Review article

Does nutrition have a role in peripheral vascular disease?

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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 under-treated 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 B₆ 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; Mulak 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;...
Brevetti et al. (1998a), 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 1998b). 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, leukocyte 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. 2000b), tissue factor (Blann et al. 2000a), 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 O2 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. 2005b). 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-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 leukotriene B₄ in PVD patients, while leukotriene B₄ 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 leukotriene B₄ and prostaglandin E₂ 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. 2004b). 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.

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

Fig. 1. n-3 PUFA metabolism. LA, linoleic acid; ALA, α-linolenic acid; AA, arachidonic acid; PG, prostaglandin; TX, thromboxane; LT, leukotriene.
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 B2 (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. 1999a). 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. 2005a). 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. 1999b). Basically, the oils differed in their antioxidant profile (α-tocopherol 300 ν. 200 mg/kg; phenolic compounds 800 ν. 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 (Morrissette & 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 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 thrombogenic 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 & Llosalzo, 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 B₉ and B₁₂ 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...
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. 2004a). Vitamin B₆ is present in foods such as potatoes, breakfast cereals, bread, meat, fish, eggs, bananas, nuts and seeds. Vitamin B₁₂ is naturally found in all animal foods – meat, meat products, milk, fish, eggs – and certain algae, such as seaweed. Recently, folate and B₆ intake have been identified as independent predictors of PVD in men aged over 50 years (Wilmink et al. 2003). 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 B₆ and PVD risk (P=0.06; Merchant et al. 2003a). 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 B₁₂ and B₆ 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, suppression of carcinogenesis, lower cholesterol, improved glycemic 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 cerebral 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. 2003b).

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 I 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 cytotoxic triacylglycerolcerols are associated with oxidative stress and can cause endothelial dysfunction (Bakker et al. 2000). Thus, increased serum HDL and triacylglycerolcerols 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.

\[ \text{L-carnitine} \]

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

\[ \text{CPT-1} \]

\[ \text{CPT-2} \]

\[ \text{PDH} \]

\[ \text{Saroplasm} \]

\[ \text{Mitochondrial membrane} \]

\[ \text{Inside the mitochondria} \]

\[ \text{Translocase} \]

\[ \text{CoASH} \]

\[ \text{Acyl-CoA} \]

\[ \text{Acetyl-CoA} \]

\[ \text{β-Oxidation} \]

\[ \text{Glycolysis} \]

\[ \text{Mitochondria} \]

\[ \text{PDH complex} \]

\[ \text{Fig. 3. Role of L-carnitine in oxidative metabolism. CoASH, acetyl-CoA; CPT, carnitine palmitoyltransferase; PDH, pyruvate dehydrogenase.} \]
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 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 tri lactones, 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). Phytolalexin, 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. 2005a). 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 these nutrients, and not the sole intake of 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.

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