Functional food science and the cardiovascular system

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Abbreviations: ALA, α-linolenic acid; apo, apolipoprotein; AT-III, antithrombin-III; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FVIIc, factor VII coagulant activity; ICAM, intracellular adhesion molecule; Lp(a), lipoprotein(a); MI, myocardial infarction; MTHFR, 5,10-methylenetetrahydrofolate reductase; NIDDM, non-insulin-dependent diabetes mellitus; oxLDL, oxidized LDL; PAI-1, plasminogen activator inhibitor-1; PG, prostaglandin; P : S, polyunsaturated : saturated fatty acid ratio; SMC, smooth-muscle cells; TGF-β, transforming growth factor-β; t-PA, plasminogen activator; Tx, thromboxane; VCAM, vascular cell adhesion molecule; vWf, von-Willebrand factor.

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Abstract

Cardiovascular disease has a multifactorial aetiology, as is illustrated by the existence of numerous risk indicators, many of which can be influenced by dietary means. It should be recalled, however, that only after a cause-and-effect relationship has been established between the disease and a given risk indicator (called a risk factor in that case), can modifying this factor be expected to affect disease morbidity and mortality. In this paper, effects of diet on cardiovascular risk are reviewed, with special emphasis on modification of the plasma lipoprotein profile and of hypertension. In addition, dietary influences on arterial thrombotic processes, immunological interactions, insulin resistance and hyperhomocysteinaemia are discussed. Dietary lipids are able to affect lipoprotein metabolism in a significant way, thereby modifying the risk of cardiovascular disease. However, more research is required concerning the possible interactions between the various dietary fatty acids, and between fatty acids and dietary cholesterol. In addition, more studies are needed with respect to the possible importance of the postprandial state. Although in the aetiology of hypertension the genetic component is definitely stronger than environmental factors, some benefit in terms of the development and coronary complications of atherosclerosis in hypertensive patients can be expected from fatty acids such as α-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid. This particularly holds for those subjects where the hypertensive mechanism involves the formation of thromboxane A_2 and/or α1-adrenergic activities. However, large-scale trials are required to test this contention. Certain aspects of blood platelet function, blood coagulability, and fibrinolytic activity are associated with cardiovascular risk, but causality has been insufficiently proven. Nonetheless, well-designed intervention studies should be initiated to further evaluate such promising dietary components as the various *n*-3 and *n*-6 fatty acids and their combination, antioxidants, fibre, etc. for their effect on processes participating in arterial thrombus formation. Long-chain polyenes of the *n*-3 family and antioxidants can modify the activity of immunocompetent cells, but we are at an early stage of examining the role of immune function on the development of atherosclerotic plaques. Actually, there is little, if any, evidence that dietary modulation of immune system responses of cells participating in atherogenesis exerts beneficial effects. Although it seems feasible to modulate insulin sensitivity and subsequent cardiovascular risk factors by decreasing the total amount of dietary fat and increasing the proportion of polyunsaturated fatty acids, additional studies on the efficacy of specific fatty acids, dietary fibre, and low-energy diets, as well as on the mechanisms involved are required to understand the real function of these dietary components. Finally, dietary supplements containing folate and vitamins B_6 and/or B_{12} should be tested for their potential to reduce cardiovascular risk by lowering the plasma level of homocysteine.

1. Some aspects of coronary heart disease (CHD) aetiology

Major health risks with respect to the cardiovascular system are CHD and hypertension. In addition, cardiovascular complications of diabetes mellitus are important in this respect. The main aetiological processes involved comprise disturbances in lipoprotein metabolism, a prothrombotic shift in the arterial thrombogenic balance, derangements of the immunological system, insulin resistance and hyperhomocysteinaemia. In the present paper, the potential effects of nutritive and non-nutritive food components on these processes will be reviewed. Epidemiological studies have provided important information on the factors involved in the aetiology of CHD, which has been used as a basis for preventive strategies. Nethertheless, only approximately 50% of the incidence of cardiovascular disease can be explained by the major risk factors, leaving space for substantial, largely unexplored, contributing factors.

1.1. Lipoprotein metabolism

The pathological changes in the coronary arteries that lead to the development of atherosclerotic plaques are now better understood. One of the earliest changes may be endothelial dysfunction (Healy, 1990) followed by the development of fatty streaks due to the formation and accumulation of oxidized lipoprotein particles in the subendothelial space (Steinberg *et al.* 1989). A critical role for antioxidant vitamins, such as α-tocopherol, ascorbic acid and (perhaps) β-carotene in the prevention of endothelial dysfunction and/or LDL oxidation has been hypothesized (Gey *et al.* 1993).

Many studies have found an association between serum lipoprotein concentrations and the risk of CHD. Associations, however, do not necessarily reflect a causal relationship; such a relationship can only be established by well-controlled intervention studies. Formally, causality has only been proven for the positive relationship between LDL-cholesterol levels and the risk of CHD (Frick *et al.* 1987). However, strong evidence also exists that a high concentration of HDL-cholesterol or a low LDL- (or total) : HDL-cholesterol ratio protects against CHD (Shaten *et al.* 1991; Castelli *et al.* 1992). Further, raised fasting triacylglycerol (Hokanson & Austin, 1996) and lipoprotein(a) (Lp(a)) concentrations (Bostom *et al.* 1996), as well as the presence of small LDL particles (Austin, 1992) and postprandial lipidaemia (Karpe *et al.* 1994) may be positively associated with an increased CHD risk.

1.2. Arterial thrombosis

Arterial thrombosis starts within seconds after vascular damage and involves the participation of blood platelets and leucocytes, and of coagulation and fibrinolysis. The process results in the formation of mural, embolizing and, ultimately, occlusive thrombi, thereby promoting the progress of atherosclerotic disease, tissue and organ infarction and sudden death (Fuster *et al.* 1990). Under normal physiological conditions, the cellular components and proteins involved (e.g. proenzymes and procofactors) are mostly present in an inactive form and become activated as a result of vascular injury. Platelets undergoing a series of biochemical and morphological changes express proteins and cell receptors, adhere and form aggregates, and bind to neutrophils and monocytes. Similarly, endothelial cells express intercellular adhesion molecules after stimulation.

1.3. Immunological interactions

The atherosclerotic lesion is associated with multiple interactions between immuno-competent cells in the blood (monocytes, T-lymphocytes and platelets) together with the two major cell types in the artery wall, endothelial cells and smooth-muscle cells (SMC). Thus, circulating blood monocytes and T-lymphocytes interact with 'damaged' endothelium, enter the subendothelial space of the artery wall and release bioactive molecules. SMC can produce and secrete proteoglycans and facilitate the formation of connective tissue and, thereby, contribute to the formation of advanced atherosclerotic lesions which are also promoted by a thrombotic process (McGill, 1984).

1.4. Hypertension

CHD is strongly related to both systolic and diastolic blood pressure in a graded fashion as demonstrated in an analysis of nine large prospective studies (MacMahon *et al.* 1990). There are multiple causes for primary hypertension with a strong genetic component. Treatment of hypertension and isolated hypertension (without an increased diastolic blood pressure) results in a reduction in coronary disease-related events (Collins *et al.* 1990).

Increased blood pressure *per se* appears to increase atherosclerosis, presumably by promoting the entry of LDL into the subendothelial space (Curmi *et al.* 1990). Haemodynamic factors appear to play a role and it is a well-known fact that certain arteries and sites near vessel bifurcations have a predilection to develop atherosclerosis.

1.5. Insulin resistance

Although the phenomenon of insulin resistance has been known for a long time (Himsworth, 1936), the link with atherogenesis was first hypothesized by Stout & Vallance-Owen (1969). This theory was revived by Reaven (1988) when he proposed the existence of a syndrome characterized by obesity, hypertension, dyslipidaemia and glucose intolerance, in which insulin resistance was the common link (metabolic syndrome X). Since then, several excellent reviews regarding the link between insulin resistance, metabolic abnormalities and diseases have been published (Ferrannini *et al.* 1991; Elliott & Viberti, 1993; Laws & Reaven, 1993; Després & Marette, 1994).

1.6. Hyperhomocysteinaemia

Compelling evidence is now available suggesting that homocyst(e)ine is implicated in cardiovascular disease. This view is based on a large number of epidemiological studies, recently summarized by Boushey *et al.* (1995) and by Malinow (1996) and supporting the hypothesis that the

plasma homocysteine concentration is an independent graded risk indicator for arteriosclerotic vascular diseases (coronary, cerebral, and peripheral arterial occlusive diseases, as well as carotid thickening). From the meta-analysis by Boushey *et al.* (1995) a total of 10 % of the population's coronary artery disease risk was suggested to be attributable to homocysteine. Recent studies by Tonstad *et al.* (1996) demonstrate that a modest elevation in plasma homocysteine level in children is related to premature cardiovascular death in their male relatives and may partly account for the contribution of family history to risk of cardiovascular disease.

2. Dietary components and serum lipoproteins

The association between serum lipoprotein concentrations and the risk of CHD is generally acknowledged. As diet plays an important role in the modulation of lipoprotein metabolism, the purpose of this section is to briefly summarize effects of various dietary components on lipoprotein metabolism. Attention will also be given to the role of the genetic background of individuals in modulating these dietary effects.

2.1. Effects of dietary components on fasting lipid and lipoprotein concentrations

2.1.1. Fatty acids. For the purpose of this discussion, the fatty acids are categorized into four classes: saturated fatty acids, monounsaturated fatty acids (mainly oleic acid, 18 : 1*n*-9), polyunsaturated fatty acids (mainly linoleic acid, 18 : 2*n*-6), and *trans* fatty acids (mainly 18 : 1*trans*).

In a meta-analysis of twenty-seven well-controlled dietary studies (Mensink & Katan, 1992), it was found that, relative to an isoenergetic amount of carbohydrates, a mixture of saturated fatty acids increases LDL-cholesterol concentrations. Polyunsaturated fatty acids, however, lower LDL-cholesterol, but to a lesser extent, as estimated by Keys *et al.* (1965*b*). The effect of oleic acid was between that of carbohydrates and polyunsaturated fatty acids. Further, it was demonstrated that all fatty acids increase HDL-cholesterol, but this effect appeared to diminish with increasing unsaturation of the fatty acid. Therefore, it was concluded that under isoenergetic metabolic-ward conditions, the most favourable lipoprotein risk profile for CHD was achieved if saturated fatty acids were replaced by unsaturated fatty acids. However, no distinction was made between the effects of the individual saturated fatty acids.

As already indicated by the studies of Keys *et al.* (1965*b*), the cholesterolaemic effects of the various saturated fatty acids are not equal. It was therefore suggested that saturated fatty acids should be divided into those with less than twelve C atoms, those with twelve to sixteen C atoms (lauric acid, 12 : 0, myristic acid, 14 : 0, and palmitic acid, 16 : 0) and those with eighteen C atoms (stearic acid, 18 : 0). For statistical reasons, the data from the meta-analysis do not allow estimation of the impact of all the various saturated fatty acids, but it is possible to calculate the separate effects of palmitic and stearic acids, the two most abundant saturated fatty acids in the diet. In agreement with the findings of others, these analyses clearly show that, in contrast with other saturated fatty acids, stearic acid affects neither serum LDL- nor HDL-cholesterol levels (Fig. 1). Other studies suggest that lauric and myristic acids are more cholesterolaemic than palmitic acid, due to increases in both LDL- and HDL-cholesterol (Zock *et al.* 1994; Temme *et al.* 1996). In a recent study, it was demonstrated that a mixture of caprylic acid (8 : 0) and capric acid (10 : 0), two medium-chain fatty acids (which are fatty acids with a chain length between four and ten C atoms) slightly increased LDL-cholesterol relative to oleic acid, and had no effect on HDL-cholesterol (Cater *et al.* 1997). Taking these studies together, it appears that, despite different effects on HDL- and LDL-cholesterol, all saturated fatty acids, including stearic acid, increase the LDL : HDL ratio to a comparable degree.

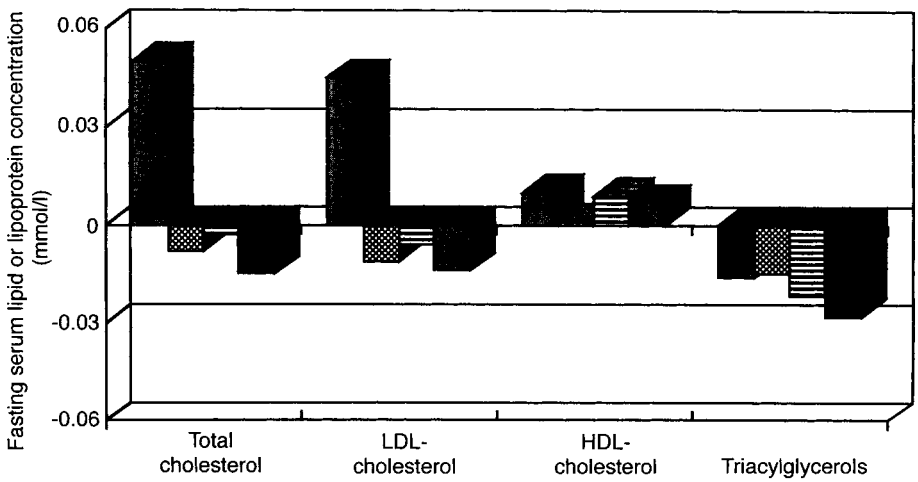

Fig. 1. Relative effects of palmitic acid (▨), stearic acid (▩), *cis*-monounsaturated fatty acids (▤) and *cis*-polyunsaturated fatty acids (▦) on fasting serum lipid and lipoprotein concentrations in human subjects. (From Mensink & Katan, 1992.)

Results from various studies have shown that *trans* monoenoic acids increase LDL- and decrease HDL-cholesterol relative to oleic acid (Katan *et al.* 1995; Aro *et al.* 1997). The effect of *trans* polyunsaturated fatty acids, which can be formed on treatments as mild as deodourization of vegetable oils, has not been properly examined as yet.

A few recent studies do suggest that in normolipidaemic subjects palmitic acid is not always an LDL-cholesterol-raising saturated fatty acid (Ng *et al.* 1992; Choudhury *et al.* 1995). This finding, of course, would be of great practical significance if it proved to be correct that under certain conditions palmitic acid can replace oleic acid without affecting LDL-cholesterol levels. Therefore, these results need to be confirmed under various experimental conditions, before any solid conclusions can be drawn.

Although linoleic acid is the most abundant polyunsaturated fatty acid in the diet, a small part of the dietary polyunsaturates is provided by α-linolenic acid (ALA; 18 : 3*n*-3) and by the very-long-chain fatty acids eicosapentaenoic acid (EPA; 20 : 5*n*-3) and docosahexaenoic acid (DHA; 22 : 6*n*-3) from fish oils. Effects on plasma lipoproteins seem comparable between ALA and linoleic acid (Chan *et al.* 1991). Fish oils, however, have a hypotriacylglycerolaemic effect and have, in normolipidaemic subjects, no effects on LDL and HDL levels. In hyperlipidaemic subjects, fish oils also lower triacylglycerols, but they can increase LDL- and HDL-cholesterol concentrations (Harris, 1997). Controversy still exists with respect to the question of whether EPA is the major triacylglycerol-lowering component of fish oil (Rambjor *et al.* 1996; Frøyland *et al.* 1997), or whether DHA has the same property (Ågren *et al.* 1996; Davidson *et al.* 1997; Grimsgaard *et al.* 1997).

The structure of dietary triacylglycerols may also affect serum lipid levels. Each natural triacylglycerol has a unique distribution of the three fatty acids over the glycerol molecule. However, the fatty acid configuration of dietary triacylglycerols is sometimes modified to produce fats that have features desired by food manufacturers and consumers. As human lipases preferentially remove fatty acids from the 1 and 3 positions of triacylglycerols, it is possible that changing the positional distribution of fatty acids could have an effect on serum lipoprotein concentrations. However, results from different studies (Grande *et al.* 1970; Nestel *et al.* 1995; Zock *et al.* 1995) have demonstrated that the position of dietary stearic acid or palmitic acid on the glycerol molecule is not an important determinant of the fasting serum lipoprotein profile. Nevertheless, animal studies carried out by Kritchevsky *et al.* (1973) suggested that randomization of peanut oil may prevent the promoting effect of peanut oil on cholesterol-induced atherosclerosis. These effects could not be explained by differences in absorption or transport of dietary cholesterol (Tso *et al.* 1984). Randomized butter or lard, however, did not protect against atherosclerosis (Kritchevsky & Tepper, 1977) and the interpretation and relevance of these studies for the human situation are not clear and need further investigation.

2.1.2. Fat replacers. Cholesterol absorption is decreased when human subjects consume diets containing non-absorbable fat replacers (Jandacek *et al.* 1990) and therefore these compounds do lower serum LDL-cholesterol concentrations (Mellies *et al.* 1983). When fat intake is decreased due to replacement by these fat substitutes, HDL-cholesterol concentrations may also be lowered (Widhalm *et al.* 1994).

2.1.3. Soyabean protein preparations. Anderson *et al.* (1995) have recently published the results of a meta-analysis concerning the effects of soyabean protein on serum lipid concentrations in human subjects. It was estimated that a daily intake of 47 g soyabean protein would lower serum total cholesterol concentrations by 0·60 mmol/l, which was mainly explained by a decrease of 0·56 mmol/l in LDL-cholesterol. The estimated reduction in serum total cholesterol concentrations in subjects with total cholesterol below 6·5 mmol/l was about 4 % (0·20 mmol/l) and about 20 % (1·85 mmol/l) in subjects with cholesterol levels above 8·7 mmol/l. Triacylglycerol levels decreased by 0·15 mmol/l, while no significant changes were seen in HDL-cholesterol levels. No difference in effect could be demonstrated between isolated soyabean protein and/or textured soyabean protein. However, only the individual results from seven out of thirty-one studies reached statistical significance. In those studies, all carried out in Italy by four different groups, subjects were hyperlipidaemic, and textured soyabean protein was used as the source of soyabean protein. Thus, it cannot be excluded that some special, unknown, characteristic of the textured soyabean protein explains the findings, and results cannot be extrapolated to all types of subjects *per se*. Further, it remains to be determined whether possible beneficial effects of soyabean protein are due to soyabean protein *per se* or to, for example, phyto-oestrogens, as suggested by the authors.

2.1.4. Mono- and disaccharides. Blaak & Saris (1995) have published a comprehensive review on health aspects of various digestible carbohydrates. It was concluded that, in the majority of studies with normolipidaemic, hypertriacylglycerolaemic or diabetic subjects, mono- and disaccharides had similar effects on the serum lipoprotein profile to those of starch, when consumed in amounts found in Western diets.

2.1.5. Resistant starch. Resistant starch is not, at least not entirely, degraded in the small intestine, and reaches the large intestine. Here, it is metabolized by the action of certain bacteria. Although it has been suggested that the metabolic products favourably affect cholesterol metabolism, neither raw nor retrograded starches appear to have a beneficial effect on the serum lipoprotein profile (Heijnen *et al.* 1996).

2.1.6. Ethanol. A moderate alcohol consumption is negatively related to the risk of CHD. This association, which may be partly explained by the ability of alcohol to increase HDL-cholesterol (Choudhury *et al.* 1994), appears not to be due to a specific alcoholic drink in particular, but rather to alcohol *per se* (Rimm *et al.* 1996).

2.1.7. Dietary cholesterol. Keys *et al.* (1965*a*) suggested that the serum total cholesterol concentration is a function of the square root of cholesterol intake. A non-linear relationship between dietary cholesterol intake and serum total cholesterol concentrations was also proposed by Hopkins (1992), but Hegsted *et al.* (1965) suggested that serum total cholesterol concentrations are linearly related to the absolute dietary cholesterol intake. Whatever the exact relationship is between dietary and serum cholesterol concentrations, lowering dietary cholesterol intake will

lower serum total cholesterol concentrations, although this effect may diminish when saturated-fat intake is low (Bronsgeest-Schoute *et al.* 1979). About 75–85 % of this effect is due to an increase in LDL- and about 15–25 % to an increase in HDL-cholesterol (Katan *et al.* 1986; Clarke *et al.* 1997).

2.1.8. Fibre. Based on a meta-analysis of ten trials, Ripsin *et al.* (1992) concluded that the daily consumption of approximately 3 g soluble fibre from oat products lowers serum total cholesterol concentrations by about 0·15 mmol/l. This effect was positively related to the initial serum cholesterol concentration. Other water-soluble fibres have also been reported to reduce total cholesterol concentrations, mainly by lowering LDL-cholesterol (Stasse-Wolthuis *et al.* 1980). Insoluble fibres have a lesser impact on serum total cholesterol levels (Glore *et al.* 1994).

2.1.9. Phytosterols. The estimated daily intake of phytosterols in Western countries is about 160–360 mg, of which campesterol, sitosterol and stigmasterol are the most common. These compounds are structurally related to cholesterol, lower cholesterol absorption, and have long been recognized as LDL-cholesterol-lowering agents (Miettinen *et al.* 1995). Saturated phytosterols are more efficient in reducing serum LDL-cholesterol concentration than unsaturated phytosterols (Ling & Jones, 1995). Esterification of sitostanols, the saturated equivalent of sitosterols, to rapeseed oil fatty acids further increases the LDL-cholesterol-lowering efficacy of phytosterols. A daily intake of about 2·0–2·5 g esterified sitostanol lowers serum LDL-cholesterol concentrations by about 10 % in hypercholesterolaemic subjects (Miettinen *et al.* 1995).

2.1.10. Tocopherols and tocotrienols. Tocopherols and tocotrienols are components with vitamin E activity. Tocopherols are present in most vegetable oils and are more common in the diet than the tocotrienols, which are found at relatively high concentrations in palm and rice-bran oils.

Tocopherols do not have any effect on serum lipoprotein concentrations (Kesaniemi & Grundy, 1982). Some studies have suggested that tocotrienols lower LDL-cholesterol concentrations (Qureshi *et al.* 1995), but other studies have not found any improvement of the serum lipoprotein profile after tocotrienol supplementation (Wahlqvist *et al.* 1992; RP Mensink, AC van Houwelingen, D Kromhout and G Hornstra, unpublished results). To explain these differences in findings, it was postulated that effective tocotrienol preparations should contain less than 150–200 mg α-tocopherol/g and 450 mg γ- plus δ-tocotrienol/g (Qureshi *et al.* 1996). This suggestion, however, awaits further confirmation. Also, the very recently reported potent LDL-cholesterol-lowering effect of a novel tocotrienol-enriched fraction (TRF25) from rice bran deserves attention in future studies (Qureshi *et al.* 1997).

2.1.11. Garlic. Two recent meta-analyses found that garlic preparations, in amounts approximately equivalent to half to one clove per day, decreased serum total and LDL-cholesterol levels by about 10 % in subjects with elevated plasma cholesterol concentrations (Warshafsky *et al.* 1993; Silagy & Neil, 1994). It was, however, noticed that many of the studies used had methodological shortcomings, which were accounted for in two very recent studies (Simons *et al.* 1995; Adler & Holub, 1997). However, results of these two studies were conflicting, despite the fact that the same garlic powder was used and in the same amount. Thus, although evidence exists that garlic may lower LDL-cholesterol concentrations, some questions still remain to be resolved. In addition, it should be emphasized that the cholesterol-lowering effect may be confined to certain fractions of garlic only. This emphasizes that each substance or preparation should be evaluated properly in well-controlled studies at more than one location, before any firm conclusions can be drawn.

2.1.12. Other components. Despite promising results in the past, very recent well-controlled studies have demonstrated that fermented milk products (Richelsen *et al.* 1996) and inulin (Pedersen *et al.* 1997) are not very likely to have beneficial effects on the fasting serum lipoprotein profile. Also, the claimed lipid-lowering effects of oligofructose in man have not yet been properly demonstrated.

2.2. *Postprandial effects*

So far, only the effects of dietary components on fasting lipid and lipoprotein concentrations have been discussed. However, lipoprotein remnant particles, which circulate in the blood after a meal, are also atherogenic.

As chylomicrons, precursors of the remnant particles, mainly transport dietary triacylglycerols (and cholesterol), it is not surprising that postprandial triacylglycerol concentrations are more pronounced on high-fat diets, even if fasting triacylglycerol levels are lower. The fatty acid composition of the habitual diet might also be an important determinant of the postprandial triacylglycerol response, as this response appears to decrease when the diet contains highly unsaturated fatty acids from fish oils (Harris, 1997). Hayford *et al.* (1979) have also reported that sucrose-containing diets induced a higher triacylglycerol response than diets containing maize-syrup. Finally, components that interfere with dietary cholesterol absorption may affect the composition of the chylomicron and its remnant particles. However, the extent and importance of these effects are difficult to quantify and the postprandial effects of diets is certainly an area that should be investigated more thoroughly in the very near future.

2.3. *Gene–diet interaction*

Apolipoprotein (apo) E, an apolipoprotein associated with chylomicrons, VLDL, HDL and remnant particles, is a ligand for both the remnant receptor and the LDL-receptor. There are three common alleles in the population, which are, in decreasing frequency: E3, E4, and E2. As apoE2 has a lower affinity for the remnant receptor than the other two isoforms, subjects with the apoE2 isoform exhibit a delayed clearance of chylomicrons and chylomicron remnant particles after a fat load. Subjects with the apoE4 isoform, however, appear to be more responsive to a reduced cholesterol and saturated fat intake than other subjects (Ordovas *et al.* 1995), which might partly be explained by the higher fractional intestinal cholesterol absorption in apoE4-subjects (Miettinen, 1991). This would also explain why apoE4-carriers benefit more from sitostanol ester intake than non-apoE4 carriers (Vanhanen *et al.*

1993). Interestingly, Dreon *et al.* (1995) have also reported that reducing fat intake caused a shift from large to smaller LDL particles, which was most pronounced in apoE4-subjects.

Although less extensively studied, it has also been suggested that common polymorphisms for apoA-I (Lopez-Miranda *et al.* 1994) and apoA-IV (Mata *et al.* 1994) may explain a part of the inter-individual response when dietary fat and/or cholesterol intake is modified. Associations between certain polymorphisms of apoB, apoC-III and lipoprotein lipase (*EC* 3.1.1.34) and lipid responses to dietary changes have also been reported, but are not very consistent (Ordovas *et al.* 1995). More research is needed to further assess the genetic impact on diet–lipoprotein interactions.

2.4. Possible mechanisms of dietary fats

Theoretically, diet may modify cholesterol metabolism in several ways at different levels: (1) cholesterol and/or fat absorption; (2) faecal sterol excretion; (3) cholesterol and/or apolipoprotein synthesis and excretion; (4) receptor-dependent and -independent lipoprotein uptake; (5) lipoprotein composition and catabolism; (6) changes in enzymes and/or proteins, like lipoprotein lipase, cholesterol-ester transfer protein, and lecithin-cholesterol acyl transferase. It should be realized, however, that these mechanisms do not operate in isolation. For example, it can be imagined that endogenous cholesterol synthesis is increased in order to compensate for a decreased cholesterol absorption.

To date, most of the studies have focused on the effects of dietary fatty acids on LDL metabolism and two competing theories will be summarized briefly.

2.4.1. Concept of Spady and colleagues. A detailed model to delineate the effects of dietary fatty acids on lipoprotein metabolism in the hamster has been described by Spady *et al.* (1993). According to their hypothesis, dietary fatty acids change LDL-receptor activity, but not the production of apoB-100 by the liver or whole-body cholesterol synthesis. Thus, if the activity of the LDL-receptor is reduced, LDL-cholesterol levels are also increased because of increased conversion of intermediate density lipoproteins to LDL. Although the results from this hamster model are very consistent, it is not known whether this concept can be extrapolated to man.

2.4.2. Concept of Hayes and colleagues. Hayes and Khosla (1992) and Hayes *et al.* (1992) have postulated that the cholesterol-raising saturated fatty acids increase the production of apoB-100 by the liver and have no effect on the activity of the hepatic LDL-receptor. This will result in an increased VLDL-output, the effect being the strongest for lauric and myristic acids; palmitic acid has a smaller effect. As the activity of the LDL-receptor is not increased, LDL-cholesterol levels must rise because of increased conversion of VLDL into intermediate-density lipoproteins and subsequently LDL. Linoleic acid lowers the LDL-cholesterol concentration, because, according to Hayes' hypothesis, it up-regulates the LDL-receptor. This effect of linoleic acid is maximal already at an intake of 6–7 % of daily energy. It is now postulated that, at adequate intakes of linoleic acid, the up-regulated LDL-receptor can counterbalance the relatively small effect of palmitic acid on VLDL production. Under these conditions, palmitic acid and oleic acid have similar effects on LDL-cholesterol concentrations. However, in situations that down-regulate the LDL receptors (for example, dietary cholesterol intake above 300 mg/d or total serum cholesterol above 6·5 mmol/l), linoleic acid cannot fully neutralize the effect of palmitic acid on apoB-100 production.

3. Some diet effects on arterial thrombotic processes: platelet and endothelial cell functions, blood coagulation and fibrinolysis

3.1. Arterial thrombosis and cardiovascular disease

Evidence that arterial thrombosis contributes to genesis and complications of cardiovascular disease is mainly based on pathological and epidemiological studies, showing significant associations between the various thrombotic processes, or the levels of factors involved in these processes, and disease morbidity or risks (Fuster *et al.* 1992; Davies, 1997). Evidence that modulation of these processes or factors affects disease risk seems strong for platelet function (Antiplatelet Trialist's Collaboration, 1994), is reasonable for coagulation (Chalmers *et al.* 1977; Loeliger, 1984; Smith *et al.* 1990), and substantial for fibrinolysis (Fibrinolytic Trialists' Collaborative Group, 1994), but is lacking for endothelial function.

The protective effect of aspirin on cardiovascular morbidity and mortality has long been considered the major evidence for a causal relationship between arterial thrombosis (and platelet function in particular) and cardiovascular disease (Steering Committee of the Physicians' Health Study Research Group, 1989), but recent evidence indicates that the beneficial effect of aspirin may be secondary to its anti-inflammatory properties (Ridker *et al.* 1997). So, for the time being, thrombotic processes and factors should be considered risk markers for cardiovascular disease, not risk factors.

3.2. Platelet function as a marker for CHD

Although platelet activation is instrumental in arterial thrombogenesis, too little is known about the predictive value of platelet function (adhesion, release and aggregation) on the incidence of CHD. It has been clearly shown that the suppression of platelet activation, either by drugs (Antiplatelet Trialist's Collaboration, 1994) or by blocking the platelet fibrinogen receptor, glycoprotein IIb/IIIa (EPIC Investigators, 1994) offers protection against myocardial infarction (MI) and other ischaemic events respectively. However, rather little is known about the association between increased platelet activation and CHD morbidity and mortality. So far, increased platelet aggregation induced by ADP or thrombin has been shown to be associated with past MI and electrocardiographic evidence of ischaemia respectively (Elwood *et al.* 1990, 1991), and prospective evidence has been presented of an association between increased platelet count and ADP-induced platelet aggregability, and long-term incidence of fatal CHD (Thaulow *et al.* 1991). However, a nearly twofold difference in the CHD rate of two Finnish cohorts was not associated with

differences in platelet aggregation induced by different agonists (Salo *et al.* 1985). Two studies have demonstrated that platelet activation, as measured by the urinary excretion of the platelet specific protein β-thromboglobulin (β-TG), is significantly associated with the risk of CHD (Ghaddar *et al.* 1995; Gorgels *et al.* 1995). Platelet volume is increased at the time of acute MI (Bath & Butterworth, 1996) and there is compelling evidence that changes in platelet volume are associated with myocardial risk (Martin *et al.* 1991; Brown & Martin, 1994).

The main method to assess platelet function in dietary studies has been the platelet aggregation test *in vitro*, with the help of which it has repeatedly been shown that changes in dietary fatty acids can modulate the platelet aggregation pattern. However, the results are far from consistent and their interpretation in terms of thrombosis tendency is difficult, if not impossible, since direct comparisons between platelet aggregation *in vitro* and arterial thrombosis *in vivo* have not been made in man. In the rat, diet-induced changes in platelet aggregation *in vitro* appeared to be negatively related to changes in arterial thrombosis *in vivo* (Hornstra *et al.* 1993).

3.3. Some diet effects on arterial thrombogenesis and platelet function

3.3.1. Fatty acids. The intake of linoleic acid (18 : 2*n*-6) is strongly correlated with the linoleic acid content of plasma phospholipids, cholesterol esters and triacylglycerols. In addition, platelet total linoleic acid, ALA (18 : 3*n*-3), arachidonic acid (20 : 4*n*-6), EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3) are significantly correlated with the concentrations of these fatty acids in plasma triacylglycerols, plasma phospholipids and/or adipose tissue. However, the concentrations of unsaturated fatty acids in e.g. adipose tissues do not predict risk for thrombosis (Kardinaal *et al.* 1995). In two Finnish cohorts, platelet aggregation induced by ADP showed a significant positive correlation with the contents of linoleic acid in adipose tissue and plasma triacylglycerols, but not with linoleic acid in platelets (Salo *et al.* 1985).

There may be a specific preventive influence of ALA on CHD, since the intake of ALA (range 0·8–1·5 g/d) was inversely associated with the risk of MI in the health professionals follow-up study (Ascherio *et al.* 1996*b*). Also in the lifestyle intervention of de Lorgeril *et al.* (1994), among several other dietary changes, about 2 g ALA/d was estimated to be protective. However, in the large Multiple Risk Factor Intervention Trial, no effect with an ALA intake of 1·7 g/d was found (Dolecek & Grandits, 1991). In this study, a positive association was observed between the dietary linoleic acid : ALA ratio and cardiovascular mortality. Serum ALA concentration appeared to be negatively associated with the risk of stroke (Simon *et al.* 1995) and in a prospective study (Miettinen *et al.* 1982), serum ALA, EPA, and DHA were all low in MI patients.

Observational studies in Norway during the Second World War and cohort studies among Greenland Eskimos and populations in Japan point to a protective effect against CHD of long-chain *n*-3 fatty acids from fish or marine mammals (Hornstra, 1989). Although later observational studies gave conflicting results (Hornstra, 1989), there are now five large-scale prospective studies demonstrating a negative association between fish consumption and cardiovascular mortality (Kromhout *et al.* 1985; Norell *et al.* 1985; Shekelle *et al.* 1985; Dolecek & Grandits, 1991; Daviglus *et al.* 1997). In five other studies of about similar size and design, however, no significant relationship was found (Curb & Reed, 1985; Vollset *et al.* 1985; Lapidus *et al.* 1986; Morris *et al.* 1992; Ascherio *et al.* 1995). This has been suggested to be due to the rather high habitual fish consumption in the 'low-fish' group in these latter studies (Kromhout, 1985). However, since all processes that are thought to be involved in the cardio-protective effects of fish (oil) show clear dose–response relationships over a wide range of fish (oil) intakes, this is a rather unlikely explanation.

Evidence has also been reported for a positive association between fish consumption and cardiovascular risk. Thus, in two cohort studies a higher mortality from CHD was observed in areas with a relatively high fish consumption as compared with low-fish regions (Simonsen *et al.* 1987; Hunter *et al.* 1988). In two large-scale prospective studies in Finland, fish consumption was also positively related to cardiovascular mortality (Salonen *et al.* 1995; Piettinen *et al.* 1997). In the study of Salonen *et al.* (1995) it was suggested that the high intake of Hg from the freshwater fish may have caused increased cardiovascular risk by promoting lipid peroxidation.

In conclusion, results of epidemiological studies with respect to the importance of dietary fish (oil) for the prevention of IHD are equivocal and not conclusive. Moreover, it should be reiterated that epidemiological studies can only indicate associations between two phenomena; they can never discriminate between causal and casual relationships. The final proof for the effectiveness of a fish (oil)-enriched diet for the prevention of cardiovascular disease has to be obtained via long-term, well-controlled, prospective primary intervention trials, which have not yet been reported. So far, only one secondary intervention study has been published (Burr *et al.* 1989), demonstrating that subjects who were advised to eat fatty fish at least twice a week had a 29 % reduction in 2-year cardiovascular mortality as compared with volunteers whose diet advice did not include fish.

The effect of *cis* unsaturated fatty acids on platelet function including *in vitro* aggregation data has recently been reviewed (Mutanen, 1997). From this review it appears that the results are very inconsistent which, at least in part, may have methodological reasons.

A promising approach to assess platelet activation *in vivo* is the measurement of thromboxane (Tx) metabolites (2,3-dinor-TxB_2 and 11-dehydro-TxB_2) in urine, or of the concentration of the platelet-specific protein β-thromboglobulin, released from α-granules. Dietary fish oil or long-chain *n*-3 fatty acids lower high basal Tx excretion rate, while only a modest effect is found at a low basal excretion rate. Results concerning the effects of other unsaturated fatty acids on urinary Tx metabolites are almost totally lacking. Preliminary results indicate that two diets with the same saturated fat content but differing in their linoleic acid contents (5 and 12 % energy) similarly increased 2,3-dinor-TxB_2 in urine; Turpeinen *et al.* 1997), which indicates

enhanced platelet activation *in vivo*. High stearic acid and *trans* fatty acid diets also stimulated 2,3-dinor-TxB_2 excretion (Turpeinen *et al.* 1998). Furthermore, the results from these studies indicate that platelet β-thromboglobulin release *in vivo* was not affected by changes in dietary fatty acids.

A general shortcoming in most of the studies to explain the effects of dietary fatty acids has been a lack of information on the fatty acid composition of individual platelet phospholipids. In addition, little is known about the role of the baseline diet with respect to the incorporation of fatty acids into platelets. Platelet membrane fatty acid composition can be changed by dietary means to some extent. The total amount of a given fatty acid in the platelet is probably less important than the factors regulating free fatty acid levels and types in the membrane and in the platelet interior. Platelet receptor responsiveness to physiological stimuli and subsequent signal transduction and fatty acid liberation for eicosanoid synthesis are probably highly dependent on membrane fatty acid composition. However, one can only speculate as to the precise underlying mechanisms.

A potentially important second messenger during platelet activation is protein kinase C, the activation of which can be modulated by *cis* unsaturated fatty acids, while saturated and *trans* unsaturated fatty acids are inactive (Khan *et al.* 1995). Six isoenzymes of protein kinase C have now been identified in human platelets, and these may be involved in various aspects of platelet activation.

Other mechanisms by which fatty acids, especially *n*-3 fatty acids, might regulate platelet function involve changes in TxA_2/prostaglandin(PG)H_2 receptor affinity following changes in membrane phospholipid composition (Bayon *et al.* 1995). An alteration of the platelet redox state and the resulting modulation of the expression of certain enzymes could also be involved (M Lagarde, F Achard, M Gilbert, C Bénistant, D Lemaitre and E Véricel, unpublished results). The enhanced sensitivity of platelets from hypercholesterolaemic patients indicates that LDL may also activate platelets. *In vitro* mildly oxidized LDL (ox-LDL) has been shown to activate platelets significantly while purified apoE seems to inhibit this (Weidtman *et al.* 1995; Zhao, 1996). In addition, activated platelets release substances, e.g. platelet-derived growth factor, which can modify LDL and enhance the macrophage uptake of ox-LDL (Aviram, 1995).

3.3.2. Antioxidants and platelet function. On the basis of epidemiological studies, dietary antioxidants (tocopherols, carotenoids, flavonoids and Se) have repeatedly been suggested to reduce CHD risk, but the results of intervention studies are more equivocal (Öhrval *et al.* 1996; van de Vijver, 1997).

Platelet function *in vitro*, and platelet adhesion in particular, has been shown to be inhibited by high levels of α-tocopherol which cannot be obtained from dietary sources alone (Steiner *et al.* 1995). This mechanism may partly explain the beneficial efficiency of pharmacological amounts of α-tocopherol to prevent MI in the Cambridge Heart Antioxidant Study (CHAOS) (Stephens *et al.* 1996). Se supplementation in human subjects with a low Se status decreases platelet aggregation *in vitro*, but has no effect on platelet activation in human subjects with a normal Se status. No experimental data are available as to the effects of carotenoids on platelet function in man. Data on the effects of flavonoids are from *in vitro* experiments only. The results indicate an inhibition of platelet eicosanoid synthesis and platelet aggregation (Goldberg, 1996).

3.4. Endothelial cell function

Damage of the endothelium leads to endothelial dysfunction which is characterized by enhanced expression of cytokines, cell adhesion molecules, von Willebrand factor (vWf), platelet activating factor, and endothelin, and decreased synthesis of PGI_2 (prostacyclin) and transforming growth factor-β (TGF-β). The level of vWf has been related to the risk of MI and sudden death in patients with angina pectoris (Thompson *et al.* 1995). The soluble form of the vascular cell adhesion molecule (VCAM) and vWf were both shown to be raised in stroke patients, while the intracellular adhesion molecule (ICAM) was raised in patients at risk of stroke only (Blann *et al.* 1996). Latent TGF-β in human vascular SMC is activated by plasmin which is produced from plasminogen by plasminogen activator (t-PA). *In vitro* Lp(a) impairs this activation. Active TGF-β inhibits SMC migration, proliferation and activation. Suppression of TGF-β led to increased *in vitro* expression of ICAM-1 in endothelial cells incubated with Lp(a) (Grainger & Metcalfe, 1995). The question of whether cell adhesion molecules are regulators of platelet function is far from clear at the moment and requires further study.

3.4.1. Dietary fatty acids and endothelial cell function. Dietary fatty acids are able to regulate prostacyclin production to some extent. Studies in Greenland Eskimos (Fischer *et al.* 1986) and in a Japanese fishing village (Hamazaki *et al.* 1989), as well as various intervention studies with fish or fish oil (for reviews, see Hornstra, 1989; Hornstra *et al.* 1990) have led to the conclusion that *n*-3 fatty acids of marine origin increase both PGI_2 and PGI_3 production in man. However, the methods used (measurement of major PGI metabolites in urine) are very complicated and this is probably the reason why data from various other studies with respect to PGI_2 are not consistent (Knapp *et al.* 1986) and effects of other dietary fatty acids have not been reported. The experimental evidence with respect to the effect of dietary fatty acids on NO synthase regulation is not clear at present.

In two cohort studies, negative associations were found between dietary consumption of *n*-3 fatty acids and plasma levels of vWf (Shahar *et al.* 1993). In an intervention study, a low-fat (28 % energy), low-saturated fatty acid (9 % energy), and low-cholesterol (215 mg/d) diet for 3 years resulted in significantly lower plasma vWf levels than the control diet. Moreover, a negative correlation between plasma vWf and dietary *n*-3 and *n*-6 fatty acids was found (Blann *et al.* 1995). In patients suffering from non-insulin-dependent diabetes mellitus (NIDDM), a diet enriched in monoenoic fatty acids (30 % energy) decreased plasma vWf when compared with a diet high in carbohydrate (11 % energy as monoenes) (Rasmussen *et al.* 1994).

In human endothelial cell cultures, both DHA and EPA attenuated the induction of ICAM-1, VCAM-1 or E-selectin

in interleukin-1β-activated cells (Collie-Duguid & Wahle, 1996). On the other hand, DHA, but not EPA or arachidonic acid, was shown to inhibit the cytokine-induced expression of VCAM-1 (Weber *et al.* 1995) by blocking the activation of nuclear factor κB, an inducible transcription factor which specifically activates transcription of cell adhesion molecules. Activation of nuclear factor-κB is significantly enhanced *in vitro* by linoleic acid (Henning *et al.* 1996) and recent results with rabbits suggest that monounsaturated fatty acids might inhibit VCAM-1 expression *in vivo* (De Caterina *et al.* 1995*b*).

Availability of arachidonic acid is an important determinant of PGI_2 synthesis by endothelial cells but recent results with respect to the effects of DHA and EPA on PGI_2 production suggest that alteration of the expression of the enzymes responsible for formation of PGI_2 may also be crucial (M Lagarde, F Achard, M Gilbert, C Bénistant, D Lemaitre and E Véricel, unpublished results). The regulation of the expression of cell adhesion molecules probably includes oxidant–antioxidant sensitive mechanisms, since *in vitro* VCAM-1 gene expression can be inhibited by synthetic antioxidants. Conversely, LysoPC, a component in oxidized LDL, has been shown to upregulate VCAM-1 and ICAM-1 expression in endothelial cells and rapidly induces P-selectin expression in both platelets and endothelial cells (Ochi *et al.* 1995; Murohara *et al.* 1996). This latter effect is probably the basis of leucocyte deposition.

3.5. *Coagulation and fibrinolysis*

In the circulation, coagulation and fibrinolysis factors balance each other. The main markers used to evaluate blood coagulability are fibrinogen, factor VII (and other coagulation factors), antithrombin III (AT-III), fibrinopeptide A released from fibrinogen by thrombin, and prothrombin fragment F_{1+2}. Today, prothrombin F_{1+2} is considered a sensitive marker of clotting activation. Fibrinolytic potential is assessed by measuring plasminogen, tPA, its inhibitor plasminogen activator inhibitor-1 (PAI-1), and cross-linked fibrinogen degradation products (D-dimers). The latter indicator reflects both coagulation and fibrinolysis.

The early results from the Northwick Park Heart Study (Meade *et al.* 1986) indicated strong independent associations between baseline plasma fibrinogen and factor VII coagulant (FVIIc) activity levels and the risk of CHD. In the same population, a U-shaped association between ATIII and the risk for CHD was found. Low fibrinolytic activity predicted a higher risk for CHD in a later analysis (Meade *et al.* 1993). Fibrinogen is also generally accepted as an independent risk factor for CHD, while the predictive value of PAI-1 and tPA levels as risk factors is still contradictory (Hamsten, 1995; Ridker & Vaughan, 1995). In patients with angina pectoris, the levels of fibrinogen and tPA antigen have been shown to be independent predictors of subsequent MI or sudden death (Thompson *et al.* 1995). Elevated plasma levels of D-dimers have been shown to be associated with early atherosclerosis (Salomaa *et al.* 1995) and increased risk of future MI (Ridker *et al.* 1994), although in the latter study the D-dimer level did not appear to be an independent predictor. Recent results from 2952 men clinically free from CHD show that six markers of the hypercoagulable state (FVIIc, FVII antigen, activated factor VIII and factor IX, prothrombin fragment F_{1+2}, and fibrinopeptide A) are all positively associated with CHD risk (Miller *et al.* 1996).

3.5.1. Effect of dietary factors on coagulation and fibrinolysis. According to several studies, dietary fatty acids hardly influence plasma fibrinogen. There is one study from Denmark (Bladbjerg *et al.* 1995) showing increased plasma fibrinogen level after an extremely high stearic acid (about 15% energy) diet when compared with a diet high in myristic and lauric acids. In a recent study, plasma fibrinogen concentration increased slightly during the stearic acid (9·3% energy) diet, but the biological significance of this is questionable (Mutanen & Aro, 1997). In the population-based cross-sectional atherosclerosis risk in communities (ARIC) study, a negative association between the intake of long-chain *n*-3 fatty acids and plasma fibrinogen levels was found (Shahar *et al.* 1993). However, intervention studies with long-chain *n*-3 fatty acids have given very inconsistent results (Hornstra, 1992).

Current knowledge about diet and factor VII (FVIIc activity or FVII antigen levels) indicates that fasting FVIIc can be reduced by low-fat diets. The fatty acid composition of the diet, i.e. saturated, monounsaturated or *n*-3 and/or *n*-6 polyunsaturated fatty acid contents, have not been found to be important in short-term experiments (Mennen *et al.* 1996). Habitual high-fat diets seem to increase both FVIIc and FVII antigen. Increased postprandial responses of FVIIc are seen after high-fat test meals regardless of the type of fat. It seems that the change in fasting FVIIc is part of a general change in concentrations of vitamin K-dependent proteins, while changes in non-fasting FVIIc activities are primarily mediated by activation of the factor VII zymogen (Bladbjerg *et al.* 1995). The activation of factor VII has been suggested to be related to free fatty acid production during lipolysis of triacylglycerol-rich lipoproteins (Silveira *et al.* 1994).

There are only a few reports about the effects of diet on AT-III. Early studies indicate that *n*-6 polyunsaturates might increase plasma AT-III, while long-chain *n*-3 have either no effect or may increase it. A recent study comparing ALA with EPA+DHA indicated that ALA might have a beneficial effect on plasma AT-III levels (Freese & Mutanen, 1997).

Supplementation for 16 weeks with long-chain *n*-3 fatty acids of patients with chronic atherosclerotic disease induced a significant increase in plasma levels of tissue factor pathway inhibitor, indicating down-regulation of the extrinsic pathway of blood coagulation (Berrettini *et al.* 1996). In an earlier study, a shorter supplementation period did not produce such an effect (Hansen *et al.* 1994). In the study of Berrettini *et al.* (1996) a significant reduction of F_{1+2} plasma levels was found also. A slight but significant decrease in F_{1+2} has been reported after a high-stearic-acid diet when compared with a high-myristic and -lauric acid diet (Bladbjerg *et al.* 1995). Circulating amounts of F_{1+2} were not different between low-fat and high-monoene diets (Lopez-Seguara *et al.* 1996).

In a long-term study with a low-fat (26% energy) high-fibre diet, tPA activity increased significantly in healthy subjects, while no change in tPA antigen was found (Marckmann *et al.* 1993). However, the reduction of the

total dietary fat content alone (from 39 to 31 % energy) had no effect. Several studies have found no effect on plasma tPA activity of dietary long-chain *n*-3 fatty acids (Eritsland *et al.* 1994), olive oil (Lopez-Segura *et al.* 1996), maize oil (Hellsten *et al.* 1993) or stearic acid or *trans* fatty acids (Mutanen & Aro, 1997).

Effects of the dietary composition on PAI-1, either antigen or activity, are not consistent (Hornstra, 1992; Hellsten *et al.* 1993). Long-chain *n*-3 fatty acid supplementation mainly increases PAI-1 antigen and either increases, or has no effect on PAI-1 activity. In a recent study, PAI-1 activity increased similarly with either ALA or EPA+DHA supplementation (Freese & Mutanen, 1997). There are only a few studies addressing the effects of other dietary fatty acids on PAI-1. A high-oleic-acid diet (fat 38 % energy, monoenes 24 % energy) decreased both PAI-1 activity and antigen when compared with a high-carbohydrate diet (fat 27 % energy, monoenes 13 % energy). The decrease was accompanied by a parallel decrease in plasma insulin levels (Lopez-Segura *et al.* 1996). Maize oil supplementation resulted in decreased PAI-1 activity (Hellsten *et al.* 1993), but in another study olive oil did not have an effect (Oosthuizen *et al.* 1994). Changes in total fat and fibre intake did not affect PAI-1 either (Marckmann *et al.* 1993).

No changes in D-dimer concentrations have been detected in some recent studies with long-chain *n*-3 fatty acids (Eritsland *et al.* 1994, 1995), *trans* fatty acids (Almendingen *et al.* 1996; Mutanen, 1997) or stearic acid (Mutanen & Aro, 1997). A decrease in D-dimer level was found in the study of Mutanen & Aro (1997) when the subjects changed from their habitual diet (polyunsaturated : saturated fatty acid ratio (P : S) 0·36) to more saturated type of diet (P : S 0.24).

Data concerning the effects of other dietary factors on coagulation and fibrinolysis are scarce. The results from a low-fat, high-fibre experiment by Marckman *et al.* (1993), however, indicate that some components of dietary fibre may affect coagulation and fibrinolysis. Two other studies support this assumption (Nilsson *et al.* 1990; Sundell & Ranby, 1993). Recently, in a large Finnish cohort of middle-aged men an inverse association was observed between the intake of dietary fibre and the risk of CHD. Adjustment for serum cholesterol did not change the results, indicating that in the mechanism lipoprotein metabolism is not involved (Pietinen *et al.* 1996).

Platelets are important contributors to both coagulation and fibrinolysis. Although tissue factor present in monocytes and the blood vessel wall, in combination with activated factor VII (FVIIa), is the main initiator of coagulation, activated platelets, by exposing phosphatidyl serine at their surface, provide the preferred surface on which coagulation occurs. This platelet procoagulant activity, also called platelet factor 3, is closely related to platelet aggregation. AT-III can rapidly inhibit FVIIa that is bound to tissue factor, thus inhibiting the start of coagulation (Rapaport & Rao, 1995). How platelet membrane fatty acid composition affects the exposure of phosphatidyl serine, or how tightly the fatty acid composition of phosphatidyl serine is regulated is not known at present. The functional association between fatty acids and tissue factor presentation in tissue-factor-containing cells is not known either.

There is some evidence that long-chain saturated fatty acids might provide a contact surface for activation of clotting factors XII and IX (Mitropoulos, 1994). Activation of these factors can cause the activation of factor VII and thus increase FVIIc.

Fibrinolysis also occurs at the platelet surface after direct binding of plasminogen, tPA and plasmin. Once bound, tPA manifests enhanced catalytic activity to convert plasminogen to plasmin, thereby enhancing thrombolysis. Formed plasmin also binds to the platelet surface and, at low concentrations, reduces fibrinogen binding which results in reduced platelet aggregation. At high concentrations, however, plasmin activates platelets (Loscalzo *et al.* 1995). How fatty acids would regulate either the production of plasminogen or tPA in the endothelium or their activation on the surface of platelet membranes is not clear as yet. In endothelial cells, both tPA and PAI-1 productions seem to be mediated by protein kinase C activation (Rydholm *et al.* 1995) and thus may be influenced by fatty acids.

There are two recent reviews on lipoprotein metabolism and thrombosis (Mitropoulos, 1994; Miller, 1995). The current opinion is that fatty acid composition of lipoprotein particles may be important for the activation of the contact system of coagulation. Furthermore, high blood lipid levels may change platelet function by influencing platelet membrane composition and fluidity.

4. Immune-mediated processes underlying CHD

Maintaining the vascular integrity and defending the circulatory system against pathogenic processes require regulatory interactions among blood cells and between blood cells and the vessel wall. The interacting cells are leucocytes (monocytes and T-lymphocytes) and platelets in the circulation, and endothelial cells and SMC in the vessel wall (Ross, 1995). These processes are controlled through activation of adhesion receptors already present on resting blood cells and endothelium, or through the expression of new receptors on the cell surface (Frenette & Wagner, 1996).

Cell activation, production of chemoattractants and cell growth factors are key components in these events, which are involved in repair and defence systems, but also, under certain conditions, in tissue injury and disruption in the cardiovascular compartment. Long-term processes also trigger the participation of key components in cellular immunity, such as T-lymphocytes and macrophages (Lodish *et al.* 1995). These cells are recognized to play a role in inflammatory and immune-mediated processes in atherosclerosis, since T-lymphocytes are present in the arterial plaque (Jonasson *et al.* 1986), and antibody responses to plaque constituents have been detected (Palinski *et al.* 1989).

4.1. Immunocompetent cells involved in the atherosclerotic lesion

4.1.1. Endothelial cells. The endothelium, which lines vessel walls and acts as a permeability barrier controlling the exchange of nutrients and fluids, is a dynamic component of the artery. It provides a non-adherent surface for leucocytes and platelets, maintains the vascular tone

by releasing vasoactive molecules such as NO, PGI_2, endothelin and angiotensin II, and produces and secretes growth factors and cytokines. The endothelium also forms and maintains the connective tissue matrix, has the capacity to modify plasma lipoproteins, and provides anti- and procoagulant activities. When these functions are altered, in the initiation of the atherosclerotic lesions, leucocytes adhere to the vessel wall, following the formation of cell adhesion proteins (ICAM-1, VCAM-1 etc.) (Springer, 1990; Poston *et al.* 1992). There is formation of oxidatively-modified particles and an accumulation of lipoproteins in the subendothelial space (Simionescu *et al.* 1986). Associated modifications take place, such as altered vascular tone, the inability to regenerate wound sites and to prevent platelet adhesion, thrombosis and coagulation. In addition, growth factors and cytokines are released after cell stimulation.

4.1.2. Smooth-muscle cells. The second major type of cell in the arterial wall is the SMC. During the formation of arterial lesions, SMC, monocyte-derived macrophages and T-lymphocytes accumulate in the lesion, and this process is associated with deposition of connective tissue matrix and lipid. SMC, which are activated to migrate from the media into the intima and to proliferate there, produce a variety of growth factors, and the genes for these molecules and for cytokines (e.g. interleukin-1, tumour necrosis factor-α) are induced by various agents (Stemme & Hansson, 1994). SMC are present in two phenotypic states: the contractile and the synthetic. In the contractile state, they respond to vasoactive agents, whereas in the synthetic state they express genes for growth factors and cytokines and also produce various forms of connective tissue matrix.

4.1.3. Immunocompetent leucocytes. In addition to the constitutive cells of the vessel wall, all forms of lesions contain elements of specialized chronic inflammation, e.g. monocyte-derived macrophages and T-lymphocytes. The macrophages, in addition to acting as scavengers and as antigen-presenting cells, produce growth-regulatory proteins and could contribute to lipoxygenase-mediated generation of oxLDL. Macrophages are the main source of foam cells, since they take up oxLDL through scavenger receptors and a putative oxLDL receptor (Stemme & Hansson, 1994). They can also produce growth factors and chemotactic molecules for other monocytes, endothelial cells and SMC.

T-lymphocytes represent the second type of cells derived from the circulation and found in common atherosclerotic lesions (Jonasson *et al.* 1986). These cells appear to be in a low degree of activation, and have a low proliferation rate (Gordon *et al.* 1990). Large numbers of T-lymphocytes are generally found in lesions associated with risk factors, e.g. hyperlipidaemia, diabetes, and hypertension.

4.1.4. Mechanisms for the recruitment of blood cells in arterial lesions. Recruitment of lymphocytes and monocytes, their binding to the endothelium, and adhesion of activated platelets to monocytes and endothelium, all these processes are mediated by cell–cell adhesion molecules. Movement of leucocytes from the blood into tissues, contributing to tissue oedema and necrosis following ischaemia, involves additional adhesion molecules, such as ICAM-1 and VCAM-1.

Growth factors and cytokines participate in cell interactions and in the development of the arterial lesions. They have been detected in atherosclerotic plaques *in vivo*, by *in situ* detection methods. Evidence for the activation of cell–cell interactions in atherosclerotic disease is now obtained from the assessment of plasma levels of cell adhesion molecules in atherosclerotic patients (Blann & McCollum, 1994). These levels were found to differ depending on the type of dyslipidaemia (Hackman *et al.* 1996).

4.2. The immune system response modulates atherosclerosis progression

One of the products of activated T-cells, interferon-γ inhibits SMC proliferation *in vitro* and *in vivo*. Therefore, reduced plaque growth would be expected following increased interferon-γ production by cells in the plaque. It has been found, in fact, that T-cell depletion leads to increased lesion size after experimental arterial injury (Hansson *et al.* 1991), and that cyclosporin A, an inhibitor of T-cell functions, accelerates atherosclerosis in hypercholesterolaemic mice (Roselaar *et al.* 1995). Interferon-γ also down-regulates the expression of the scavenger receptor by human macrophages, inhibiting foam-cell formation *in vitro* (Geng & Hansson, 1992). The following additional observations indicate protective effects of the immune system with respect to the progression of atherosclerosis: in LDL-receptor-deficient rabbits hyperimmunized with homologous oxLDL, there is a substantial reduction in the progression of the lesions (Palinski *et al.* 1995); elimination of T-lymphocytes with monoclonal antibodies results in larger proliferative lesions in balloon-catheterized rat aortas (Hansson *et al.* 1991), and mice lacking cytotoxic T-cells develop much larger lesions in the aorta (Fyfe *et al.* 1994). On the other side, early studies have shown that immunization of rabbits with HSP/65 induces an inflammatory type of lesion (Xu *et al.* 1992).

It is too early, at this point, to conclude what would be the net effect on atherogenesis of a local immune response in the plaque. The same holds for the effect of systemic immune responses, although the systemic antibody response to the plaque autoantigens against oxLDL tends to correlate with aggravation of the disease.

4.3. Nutrition and the immunological aspects of atherosclerosis

An array of major and minor components of the diet is able to modulate some functional factor of various types of cells, including those that participate in the formation of the arterial plaque and involve the immune system in atherosclerotic disease.

Among the major components of the diet, polyunsaturated fatty acids play an important role in atherogenesis. Among the minor components of the diet, antioxidants have been reported to affect the atherosclerotic process. This heterogeneous class of compounds includes antioxidant vitamins and a large number of molecules, e.g. flavonoids and polyphenols, present in several foods. While some of these effects may be attributed to typical antioxidant

activities, such as reduced production of reactive O species and of the compounds generated by them (e.g. oxLDL), other effects appear to be mediated by effects on cellular functions. We will consider only the activities of antioxidants on cell-mediated processes. Those concerning the direct effects on the production of reactive O species in biological systems are discussed by Diplock *et al.* (1998).

4.3.1. Effects of n-*3 fatty acids on cellular immune response and inflammatory events in atherogenesis.* As recently reviewed by Calder (1996), polyunsaturated fatty acids such as arachidonic acid (20 : 4*n*-6), EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3) affect functional variables in various types of cells, including those involved in inflammation and immunity. The compounds of the *n*-3 series have been shown to be particularly potent and, therefore, their effects will be more specifically discussed.

In vitro *effects.* ALA (18 : 3*n*-3), EPA and DHA have been shown to reduce the proliferation of human lymphocytes (Kelly & Parker, 1979; Santoli *et al.* 1990). They also inhibit the response to antigens and the ability of antigen-presenting cells to present antigen (Fujikawa *et al.* 1992), and suppress the production of interleukin-2 (Calder & Newsholme, 1992), a major stimulator of the proliferation of lymphocytes and regulator of cytotoxic T-lymphocytes, natural killer cells and B-cells. This type of action suggests that *n*-3 polyunsaturated fatty acids may play a role in controlling cellular immune processes in atherogenesis. EPA appears to be more active, but ALA has also been shown to exert some of the effects mentioned. However, the experimental conditions in these studies are often far from physiological and, consequently, the relevance of these studies is questioned.

Ex vivo *effects.* Feeding fish oils, often in large amounts, to animals suppresses the response of spleen, thymus, lymph node and peripheral blood lymphocytes to mitogenic stimuli (Kelley *et al.* 1988) and reduces the proportion of spleen lymphocytes bearing the interleukin-2 receptor (Yaqoob & Calder, 1993). Results concerning the effects on the phagocytic activity of macrophages are conflicting, but reduction of macrophage function has consistently been reported in various animal models and in human subjects. Finally, chemotaxis of blood neutrophils and monocytes towards a variety of chemoattractants is reduced after *n*-3 administration (Sperling *et al.* 1993).

In feeding studies, *n*-3 fatty acids appeared to directly affect cellular adhesion processes, as shown by the reduction in the expression of adhesion molecules in T-lymphocytes (Sanderson *et al.* 1995). These effects have been further investigated in *in vitro* systems, where *n*-3 fatty acids appeared to reduce the expression of various adhesion molecules, as well as the binding between monocytes and endothelial cells (De Caterina *et al.* 1995*a*).

A reduced production of various cytokines by peripheral blood monocytes after *n*-3 fatty acid supplementation has been reported in human subjects (Endres *et al.* 1993, 1995*b*), but later studies by others have not always given similar results. Studies with respect to lymphocyte-derived cytokines are limited and the results are somewhat contradictory too.

Clinical aspects of immunomodulation by n-*3 fatty acids.* Clinical studies aiming to assess the effects on functional variables of the immune system in human subjects have shown that diets rich in *n*-3 long-chain polyunsaturates *v.* diets rich in *n*-6 fatty acids (mainly linoleic acid) decrease the *ex vivo* synthesis of the cytokines interleukin-1β and tumour necrosis factor-α and reduce T-cell proliferation (Meydani *et al.* 1993). Suppression of *ex vivo* interleukin-2 production and of mononuclear cell proliferation was observed in a cohort of the same study (Endres *et al.* 1993). These effects on cytokine synthesis may take place at the level of transcription, as is suggested from the observed reduction of mRNA (Kaminski *et al.* 1993). The potential benefits of *n*-3 fatty acids in controlling cellular events in atherogenesis are indirectly supported by *in vitro* studies showing reduction of the expression of cytokine-induced pro-atherogenic and pro-inflammatory proteins in human endothelial cells by DHA (De Caterina *et al.* 1994). These studies concerning the effects of *n*-3 fatty acids on the function of cells involved in the remodelling of vessel walls and in the atherosclerotic process under pathogenic conditions suggest that these fatty acids exert a protective action with respect to the arterial wall. It should be kept in mind, however, that the *in vitro* results should be carefully evaluated in the context of the experimental conditions in order to make comparisons with the *in vivo* situation. Moreover, the *in vivo* or *ex vivo* effects have been obtained in general with high doses of the *n*-3 fatty acids, for relatively short periods of treatment, whereas few studies have been made mimicking the dietary situation (relatively low intakes of long duration).

Mechanisms of action. Long-chain polyunsaturates of the *n*-3 family may act at the cellular level through various mechanisms which can be summarized as follows.

(a) Modulation of eicosanoid production by cells of the immune system, especially reduction of proinflammatory PGE_2 and leukotriene B_4.
(b) Modulation of membrane fluidity.
(c) Modulation of signal transduction pathways, especially those involving lipid mediators, protein kinase C and Ca^{2+} mobilization.
(d) Modulation of the expression of genes involved in cytokine production or in peroxisomal proliferation, fatty acid oxidation and lipoprotein assembly.

Safety. Although the issue of safety of *n*-3 fatty acids has not been specifically addressed, there are some reports of alterations of liver function in rodents, at high levels of intake. On the other hand, there is no evidence of negative effects even at relatively high levels of intake, in population groups. It is recommended however, on the basis of some reports of enhanced susceptibility of LDL enriched in *n*-3 fatty acids to oxidation *in vitro* (a marker which has a somewhat disputable significance), to increase the intake of antioxidants, such as vitamin E, as a preventive measure.

4.3.2. Antioxidants. Uncontrolled oxidative stress in the cardiovascular system is considered to promote the progression of arterial wall lesions through various mechanisms, the major ones being the enhanced oxidation of lipoproteins (resulting in greater atherogenicity and enhanced accumulation in the cells of the vascular wall) and the activation of cells involved in the pathogenesis of

atherosclerosis (monocytes, endothelium, SMC, platelets), as a consequence of enhanced formation of activators (oxLDL, cytokines, eicosanoids, etc.).

In various types of cells, reactive O species may act at the transcriptional level through the activation by cytokines of the transcription factor nuclear factor-κB (Schreck *et al.* 1992). The major antioxidants in the diet, found also in plasma, are tocopherols, mainly α, but also β and γ, β-carotene and other carotenoids, ubiquinone (coenzyme Q_{10}), flavonoids, and other plant polyphenols (all lipid-soluble), and vitamin C, which is water-soluble.

In vitro and *ex vivo* studies have shown effects of natural antioxidants on immune competent cells (Middleton & Kandaswami, 1992; Faruqi *et al.* 1994) and cells in the cardiovascular system. In addition, potent synthetic antioxidants have been demonstrated to inhibit the expression of genes coding for cytokines (DeForge *et al.* 1992) and to reduce VCAM-1 gene expression in human vascular endothelial cells (Marui *et al.* 1993).

5. Diet, hypertension and heart function

CHD is strongly related to both systolic and diastolic blood pressure in a graded fashion (MacMahon *et al.* 1990) and treatment of hypertension results in a reduction in coronary disease-related events (Collins *et al.* 1990). Hypertension due to known factors or diseases (i.e. secondary forms of hypertension) is distinguished from primary hypertension where no known clinical cause for the persistent elevated blood pressure can be identified (essential hypertension). In approximately 90–95% of hypertensives the causes are unknown.

5.1. Aetiology of hypertension

5.1.1. Membrane function. The role of ion transport across cell membranes in the development of hypertension has been studied extensively. Some cellular activities, such as Na^+–Li^+ counter transport, ouabain binding sites (Na^+, K^+-ATPase) or activity, Li^+–K^+ co-transport, or Li^+ leakage, are considered to reflect ion channel function and there is a strong genetic influence on the association between these markers of ion channel activity and hypertension. Depending on the specific marker, the genetic make-up can 'explain' 20–60% of the occurrence of hypertension (Williams *et al.* 1991). In contrast, environmental influences (including dietary factors) are much less important and explain between 0 and 16%. This is important when considering the effect of dietary fatty acids that may change cellular membrane fatty acid composition (see section 5.3). The alterations in ionic channel activity are assumed to lead to increased intracellular Ca^{2+} content and activity, contraction of the arterial smooth muscles and, ultimately, to vasoconstriction. The gene effect related to decreased ouabain-binding sites which are associated with increased intracellular Na^+ levels (and presumably with increased intracellular Ca^{2+} activity due to Na^+–Ca^{2+} exchange) may occur in 8% of hypertensives. Other ion-channel-related gene effects mentioned are less common: 2–3%. In contrast, some 'ion channels' are associated with obesity and the combined gene effect occurs in 10% of the hypertensive population.

5.1.2. Role of humoral mediators. A number of vasoactive substances (angiotensin, endothelin) have been implicated in the development of hypertension. Endothelin-1 is a potent vasoconstrictor produced by endothelial cells, but most endothelin is not secreted luminally and hence plasma levels may not adequately reflect local production. Inhibitors of endothelin synthesis or blockade of receptors can reduce blood pressure in genetic hypertensive animal models. Interestingly, noxious stimuli (including oxLDL and cytokines) cause endothelial cells to synthesize endothelin-1 (Boulanger *et al.* 1992). Thus oxLDL could lead to exaggerated endothelin-1 production in atherosclerotic vessels.

The renin–angiotensin system plays an important role in the homeostasis of salt and water. The conversion of angiotensinogen to angiotensin I by renin is rate limiting. Angiotensin II is produced by an angiotensin converting enzyme (*EC* 3.4.15.1). Angiotensinogen levels have been related to hypertension and a gene resulting in higher angiotensinogen levels has been described. This complex system with positive and negative feedback mechanisms has many aspects that may be important in the aetiology of hypertension. Obesity, excess energy intake and increased angiotensinogen levels have also been linked. A deletion polymorphism of the angiotensin converting enzyme gene has been thought to be connected with the development of CHD (Cambien *et al.* 1992) and increased pressor responsiveness to angiotensin I in normotensive men (Ueda *et al.* 1995). However, no linkage with essential hypertension was observed (Jeunemaitre *et al.* 1992) and apparent linkage with CHD is now strongly contested.

5.1.3. Insulin resistance. Insulin resistance is closely related to hypertension and hyperlipoproteinaemia. Much interest is focused on the metabolic syndrome X, not to be confused with the cardiac syndrome X (chest pain with normal coronary arteries on angiography). The metabolic syndrome is associated with central obesity (although not always), hyperinsulinaemia, hypertriacylglycerolaemia, maturity onset diabetes and hypertension and will be discussed in more detail in section 6. Hyperinsulinaemia has been shown to be related to the development of hypertension in prospective studies (Skarfors *et al.* 1991; Lissner *et al.* 1992). Since insulin also affects ion transport and acts as a growth factor, it is thought that these mechanisms may lead to hypertension (Stout, 1990).

5.1.4. Environmental factors. Environmental factors also play a role. Dietary intake of Na will increase blood pressure although some human subjects are more salt-sensitive than others. Yet there is a range of responses and it would be an oversimplification to dichotomize the population into those who are either salt-sensitive or not (Weinberger, 1990). On the other hand an inverse association between dietary K^+ intake and blood pressure is recognized. It may be by this mechanism that fruit and vegetable consumption (rich in K^+) helps to prevent hypertension, although the lower blood pressure in women consuming a fruit- and vegetable-rich diet and in vegetarians may be independent of K (Ascherio *et al.* 1996*a*; Beilin & Burke, 1995). In addition, fruit and vegetables may help to lower the dietary fat intake and the development of obesity. Smoking also increases blood pressure acutely for

up to 30 min and when it is considered that many smokers may smoke twenty cigarettes per day or more, blood pressure would be raised for long periods of time (Groppelli *et al.* 1992). However, earlier studies in which blood pressures were compared between smokers and non-smokers before smoking failed to document this (Groppelli *et al.* 1992). The diet of smokers differs from that of non-smokers and in the UK smokers consume more salt and fewer essential fatty acids, in particular linoleic acid (Fulton *et al.* 1988).

Epidemiological studies have generally found positive associations between alcohol consumption and blood pressure (World Hypertension League, 1991). Alcohol in large quantities will contribute significantly to energy intake and may lead to obesity and hypertriacylglycerolaemia. Alcohol intake is associated with other unfavourable lifestyle factors such as smoking and low physical activity.

5.2. *Strategies to reduce CHD by lowering blood pressure*

5.2.1. Intervention trials. Many of the early clinical trials had insufficient statistical power to evaluate the benefits of blood pressure reduction in hypertensives in terms of CHD (and stroke). Recently, despite a wide range of treatments with drugs, inclusion criteria, etc. these trials have been subjected to meta analysis. CHD was reduced by 10–14 % by hypertensive treatment. (The reduction in haemorrhagic stroke was 40 %.) The reduction in CHD events is less than was predicted from observational studies and this has led to concern about possible adverse side-effects of drugs (MacMahon *et al.* 1990). β-Blockers adversely affect lipid and glucose metabolism, whilst high doses of diuretics share some of these metabolic effects. Neither Ca nor Mg supplements reduce blood pressure in subjects that are not deficient in these minerals. Algorithms have been developed for the treatment of essential hypertension. After it has been established that blood pressure is really elevated (several readings after reasonable rest on several occasions) a non-pharmacological approach to lower blood pressure is commenced (see section 5.2.2). Only thereafter is the need for pharmacological treatment considered as part of a multiple-risk-factor approach to management. For reasons given earlier, traditional therapy with diuretics and β-blockers is often replaced by angiotensin converting enzyme inhibitors, Ca-antagonists or α1-adrenoceptor blockers.

5.2.2. Individual v. *population approach.* There are more hypertensive subjects in populations with a high median blood pressure level. Most CHD events are seen in the many patients with mildly elevated blood pressure, although their risk is less than that of the small group of severe hypertensive subjects. These arguments form the basis for the population strategy to prevent hypertension and CHD by non-pharmacological measures: reduction in salt and high alcohol intake, avoidance of obesity and increasing the dietary $K^+ : Na^+$ ratio. It is estimated that the average systolic blood pressure would be lowered by some 8 mmHg. The magnitude of this blood pressure lowering effect would be difficult to discern in an individual. Nevertheless, at the population level such a reduction in systolic blood pressure in middle-aged men has the potential to reduce CHD and stroke mortality by 16 and 23 % respectively.

5.3. *Dietary fatty acid composition and hypertension*

There are some epidemiological observations that suggest that dietary polyunsaturated fatty acids, whether *n*-3 or *n*-6, may reduce blood pressure. However, dietary intervention studies are contradictory. Diets supplemented with *n*-6 polyunsaturates (mainly linoleic acid) do not consistently reduce blood pressure. There have been many supplementation studies with mixtures of the fish-oil-derived *n*-3 long-chain polyenes EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3). Many of these studies, in normotensive, hypertensive, hypercholesterolaemic and CHD patients were primarily designed to examine the effect of *n*-3 long-chain polyenes on plasma lipids and generally the number of subjects in the study was too low. The two largest studies, examining the effect in 350 healthy or in 156 hypertensive men and women are still very small in comparison with current cardiovascular trials (Bønaa *et al.* 1990; Trials of Hypertension Collaborative Research Group, 1992). Nevertheless, they documented no benefit in healthy subjects and a reduction in hypertensives. A recent meta analysis has been carried out. There are significant differences between the studies in terms of blood pressure recording (single or repeated, automatic device or random zero sphygmomanometer, blinding of patients and observers, choice of olive or *n*-6 polyunsaturated oil as a placebo, etc.). The results suggest that *n*-3 long-chain polyenes (mainly EPA) reduce blood pressure in a dose-dependent manner in hypertensives, but have little or no effect in healthy volunteers (Morris *et al.* 1993). The mechanism is not clear, but is assumed to be by a reduction in the production of the vasoconstrictor TxA_2. It is widely held that linoleic acid and *n*-3 long-chain polyenes could affect blood pressure because they can change membrane fatty acid composition and/or membrane fluidity and thereby alter ion-channel activity and prostaglandin synthesis. The fatty acid composition of membrane phospholipids is hardly changed by diets rich in linoleic acid. Long-chain polyenes of the *n*-3 family, on the other hand, markedly reduce the level of arachidonic acid in phospholipids. A shift from TxA_2 to TxA_3, devoid of powerful vasoconstricting properties, is now accepted. Fish oil may not reduce arachidonic acid in phosphatidyl inositol, a phospholipid central to α1-adrenoceptor-mediated inositol pathway signal transduction (MacLeod *et al.* 1994). However, little is known about the effect of fish oil on the fatty acid composition of small arterial resistance vessels (MacLeod *et al.* 1994). In view of these uncertainties it is clear that a meta analysis is no substitute for a properly conducted trial. What is needed is a large double-blind controlled trial in hypertensive patients, in whom TxA_2 production or α1-adrenergic mechanisms have been implicated in their hypertension.

5.4. *Heart function*

The maintenance of blood pressure and perfusion of organs is the main function of the heart. The energy for this is derived from oxidative phosphorylation in mitochondria. As myocardial energy reserves last but a few seconds, there is a constant need of O_2 supply. Cardiac output can vary enormously, due to large changes in the emotional and

environmental influences, and changes in O_2 consumption will follow in its tract. The function of the heart is controlled by the autonomic nervous system. Increasing O_2 requirements are met by increased O_2 extraction and blood flow (vasodilatation). The lumen of coronary vessels is controlled by a tonic vasoconstriction mediated by α1-adrenergic receptors balanced by a vasodilatation under the influence of adenosine, prostaglandins and endothelial-derived relaxing factor (NO). The heart uses a variety of substrates (non-esterified fatty acids, glucose, lactate, etc.) depending on the nutritional state. Non-esterified fatty acids form the main substrate, but the importance of glucose increases during the fed state.

5.4.1. Effect of diet. The contractile function of the heart is under autonomic control and there is no evidence that myocardial function is sensitive to dietary factors under conditions of adequate perfusion and O_2 supply. However, when O_2 supply is limited due to atherosclerotic lesions in coronary vessels or microvessel disease, the function may become compromised. Oxidative metabolism of glucose requires less O_2 than that of non-esterified fatty acids to produce ATP and may help to improve myocardial function under those conditions. Relatively few experimental studies have examined whether myocardial function is sensitive to changes in the dietary fatty acid composition. Early studies in rats have limited value as control diets were deficient in essential fatty acids (Semafuko *et al.* 1984; Wince *et al.* 1984). However, a linoleic acid-rich diet may increase contractile force in isolated papillary muscles and improve the relationship between left ventricular work and filling pressure in the isolated perfused rat heart (de Deckere & ten Hoor, 1979). It should be noted that these preparations may have a limited O_2 supply and some of these effects may not apply to the well-oxygenated heart. A reduction in basal and α1-adrenoceptor-mediated peak left ventricular pressure by a large intake of fish oil has been reported, but no effect was observed in pigs. Yet, endothelium-derived relaxing factor, which was reduced in some studies by feeding fish oil, can reduce cardiac contractility (Mohan *et al.* 1994). Thus the potential of *n*-3 and perhaps also of *n*-6 fatty acids to influence cardiac contractility under conditions of limited O_2 supply or at high work loads can be envisaged. However, without a clear mechanism and understanding of conflicting reports it is too early to speculate.

5.5. Function of the ischaemic heart

Acute myocardial ischaemia occurs when coronary flow can no longer meet the O_2 requirements of the heart. Ischaemia may be precipitated by an increased O_2 demand due to severe exercise or stress and/or acute vasoconstriction or thrombus in the coronary vessel. Prolonged ischaemia results in acute MI and necrosis of the muscle that is inadequately or not perfused. The occurrence of serious ventricular arrhythmias very early is the principal cause of sudden cardiac death. In a patient who has survived this critical period, the loss of ventricular mass may result in heart failure. Early restoration of blood flow (dissolution of the thrombus) may prevent necrosis; however, reperfusion may also stimulate the production of free radicals, which may reduce the function and induce serious ventricular arrhythmias (see Diplock *et al.* 1998).

5.5.1. Dietary fatty acids and arrhythmia. Diets rich in linoleic acid protect against experimental ischaemia-induced arrhythmias (Leprán *et al.* 1981; McLennan *et al.* 1985; Riemersma *et al.* 1988). There is no consensus about the mechanism that underlies the anti-arrhythmic effect of such diets (Riemersma *et al.* 1988; Charnock, 1994). It is assumed to be mediated by prostaglandins as a result of changing phospholipid fatty acid composition. However, the protective effect may not be abolished by non-steroidal anti-inflammatory agents (Leprán *et al.* 1981; Sargent, 1990). Some, but not all, studies have also shown a protection against the so-called ischaemic reperfusion-induced arrhythmias (in contrast to ischaemia-induced arrhythmias discussed earlier) for reasons that are not clear. It is important to point out that diets rich in linoleic acid are low in saturated fatty acids. Thus, it is possible that saturated fatty acids are pro-arrhythmic (Riemersma *et al.* 1988). However, fewer arrhythmias were also observed when the control diet was rich in monoenoic fatty acids (McLennan, 1993).

Large amounts of fish oil, in quantities that would be difficult to consume in the context of a Western diet, protect against the development of serious ventricular arrhythmias in animal studies (McLennan *et al.* 1989, 1990; Sargent & Riemersma, 1990). Conflicting reports have appeared as to whether this protection extends itself to reperfusion-induced arrhythmias. The underlying mechanism is assumed to be due to increased PGI_3 and reduced TxA_2 production. Alternatively, it may be due to a direct inhibition of the voltage-sensitive Na^+ channel by non-esterified EPA (Weylandt *et al.* 1996). It is worth emphasizing again that diets rich in polyunsaturated fatty acids usually reduce the intake of saturated fat.

Saturated fat intake is not reduced when animals receive small fish-oil supplements (0·4 % energy). Long-term supplementation with small amounts of fish oil was not anti-arrhythmic, yet the well-documented biochemical effects (reduction of phospholipid arachidonate content and an increase in the amounts of EPA and DHA) were observed (Riemersma, 1995). Epidemiological and clinical data suggest that small amounts of fish oil may prevent CHD mortality, reduce the risk of lethal events following MI (Burr *et al.* 1989), and lower the chance of primary cardiac arrest (related to serious ventricular arrhythmias) in the community. It has been suggested that ALA as part of a Mediterranean diet may reduce events after an acute MI (De Lorgeril *et al.* 1994). This is an intriguing possibility (McLennan & Dallimore, 1995), but requires confirmation in a single-factor study. The convergence between experimental and human studies suggests that dietary fatty acid composition may indeed reduce the risk of atherosclerosis (via haemostatic and/or immunological effects) and lethal coronary events by prevention of serious ventricular arrhythmias.

6. Insulin resistance, obesity and non-insulin-dependent diabetes mellitus

Both experimental data and epidemiological studies suggest that abnormalities in lipid- and lipoprotein metabolism, as well as the presence of hypertension, are associated with an

increased cardiovascular risk. In addition, several other risk indicators, for example insulin resistance, obesity and NIDDM interfere with lipid metabolism and hypertension.

The relationship between insulin resistance and subsequent CHD morbidity and mortality is evident from several epidemiological and a few prospective studies. In the Helsinki Police Officers study (Pyörälä, 1979), for instance, 982 men with an age range of 30–59 years were followed over a period of 10 years. In this study, high 1 and 2 h post-glucose insulin levels were independent predictors of CHD end-points, where fasting insulin was not an independent contributor. The Paris Prospective Study (Fontbonne *et al.* 1991) examined the incidence of fatal and non-fatal CHD in 7038 males aged 43–54 years for a period of 15 years. Analysis at 15 years showed that the 2 h post-load insulin level was a significant, independent predictor of death from CHD.

6.1. Insulin resistance, cardiovascular disease and cardiovascular risk factors

Insulin resistance may be defined as a diminution of the biological response to a given concentration of insulin. It is a heterogeneous syndrome with both genetic and environmental factors playing a determinant role in the development. Several factors proposed in this respect have been reviewed by Després & Marette (1994). These include genetic factors, such as an excessive accumulation of visceral fat, and circulating factors, such as free fatty acids, sex-steroid hormones, tumour necrosis factor-α, hyperinsulinaemia and hyperglycaemia, with main target tissues muscle, adipose tissue and liver. In addition, the morphology of skeletal muscle may itself contribute to the insulin resistance syndrome, since it has been demonstrated that the proportion of oxidative fibres (type I) and capillary density are positively correlated with insulin action *in vivo*, as determined by the hyperinsulinaemic euglycaemic clamp technique (Lillioja *et al.* 1987). Environmental factors associated with the insulin resistance syndrome include lack of physical activity and intake of dietary fat (Després & Marette, 1994).

Large-scale, prospective epidemiological studies revealed a clear association between insulin resistance and CHD (Pyörälä, 1979; Fontbonne *et al.* 1991). Factors which may contribute to this association are certainly the abnormalities in lipid metabolism and hypertension.

6.1.1. Lipid abnormalities and insulin resistance. There is now abundant evidence for a relation between hyperinsulinaemia and/or insulin resistance and various lipid abnormalities which are known risk factors for CHD and other macrovascular complications. Results of the Helsinki Heart Study (Manninen *et al.* 1992) and of the PROCAM study (Assman & Schulte, 1992) both demonstrate that the characteristic dyslipidaemia associated with an insulin-resistant hyperinsulinaemic state is associated with a marked increase in CHD risk. The alterations in lipid metabolism commonly associated with insulin resistance have been reviewed by Frayn (1993). The insulin resistant state is characterized by elevated plasma triacylglycerol concentrations (particularly elevation of VLDL-triacylglycerol and VLDL-apoB), a decreased plasma HDL-cholesterol concentration (especially HDL_2-cholesterol) and the presence of small, dense LDL particles. The presence of this dense LDL phenotype may result from an overproduction of apoB, induced by an increased availability of free fatty acids (Sniderman *et al.* 1992). Although cause and effect have never been properly elucidated, it seems that hyperinsulinaemia causes dyslipidaemia, because correction of the dyslipidaemic state leaves the insulin resistance unchanged, whereas correction of the insulin resistance by weight reduction, increased physical activity, and a low-fat diet is immediately followed by the normalization of dyslipidaemia.

In most studies concerning the relationship between lipoprotein metabolism and the risk of CHD, only fasting blood lipids and lipoproteins were considered. However, as proposed by Zilversmit (1979) almost 20 years ago and more recently by Patsch (1987), postprandial hyperlipaemia may be particularly atherogenic, especially since a major part of lifetime is spent in the period between food ingestion and 6–8 h thereafter. The magnitude of postprandial lipaemia differs substantially among individuals, including those considered to be normolipidaemic on the basis of fasting blood lipid values (Patsch, 1987). Factors which have been reported to influence postprandial lipaemia include basal triacylglycerol and HDL-cholesterol concentrations (O'Meara *et al.* 1992), obesity (Lewis *et al.* 1990; Potts *et al.* 1995), diabetes mellitus (Stinson *et al.* 1993) and insulin resistance (Frayn, 1993).

Lewis *et al.* (1990) demonstrated that even normolipidaemic obese subjects have greater postprandial lipaemia and triacylglycerol enrichment of HDL after ingestion of a high-fat meal. Potts *et al.* (1995) concluded that a disturbed triacylglycerol clearance in subcutaneous adipose tissue is related to elevated plasma triacylglycerol concentrations and reduced HDL-cholesterol levels. Roust & Jensen (1993) investigated postprandial free fatty acid kinetics in obese persons and concluded that impaired suppression of adipose tissue lipolysis is a potentially important abnormality present in upper body obesity. Since obesity, and particularly abdominal obesity, is frequently associated with insulin resistance, it could be expected that insulin resistance also might interfere with the magnitude of postprandial lipaemia.

The effects of insulin on lipid metabolism occur both in adipose tissue and the liver. Insulin mediates the activation of lipoprotein lipase in adipose tissue; the consequence of a disruption at this level will result directly in an impaired postprandial triacylglycerol clearance (Frayn, 1993). Other actions of insulin comprise the suppression of non-esterified fatty acid release in adipose tissue by inactivation of the hormone-sensitive lipase (*EC* 3.1.1.3) and increased re-esterification, and the suppression of hepatic secretion of VLDL-triacylglycerol in the liver. The latter may lead to inappropriate postprandial VLDL-triacylglycerol secretion and the presence of large triacylglycerol-enriched VLDL in the postprandial period. As a consequence, neutral lipid exchange with LDL may lead to small, dense LDL particle formation (Frayn, 1993).

These observations show a clear relationship between insulin resistance and certain disturbances in lipoprotein metabolism which contribute to the development of

cardiovascular diseases. However, to ascertain a causal relationship, future research has to concentrate on intervention studies which may elucidate the sequence of events.

6.1.2. Hypertension and insulin resistance. Arterial hypertension is an established risk factor for CHD. Several large studies have already reported on the relationship between insulin resistance and hypertension. Some authors described a positive relationship between insulin concentrations and blood pressure (Welborn *et al.* 1966; Lucas *et al.* 1985; Modan *et al.* 1985) but others were unable to demonstrate such a relationship (Cambien, 1987; Asch *et al.* 1991). The reason for this discrepancy may be found in ethnicity (Saad *et al.* 1991) and in the presence of obesity (Cambien, 1987). On the basis of published reports, at least half of the patients with hypertension can be considered to have insulin resistance and hyperinsulinaemia (Reaven *et al.* 1996).

The possible mechanisms underlying a relationship between insulin and blood pressure are complex and can be divided into direct and indirect effects. Hypertension may result directly from insulin resistance through the stimulatory effect of high insulin concentrations on vascular smooth muscle proliferation (Banskota *et al.* 1989). Insulin also enhances renal Na retention directly via its effects on the proximal tubuli (DeFronzo, 1981) and indirectly through stimulation of the sympathetic nervous system and augmentation of angiotensin II-induced aldosterone secretion (Rocchini *et al.* 1990). Stimulation of the sympathetic nervous system by insulin may also have a direct hypertensive effect (Landsberg & Krieger, 1989).

Hypertension frequently occurs in combination with other metabolic alterations such as disturbances in lipid metabolism, obesity and NIDDM. Since insulin resistance seems to be the common link between these factors, the non-pharmacological treatment approach should focus on the increase of insulin sensitivity. Effective tools are weight reduction, increased physical activity, low-fat diet and perhaps consumption of foods that reduce the insulinaemic response. This strategy probably results in a lowering of the blood pressure as well.

6.2. Nutritional aspects

Although insulin resistance also occurs in persons with normal body weight, it is a common feature in obese patients with or without impaired glucose tolerance or NIDDM. In this specific patient group, diet and exercise are two common, non-pharmacological approaches for treatment. Considering the aim of the present review, only relevant dietary factors will be discussed.

In obese, insulin-resistant patients, several studies have examined the effect of overall weight loss, by diet or a diet + exercise combination, on cardiovascular risk factors such as lipid and lipoprotein abnormalities and hypertension. From the results, it is clear that weight loss is accompanied by improved insulin sensitivity and a subsequent better metabolic profile (Colman *et al.* 1995). Whether specific dietary components may influence the status of insulin resistance in obese and non-obese persons is not fully understood.

The relationship between dietary factors and physical activity with hyperinsulinaemia was examined in 389 non-diabetic men, 70–89 years of age, who participated in the Zutphen Elderly Study (Feskens *et al.* 1994). A significant, negative association was observed between insulin levels (during an oral glucose tolerance test) and the intake of dietary fibre and polyunsaturated fatty acids, which could not be accounted for by energy intake, BMI, physical activity, prescribed diets or the presence of CHD. In contrast, insulin levels increased with the increasing intake of saturated fatty acids and alcohol. Apart from overweight, physical activity and dietary factors such as the intake of fatty acids, fibre, carbohydrates and alcohol, were independently associated with hyperinsulinaemia and insulin resistance.

In a study with 544 non-diabetic women (aged 30–84 years), the habitual intake of total dietary fat was positively related to fasting insulin concentrations, particularly among sedentary women. The positive relation of dietary fat content with the percentage of body fat accounted for a substantial proportion (±30 %) of the association of dietary fat with insulin concentrations (Mayer *et al.* 1993).

In a group of male Swedish elite athletes, diet modification during 1 year resulted in decreased insulin levels in conjunction with a decreased relative fat energy content. Insulin levels returned to baseline amounts when the relative fat energy content increased again (Tegelman *et al.* 1996).

Information about the effect of specific fatty acids on insulin metabolism is scarce. The incorporation of *n*-3 fatty acids, and of DHA (22 : 6*n*-3) in particular, into phospholipids, prevents the expected insulin resistance in rats fed on a high-fat diet (Storlien *et al.* 1991). In human subjects, decreased insulin sensitivity is associated with decreased concentrations of certain long-chain polyunsaturated fatty acids (20 : 4*n*-6, 22 :4*n*-6, 22 : 5*n*-6, and 22 : 5*n*-3) in skeletal muscle phospholipids (Borkman *et al.* 1993). Specifically, decreases in C20–C22 long-chain polyunsaturated fatty acids were associated with increased insulin resistance. This raises the possibility that changes in the fatty acid composition of muscle modulate the action of insulin. The results of the study of Borkman *et al.* (1993) demonstrate that in patients with coronary artery disease, linoleic acid (18 : 2*n*-6) correlated directly with hyperinsulinaemia, but this was not the case in normal controls. Since insulin has an effect on Δ^6-desaturation, the conversion of linoleic acid to γ-linolenic acid (18 : 3*n*-6) may be impaired. In addition, Pan *et al.* (1995) demonstrated that an impaired insulin action and obesity are independently associated with reduced Δ^5-desaturase activity. In these circumstances, the direct supply of γ-linolenic acid (impairment of Δ^6-desaturase) or arachidonic acid (impairment of Δ^5-desaturase) may be of value (Horrobin, 1993). Obesity was also found to be associated with reduced elongase activity and higher Δ^9-desaturase activity (Pan *et al.* 1995). *Trans* fatty acids interfere with desaturation and elongation of 18 : 2*n*-6 and 18 : 3*n*-3 (ALA), thereby further contributing to decreases in C20–C22 long-chain polyunsaturated fatty acids. A decrease in C20–C22 polyunsaturates leads to increased fatty acid synthesis, lipogenesis, insulin resistance and hyperinsulinaemia, with the subsequent development of obesity, hypertension, NIDDM and CHD (Ostlund-Lindqvist *et al.* 1985; Simopoulos, 1994).

Further investigations are needed to evaluate if the essential fatty acids linoleic acid and ALA influence insulin resistance and, if so, whether this effect requires desaturation and elongation of these fatty acids.

As was already demonstrated in a few studies, postprandial triacylglycerol concentrations correlate with the degree of hyperinsulinaemia and/or insulin resistance, at least in obese persons. The influence of dietary factors on postprandial lipaemia was investigated by Jeppesen *et al.* (1995). The acute effects of varying amounts of fat and fructose were studied in eleven healthy, non-diabetic subjects with a wide range of plasma triacylglycerol concentrations. Increasing the dietary intake of fat from 5 to 40 to 80 g led to a significant increase in postprandial concentrations of both triacylglycerol and retinyl palmitate. Furthermore, adding 50 g fructose to 5 g fat also led to a significant increase in postprandial concentrations of triacylglycerol and retinyl palmitate. Chronic intake of fish oil (64 mg *n*-3 fatty acids/kg body weight per d) reduced postprandial lipaemia in eight normolipidaemic volunteers. This effect was not due to increased chylomicron clearance but more probably to reduced chylomicron production or secretion (Harris & Muzio, 1993). Further research is needed to elucidate whether these effects are reflected in changes in insulin sensitivity in these persons. The results suggest that it may be possible to modulate insulin sensitivity and subsequent cardiovascular risk factors by diet. However, further research on mechanisms is unavoidable to determine the real functional component in the diet.

7. Hyperhomocysteinaemia and cardiovascular risk

7.1. Causes of hyperhomocysteinaemia

Although there is some evidence that increased plasma homocysteine levels are caused by a massive export of homocysteine from tissues into plasma (Guttormsen *et al.* 1996), it is usually thought to result from impaired elimination of plasma homocysteine, either by a defective methylation to methionine or by a reduced trans-sulfuration to cystathionine and cystathione. Betaine (an oxidation product of choline) and folic acid (in the form of 5-methyltetrahydrofolate) are the methyl donors for the transmethylation of homocysteine. 5-Methyltetrahydrofolate is obtained from the reduction of 5,10-methylenetetrahydrofolate which is catalysed by the enzyme 5,10-methylenetetrahydrofolate reductase (*EC* 1.5.1.20; MTHFR). In this reaction, methylcobalamin, derived from vitamin B_{12}, serves as a cofactor. A deficiency or reduced activity of MTHFR results in increased plasma homocysteine levels. Such a reduced MTHFR activity has been shown to result from a series of mutations in the gene coding for this enzyme (Frosst *et al.* 1995; Goyette *et al.* 1995).

Transfer of a methyl group from betaine to homocysteine requires the active enzyme betaine : homocysteine methyltransferase (*EC* 2.1.1.5), whereas pyridoxal-5′-phosphate, a form of vitamin B_6, is required as a cofactor. A reduction in this pathway of homocysteine methylation has not been reported so far (Dudman *et al.* 1996).

Because folic acid is an important methyl donor for the methylation of homocysteine, methylcobalamin (derived from vitamin B_{12}) is the necessary coenzyme in this reaction and B_6-derived pyridoxal-5′-phosphate is required for homocysteine removal by trans-sulfuration, folate deficiency and/or a poor status of the vitamins B_6 or B_{12} may be a nutritional reason for hyperhomocysteinaemia.

7.2. Athero-thrombotic mechanisms of hyperhomocysteinaemia

7.2.1. Interaction with lipoproteins. In the chemical pathology of atherosclerosis, homocysteine is thought to play an important role, because the free amino groups of LDL can be thiolated by homocysteine thiolactone, causing aggregation and increased uptake of LDL by macrophages, explaining lipid deposition in atheromas. Homocysteine thiolactone, released from homocysteinylated LDL within the vascular wall, promotes intimal injury, oxidation of cholesterol and unsaturated lipids, platelet aggregation, thrombogenic factors, myointimal hyperplasia, deposition of sulfated glycosaminoglycans, fibrosis and calcification of atherosclerotic plaques (McCully, 1993). It has also been suggested (Harpel & Borth, 1992) that homocysteine increases the atherogenic and antifibrinolytic potential of Lp(a).

7.2.2. Smooth-muscle cell proliferation. Homocysteine has been shown to stimulate vascular SMC proliferation, a hallmark of arteriosclerosis, possibly by increasing the transcription rate of cyclin A (Tsai *et al.* 1996).

7.2.3. Endothelial functions. In cell culture studies, it was demonstrated that homocysteine significantly lowers endothelial cell growth (Tsai *et al.* 1994). Moreover, as demonstrated by Dudman *et al.* (1991) in *in-vitro* studies, homocysteine causes endothelial detachment, but since fibronectin greatly diminished this process, it was considered of limited relevance to atherogenesis in hyperhomocysteinaemia.

It has been suggested that high plasma homocysteine levels cause endothelial injury, largely as a consequence of facilitating the generation of H_2O_2 from O_2 (Stamler & Loscalzo, 1992; Jones *et al.* 1994; Hultberg *et al.* 1995), although the evidence is not unanimous (Clarke *et al.* 1992). H_2O_2, in turn, is presumed to induce dysfunction and damage to the endothelial cell resulting in platelet activation, coagulation and reduced fibrinolysis.

Further studies by Stamler *et al.* (1993) indicate that normal endothelium modulates the potential adverse effects of homocysteine by releasing NO and forming the adduct S-NO-homocysteine. The adverse vascular properties of homocysteine may result from an inability to sustain S-NO-homocysteine formation owing to an imbalance between the production of NO by progressively dysfunctional endothelial cells and the levels of homocysteine.

There is no evidence that homocysteine inhibits the formation of endothelial prostacyclin (Wang *et al.* 1993).

7.2.4. Functions of blood platelets. As summarized by Stamler & Slivka (1996), the effect of homocysteine on platelet function and survival is controversial. Thus, the shortened survival as measured by Harker *et al.* (1974) in homocysteinuria patients could not be reproduced by others (Uhleman *et al.* 1976; Hill-Zobel *et al.* 1982).

Homocysteine has been shown to inhibit the ecto-ADPase activity of human umbilical vein endothelial cells. Because ADP is a potent platelet aggregatory agent, this action of homocysteine may enhance platelet aggregability (Harpel *et al.* 1996).

7.2.5. Coagulation and natural anticoagulants. Hyperhomocysteinaemia has been associated with a consumption coagulopathy, resulting in reduced amounts of clotting factor VIIc and AT-III. However, there is some evidence that deficient synthesis of these substances is involved, which is normalized on treatment with pyridoxine plus folate (Schienle *et al.* 1994).

In male CHD patients, homocysteine levels were significantly correlated with fibrinogen content and plasma viscosity (von Eckardstein *et al.* 1994). Within the patient group of this study, both fibrinogen and homocysteine contents significantly increased in parallel with the number of stenosed coronary vessels.

Homocysteine was shown to induce tissue factor procoagulant activity in cultured human endothelial cells in a time- and concentration-dependent manner by tissue factor gene transcription (Fryer *et al.* 1993).

In monkeys, Lentz *et al.* (1996) demonstrated that diet-induced hyperhomocysteinaemia is associated with significantly decreased thrombomodulin anticoagulant activity. In contrast, van den Berg *et al.* (1995) noted increased plasma levels of thrombomodulin in young patients with peripheral arterial occlusive disease and hyperhomocysteinaemia after methionine loading. Since the thrombomodulin levels decreased on treatment with pyridoxine plus folic acid, in this patient group hyperhomocysteinaemia appears to be associated with enhanced thrombomodulin levels. Together with the increased levels of vWf, this is considered by the authors to be a marker of endothelial dysfunction.

Homocysteine inhibits the expression and activity of endothelial cell surface thrombomodulin, the thrombin cofactor responsible for activation of a natural anticoagulant, protein C (Harpel *et al.* 1996). Although Rodgers & Conn (1990) demonstrated homocysteine to reduce protein C activation by endothelial cells *in vivo*, this finding could not be confirmed by others (Bienvenu *et al.* 1993; Aronson *et al.* 1994). Homocysteine also lowers expression of the natural anticoagulant heparan sulfate proteoglycan on the surface of porcine aortic endothelial cells in culture, as reflected by the reduced binding of another natural anticoagulant, AT-III (Nishinaga *et al.* 1993).

Increased coagulation and fibrinolysis in hyperhomocysteinaemia *in vivo* has recently been substantiated by increased concentrations of thrombin–antithrombin complexes and D-dimers (Hamano *et al.* 1996).

7.2.6. Fibrinolysis. In stroke patients, plasma homocysteine levels appeared significantly related to the concentrations of tPA, but not to PAI-1 (Lindgren *et al.* 1996). Similar results had also been found by Bienvenue *et al.* (1993) in fifty patients with arterial and venous thrombosis. These authors failed to demonstrate a significant relationship between plasma homocysteine and plasminogen levels. Van den Berg *et al.* (1995) found normal tPA levels in young patients with peripheral arterial occlusive disease and hyperhomocysteinaemia after methionine loading. Since homocysteine has been shown to inhibit the binding of tPA to endothelial cells in culture (Hajjar, 1993), it may interfere with the fibrinolytic properties of the endothelial surface.

7.2.7. Altered gene expression. By using a modified non-radioactive differential display analysis to evaluate gene expression in cultured human umbilical vein endothelial cells, Kokame *et al.* (1996) demonstrated that homocysteine can alter the expressivity of multiple genes, including a stress protein, which may contribute to atherogenesis.

7.3. Hyperhomocysteinaemia or B-vitamin status of primary importance in cardiovascular risk?

In many studies relating hyperhomocysteinaemia to cardiovascular risk, increased plasma levels of homocysteine are associated with reduced amounts of folic acid, vitamin B_{12} (cobalamin), vitamin B_6 and/or pyridoxal-5′-phosphate (Ubbink *et al.* 1993; Jacobsen *et al.* 1994; Pancharuniti *et al.* 1994; Dalery *et al.* 1995; Robinson *et al.* 1995, 1996; Chasan-Taber *et al.* 1996; Petri, 1996). In addition, cobalamin-deficient patients usually have increased plasma levels of homocysteine (Stabler *et al.* 1990). This implies that a relative deficiency of these B-complex vitamins rather than the high homocysteine plasma levels may be the actual cardiovascular risk factor (Chasan-Taber *et al.* 1996). Findings by Schmitz *et al.* (1996) that homozygosity for the C677T mutation, associated with increased plasma homocysteine level, is not associated with increased risk of MI, irrespective of folate intake, support this contention. Comparable results were obtained by Ma *et al.* (1996), who suggest that a gene–environment interaction might increase the risk by further elevating plasma homocysteine, especially when folate intake is low. Further evidence for a primary role of folate deficiency in cardiovascular risk comes from studies by Selhub and co-workers (Selhub *et al.* 1995; Selhub, 1996) who demonstrated that plasma concentrations of folate and pyridoxal-5′-phosphate as well as folate intake were inversely related to extracranial carotid stenosis after adjustment for other known risk factors. In a group of 367 elderly patients undergoing coronary angiography, Herzlich *et al.* (1996) observed no significant trend in change in homocysteine as the extent of coronary artery disease increased. However, a low vitamin B_{12} status was shown to be associated with a lower left ventricular ejection fraction, suggesting a primary role for the cobalamin status in determining left ventricular function. Verhoef *et al.* (1996) also demonstrated that plasma levels of vitamin B_6 and folate (but not of vitamin B_{12}) were inversely associated with the risk of MI, independently of other potential risk factors. From their studies, Robinson *et al.* (1995) conclude that low pyridoxal-5′-phosphate confers an independent risk for coronary artery disease and Ellis & McCully (1995) observed that the treatment of patients with carpal tunnel syndrome and related disorders with vitamin B_6 was associated with only 27 % of the risk of developing cardiac chest pain or MI compared with patients who had not taken vitamin B_6. Dalery *et al.* (1995), however, did not observe differences for folate, vitamin B_{12} or total vitamin B_6 between CHD patients and controls.

7.4. Dietary B-vitamins lower plasma homocysteine

In numerous studies, increased plasma homocysteine levels appear to be associated with reduced plasma folate concentrations (Verhoef *et al.* 1996) and since folate is the main methyl donor in the conversion of homocysteine to methionine, folate supplementation may be the preferred way to lower homocysteine-mediated cardiovascular risk. In their meta-analysis Boushey *et al.* (1995) calculated that an additional intake of 200 μg folate/d would reduce the plasma homocysteine content by about 4 μmol/l and that by increasing dietary folate in the USA 13 500–50 000 CHD deaths per year could be avoided. As suggested by Jacques *et al.* (1996), individuals carrying a gene mutation resulting in expression of a sub-normal activity of the homocysteine transmethylation enzyme MTHFR may have a higher folate requirement. Consequently, this population may certainly require folate supplementation to prevent hyperhomocysteinaemia.

Van den Berg *et al.* (1995) demonstrated, in young patients suffering from peripheral arterial occlusive disease and hyperhomocysteinaemia after methionine loading, that treatment with pyridoxine plus folic acid resulted in normalization of homocysteine metabolism and ameliorated endothelial dysfunction as reflected by a change towards normal circulating levels of vWf and thrombomodulin. Schienle *et al.* (1994) reported a case study, demonstrating that in a patient with homocystinuria due to cystathionine-β-synthase deficiency and thromboembolic disease, treatment with pyridoxine plus folate not only led to normalization of amino acids in urine and plasma and of plasma levels of plasma coagulation and anti-coagulation factors, but also prevented further thromboembolic episodes.

8. Critical assessment of the science base

8.1. Identification of criteria

In this section, the science base presented previously will be critically evaluated, using the following criteria of decreasing importance (except criterion 5).

Criterion 1. Plausible and validated evidence does exist for the involvement of the various variables investigated as (anti)risk factors or -indicators in the aetiology of CHD. For risk factors a causal involvement in CHD aetiology should have been proven; for risk indicators such a causality is not required.

Criterion 2. Well-designed human intervention studies have been published, demonstrating that higher intakes (or body levels) of the food items considered lower the levels of the identified risk factors or indicators.

Criterion 3. Prospective, statistically validated epidemiological evidence is available that links higher intakes (or body levels) of these food items to reduced levels of the risk factors or indicators.

Criterion 4. Retrospective epidemiological data are present which demonstrate a statistically validated association between intake or body levels of the food items investigated and the levels of the risk factors or indicators identified under criterion 1.

Criterion 5. Clear evidence exists for the safety of the food items considered.

8.2. Evaluation of the present knowledge base with respect to food functionality

8.2.1. Plasma lipoproteins. Results from well-designed intervention trials clearly demonstrate that the plasma concentration of LDL-cholesterol is a causal risk factor for CHD. Most probably, plasma HDL-cholesterol concentration is an anti-risk factor, but confirmation still depends on results of intervention studies showing that an isolated increase in the plasma HDL-cholesterol concentration significantly lowers the risk of CHD. Evidence for plasma VLDL or triacylglycerol levels being associated with the risk of CHD is mainly based on epidemiological studies. Therefore, the plasma VLDL or triacylglycerol concentration can only be considered a risk marker for CHD. The same holds for the plasma concentration of Lp(a), which has been demonstrated to be a powerful risk marker in epidemiological studies.

It should be mentioned that so far the lipoprotein profile has mainly been investigated in blood sampled under fasting conditions, whereas man is usually in a state of postprandial hyperlipidaemia for at least 8 of every 24 h. Since the importance of the postprandial lipoprotein profile for determining the risk of CHD has only been superficially investigated, it will not be emphasized here.

Taking these considerations into account, dietary saturated fatty acids can be classified as CHD-risk-promoting nutrients because, as compared with carbohydrates, they increase plasma LDL-cholesterol concentrations more strongly than plasma HDL-cholesterol levels (chain lengths up to sixteen C atoms) or reduce the plasma HDL-cholesterol concentration (stearic acid, 18 : 0), even if they seem to lower slightly the plasma Lp(a) concentration. Dietary *trans*-monounsaturated fatty acids increase LDL- and reduce HDL-cholesterol levels in plasma; moreover they increase the plasma Lp(a) concentration. Foods low in saturated and *trans* fatty acids and high in linoleic acid and ALA can, therefore, be classified as functional with respect to lowering the lipoprotein-associated risk of CHD.

The *cis*-unsaturated fatty acids oleic acid, linoleic acid and ALA reduce the plasma concentration of LDL-cholesterol, whereas they hardly affect plasma HDL-cholesterol and Lp(a) concentrations. Therefore, foods enriched in these unsaturated fatty acids can be classified as functional in reducing CHD risk. Oils rich in the highly unsaturated fatty acids EPA and DHA have consistently been shown to lower plasma VLDL concentrations and may, therefore, reduce CHD risk. However, in certain population groups they increase the plasma LDL-cholesterol concentration. So, with respect to lipoprotein effects, foods enriched in EPA and/or DHA cannot be classified as functional in reducing CHD risk by virtue of their effect on the plasma lipoprotein profile.

Dietary soluble fibre and certain phytosterols can be classified as functional in lowering CHD risk, because they improve the plasma lipoprotein profile. Although ethanol and a number of fat replacers have similar effects, their side-effects may hamper their use as functional foods. Insufficient evidence is available with respect to soyabean protein preparations, garlic, inulin and oligofructose. Finally, the available evidence does not support the

classification of mono- and disaccharides, resistant starch, fermented milk products, tocopherols and tocotrienols as functional food components.

8.2.2. Arterial thrombosis. In Western societies with ageing populations the modulation of thrombosis tendency is likely to become an important approach to the prevention of CHD. The main problem today, however, is the lack of reliable variables to measure the prothrombotic state in human subjects. In addition, there is a considerable lack of indicators reliably reflecting thrombotic risk in man. So far it has not been shown that changes found in platelet function measured *in vitro* significantly predict changes in thrombosis tendency *in vivo*. The plasma levels of factors involved in coagulation and fibrinolysis do not necessarily reflect the degree to which these phenomena really occur. Similarly, the predictive value of endothelial cell function for CHD has been insufficiently evaluated.

Diet, especially dietary fatty acids, has been shown to affect many of the previously mentioned variables, but the mechanisms involved are largely unknown. Consequently, increasing mechanistic knowledge about the influence of dietary factors on platelet, leucocyte and endothelial functions and on coagulation and fibrinolysis *in vivo*, is required for improving dietary strategies to control the prothrombotic state. According to current knowledge, long-chain *n*-3 and *n*-6 fatty acids are particularly able to modulate both endothelial cell and platelet functions. However, the optimal *n*-6 : *n*-3 fatty acid ratio and the effect of these fatty acids on the antioxidant status of the body is not clear. The same holds for the mechanisms by which platelet and/or leucocyte fatty acid composition affect coagulation and fibrinolysis in man. Also the role of dietary factors as regulators of the interaction between different cell types involved in thrombogenesis has been insufficiently studied so far. Because of all these uncertainties, there is no solid evidence for any food item to be considered 'functional' with respect to lowering platelet and endothelial functions, coagulation and fibrinolysis.

8.2.3. Immunological interactions. The immune system responses in the cardiovascular system cannot be considered risk factors with respect to the atherosclerotic process, because of a lack of evidence for the causal involvement of these responses in atherogenesis. Therefore, the term risk indicators should be used.

Although various studies have shown that a high intake of *n*-3 fatty acid-rich foods (fish), or of *n*-3-rich preparations (fish oils) may exert antiatherosclerotic activities, there is no direct evidence that these effects are mediated by modifications of immune responses participating in the atherogenic process. Some indirect evidence may be provided by the results of some, but not all, studies showing favourable effects of *n*-3 intake on the rate of re-stenosis of dilated coronary arteries (for reviews, see Gapinski *et al.* 1993 and Cairns *et al.* 1996), a process which appears to involve cells of the immune system and the proliferation of cells of the arterial walls (Westerband *et al.* 1997). Additional studies are required to substantiate the effects of *n*-3 fatty acids on re-stenosis following angioplasty. However, these results may not disclose the mechanism(s) of *n*-3 activities.

Diets rich in antioxidants have been shown to exert protective effects with respect to the atherogenic process. They have also been shown to affect the activities of immune competent cells and to inhibit the expression of genes coding for cell–cell adhesion molecules, which play a role in the development of the arterial lesions. As for the *n*-3 long-chain polyenes, however, there are no statistically validated epidemiological, prospective, or intervention data indicating that these effects may be mediated by modulation of immune system responses.

8.2.4. Hypertension. CHD is strongly related to both systolic and diastolic blood pressure in a graded fashion and treatment of hypertension results in a reduction in coronary disease-related events. Therefore, hypertension is a risk factor for coronary artery disease.

Reports on the blood pressure-reducing effect of linoleic acid are inconsistent. With respect to *n*-3 long-chain polyenes meta-analyses suggest that these fatty acids may reduce blood pressure in hypertensive, but not in normotensive, subjects. Consequently, *n*-3 long-chain polyenes may be considered 'functional' with respect to reducing increased blood pressure. Since it is not known whether these fatty acids will prevent normotensive people from becoming hypertensive, blood pressure-related functionality of these fatty acids is restricted to hypertensive subjects. A diet rich in fruit and vegetables also helps to lower blood pressure; however, the mechanism involved has not yet been elucidated.

The potential of *n*-3 and perhaps also of *n*-6 fatty acids to influence cardiac contractility under conditions of limited O_2 supply or at high work loads can be envisaged, but evidence is available from *in vitro* and animal studies only, results are not consistent and mechanisms involved remain controversial. The same holds for the reported preventive or reducing effects of *n*-6 and *n*-3 fatty acids on arrhythmia: the data are largely based on animal studies and underlying mechanisms are hardly known. Therefore, insufficient evidence is available for the classification of unsaturated fatty acids as functional with respect to cardiac contractility and prevention of arrhythmia.

8.2.5. Insulin resistance. Although several excellent studies are available, demonstrating a link between insulin resistance, obesity, NIDDM, metabolic abnormalities and coronary artery disease, cause-and-effect relationships have not been proven by statistical means. Consequently, these conditions can only be regarded as risk indicators, not risk factors.

From epidemiological studies it is suggested that the intake of dietary fibre (positively) and the intake of dietary fat (negatively) affect insulin sensitivity. However, well-designed intervention trials of sufficient size and duration concerning the effect of either of these dietary components on insulin sensitivity have not yet been performed. The relatively short-term and mostly small studies that have been reported were largely carried out in obese subjects in which many physiological variables (e.g. general food habits, body weight, insulin sensitivity, blood pressure) are different from normal-weight subjects.

There are some data from intervention studies about the effect of specific fatty acids on insulin metabolism. However, mechanisms underlying these associations have not yet been elucidated. Further intervention studies will be important in determining the sequence of events.

In these studies, the use of stable isotopes will be instrumental.

Taken together, there is insufficient evidence to classify any of the food items 'functional' with respect to insulin resistance and related conditions.

8.2.6. Hyperhomocysteinaemia. The view that the level of homocysteine is a risk factor for cardiovascular disease is exclusively based on epidemiological investigations, most of which were case–control studies. *In-vitro* studies with, mainly, endothelial cell cultures clearly demonstrate an endothelium-activating effect of homocysteine, possibly resulting in thrombogenic conditions. However, *in vivo* data to confirm this thrombogenic potential of plasma homocysteine are not available as yet. Because of the rather consistent inverse relationship between plasma levels of homocysteine and of folate, vitamin B_{12} and/or vitamin B_6, no final answer can be given to the question of whether hyperhomocysteinaemia or a reduced vitamin status is ultimately associated with an increased cardiovascular risk. Since no well-designed intervention studies have been reported showing that reducing hyperhomocysteinaemia or increasing the folate and/or B-vitamin status causes a reduction in cardiovascular risk, plasma homocysteine, folate, vitamin B_6 and vitamin B_{12} levels can be considered (anti)risk indicators at best.

Increasing the consumption of folate and/or vitamins B_{12} or B_6 lowers plasma homocysteine quite consistently, but whether this will result in a reduced cardiovascular risk remains to be proven.

In principle, improvement of the folic acid status by dietary folate supplementation may mask or even precipitate clinical manifestations related to vitamin B_{12} deficiency. However, extensive studies in more than 700 elderly participants in the Framingham Heart Study revealed that the benefit of folate fortification through projected decreases in homocysteine level and heart disease risk greatly outweigh this risk (Tucker *et al.* 1996). Moreover, concerns about masking cobalamin deficiency by folic acid supplementation could be lessened by adding cobalamin to folic acid supplements (Boushey *et al.* 1995).

9. Conclusions and recommendations for further research

9.1. Plasma lipoproteins

Dietary lipids are able to affect lipoprotein metabolism in a significant way, thereby modifying the risk of cardiovascular disease. Although effects of the individual dietary fatty acids and dietary cholesterol on fasting serum lipids and lipoproteins have been studied extensively, possible interactions among fatty acids or with dietary cholesterol, as well as postprandial effects, are only poorly understood. This should be investigated more thoroughly in well-controlled dietary trials, using recently developed techniques. For example, stable-isotope methodology should be used to measure apoprotein metabolism, or to measure in mononuclear cells mRNA levels of the LDL receptor and of hydroxymethylglutaryl CoA reductase. Also, effects on other lipid variables like, for example, cholesterol ester transfer protein-activity and lipoprotein particle sizes, should then be taken into account so as to increase our understanding of the dietary effects on lipoprotein metabolism. In addition, special attention should be paid to (potential) gene–diet interactions. These remarks, of course, also apply to other dietary components that interfere with cholesterol absorption.

9.2. Arterial thrombosis

Platelet function may possibly affect cardiovascular risk and the relatively low platelet content of *n*-3 polyunsaturated fatty acids may present a risk for platelet hyperactivity. Insufficient evidence is available to reliably link endothelial cell function to cardiovascular risk. Increased blood coagulability and reduced fibrinolytic activity are associated with increased risk for cardiovascular disease, but causality has not been proven and, consequently, 'functional foods' cannot be identified.

Further research is needed in the following areas.

(1) Prospective validation studies should be performed to find out to what extent the presently available putative indicators of arterial thrombosis tendency (i.e. platelet aggregation *in vitro*, urinary excretion of thromboxane- and prostacyclin metabolites and of specific platelet proteins, plasma concentrations of soluble forms of cell adhesion molecules, activation fragments of clotting factors, and fibrin degradation products) reflect the risk for arterial thrombosis.
(2) Depending on the results, it may be necessary to develop and validate new methods to measure *in vivo* arterial thrombosis tendency in human subjects and to search for and prospectively validate more specific *in vivo* activation markers for platelets, endothelial cells, leucocytes, clotting factors and the fibrinolytic process.
(3) Well-designed intervention studies should be initiated to investigate the effect of selected dietary components (e.g. the various *n*-3 and *n*-6 fatty acids and their combination, antioxidants, fibre) on the processes participating in arterial thrombus formation. These studies should not only measure effects, but should also try and unravel the mechanisms involved.

9.3. Immunological interactions

Long-chain polyenes of the *n*-3 family and antioxidants are examples of food components endowed with various biological activities, which can be assessed in *in vitro* and in *ex vivo* experiments. These activities include modification of immune system responses of cells participating in atherogenesis, which may thus be considered markers of an active state of this process. Certain foods are rich in *n*-3 fatty acids (e.g. fish rich in the *n*-3 long-chain polyenes and some vegetable oils, such as soyabean and low-erucic acid rapeseed, rich in ALA). Other foods (e.g. vegetables and vegetable oils, fruits) are rich in various types of antioxidants (vitamins, flavonoids, polyphenols, etc.). Diets based on high intakes of these foods are, therefore, expected to exert beneficial health effects on the atherosclerotic process, as shown by various studies. However, the variable contents, from both a quantitative and qualitative point of

view, of these bioactive components in these foods make it difficult to define them as 'functional foods'. In addition, although beneficial effects, for instance on the cardiovascular system, have been shown in human studies, there is little evidence, from statistically validated epidemiological, prospective, or intervention studies, that these effects may be mediated by modulation of immune system responses.

As to the safety of high intakes of foods rich in *n*-3 long-chain polyenes, the absence of detrimental effects, except for possible minor intestinal dysfunctions, in the reported studies, indicates that they should be considered safe. The same holds for foods rich in antioxidants, since there is no evidence that a high consumption of these foods results in detrimental effects.

We are at an early stage of examining the role of immune function on the development of atherosclerotic plaques and there is a great need to develop strategies for studying the effects of macro- and micronutrients on the function of the immune system. These should take place at different levels of complexity and biological organization, and using dietary investigations that are relevant to the diets consumed in the Western world.

Strictly standardized *in vitro* experiments are required to obtain new information on the role of the cells involved in the onset of the arterial lesions, and main research areas are functional activities, and their controlling factors, of the main cell types participating in the formation of atherosclerotic plaques. These activities need to be tested either alone or during cell–cell interactions, and should involve the assessment of the factors responsible for these events (expression of cell-adhesion molecules, cytokines and growth factors). As to the underlying mechanisms, both short-term effects, mediated by fast cell-signalling pathways, and long-term processes, generally mediated by gene activation and transcription, need to be studied in detail. In this context, special attention should be paid to the interplay between functionally specialized cells in the vessel wall, e.g. endothelial cells and SMC, and inflammatory and immune cells. Recruitment of these latter cells from the circulation into the vessel wall is a major factor in controlling locally the progression of the lesions, whereas systemic immune responses may differently modulate the process. Clearly, studies of the *in vivo* effects of nutrients on these steps represent the first approach in the identification of active components and they will also shed some light on potential and most promising mechanisms of action.

While animal studies allow the assessment of pathological events at the organ and tissue level and of the effects of treatments on these processes, this can obviously not be done in human subjects. The most important aspects of research on cell-mediated processes in atherogenesis, i.e. human studies, and on the effects of drugs and nutrients, are therefore also the most difficult ones and completely rely on specific markers of the disease state. Therefore, a most important area of research is the assessment of clear relationships between different stages and forms of the disease and selected markers of cellular and immune activation, to be detected in the circulation and possibly in urine. More specifically, measurements of soluble adhesion molecules in plasma and, possibly, of cleavage products in urine, may improve the diagnosis and the evaluation of prognosis of the disease. In addition this may help to establish and follow the impact of nutrient supplementation.

9.4. Hypertension

There are many different reasons for hypertension in individuals. In the aetiology of hypertension, the genetic component is definitely stronger than environmental factors, including diet. Future work should consider the multiple reasons that may lead to hypertension. The effect of dietary fatty acids on blood pressure should be examined in patients in whom TxA2 production and/or α1-adrenergic mechanisms are implicated in hypertension. The effect of individual fatty acids, such as ALA, EPA and DHA, on the development of atherosclerosis (via haemostatic or immunological effects) and lethal coronary events should be examined in large well-designed trials in man.

9.5. Insulin resistance

Several studies indicate an existing relationship between insulin resistance and cardiovascular disease. Factors which may contribute are fasting and postprandial lipoprotein levels in plasma, as well as hypertension. Environmental factors include the lack of physical activity and the intake of dietary fat. It may be possible to modulate insulin sensitivity and subsequent cardiovascular risk factors by diet, more specifically by decreasing the total amount of dietary fat and increasing the proportion of polyunsaturated fatty acids. However, additional studies on the mechanisms involved are required to understand the real function of these dietary components.

Further research should also focus on intervention studies, not only to test the efficacy of specific fatty acids, dietary fibre, low-energy diets, etc., but also to try and explain the mechanisms underlying the observed changes. Moreover, these studies will be helpful in determining the sequence of events.

Further investigations are also needed to evaluate whether the essential fatty acids linoleic acid and ALA ameliorate insulin resistance and, if so, whether this effect requires desaturation and elongation of these fatty acids. For these studies, the use of stable isotopes is instrumental.

9.6. Hyperhomocysteinaemia

Compelling evidence is now available for the association between the plasma level of homocysteine and the risk of cardiovascular disease, although further studies are needed to substantiate the causality of this relationship. In addition, well-designed intervention trials are required to prove the beneficial role of dietary supplements containing folate and vitamins B_6 and B_{12} in reducing the risk of CHD.

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