal fibre, alcohol and vitamins E and C. In the last few decades, several nutritional intervention studies in PVD patients have reported interesting findings for isolated nutrients, but the studies do not provide consistent evidence such as to suggest a specific dietetic guideline (Hooper *et al.* 2004). The purpose of the present review is to provide an overview of our present understanding of how foods and specifically certain health-promoting food components could possibly benefit PVD. Furthermore, the potential mechanisms by which this nutrient interaction contributes to the reduction of PVD will be briefly explored.

Nutritional targets

Atherosclerosis, underlying PVD complications, is an inflammatory disease, not merely the passive accumulation of lipids within lower limb artery walls. A variety of initiating agents and multiple pathogenic mechanisms (for example, hyperlipidaemia) initiate an inflammatory response that contributes to the development of atheromatous plaques (Ross, 1999). Endothelial dysfunction, by predisposing to thrombosis, impairment of the flow, leucocyte adhesion, and smooth muscle cell proliferation, plays a pivotal role in the development, progression, and clinical manifestations of atherosclerosis. Endothelial dysfunction originates an inflammatory cascade that includes the interaction of pro- and anti-inflammatory cytokines within the arterial wall.

Patients with PVD have impaired endothelial function, which is related to the severity of the circulatory failure in the affected limb and to increased plasma markers of inflammation (Yataco *et al.* 1999; Brevetti *et al.* 2003; Gokce *et al.* 2003). PVD patients have been reported to have elevated levels of cytokines, adhesion molecules (Signorelli *et al.* 2003), selectins (Blann *et al.* 1997; Signorelli *et al.* 2003), von Willebrand factor (Philipp *et al.* 1997; Blann *et al.* 2000*b*), tissue factor (Blann *et al.* 2000*a*), C-reactive protein (Rossi *et al.* 2002) and fibrinogen (Violi *et al.* 1996).

Moreover, PVD patients also have reduced erythrocyte deformability, increased erythrocyte aggregation and increased blood viscosity (Lowe *et al.* 1993), which may impede blood flow through various regions of the microcirculation in the lower limbs (Simchon *et al.* 1987). Pathological changes in erythrocyte structure and haemodynamic functions may hinder blood flow in large vessels and occlude microvessels, facilitating endothelial and platelet activation by modulating shear stress and attenuating flow rate, and promoting white cell migration and adhesion to vessel wall endothelium.

Finally, systemic atherosclerosis narrows limb vessels and impairs blood flow to exercising leg muscles causing claudication, which is brought on by exercise and relieved by rest. As vessel narrowing increases, critical limb ischaemia can develop when the blood flow does not meet the metabolic demands of tissue at rest. A reduction of the symptoms produced by this ischaemic process would also ameliorate PVD progression.

Three major targets in PVD for which nutrients could play an important role have been summarised: (1) restoring the endothelial dysfunction; (2) improving erythrocyte deformability, aggregation and blood flow; (3) improving O_2 perfusion in atherosclerosis-induced muscle ischaemia. The existing literature suggests certain evidence of how selected nutrients could diminish PVD symptoms by affecting these three targets.

Dietary fats

Fish oil

n-3 Long-chain PUFA, namely EPA and DHA, are found in fatty fish and in fish oils. Evidence from epidemiological and case–control studies indicate that consumption of fish, fatty fish and long-chain *n*-3 PUFA reduces the risk of cardiovascular mortality (Carrero *et al.* 2005*b*). Studies using *n*-3 PUFA in post-myocardial infarction patients have shown a reduction in total and cardiovascular mortality. *n*-3 PUFA have been shown to decrease blood triacylglycerol concentrations, to decrease production of chemoattractants, growth factors, adhesion molecules, inflammatory eicosanoids and inflammatory cytokines, to lower blood pressure, to increase NO production, endothelial relaxation and vascular compliance, to decrease thrombosis and cardiac arrhythmias and to increase heart rate variability (Calder, 2004). These mechanisms most probably explain the primary and secondary CVD protection afforded by long-chain *n*-3 PUFA consumption, suggesting that long-chain *n*-3 PUFA consumption may be beneficial in PVD too.

Modern research regarding *n*-3 PUFA supplementation on other atherosclerotic disorders suggests mechanisms by which these fatty acids could play a potential role in the specific case of PVD. EPA and DHA exert an anti-inflammatory effect within the vessel wall (Calder, 2004). EPA and DHA compete with arachidonic acid for the insertion at the sn-2 position of membrane phospholipids, producing less potent eicosanoids than those produced by arachidonic acid (Fig. 1). In fact, fish oil decreased neutrophil leucotriene B_4 in PVD patients, while leucotriene B_5 levels increased significantly (Mori *et al.* 1992). Supporting this idea we have recently suggested how fish oil might influence inflammation in PVD patients by altering the balance between leucotriene B_4 and prostaglandin E_2 production. As the former stimulates and the latter inhibits pro-inflammatory cytokine production respectively, a fall in the ratio will lead to a decrease in cytokine production and *vice versa* (Carrero *et al.* 2004*b*). Also, EPA is able to promote vascular endothelial cell migration and simultaneously block the smooth muscle cell migration (Kanayasu-Toyoda *et al.* 1993, 1996), which is beneficial for repair of blood vessel injuries and inhibition of atherosclerotic plaque formation, respectively. Moreover, we also reported a rapid incorporation of dietary EPA and DHA in atherosclerotic plaques, resulting in increased plaque stability and reduced macrophage infiltration, slowing the progression of the vascular lesion (Thies *et al.* 2003) and perhaps the onset of clinical events.

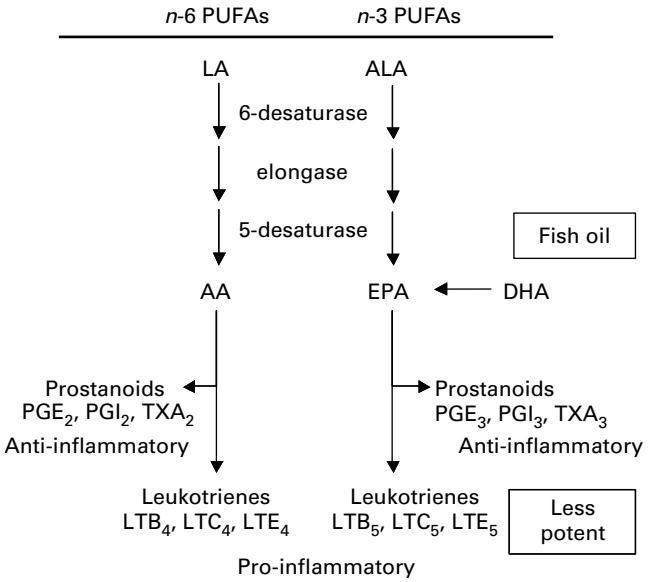

Fig. 1. *n*-3 PUFA metabolism. LA, linoleic acid; ALA, α-linolenic acid; AA, arachidonic acid; PG, prostaglandin; TX, thromboxane; LT, leucotriene.

In addition, EPA and DHA have been shown to increase erythrocyte deformability (Ernst, 1989) and reduce their aggregation (Ho *et al.* 1999), maybe as a result of modifying the cell membrane lipid content. Erythrocyte aggregation results in a net increase in 'cell' size (of the aggregate) and increased sludging in capillaries. Therefore, reduced platelet and erythrocyte aggregation can potentially increase blood flow (Vicaut, 1995).

Despite this existing evidence regarding the protective effect of fish oil on CVD, few randomised controlled trials have studied the relationship between *n*-3 PUFA and PVD. The very first of them described rheological changes (a decrease in whole-blood viscosity) and a fall in triacylglycerols after supplementation for 7 weeks with 1·8 g EPA/d in the form of fish oil capsules (Woodcock *et al.* 1984). No effect on clinical outcomes was reported in such a short study though, and the changes in blood viscosity were not such as to give consistency to the hypothesis. A later double-blind study (Gans *et al.* 1990) reported that 4-month daily administration of fish oil capsules, containing 1·8 g EPA and 1·2 g DHA, significantly increased HDL-cholesterol and decreased triacylglycerols, blood viscosity and blood pressure in the intervention group. However, clinical outcomes were controversial: the mean PFWD increased by 18 % in the intervention group and by 41 % in the control group. Large standard deviations prevented results from being statistically significant. This also happened in another trial (Leng *et al.* 1998) that reported a decrease in blood pressure and a non-significant 81 % increase of the mean PFWD in the intervention group compared with a 26 % increase in the control group after 2-year treatment with a combination of γ-linolenic acid and EPA (280 mg GLA + 45 mg EPA/d).

The effects reported from these studies should theoretically reduce the elevated risk of CHD possessed by individuals with PVD, but further research is needed to evidence the clinical implications of this supplementation. Methodological shortcomings in these trials may have resulted in a failure to detect significant clinical effects (Sommerfield & Hiatt, 2004). We believe that the use of higher dosages and bigger cohorts in these studies would have possibly resulted in positive clinical effects after fish oil intake. On the other hand, duration of the study might have been insufficient in some of them, as we have recently reported that a minimum of 10–12 weeks of intervention are necessary to allow EPA and DHA to effectively incorporate into membrane and therefore expect any kind of effect (Thies *et al.* 2003). Finally, the heterogeneous genetic background of the subjects in each study may have contributed to the variability in response as well. We have previously demonstrated that individuals who exhibit an inherently high capacity for TNF-α production

were more sensitive to the anti-inflammatory effects of fish oil than individuals who exhibit a low capacity (Grimble *et al.* 2002). This sensitivity might be influenced by the BMI and the possession of certain alleles (Markovic *et al.* 2004). All these aspects should be taken into consideration when designing new intervention studies with fish oil. We are currently undertaking in our laboratory an intervention trial administering fish oil capsules for 3 months in a large cohort of PVD patients and healthy controls (*n* 250). Special emphasis will be on the inflammation and endothelial dysfunction markers and whether or not our genotype influences our response to fish oil. We believe that this study will demonstrate the potential of fish oil to ameliorate atherosclerosis at an individual level and will contribute as well to the possibility of developing a 'tailor-made' nutritional therapy for this condition.

Olive oil

Olive oil in the Mediterranean diet partially replaces a range of fats consumed in Western countries, including those rich in saturated fatty acids and *n*-6 PUFA. Accumulating evidence suggests that it may have health benefits that include reduction of risk factors of CHD and modification of immune and inflammatory responses. The therapeutic properties of olive oil are often attributed to its high levels of MUFA, but other minor components (for example, antioxidants and phytochemicals) are also responsible for its health effects. It has the advantage of being less susceptible to oxidation than oils rich in PUFA and in most studies olive oil has been shown to lower plasma total and LDL-cholesterol to a similar degree as PUFA, combined with a possible protective antioxidant effect (Stark & Madar, 2002). Olive oil has also been shown to have beneficial effects on blood pressure (Ruiz-Gutierrez *et al.* 1996; Ferrara *et al.* 2000) and to modify the immune response (Alarcon de la Lastra *et al.* 2001).

This evidence suggests that olive oil consumption could be indicated in PVD management. However, very few trials have tested its potential role in IC. Olive oil reduced total cholesterol levels after 4 weeks of supplementation in PVD patients, due to a significant decrease in LDL-cholesterol levels; there was also an increase in serum thromboxane B_2 (Mori *et al.* 1992). Another small 3-month trial in Spanish PVD patients, using a combination of olive oil and fish oil, resulted in a fall in triacylglycerols and a lower susceptibility of LDL to oxidation (Ramirez-Tortosa *et al.* 1999*a*). In both trials, no effect was found in clinical outcomes. We believe that duration and sample sizes in both studies were perhaps insufficient to denote an effect in clinical outcomes, as incorporation into tissue could not fully occur and large standard deviations prevented results from reaching any statistical significance. Finally, the selection of the population group might have also played a part in the lack of clinical effects, as Mediterranean-border countries have a high intake of olive oil in their diets, and estimates for olive oil intake in the south-Spanish population are about 23 g/d (Carrero *et al.* 2005*a*). Habitual olive oil intake might have masked further effects.

A similar randomised, two-period, cross-over trial compared the effects of extra-virgin and refined olive oils in PVD men (Ramirez-Tortosa *et al.* 1999*b*). Basically, the oils differed in their antioxidant profile (α-tocopherol 300 *v.* 200 mg/kg; phenolic compounds 800 *v.* 60 mg/kg) and concentration, but not in their fatty acid composition. After the intervention period, resistance of LDL to oxidation was higher after consumption of extra-virgin olive oil but not refined olive oil, suggesting that antioxidants present in extra-virgin olive oil may be responsible for this LDL protection against oxidation. Since well-sustained evidence recommends its consumption for cardiovascular risk prevention and no deleterious or side effects have been reported, it might be interesting to contemplate the use of virgin olive oil in future trials in order to assess its potential in PVD management.

Antioxidants

Free radical activity and oxidative damage have been implicated in the initiation of vascular disease, and antioxidants provide the first line of defence against free radicals. Several studies have shown that episodes of ischaemia–reperfusion can reduce the total antioxidant capacity (Khaira *et al.* 1995, 1996). It is possible, therefore, that the longer or more severe the bouts of ischaemia, the greater the reduction in the total antioxidant capacity and subsequent increase in the risk of developing infective complications. In fact, it has been shown that antioxidant defences in PVD patients are lower than age-matched controls (Duthie *et al.* 1989; Spark *et al.* 2002). If these patients have an unimpaired nutritional status with low total antioxidant capacity, they may benefit from antioxidant supplementation. If their nutritional status is impaired, then nutritional supplementation could also be required.

Vitamin E

Vitamin E (tocopherol) is a fat-soluble vitamin which functions solely as a membrane-bound antioxidant that prevents cell membrane damage by inhibiting peroxidation of membrane phospholipids and disrupting free radical chain reactions induced by formation of lipid peroxides. As the only membrane-bound lipid-soluble antioxidant, vitamin E plays a key role in preventing cellular injury from oxidative stress associated with premature ageing, cataracts, uncontrolled diabetes, CVD, inflammation, and infection (Morrissey & Sheehy, 1999).

It has been hypothesised that vitamin E consumption could benefit PVD. Vitamin E might improve tolerance to the ischaemia that occurs in the lower limbs, if indeed it eliminates free radicals (Ferrari *et al.* 1983). It also might influence the process of atherosclerosis by stopping further deterioration. It has been shown that patients with IHD and patients with PVD have higher plasma lipid peroxide concentrations than controls (Stringer *et al.* 1989). Inhibition of peroxidation by vitamin E might influence beneficially the balance between peroxidative damage and the body's repair mechanisms. Finally, it may influence platelet aggregation (Steiner & Mower, 1982) and affect erythrocytes (Farrell *et al.* 1977), improving blood flow, which might account for some beneficial effect on the symptoms of IC (Kleijnen & Mackerras, 2004).

The treatment of IC with vitamin E was originally proposed in the 1940s by Shute *et al.* (1948), and led to several

controlled trials in the following decades. These trials lasted for between 12 weeks and 18 months and they all showed increase in both PFWD and blood flow through arteries (in most of the cases measured as ABI) of the lower legs in individuals with IC (Hamilton *et al.* 1953; Livingstone & Jones, 1958; Boyd & Marks, 1963; Williams *et al.* 1971; Haeger, 1974; Westheim *et al.* 1975). The dosage used varied from 180 to 270 mg/d, although one study used 1080 mg/d. Increasing dietary intake of vitamin E was also associated with better blood flow to the legs (Donnan *et al.* 1993). Possibly, more effect would have been noticed with longer duration of the studies (Livingstone & Jones, 1958), as one review article suggested that a minimum of 4–6 months of vitamin E supplementation may be necessary before significant improvement is seen (Piesse, 1984). In the Rotterdam Study, vitamin E intake was inversely associated with PVD in men, and a 10 mg increase in intake was associated with a 0·015 ABI increase (Klipstein-Grobusch *et al.* 2001).

Big methodological differences in these trials make comparison of studies and meta-analysis difficult (Kleijnen & Mackerras, 2004); the trials had different study lengths and dosages, they measured four different physical outcomes in small sample sizes, which means that baselines were not necessarily equal between the trials, and finally the active period for conducting vitamin E trials in IC was about 30 years ago, with different techniques and understanding of the disease. There might not be enough consistent data still to recommend using vitamin E in patients with IC. However, the existing evidence always shows a positive beneficial effect and is in favour of vitamin E consumption. Further trials in larger PVD cohorts would contribute to the assessment of the exact role of vitamin E on PVD progression.

A combined strategy of *n*-3 PUFA and vitamin E supplementation has proved to be effective in CVD prevention, as an important function of vitamin E in the body is the protection of PUFA from oxidation. Vitamin E could improve the role of *n*-3 PUFA through protection from lipid peroxidation, by acting independently on the same or closely related atherogenic and thrombotic mechanisms, or both (Meydani *et al.* 1991; Steinberg, 1991; Morrissey & Sheehy, 1999). *n*-3 PUFA are highly susceptible to oxidation by endogenous free radicals which are formed and needed in normal cell metabolism. In PVD, the cell damage that occurs in ischaemic periods in the calf muscle and lower limbs is probably caused by free radicals. Deformability of erythrocytes, for instance, may be enhanced by vitamin E, since *n*-3 PUFA incorporated in the membranes are protected from oxidation. However, there are no studies available with this combination of nutrients in PVD patients.

Vitamin C

Vitamin C (ascorbic acid) is a water-soluble antioxidant capable of scavenging free radicals and is the first-line defence in the control of the redox state, sparing other antioxidants from consumption. Vitamin C preferentially concentrates in leucocytes and attenuates reperfusion-induced muscle injury (Kearns *et al.* 2004). It has been postulated that vitamin C may be effective in PVD management by restoring endothelial function. A small study showed that vitamin C prevented endothelial function induced by exercise in PVD patients with IC, together with a decrease in thiobarbituric acid reactive substance and soluble intracellular adhesion molecule-1 levels (Silvestro *et al.* 2002). Pre-treatment with vitamin C preserved muscle function and reduced the expression of soluble intracellular adhesion molecule-1, infiltration of the neutrophils and oedema in a study with skeletal muscle ischaemic patients (Kearns *et al.* 2004), and acted as a potent acute vasodilator in the radial arteries of pre-operative coronary patients (Drossos *et al.* 2003). In fact, a prospective study (Langlois *et al.* 2001) showed that vitamin C status is depleted in PVD patients, and is associated with the grade of inflammation (measured as C-reactive protein levels) and the severity of the disease (measured as shorter PFWD).

The question arises whether antioxidant vitamin supplements would be useful to address reduction of oxidative stress in PVD. The studies reviewed in this section give certain evidence to recommend their use or at least to suggest the necessity of more specific trials to clarify this topic. However, not all trials describe positive effects. A pro-oxidant effect of dietary vitamin C has been described in human subjects, which may give rise to paradoxical effects in clinical intervention trials (Podmore *et al.* 1998). Recently, the long-term effect of combined vitamins E and C did not show any improvement on peripheral endothelial function (Kinlay *et al.* 2004). An ongoing multicentre European trial, the Critical Leg Ischemia Prevention Study (CLIPS), is investigating the effectiveness of low-dose aspirin and antioxidant vitamins (vitamin E, vitamin C, β-carotene) with a 2×2 factorial design.

Folates and other B vitamins

Elevated levels of plasma homocysteine (Hcy) increase platelet aggregation (Welch & Loscalzo, 1998), oxidative stress (Nappo *et al.* 1999) and vascular smooth muscle proliferation, decrease NO production (Tsai *et al.* 2000), and impair endothelial function. Consistent with these adverse cardiovascular effects, elevated concentrations of Hcy have been positively associated with the risk of CHD (Cleophas *et al.* 2000) and PVD (Cheng *et al.* 1997).

Hyperhomocysteinaemia (HHcy) is associated with an increased risk of developing PVD independent of established risk factors (smoking, hypercholesterolaemia, diabetes and hypertension) (Kuan *et al.* 2002). The relative risk of developing PVD has been estimated to vary from 2·0 to 11·0 for elevated fasting Hcy levels in several studies (Boers, 1997; de Jong *et al.* 1999; Taylor *et al.* 1999). In addition, there is evidence that post-methionine load HHcy is an independent risk factor for PVD (Refsum *et al.* 1998; Graham *et al.* 1997). This risk increases when other factors such as hypertension, smoking and hypercholesterolaemia are included. HHcy is present in 30 % of PVD patients (Taylor *et al.* 1991), and the existing plasma levels of Hcy are associated with the severity of the PVD disease in type 2 diabetic patients, since PVD is the most prevalent expression of vascular atherosclerosis in this kind of patient (Ciccarone *et al.* 2003).

Fig. 2 shows Hcy metabolism. A low intake of folate limits the remethylation of Hcy to methionine and increases the concentration of plasma Hcy (Verhoef *et al.* 1996). Vitamins B_6 and B_{12} are cofactors that contribute to the conversion of Hcy to cysteine or methionine, respectively (Verhoef *et al.* 1996); low intakes of these vitamins can potentially increase

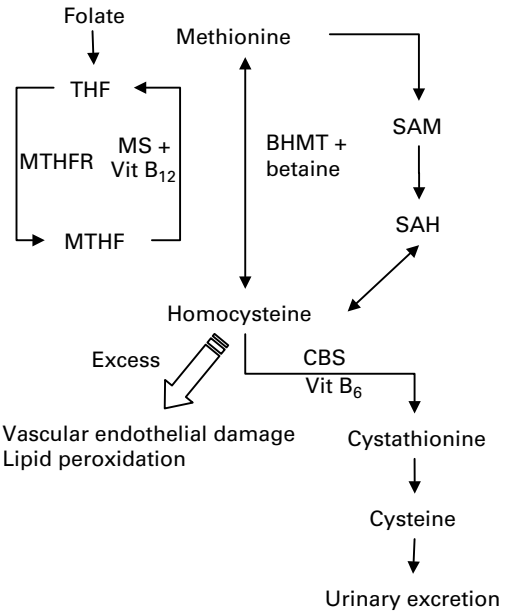

Fig. 2. Homocysteine metabolism. THF, tetrahydrofolate; MTHFR, methyltetrahydrofolate reductase; MS, methionine synthase; Vit, vitamin; MTHF, methyltetrahydrofolate; BHMT, betaine homocysteine methyltransferase; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; CBS, cystathionine β synthase.

Hcy. Therefore potential contributions to the regression of PVD can be made by indirectly reducing Hcy levels and their effects.

Folate is a water-soluble vitamin that mammals cannot synthesise and have to obtain from their diets. Folate is present in selected foods such as orange juice, dark green leafy vegetables, dried beans and peas, asparagus, strawberries and groundnuts (Carrero *et al.* 2004*a*). Vitamin B_6 is present in foods such as potatoes, breakfast cereals, bread, meat, fish, eggs, bananas, nuts and seeds. Vitamin B_{12} is naturally found in all animal foods – meat, meat products, milk, fish, eggs – and certain algae, such as seaweed. Recently, folate and B_6 intake have been identified as independent predictors of PVD in men aged over 50 years (Wilmink *et al.* 2004).

Very few intervention studies on PVD patients have been found in the literature. The existing data refer to nutritional epidemiology. A study recently held in the Health Professionals' Follow-up Study population revealed that men in the top category of folate intake (median 840 μg/d) were at 33 % lower risk of PVD than men in the bottom category (median 244 μg/d). Besides, there was a weak inverse association between intake of vitamin B_6 and PVD risk ($P=0{\cdot}06$; Merchant *et al.* 2003*a*). These results suggest that higher consumption of folate and B vitamins may contribute to the prevention of PVD.

The Hcy-lowering effects of folate have been well documented (van den Berg *et al.* 1994), and the addition of vitamins B_{12} and B_6 to folic acid supplements or enriched foods may maximise the reduction of Hcy in about 7 % of healthy individuals (Anonymous, 1998; Bronstrup *et al.* 1998). To give an idea of the importance of Hcy reduction, a recent meta-analysis suggests that lowering Hcy concentrations by 3 μmol/l from current levels would reduce the risk of IHD by 16 %, deep-vein thrombosis by 25 % and stroke by 24 % (Wald *et al.* 2002).

The mechanisms by which Hcy exerts its deleterious effects are not fully known, and *in vitro* studies demonstrate the multi-factorial nature of Hcy-induced vascular disease. Regarding PVD symptoms, a reduction of Hcy could be related to reduced endothelial cell injury (Wall *et al.* 1980), reduced adhesion molecule expression (Silverman *et al.* 2002), reduced monocyte and T-cell binding to endothelial cells (Koga *et al.* 2002), reduced endothelium-dependent relaxation (Weiss *et al.* 2002), or reduced factor V activation (Rodgers & Kane, 1986). In addition, several studies have highlighted Hcy-induced changes in coagulation response (Lentz & Sadler, 1991; Mujumdar *et al.* 2001), and a recent trial *ex vivo* suggests that the presence of IC alone does not influence platelet function, but if complicated with mild HHcy, there appears an increased platelet activation (Riba *et al.* 2004).

In PVD patients, flow-mediated endothelium-dependent dilatation of the peripheral arteries is decreased (Poredos *et al.* 2003), and a reduction of Hcy is associated with an improved endothelial function by mediating in the three vasodilator pathways. The pathways involve NO synthesis (Stamler *et al.* 1993), prostacyclin production (Wang *et al.* 1993) and the endothelium-derived hyperpolarising factor, which is a major determinant of vascular tone in small resistance cells (de Vriese *et al.* 2004). In summary, an Hcy decrease is strongly related to vasodilatation, reduced endothelial dysfunction and reduced platelet reactivity, and can potentially be useful in ameliorating the flow in the lower limbs when in the context of PVD. However, more evidence is needed again, such as to suggest dosages of these nutrients, fortification levels or suitability of supplements.

Fibre

Dietary fibre is a heterogeneous food component consisting of a variety of plant substances. Dietary fibre usually includes the indigestible NSP, cellulose and hemicellulose, oligosaccharides, pectins, gums and waxes (James *et al.* 2003). The heterogeneous nature of fibre means that the physiological effects of raising the intake of each type might be expected to exert different physiological effects; not all will be of direct relevance to preventing PVD. Fibre results, for instance, in improved bowel motion, frequency and consistency, suppressed carcinogenesis, lower cholesterol, improved glycaemic control, or increased satiety and therefore lower weight (for a review, see James *et al.* 2003).

In recent years, the role of dietary fibre in the prevention of CVD has been a subject of considerable attention. A recent pooled analysis of no fewer than ten cohort studies addressing this topic has shown a consistent protective effect of dietary fibre on stroke, myocardial infarction and cardiac death (Pereira *et al.* 2004). The available evidence indicates that the consumption of foods high in dietary fibre (wholegrain cereals, fruits and vegetables) is associated with a lower prevalence of important risk factors for CVD, including hypertension, obesity and type 2 diabetes (Bazzano *et al.* 2003, Liese *et al.* 2003). Large, prospective studies also show a direct inverse association between high-fibre food intake and the development of CHD and stroke (Diehr & Beresford, 2003). While several

aetiologies have been considered, the biological mechanisms whereby a diet of high-fibre foods may exert beneficial cardiovascular effects are not entirely known.

To date, few studies have studied the epidemiological association between fibre and PVD. The Edinburgh Artery Study (Donnan *et al.* 1993) was pioneer in describing a positive association between higher ABI, indicating less evidence of PVD, in men with higher cereal fibre intake in a randomly selected population after adjustment for age, sex, height, smoking and total energy. Later on, a Greek case–control study in 100 PVD patients showed the same association with total fibre (Katsouyanni *et al.* 1991). In another prospective study conducted among Finnish smokers (Tornwall *et al.* 2000), an inverse association with PVD was found comparing the top and bottom quintile of fibre intake; separate estimates for cereal, fruit and vegetable fibre were not available. The main food source of dietary fibre in that study was rye bread, so that the results are also probably due to cereal fibre. Finally, based in the male population of the Health Professionals' Follow-up Study, an inverse association with PVD risk was found for cereal fibre intake and not for total fibre intake, suggesting that it is important to evaluate the different types of fibre in relation to PVD risk, because associations vary considerably (Merchant *et al.* 2003*b*).

Unfortunately, no intervention studies in PVD patients are available such as to provide an idea of possible improvements in clinical outcomes. Several mechanisms of action, though, have been highlighted to explain the potential protective effect of fibre in PVD. It has been hypothesised that the apparent beneficial effect of fibre intake against CHD is mediated by lowered cholesterol, especially due to soluble fibre (Hunninghake *et al.* 1994), and lowered plasminogen activator inhibitor type 1 and factor VII activity (Marckmann *et al.* 1993). PVD results mainly from atherosclerotic narrowing of the blood vessel lumen. LDL is taken up by monocytes in the intima of the blood vessels, becoming foam cells, and leading to the formation of plaque. Increased cytosolic triacylglycerols are associated with oxidative stress and can cause endothelial dysfunction (Bakker *et al.* 2000). Thus, increased serum HDL and triacylglycerols increase the risk of PVD (Drexel *et al.* 1996). Other described effects by which fibre intake might protect against PVD risk are: (1) increased insulin sensitivity, as fibre intake improves insulin sensitivity by slowing the absorption of nutrients from the gut (Jenkins & Jenkins, 1985); (2) reduced serum glucose levels (Jenkins *et al.* 2000); (3) production of SCFA by gut bacteria consequently improving glucose metabolism (Thorburn *et al.* 1993), which is associated with lower LDL, blood pressure and triacylglycerols, and higher HDL (Hunninghake *et al.* 1994).

Recently, a strong association has been shown in the 1999–2000 US National Health and Nutrition Examination Survey between dietary fibre and levels of C-reactive protein (King *et al.* 2003), after controlling for age, sex, race, education, smoking, physical activity, BMI, total energy consumed and fat intake. This association, among 4900 adults aged 40–65 years, is a strong indicator that the effect of dietary fibre on the risk of CVD may be mediated in part by the inflammatory process. This hypothesis could contribute to complement the mechanisms at work in reducing PVD risk above exposed. However, it should not be forgotten that foods naturally containing fibre also contain other positive nutrients in PVD management such as vitamin E or folate, whose benefits are discussed in the present review. It might be plausible to say that increasing cereal fibre intake in the diet could contribute, to some extent, to the prevention of PVD. Intervention trials are needed though to prove these hypotheses. The Trial to Reduce Inflammatory Markers (TRIM) is an ongoing trial in South Carolina University (USA) that is investigating whether fibre supplementation in 180 healthy volunteers can reduce markers of inflammation. This trial, that includes a strictly controlled high-fibre diet, will hopefully provide important information to isolate the impact of fibre and type of fibre on inflammation as well as lipids and oxidative markers, providing more consistent data of the potential use of this food component in the treatment of PVD.

L-Carnitine

L-Carnitine (LC) or propionyl-L-carnitine (PLC) is a non-essential dietary amino acid that man synthesises from lysine and methionine, and is mainly found in meat, poultry, fish, avocados and dairy products.

LC is a metabolic agent and an important cofactor for normal skeletal muscle bioenergetics (Fig. 3): First, LC is required for long-chain fatty acid oxidation. LC is a cofactor of carnitine, acyltransferases transporting long-chain fatty acids across the mitochondrial inner membrane. In the absence of LC, the inner mitochondrial membrane would be impermeable to long-chain fatty acids and fatty acyl-CoA esters. Once inside the mitochondria, these compounds can be degraded to acetyl-CoA through a process known as β-oxidation; second, it assists in removing accumulated acetyl groups from the mitochondria contributing to maintain the acetyl-CoA:CoA ratio in the cell. During high-intensity exercise, there is a large production of acetyl-CoA. This increase in turn inhibits the pyruvate dehydrogenase complex and reduces flux through the pyruvate dehydrogenase complex. Acetyl-CoA reacts with free carnitine to form acetyl-carnitine and CoA. As a consequence, acetyl-CoA gives rise to lactate as opposed to CoA; carnitine therefore may suppress the accumulation of lactic

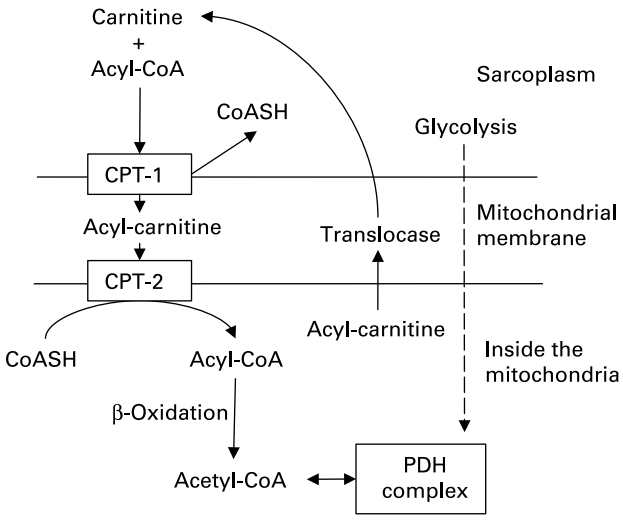

Fig. 3. Role of L-carnitine in oxidative metabolism. CoASH, acetyl-CoA; CPT, carnitine palmitoyltransferase; PDH, pyruvate dehydrogenase.

acid, which can mean delayed fatigue, thereby enhancing exercise performance (Jeukendrup, 2002).

Muscles require optimum performance of these metabolic processes during impaired exercise. Theoretically, carnitine availability may be the limiting factor for fatty acid oxidation, or the removal of acyl-CoA during exercise (Karlic & Lohninger, 2004). Patients with PVD have been shown to accumulate acylcarnitines in their skeletal muscle, and this abnormal accumulation of acylcarnitines is directly correlated with impaired exercise performance (Smit, 1992; Hiatt, 1994). Oral ingestion of LC would result in an increase of the total LC concentration in muscle. This increase in the muscle would result in increased rate of oxidation of intramuscular fatty acids and triacylglycerols during exercise, thereby reducing muscle glycogen breakdown and postponing fatigue. It has therefore been suggested that oral supplementation with LC in PVD patients could help to improve their walking distance.

In double-blind trials, supplementation with either LC or PLC has increased PFWD in individuals with IC. PFWD was 75 % greater after 3 weeks of 2 g twice per d LC supplementation (Brevetti *et al.* 1998*b*). In the study using PLC, improvement occurred only in those with severely limited walking capacity (PFWD $<$ 250 m), where PFWD increased by 78 % with PLC supplementation compared with a 44 % increase in the placebo group (Brevetti *et al.* 1997). The amount of PLC used was 1 g/d, increasing to 2 g/d after 2 months, and 3 g/d after an additional 2 months, if needed. The results of this trial have been confirmed in a large European trial involving 485 IC patients, where PLC supplementation in the subgroup of severe PVD patients resulted in a 98 % PFWD increase compared with a 54 % PFWD increase in the placebo group (Brevetti *et al.* 1999). Oral LC has been associated with increased leg muscle strength (Barker *et al.* 2001), increased blood flow velocity, plasminogen activator inhibitor-1 activity and erythrocyte deformity (Dal Lago *et al.* 1999).

Studies with athletes show, in the majority of the cases, an improvement in muscle function exercise performance and/or recovery after dietary LC (Karlic & Lohninger, 2004). Though there have not been later studies with oral LC, evidence suggests a potential use of this nutrient in diminishing IC symptoms by targeting the ischaemia induced in the calf muscle.

Other nutritional approaches

Several studies have used *Ginkgo biloba* extracts for treatment of IC (Schneider, 1992; Peters *et al.* 1998). *Ginkgo biloba* is rich in flavonoids and terpene trilactones, such as ginkgolide B, which inhibits platelet activation factor, releases NO, decreases aggregation and blood viscosity and shows anti-ischaemic effects (Pittler & Ernst, 2000). Oral *Ginkgo biloba* extract tablets increased PFWD in three controlled but not randomised studies (Bauer, 1984; Blume *et al.* 1996, 1998) and these improvements are more pronounced when the dose is higher (160 mg/d; Schweizer & Hautmann, 1999). Non-randomisation, however, may have resulted in a substantial overestimation of the effect size (Kleijnen & Knipschild, 1992), and the size of the overall treatment effect is modest and of uncertain clinical relevance such as to recommend its general consumption for this disease, as suggested in a meta-analysis (Pittler & Ernst, 2000).

Garlic has also been tested as a treatment for IC, since its primary active component, allicin, has been reported to have some beneficial effects on serum cholesterol and platelet aggregation (Jepson *et al.* 2005). Only one study tested this hypothesis, supplementing 400 mg garlic powder extract twice per d for 12 weeks. Although no significant improvement was found overall, the authors report that there was a significant increase in PFWD, but this only occurred in the last weeks of therapy (Kiesewetter *et al.* 1993).

It has been hypothesised that moderate alcohol consumption exerts a protective effect on IC risk. The rationale for this hypothesis lies in the fact that alcohol raises HDL-cholesterol (van Tol *et al.* 1998). HDL plays an important role in LDL transport from the bloodstream to the liver, where it is degraded (Reichl & Miller, 1989). Oxidised LDL is a key element in the pathophysiology of atherosclerosis, and an inverse association between HDL and IC has also been reported (Fowkes *et al.* 1992). Alcohol intake favourably influences fibrinogen (Mennen *et al.* 1999), plasminogen activator inhibitor 1 (Ridker *et al.* 1994) and factor VII (Gorinstein *et al.* 1997), and lowers platelet aggregation (Renaud & Ruf, 1996), all of them mechanisms that may prevent thrombogenesis or improve fibrinolysis in PVD patients. However, limited data are available on the effects of alcohol on IC. Most of the few observational studies that have evaluated this relationship have yielded weak and inconsistent results. In the Edinburgh Artery Study, alcohol was positively associated with the ABI in males (Jepson *et al.* 1995), and another prospective study showed that moderate alcohol consumption was associated with decreased risk in male PVD patients (Camargo *et al.* 1997). Finally, this relationship was studied in the Framingham cohort (Djousse *et al.* 2000), finding a protective effect of moderate alcohol consumption on IC risk, with lowest risk observed in men consuming 13 to 24 g/d (1–2 drinks/d) and in women consuming 7–12 g/d (0·5–1 drink/d). However, non-alcoholic components of certain drinks such as wine and beer may also contribute to IC risk reduction. Wine and beer contain polyphenols with antioxidant properties; phenolic compounds may delay the onset of atherosclerosis by preventing the oxidation of LDL (Frankel *et al.* 1993). Phytoalexin, an antifungal compound found in grape skin (found in higher concentration in red wine), may raise HDL and reduce platelet aggregation (Jepson *et al.* 1995).

Finally, L-arginine has been shown to induce NO formation and improve endothelial-dependent vasodilatation in patients with atherosclerosis. Intravenous injections of L-arginine have been shown to be effective at improving IC (Boger *et al.* 1998). However, to date no trials have examined the effects of oral arginine supplementation, except for a nutritional food bar enriched with L-arginine. In this 2-week double-blind controlled trial, IC patients who consumed the food bar improved their PFWD in about 66 % (Maxwell *et al.* 2000).

Conclusion

The potential role of nutrition in PVD is important. Data referred to in the present review suggest that quite modest

levels of dietary modification could have significant effects. We have identified several nutrients that, together with appropriate lifestyle changes (such as daily exercise or smoking cessation), could be suitable in the prevention of this disease. It could be beneficial for PVD patients to increase in their diets the intake of fish or fish oil (*n*-3 PUFA) and replace saturated fat by monounsaturated fat (olive oil). A bigger consumption of fruits (antioxidants) and vegetables (fibre, folic acid and other vitamins) is also recommended. However, the limited data available in this patient group provide no clear-cut evidence in favour of the clinical benefit of nutritional interventions aimed at reducing risk factors and ameliorating symptoms in PVD. No practical recommendations can be given at this stage, and further studies are clearly needed to determine their plausible benefit and to work towards a nutritional recommendation strategy for this impairment condition.

It should be noticed that these observations do not differ much from the nutritional consensus made for CHD. Though Adult Treatment Panel III recommendations emphasise lifestyle and dietary changes in CVD prevention (National Cholesterol Education Programme, 2002), it has been evidenced in recent years that more attention should be paid to dietary approaches in the management of PVD patients, as PVD risk factors are still and often mismanaged (Henke *et al.* 2004). For example, after a hospital discharge, only 50 % of the PVD patients would modify their diet for lipid control (Mukherjee *et al.* 2002) and only 18 % of the general practitioners would consider cholesterol-lowering therapy to be primary prevention (Cassar *et al.* 2003).

Following these observations, we recently performed a small 1-year randomised controlled trial in which PVD patients were dietary supplemented with small daily doses of fish oil, olive oil, and B and E vitamins (Carrero *et al.* 2005*a*). The purpose of this study was to identify some evidence for a possible dietary guideline for this patient group. We hypothesised that it is the long-term daily consumption of small amounts of these nutrients, and not the sole intake of one of them in pharmacological amounts, that is responsible for the beneficial effects on the development of this atherosclerotic condition. Almost no dietary intervention trials in PVD have considered a combination with a full mix of nutrients before. After 1 year consuming these small amounts of nutrients, the plasma concentrations of EPA, DHA, oleic acid, folic acid, and vitamins B_6 and E significantly increased, improving nutritional status. Several traditional risk factors, such as total cholesterol and apo B concentrations, decreased in the intervention group, and total Hcy was decreased in those patients with initial HHcy. But importantly, a remarkable 3-fold increase in PFWD was observed in the intervention group when compared with the placebo group and followed by a significant increase in the ABI. We believe that this study confirms the ideas postulated in the present review, and shows how the inclusion of certain health-promoting nutrients in the everyday diet of PVD patients has not only a potential but also clearly an effective role in the amelioration of the symptoms associated with this impaired condition. Though the small sample size precludes firm conclusions, a new field of action is suggested where nutritionists could play an important part towards the development of specific dietary guidelines.

Acknowledgements

J. J. C. has a PhD educational grant from University of Granada, Spain. We are grateful to Puleva Biotech S.A. for supporting the visit of J. J. C. to Southampton University.

References

Alarcon de la Lastra C, Barranco MD, Motilva V & Herrerias JM (2001) Mediterranean diet and health: biological importance of olive oil. *Curr Pharm Des* **7**, 933–950.

Anonymous (1998) Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. Homocysteine Lowering Trialists' Collaboration. *BMJ* **316**, 894–898.

Anonymous (2000) Management of peripheral arterial disease (PAD). TransAtlantic Inter-Society Consensus (TASC). *Eur J Vasc Endovasc Surg* **19**, S1–S250.

Bakker SJ, Ijzerman RG, Teerlink T, Westerhoff HV, Gans RO & Heine RJ (2000) Cytosolic triglycerides and oxidative stress in central obesity: the missing link between excessive atherosclerosis, endothelial dysfunction, and beta-cell failure?" *Atherosclerosis* **148**, 17–21.

Barker GA, Green S, Askew CD, Green AA & Walker PJ (2001) Effect of propionyl-L-carnitine on exercise performance in peripheral arterial disease. *Med Sci Sports Exerc* **33**, 1415–1422.

Bauer U (1984) Six-month double-blind randomised clinical trial of *Ginkgo biloba* extract versus placebo in two parallel groups in patients suffering from peripheral arterial insufficiency. *Arzneimittelforsschung* **34**, 716–720.

Bazzano LA, He J, Ogden LG, Loria CM & Whelton PK (2003) Dietary fiber intake and reduced risk of coronary heart disease in US men and women: the National Health and Nutrition Examination Survey I Epidemiologic Follow-up Study. *Arch Intern Med* **163**, 1897–1904.

Blann AD, Amiral J, McCollum CN & Lip GY (2000*a*) Differences in free and total tissue factor pathway inhibitor, and tissue factor in peripheral artery disease compared to healthy controls. *Atherosclerosis* **152**, 29–34.

Blann AD, Farrell A, Picton A & McCollum CN (2000*b*) Relationship between endothelial cell markers and arterial stenosis in peripheral and carotid artery disease. *Thromb Res* **97**, 209–216.

Blann AD, Seigneur M, Steiner M, Boisseau MR & McCollum CN (1997) Circulating endothelial cell markers in peripheral vascular disease: relationship to the location and extent of atherosclerotic disease. *Eur J Clin Invest* **27**, 916–921.

Blume J, Kieser M & Hölscher U (1996) Placebo-controlled, double-blind study on the efficacy of Ginkgo biloba special extract EGb 761 in maximum-level trained patients with intermittent claudication. *Vasa* **25**, 265–274.

Blume J, Kieser M & Hölscher U (1998) Efficacy of *Ginkgo biloba* special extract EGb 761 in peripheral occlusive disease. *Fortschr Med* **116**, 137–143.

Boers GH (1997) Hyperhomocysteinemia as a risk factor for arterial and venous disease. A review of evidence and relevance. *Thromb Haemost* **78**, 520–522.

Boger RH, Bode-Boger SM, Thiele W, Creutzig A, Alexander K & Frolich JC (1998) Restoring vascular nitric oxide formation by L-arginine improves the symptoms of intermittent claudication in patients with peripheral arterial occlusive disease. *J Am Coll Cardiol* **32**, 1336–1344.

Boyd AM & Marks J (1963) Treatment of intermittent claudication. A reappraisal of alpha-tocopherol. *Angiology* **14**, 198–208.

Brevetti G, Chiariello M, Ferulano G, Policicchio A, Nevola E, Rossini A, Ambrosio G, Siliprandi N & Angelini C (1998*a*) Increases in walking distance in patients with peripheral vascular disease treated with L-carnitine: a double-blind, cross-over study. *Circulation* **77**, 767–773.

Brevetti G, Diehm C & Lambert D (1999) European multicenter study on propionyl-L-carnitine in intermittent claudication. *J Am Coll Cardiol* **34**, 1618–1624.

Brevetti G, Martone VD, Perna S, Cacciatore F, Corrado S, Di Donato A & Di Iorio A (1998*b*) Intermittent claudication and risk of cardiovascular events. *Angiology* **49**, 843–848.

Brevetti G, Perna S, Sabba C, Martone VD, Di Iorio A & Barletta G (1997) Effect of propionyl-L-carnitine on quality of life in intermittent claudication. *Am J Cardiol* **79**, 777–780.

Brevetti G, Silvestro A, Di Giacomo S, Bucar R, Di Donato A, Schiano V & Scopacasa F (2003) Endothelial dysfunction in peripheral arterial disease is related to increased plasma markers of inflammation and severity of peripheral circulatory impairment, but not to classic risk factors and atherosclerotic burden. *J Vasc Surg* **38**, 374–379.

Bronstrup A, Hages M, Prinz-Langenohl R & Pietrzik K (1998) Effects of folic acid and combinations of folic acid and vitamin B-12 on plasma homocysteine concentrations in healthy, young women. *Am J Clin Nutr* **68**, 1104–1110.

Calder PC (2004) *n*-3 Fatty acids and cardiovascular disease: evidence explained and mechanisms explored. *Clin Sci (Lond)* **107**, 1–11.

Camargo CA Jr, Stampfer MJ, Glynn RJ, Gaziano JM, Manson JE, Goldhaber SZ & Hennekens CH (1997) Prospective study of moderate alcohol consumption and risk of peripheral arterial disease in US male physicians. *Circulation* **95**, 577–580.

Carrero JJ, Fonolla J, González-Santiago M, Baro L, Jimenez J, Boza J & Lopez-Huertas E (2004*a*) Health effects due to folic acid supplementation. Food fortification strategies to achieve adequate folate status. *Nutrafoods* **3**, 5–13.

Carrero JJ, Lopez-Huertas E, Salmeron LM, Baro L & Ros E (2005*a*) Daily supplementation with (*n*-3) PUFAs, oleic acid, folic acid, and vitamins B-6 and E increases pain-free walking distance and improves risk factors in men with peripheral vascular disease. *J Nutr* **135**, 1393–1399.

Carrero JJ, Madden J, Hadley J, *et al.* (2004*b*) Fish oil alters the balance between LPS stimulated leukotriene B4 and prostaglandin E2 production by PBMCs in patients with peripheral vascular disease but not in healthy controls. *Clin Nutr* **23**, 789–790.

Carrero JJ, Martin-Bautista E, Baro L, Fonolla J, Jimenez J, Boza JJ & López-Huertas E (2005*b*) Cardiovascular effects of omega-3 fatty acids and alternatives to increase intake. *Nutr Hosp* **20**, 56–62.

Cassar K, Coull R, Bachoo P, Macaulay E & Brittenden J (2003) Management of secondary risk factors in patients with intermittent claudication. *Eur J Vasc Endovasc Surg* **26**, 262–266.

Cheng SW, Ting AC & Wong J (1997) Fasting total plasma homocysteine and atherosclerotic peripheral vascular disease. *Ann Vasc Surg* **11**, 217–223.

Ciccarone E, Di Castelnuovo A, Assanelli D, Archetti S, Ruggeri G, Salcuni N, Donati MB, Capani F, Laciovalli L & GENDIABE Investigators (2003) Homocysteine levels are associated with the severity of peripheral arterial disease in type 2 diabetic patients. *J Thromb Haemost* **1**, 2540–2547.

Cleophas TJ, Hornstra N, van Hoogstraten B & van der Meulen J (2000) Homocysteine, a risk factor for coronary artery disease or not? A meta-analysis. *Am J Cardiol* **86**, 1005–1009.

Criqui MH, Langer RD, Fronek A, Feigelson HS, Klauber MR, McCann TJ & Browner D (1992) Mortality over a period of 10 years in patients with peripheral arterial disease. *N Engl J Med* **326**, 381–386.

Dal Lago A, De Martini D, Flore R, *et al.* (1999) Effects of propionyl-L-carnitine on peripheral arterial obliterative disease of the lower limbs: a double-blind clinical trial. *Drugs Exp Clin Res* **25**, 29–36.

de Jong SC, Stehouwer CD, van den Berg M, Geurts TW, Bouter LM & Rauwerda JA (1999) Normohomocysteinaemia and vitamin-treated hyperhomocysteinaemia are associated with similar risks of cardiovascular events in patients with premature peripheral arterial occlusive disease. A prospective cohort study. *J Intern Med* **246**, 87–96.

de Vriese AS, Blom HJ, Heil SG, Mortier S, Kluijtmans LA, Van de Voorde J & Lameire NH (2004) Endothelium-derived hyperpolarizing factor-mediated renal vasodilatory response is impaired during acute and chronic hyperhomocysteinemia. *Circulation* **109**, 2331–2336.

Diehr P & Beresford SA (2003) The relation of dietary patterns to future survival, health, and cardiovascular events in older adults. *J Clin Epidemiol* **56**, 1224–1235.

Djousse L, Levy D, Murabito JM, Cupples LA & Ellison RC (2000) Alcohol consumption and risk of intermittent claudication in the Framingham Heart Study. *Circulation* **102**, 3092–3097.

Donnan PT, Thomson M, Fowkes FG, Prescott RJ & Housley E (1993) Diet as a risk factor for peripheral arterial disease in the general population: the Edinburgh Artery Study. *Am J Clin Nutr* **57**, 917–921.

Drexel H, Steurer J, Muntwyler J, Meienberg S, Schmid HR, Schneider E, Grochenig E & Amann FW (1996) Predictors of the presence and extent of peripheral arterial occlusive disease. *Circulation* **94**, 199–205.

Drossos GE, Toumpoulis IK, Katritsis DG, Ioannidis JP, Kontogiorgi P, Svarna E & Anagnostopoulos CE (2003) Is vitamin C superior to diltiazem for radial artery vasodilation in patients awaiting coronary artery bypass grafting?" *J Thorac Cardiovasc Surg* **125**, 330–335.

Duthie GC, Wahle KWJ & James PWT (1989) Oxidants, antioxidants and cardiovascular disease. *Nutr Res Rev* **2**, 51–62.

Ernst E (1989) Effects of *n*-3 fatty acids on blood rheology. *J Intern Med* **225**, Suppl., 129–132.

Farrell PM, Bieri JG, Fratantoni JF, Wood RE & di Sant'Agnese PA (1977) The occurrence and effects of human vitamin E deficiency. A study in patients with cystic fibrosis. *J Clin Invest* **60**, 233–241.

Ferrara LA, Raimondi AS, d'Episcopo L, Guida L, Dello Russo A & Marotta T (2000) Olive oil and reduced need for antihypertensive medications. *Arch Intern Med* **160**, 837–842.

Ferrari R, Visioli O, Guarnieri C & Caldarera M (1983) Vitamin E and the heart: possible role as antioxidant. *Acta Vitaminol Enzymol* **5**, 11–22.

Fowkes FG, Housley E, Riemersma RA, Macintyre CC, Cawood EH, Prescott RJ & Ruckley CV (1992) Smoking, lipids, glucose intolerance, and blood pressure as risk factors for peripheral atherosclerosis compared with ischemic heart disease in the Edinburgh Artery Study. *Am J Epidemiol* **135**, 331–340.

Frankel EN, Kanner J, German JB, Parks E & Kinsella JE (1993) Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet* **341**, 454–457.

Gans RO, Bilo HJ, Weersink EG, Rauwerda JA, Fonk T, Popp-Snijders C & Donker AJ (1990) Fish oil supplementation in patients with stable claudication. *Am J Surg* **160**, 490–495.

Gokce N, Keaney JF Jr, Hunter LM, Watkins MT, Nedelijkovic ZS, Menzoian JO & Vita JA (2003) Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. *J Am Coll Cardiol* **41**, 1769–1775.

Gorinstein S, Zemser M, Berliner M, Goldstein R, Libman I, Trakhtenberg S & Caspi A (1997) Moderate beer consumption and positive biochemical changes in patients with coronary atherosclerosis. *J Intern Med* **242**, 219–224.

Graham IM, Daly LE, Refsum HM, *et al.* (1997) Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. *JAMA* **277**, 1775–1781.

Grimble RF, Howell WM, O'Reilly G, Turner SJ, Markovic O, Hirrell S, East JM & Calder PC (2002) The ability of fish oil to suppress tumor necrosis factor-alpha production by peripheral

blood mononuclear cells in healthy men is associated with polymorphisms in genes which influence TNF-alpha production. *Am J Clin Nutr* **76**, 454–459.
Haeger K (1974) Long-time treatment of intermittent claudication with vitamin E. *Am J Clin Nutr* **27**, 1179–1181.
Halperin JL (2002) Evaluation of patients with peripheral vascular disease. *Thrombosis Research* **106**, 303–311.
Hamilton M, Wilson GM, Armitage P & Boyd JT (1953) The treatment of intermittent claudication with vitamin E. *Lancet* **i**, 367–370.
Henke PK, Blackburn S, Proctor MC, Stevens J, Mukherjee D, Rajagopalin S, Upchurch GR Jr, Stanley JC & Eagle KA (2004) Patients undergoing infrainguinal bypass to treat atherosclerotic vascular disease are underprescribed cardioprotective medications: effect on graft patency, limb salvage, and mortality. *J Vasc Surg* **39**, 357–365.
Hertzer NR (1991) The natural history of peripheral vascular disease: implications for its management. *Circulation* **83**, 12–19.
Hiatt WR (1994) Medical treatment of the patient with intermittent claudication. *J Vasc Tech* **18**, 311–315.
Hiatt WR (2001) Medical treatment of peripheral arterial disease and claudication. *N Engl J Med* **344**, 1608–1621.
Hiatt WR, Hoag S & Hamman RF (1995) Effect of diagnostic criteria on the prevalence of peripheral arterial disease: the San Luis Valley Diabetes Study. *Circulation* **91**, 1472–1479.
Hirsch AT & Hiatt WR; PARTNERS Steering Committee (2001) PAD awareness, risk, and treatment: new resources for survival – the USA PARTNERS program. *Vasc Med* **6**, 9–12.
Ho M, Maple C, Bancroft A, McLaren M & Belch JJ (1999) The beneficial effects of omega-3 and omega-6 essential fatty acid supplementation on red blood cell rheology. *Prostaglandins Leukot Essent Fatty Acids* **61**, 13–17.
Hooper L, Griffiths E, Abrahams B, *et al.* (2004) Dietetic guidelines: diet in secondary prevention of cardiovascular disease (first update June 2003). *J Hum Nutr Diet* **17**, 337–349.
Hunninghake DB, Miller VT, LaRosa JC, Kinosian B, Brown V, Howard WJ, DiSerio FJ & O'Connor RR (1994) Hypocholesterolemic effects of a dietary fibre supplement. *Am J Clin Nutr* **59**, 1050–1054.
James SL, Muir JG, Curtis SL & Gibson PR (2003) Dietary fibre: a roughage guide. *Intern Med J* **33**, 291–296.
Jenkins DJ, Axelsen M, Kendall CW, Augustin LS, Vuksan V & Smith U (2000) Dietary fibre, lente carbohydrates and the insulin-resistant diseases. *Br J Nutr* **83**, 157–163.
Jenkins DJ & Jenkins AL (1985) Dietary fibre and the glycemic response. *Proc Soc Exp Biol Med* **180**, 422–431.
Jepson RG, Fowkes FG, Donnan PT & Housley E (1995) Alcohol intake as a risk factor for peripheral arterial disease in the general population in the Edinburgh Artery Study. *Eur J Epidemiol* **11**, 9–14.
Jepson RG, Kleijnen J & Leng GC (2005) Garlic for peripheral arterial occlusive disease (Cochrane Review). In *The Cochrane Library*, issue 3. Chichester, UK: John Wiley & Sons, Ltd.
Jeukendrup AE (2002) Regulation of fat metabolism in skeletal muscle. *Ann N Y Acad Sci* **967**, 217–235.
Kanayasu-Toyoda T, Morita I, Hibino H, Nakao-Hayashi J, Ito H & Murota S (1993) Eicosapentaenoic acid abolishes the proatherogenic effects of cholesterol: effects on migration of bovine smooth muscle and endothelial cells in vitro. *Prostaglandins Leukot Essent Fatty Acids* **48**, 463–468.
Kanayasu-Toyoda T, Morita I & Murota S (1996) Docosapentaenoic acid (22: 5, *n*-3), an elongation metabolite of eicosapentaenoic acid (20: 5, *n*-3), is a potent stimulator of endothelial cell migration on pretreatment in vitro. *Prostaglandins Leukot Essent Fatty Acids* **54**, 319–325.
Karlic H & Lohninger A (2004) Supplementation of L-carnitine in athletes: does it make sense?" *Nutrition* **20**, 709–715.
Katsouyanni K, Skalkidis Y, Petridou E, Polychronopoulou-Trichopoulou A, Willett W & Trichopoulos D (1991) Diet and peripheral arterial occlusive disease: the role of poly-, mono-, and saturated fatty acids. *Am J Epidemiol* **133**, 24–31.
Kearns SR, Daly AF, Sheehan K, Murray P, Kelly C & Bouchier-Hayes D (2004) Oral vitamin C reduces the injury to skeletal muscle caused by compartment syndrome. *J Bone Joint Surg Br* **86**, 906–911.
Khaira HS, Maxwell SR & Shearman CP (1995) Antioxidant consumption during exercise in intermittent claudication. *Br J Surg* **82**, 1660–1662.
Khaira HS, Maxwell SR, Thomason H, Thorpe GH, Green MA & Shearman CP (1996) Antioxidant depletion during aortic aneurysm repair. *Br J Surg* **83**, 401–403.
Kiesewetter H, Jung F, Jung EM, Blume J, Mrowietz C, Birk A, Koscielny J & Wenzel E (1993) Effects of garlic coated tablets in peripheral arterial occlusive disease. *Clin Investig* **71**, 383–386.
King DE, Egan BM & Geesey ME (2003) Relation of dietary fat and fiber to elevation of C-reactive protein. *Am J Cardiol* **92**, 1335–1339.
Kinlay S, Behrendt D, Fang JC, Delegrange D, Morrow J, Witztum JL, Rifai N, Selwyn NP, Creager MA & Ganz P (2004) Long-term effect of combined vitamins E and C on coronary and peripheral endothelial function. *J Am Coll Cardiol* **43**, 629–634.
Kleijnen J & Knipschild P (1992) Ginkgo biloba. *Lancet* **340**, 1136–1139.
Kleijnen J & Mackerras D (2004) Vitamin E for intermittent claudication (Cochrane Review). In *The Cochrane Library*, issue 3. Chichester, UK: John Wiley & Sons, Ltd.
Klipstein-Grobusch K, den Breeijen JH, Grobbee DE, Boeing H, Hofman A & Witteman JC (2001) Dietary antioxidants and peripheral arterial disease: the Rotterdam Study. *Am J Epidemiol* **154**, 145–149.
Koga T, Claycombe K & Meydani M (2002) Homocysteine increases monocyte and T-cell adhesion to human aortic endothelial cells. *Atherosclerosis* **161**, 365–374.
Kuan YM, E Dear A & Grigg MJ (2002) Homocysteine: an aetiological contributor to peripheral vascular arterial disease. *ANZ J Surg* **72**, 668–671.
Langlois M, Duprez D, Delanghe J, De Buyzere M & Clement DL (2001) Serum vitamin C concentration is low in peripheral arterial disease and is associated with inflammation and severity of atherosclerosis. *Circulation* **103**, 1863–1868.
Leng GC & Fowkes FGR (1993) The epidemiology of peripheral arterial disease. *Vasc Med Rev* **4**, 5–18.
Leng GC, Fowkes FG, Lee AJ, Dunbar J, Housley E & Ruckley CV (1996) Use of ankle brachial pressure index to predict cardiovascular events and death: a cohort study. *BMJ* **313**, 1440–1444.
Leng GC, Lee AJ, Fowkes FG, Jepson RG, Lowe GD, Skinner ER & Mowat BF (1998) Randomized controlled trial of gamma-linolenic acid and eicosapentaenoic acid in peripheral arterial disease. *Clin Nutr* **17**, 265–271.
Lentz SR & Sadler JE (1991) Inhibition of thrombomodulin surface expression and protein C activation by the thrombogenic agent homocysteine. *J Clin Invest* **88**, 1906–1914.
Liese AD, Roach AK, Sparks KC, Marquart L, D'Agostino RB Jr & Mayer-Davis EJ (2003) Whole-grain intake and insulin sensitivity: the Insulin Resistance Atherosclerosis Study. *Am J Clin Nutr* **78**, 965–971.
Livingstone PD & Jones C (1958) Treatment of intermittent claudication with vitamin E. *Lancet* **ii**, 602–664.
Lowe GD, Fowkes FG, Dawes J, Donnan PT, Lennie SE & Housley E (1993) Blood viscosity, fibrinogen, and activation of coagulation and leukocytes in peripheral arterial disease and the normal population in the Edinburgh Artery Study. *Circulation* **87**, 1915–1920.
McDermott MM, Mehta S, Ahn H & Greenland P (1997) Atherosclerotic risk factors are less intensively treated in patients with

peripheral arterial disease than in patients with coronary artery disease. *J Gen Intern Med* **12**, 209–215.
Marckmann P, Sandstrom B & Jespersen J (1993) Dietary effects on circadian fluctuation in human blood coagulation factor VII and fibrinolysis. *Atherosclerosis* **101**, 225–234.
Markovic O, O'Reilly G, Fussell HM, Turner SJ, Calder PC, Howell WM & Grimble RF (2004) Role of single nucleotide polymorphisms of proinflammatory cytokine genes on the relationship between serum lipids and inflammatory parameters, and the lipid-lowering effect of fish oil in healthy males. *Clin Nutr* **23**, 1084–1095.
Maxwell AJ, Anderson BE & Cooke JP (2000) Nutritional therapy for peripheral arterial disease: a double-blind, placebo-controlled, randomized trial of HeartBar. *Vasc Med* **5**, 11–19.
Mennen LI, Balkau B, Vol S, Caces E & Eschwege E (1999) Fibrinogen: a possible link between alcohol consumption and cardiovascular disease? DESIR Study Group. *Arterioscler Thromb Vasc Biol* **19**, 887–892.
Merchant AT, Hu FB, Spiegelman D, Willett WC, Rimm EB & Ascherio A (2003*a*) The use of B vitamin supplements and peripheral arterial disease risk in men are inversely related. *J Nutr* **133**, 2863–2867.
Merchant AT, Hu FB, Spiegelman D, Willett WC, Rimm EB & Ascherio A (2003*b*) Dietary fibre reduces peripheral arterial disease risk in men. *J Nutr* **133**, 3658–3663.
Meydani M, Natiello F, Goldin B, Free N, Woods M, Schaefer E, Blumberg JB & Gorbach SL (1991) Effect of long-term fish oil supplementation on vitamin E status and lipid peroxidation in women. *J Nutr* **121**, 484–491.
Mori TA, Vandongen R, Mahanian F & Douglas A (1992) Plasma lipid levels and platelet and neutrophil function in patients with vascular disease following fish oil and olive oil supplementation. *Metabolism* **41**, 1059–1067.
Morrissey PA & Sheehy PJ (1999) Optimal nutrition: vitamin E. *Proc Nutr Soc* **58**, 459–468.
Mujumdar VS, Aru GM & Tyagi SC (2001) Induction of oxidative stress by homocyst(e)ine impairs endothelial function. *J Cell Biochem* **82**, 491–500.
Mukherjee D, Lingam P, Chetcuti S, Grossman PM, Moscucci M, Luciano AE & Eagle KA (2002) Missed opportunities to treat atherosclerosis in patients undergoing peripheral vascular interventions: insights from the University of Michigan Peripheral Vascular Disease Quality Improvement Initiative, (PVD-QI2). *Circulation* **106**, 1909–1912.
Muluk SC, Muluk VS, Kelley ME, Whittle JC, Tierney JA, Webster MW & Makaroun MS (2001) Outcome events in patients with claudication: a 15-years study in 2777 patients. *J Vasc Surg* **33**, 251–257.
Nappo F, De Rosa N, Marfella R, De Lucia D, Ingrosso D, Perna AF, Farzati B & Giugliano D (1999) Impairment of endothelial functions by acute hyperhomocysteinemia and reversal by antioxidant vitamins. *JAMA* **281**, 2113–2118.
National Cholesterol Education Programme (2002) *Third Report of the Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III)*, National Institutes of Health publication number 02–5215. Washington, DC: NIH.
Pereira MA, O'Reilly E, Augustsson K, *et al.* (2004) Dietary fiber and risk of coronary heart disease: a pooled analysis of cohort studies. *Arch Intern Med* **164**, 370–376.
Peters H, Kieser M & Hölscher U (1998) Demonstration of the efficacy of Ginkgo biloba special extract EGb 761® on intermittent claudication: a placebo-controlled, double-blind multicenter trial. *VASA* **27**, 106–110.
Philipp CS, Cisar LA, Kim HC, Wilson AC, Saidi P & Kostis JB (1997) Association of hemostatic factors with peripheral vascular disease. *Am Heart J* **134**, 978–984.
Piesse JW (1984) Vitamin E and peripheral vascular disease. *Int Clin Nutr Rev* **4**, 178–182.
Pittler MH & Ernst E (2000) Ginkgo biloba extract for the treatment of intermittent claudication: a meta-analysis of randomized trials. *Am J Med* **108**, 276–281.
Podmore ID, Griffiths HR, Herbert KE, Mistry N, Mistry P & Lunec J (1998) Vitamin C exhibits pro-oxidant properties. *Nature* **392**, 559.
Poredos P, Golob M & Jensterle M (2003) Interrelationship between peripheral arterial occlusive disease, carotid atherosclerosis and flow mediated dilation of the brachial artery. *Int Angiol* **22**, 83–87.
Ramirez-Tortosa C, Lopez-Pedrosa JM, Suarez A, Ros E, Mataix J & Gil A (1999*a*) Olive oil- and fish oil-enriched diets modify plasma lipids and susceptibility of LDL to oxidative modification in free-living male patients with peripheral vascular disease: the Spanish Nutrition Study. *Br J Nutr* **82**, 31–39.
Ramirez-Tortosa MC, Urbano G, Lopez-Jurado M, Nestares T, Gomez MC, Mir A, Ros E, Mataix J & Gil A (1999*b*) Extra-virgin olive oil increases the resistance of LDL to oxidation more than refined olive oil in free-living men with peripheral vascular disease. *J Nutr* **129**, 2177–2183.
Refsum H, Ueland PM, Nygard O & Vollset SE (1998) Homocysteine and cardiovascular disease. *Annu Rev Med* **49**, 31–62.
Reichl D & Miller NE (1989) Pathophysiology of reverse cholesterol transport. Insights from inherited disorders of lipoprotein metabolism. *Arteriosclerosis* **9**, 785–797.
Renaud SC & Ruf JC (1996) Effects of alcohol on platelet functions. *Clin Chim Acta* **246**, 77–89.
Riba R, Nicolaou A, Troxler M, Homer-Vaniasinkam S & Naseem KM (2004) Altered platelet reactivity in peripheral vascular disease complicated with elevated plasma homocysteine levels. *Atherosclerosis* **175**, 69–75.
Ridker PM, Vaughan DE, Stampfer MJ, Glynn RJ & Hennekens CH (1994) Association of moderate alcohol consumption and plasma concentration of endogenous tissue-type plasminogen activator. *JAMA* **272**, 929–933.
Rodgers GM & Kane WH (1986) Activation of endogenous factor V by homocysteine-induce vascular endothelial cell activator. *J Clin Invest* **11**, 1909–1916.
Ross R (1999) Related atherosclerosis – an inflammatory disease. *N Engl J Med* **340**, 115–126.
Rossi E, Biasucci LM, Citterio F, Pelliccioni S, Monaco C, Ginnetti F, Angiolillo DJ, Grieco G, Liuzzo G & Maseri A (2002) Risk of myocardial infarction and angina in patients with severe peripheral vascular disease: predictive role of C-reactive protein. *Circulation* **105**, 800–803.
Ruiz-Gutierrez V, Muriana FJ, Guerrero A, Cert AM & Villar J (1996) Plasma lipids, erythrocyte membrane lipids and blood pressure of hypertensive women after ingestion of dietary oleic acid from two different sources. *J Hypertens* **14**, 1483–1490.
Schneider B (1992) Ginkgo biloba extract in peripheral arterial disease. Meta-analysis of controlled clinical trials. *Arzneimittelforschung* **42**, 428–436.
Schweizer J & Hautmann C (1999) Comparison of two dosages of Ginkgo biloba extract EGb 761 in patients with peripheral arterial occlusive disease Fontaine's stage IIb. *Arzneimittelforschung* **49**, 900–904.
Shute EV, Vogelsang AB, Skelton FR & Shute WE (1948) The influence of vitamin E on vascular disease. *Surg Gynaecol Obst* **86**, 1–8.
Signorelli SS, Mazzarino MC, Di Pino L, *et al.* (2003) High circulating levels of cytokines (IL-6 and TNFalpha), adhesion molecules (VCAM-1 and ICAM-1) and selectins in patients with peripheral arterial disease at rest and after a treadmill test. *Vasc Med* **8**, 15–19.
Silverman MD, Tumuluri RJ, Davis M, Lopez G, Rosenbaum JT & Lelkes PI (2002) Homocysteine upregulates vascular cell adhesion molecule-1 expression in cultured human aortic endothelial cells

and enhances monocyte adhesion. *Arterioscler Thromb Vasc Biol* **22**, 587–592.
Silvestro A, Scopacasa F, Oliva G, de Cristofaro T, Iuliano L & Brevetti G (2002) Vitamin C prevents endothelial dysfunction induced by acute exercise in patients with intermittent claudication. *Atherosclerosis* **165**, 277–283.
Simchon S, Jan KM & Chien S (1987) Influence of reduced red cell deformability on regional blood flow. *Am J Physiol* **253**, 898–903.
Smit AJ (1992) Medical treatment of peripheral vascular disease: are there new perspectives?" *Vasa Supplemental* **34**, 20–24.
Sommerfield T & Hiatt WR (2004) Omega-3 fatty acids for intermittent claudication (Cochrane review). In *The Cochrane Library*, issue 3, Chichester, UK: John Wiley & Sons, Ltd.
Spark JI, Robinson JM, Gallavin L, Gough MJ, Homer-Vanniasinkam S, Kester RC & Scott DJ (2002) Patients with chronic critical limb ischemia have reduced total antioxidant capacity and impaired nutritional status. *Eur J Vasc Endovasc Surg* **24**, 535–539.
Stamler JS, Osborne JA, Jaraki O, Rabbani LE, Mullins M, Singel D & Loscalzo J (1993) Adverse vascular effects of homocysteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. *J Clin Invest* **91**, 308–318.
Stark AH & Madar Z (2002) Olive oil as a functional food: epidemiology and nutritional approaches. *Nutr Rev* **60**, 170–176.
Steinberg D (1991) Antioxidants and atherosclerosis: a current assessment. *Circulation* **84**, 1420–1425.
Steiner M & Mower R (1982) Mechanism of action of vitamin E on platelet function. *Ann N Y Acad Sci* **393**, 289–299.
Stringer MD, Gorog PG, Freeman A & Kakkar VV (1989) Lipid peroxides and atherosclerosis. *BMJ* **298**, 281–284.
Tabet S & Berg AO, for the US Preventive Services Task Force (1996) Screening for peripheral arterial disease. In *Guide to Clinical Preventive Services: Report of the US Preventive Services Task Force*, pp. 63–66. Baltimore, MD: Williams & Wilkins.
Taylor LM Jr, DeFrang RD, Harris EJ Jr & Porter JM (1991) The association of elevated plasma homocyst(e)ine with progression of symptomatic peripheral arterial disease. *J Vasc Surg* **13**, 128–136.
Taylor LM Jr, Moneta GL, Sexton GJ, Schuff RA & Porter JM (1999) Prospective blinded study of the relationship between plasma homocysteine and progression of symptomatic peripheral arterial disease. *J Vasc Surg* **29**, 8–21.
Thies F, Garry JM, Yaqoob P, Rerkasem K, Williams J, Shearman CP, Gallagher PJ, Calder PC & Grimble RF (2003) Association of *n*-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomised controlled trial. *Lancet* **361**, 477–485.
Thorburn A, Muir J & Proietto J (1993) Carbohydrate fermentation decreases hepatic glucose output in healthy subjects. *Metabolism* **42**, 780–785.
Tornwall ME, Virtamo J, Haukka JK, Aro A, Albanes D & Huttunen JK (2000) Prospective study of diet, lifestyle, and intermittent claudication in male smokers. *Am J Epidemiol* **151**, 892–901.
Tsai MY, Arnett DK, Eckfeldt JH, Williams RR & Ellison RC (2000) Plasma homocysteine and its association with carotid intimal-medial wall thickness and prevalent coronary heart disease: NHLBI Family Heart Study. *Atherosclerosis* **151**, 519–524.
van den Berg M, Franken DG, Boers GH, Blom HJ, Jakobs C, Stehouwer CD & Rauwerda JA (1994) Combined vitamin B6 plus folic acid in young patients with atherosclerosis and hyperhomocysteinaemia. *J Vasc Surg* **20**, 933–940.
van Tol A, van der Gaag MS, Scheek LM, van Gent T & Hendriks HF (1998) Changes in postprandial lipoproteins of low and high density caused by moderate alcohol consumption with dinner. *Atherosclerosis* **141**, 101–103.
Verhoef P, Stampfer MJ, Buring JE, Gaziano JM, Allen RH, Stabler SP, Reynolds RD, Kok FJ, Hennekens CH & Willett WC (1996) Homocysteine metabolism and risk of myocardial infarction: relation with vitamins B6, B12, and folate. *Am J Epidemiol* **143**, 845–859.
Vicaut E (1995) Opposite effects of red blood cell aggregation on resistance to blood flow. *J Cardiovasc Surg* **36**, 361–368.
Violi F, Criqui M, Longoni A & Castiglioni C (1996) Relation between risk factors and cardiovascular complications in patients with peripheral arterial disease: results from the A.D.E.P. study. *Atherosclerosis* **120**, 25–35.
Vogt MT, Wolfson SK & Kuller LH (1993) Segmental arterial disease in the lower extremities: correlates of disease and relationship to mortality. *J Clin Epidemiol* **46**, 1267–1276.
Wald DS, Law M & Morris JK (2002) Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. *BMJ* **325**, 1202–1206.
Wall RT, Harlan JM, Harker LA & Striker GE (1980) Homocysteine-induced endothelial cell injury in vitro: a model for the study of vascular injury. *Thromb Res* **18**, 113–121.
Wang J, Dudman NP & Wilcken DE (1993) Effect of homocysteine and related compounds on prostacyclin production by cultured human vascular endothelial cells. *Thromb Haemost* **70**, 1047–1052.
Weiss N, Keller C, Hoffmann U & Loscalzo J (2002) Endothelial dysfunction and atherothrombosis in mild hyperhomocysteinemia. *Vasc Med* **7**, 227–239.
Welch GN & Loscalzo J (1998) Homocysteine and atherothrombosis. *N Engl J Med* **338**, 1042–1050.
Westheim AS, Brox D & Selvaag OAW (1975) D-alpha-tokoferol ved claudication intermittens (D-alpha-Tocopherol in intermittent claudication; article in Norwegian). *Tidsskr Norske Laegeforen* **95**, 13–15.
Williams HT, Fenna D & Macbeth RA (1971) Alpha tocopherol in the treatment of intermittent claudication. *Surg Gynecol Obstet* **132**, 662–666.
Wilmink AB, Welch AA, Quick CR, Burns PJ, Hubbard CS, Bradbury AW & Day NE (2004) Dietary folate and vitamin B6 are independent predictors of peripheral arterial occlusive disease. *J Vasc Surg* **39**, 513–516.
Woodcock BE, Smith E, Lambert WH, Jones WM, Galloway JH, Greaves M & Preston FE (1984) Beneficial effect of fish oil on blood viscosity in peripheral vascular disease. *Br Med J (Clin Res Ed)* **288**, 592–594.
World Health Organization Study Group (2003) *Diet, Nutrition and Prevention of Chronic Diseases*. Report of the Joint WHO/FAO Expert Consultation. WHO Technical Report Series, no. 916. Geneva: WHO.
Yataco AR, Corretti MC, Gardner AW, Womack CJ & Katzel LI (1999) Endothelial reactivity and cardiac risk factors in older patients with peripheral arterial disease. *Am J Cardiol* **83**, 754–758.