Upsala J Med Sci 98: 89–148, 1993

Effects of Fish Oil on Risk Factors for Cardiovascular Disease

Minireview Based on a Doctoral Thesis

Olle Haglund

Department of Forensic Medicine, University of Uppsala, Uppsala, Sweden

INTRODUCTION

By cardiovascular disease (CVD) is meant in the following mainly ischaemic CVD, i.e. coronary heart disease (CHD), cerebrovascular disease, hypertension and peripheral vascular disease. Although the incidence of CVD has shown some decline during the last decades in many countries, it is still the most common cause of death in the middle-aged and elderly in most Western societies. It is a major contributor to chronic debilitation and has enormous economic consequences. In most industrialised countries CVD is closely related to the life-style, and is therefore highly modifiable. One example of populations with a low rate of ischaemic heart disease (IHD) is that of the Mediterranean countries, where there is epidemiological support for a protective role of monounsaturated fatty acids, e.g. oleic acid. Other examples, highlighted in this thesis, are the Greenland Eskimos and coastland Japanese, where the incidence of atherosclerosis and cardiac death is low (92,108). Early epidemiological investigations in Eskimos suggested that this was due to the high consumption of (n-3) fatty acids from seafood (8). Since then a large number of epidemiological, clinical and experimental studies on these fatty acids have been published.

RISK FACTORS FOR CARDIOVASCULAR DISEASE

The aetiopathogenesis of ischaemic CVD is multifactorial and its details are still unclear. It has been calculated that only about 30% of all cardiovascular events can be explained on the basis of the major "accepted" risk factors (87). One review included 246 suggested coronary risk factors (95), of which 46 were related to the diet. Some important risk factors are listed below, and some of these, relevant for this thesis, are commented on.

- Positive family history
- Male sex
- Increasing age
- Elevated total cholesterol
- Elevated LDL cholesterol
- Elevated homocysteine
- Hypertension
- Cigarette smoking
- Stress and type A personality
- Diabetes mellitus

Low HDL cholesterol	Metabolic syndrome
High level of triglycerides	Physical inactivity
Elevated lipoprotein (a)	Obesity, mainly central
Elevated antithrombin III	Increased serum uric acid
Elevated factor VII	Increased serum calcium
Elevated fibrinogen	High white cell count
Decreased fibrinolytic capacity	Antibodies against Chlamydia

Total cholesterol and LDL cholesterol. There seems to be little doubt that an elevated concentration of serum cholesterol, specifically LDL cholesterol, increases the risk for CVD (176), although in recent years this subject has been disputed. There is strong evidence in favour of a causal relationship. The relation between CHD and serum cholesterol is continuous, graded and curvilinear. The risk for CHD spans a very wide range of serum cholesterol concentrations, increasing progressively from a concentration of about 3.5 mmol/L, and becomes increasingly steep in the upper part of the distribution. Elevated serum cholesterol levels are most often the result of a diet rich in saturated fat and cholesterol. Saturated fatty acids, except stearic acid and fatty acids shorter than 12 carbons, have been shown to raise the serum cholesterol (122). A reduction of high cholesterol levels by dietary and pharmacological means also results in a reduction in CHD (94). Recently it has been reported that the relation between serum cholesterol and mortality is a U-shaped one (37). However, the difference between the effects of lowering cholesterol by drugs and by diet and other life-style changes has been stressed. The importance of oxidative modification of LDL cholesterol has recently received great attention (178). Modified LDL particles lose the capacity for regulated uptake by the LDL (B/E) receptor, and instead are taken up by macrophage scavenger receptors, leading to an uncontrolled uptake of cholesterol in these cells.

HDL cholesterol. There is an inverse relationship between HDL cholesterol and the incidence of atherosclerotic CAD. This is thought to be due mainly to reverse cholesterol transport caused by HDL, but also to antioxidative properties of HDL preventing LDL modification and uptake of LDL particles in monocytes and endothelial cells, and antithrombotic functions with stimulation of the fibrinolytic system and inhibition of platelet aggregation (23). The preventive effects are mainly attributable to the HDL_2 fraction, which contains most Lp A1-lipoprotein particles with only apolipoprotein A1. Examples of factors that increase HDL cholesterol are physical activity, weight reduction, smoking cessation, oestrogens and drugs of the fibrate group. There are also several reports on an HDL increasing effect of very long chain (n-3) fatty acids (86), which are claimed to lead to a significant size redistribution of HDL particles from smaller, denser particles to larger ones; that is, the proportion of HDL_2 to HDL_3 rises significantly (1,60).

Triglycerides. The importance of triglycerides in serum as a risk factor for IHD is somewhat controversial. Many studies have shown a univariate association between an elevated serum triglyceride level and the risk for IHD (6,73). After adjustment for other lipid values, however, in particular HDL cholesterol, this association is often no longer statistically significant (6,181). There are, however, several statistical properties which will wrongly attenuate measures of association between triglyceride levels and disease (6). Both the intraindividual and the interindividual variation in triglyceride concentrations generally show larger variability than those in other lipids. The strong inverse correlation between triglycerides and HDL cholesterol also contributes to an underestimation of the former as a risk factor. Recently the value of multiple lipid measurements simultaneous with an assessment of their joint effect has been emphasized (7). The reason for the connection between triglycerides and IHD is not well understood (71,84). First, triglyceride-rich lipoproteins, particularly very low density lipoproteins (VLDL), may be atherogenic and thrombogenic. Secondly, the metabolic consequences of hypertriglyceridaemia may account for the triglyceride-IHD relationship. These consequences include an increase in postprandial lipoproteins, large VLDL particles, small, dense LDL particles, low levels of HDL cholesterol, and possibly a procoagulant state.

Lipoprotein (a). An elevated level of Lp (a) has recently been suggested as an important independent risk factor for IHD (116). The plasma levels of Lp (a) are genetically determined and there are only few reports on successful ways of reducing them (28). Lp (a) has been shown to compete with both plasminogen and tissue plasminogen activator (t-PA) for fibrin binding, and the injurious effect may be partly exerted through inhibition of the fibrinolytic system (49).

Haemostatic factors. In longitudinal studies in the population at large and in atherosclerotic patients, an increased risk of major cardiovascular events has been found to be related to baseline abnormalities in several coagulation, fibrinolytic and rheological variables, including the concentrations of fibrinogen, factor VII, antithrobin III, von Willebrand factor, factor VIII, plasminogen activator inhibitor, and plasma viscosity (85).

Plasma fibrinogen. There is growing evidence to indicate that elevated plasma fibrinogen is an important independent risk factor for IHD (196). This topic was very recently reviewed by Ernst (54). Several epidemiological studies have yielded longitudinal data identifying fibrinogen as a major cardiovascular risk factor. Cross-sectional results show strong associations between fibrinogen and a variety of demographical variables and cardiovascular risk factors or diseases. Clinical cohort studies indicate that fibrinogen might also be a risk factor for the sequelae of cardiovasc-

ular disease. Knowledge about the determinants of the plasma level of fibrinogen in health and disease is as yet incomplete, as is our understanding of the mechanisms leading to the atherothrombogenic action of fibrinogen.

Decreased fibrinolytic capacity. Defective fibrinolysis is mainly caused by low activity of tPA or by an increased concentration of plasminogen activator inhibitor-1 (PAI-1) (137,197). In prospective studies increased amounts of tPA antigen have been found to be associated with the occurrence of future myocardial infarction (153). Increased PAI-1 has been shown to be associated with IHD and peripheral vessel disease (83). In a recent critical review of the relationship between impaired fibrinolysis and myocardial infarction, Prins et al. (149) state that high levels of PAI-1 are associated with an increased risk of reinfarction in survivors of a first myocardial infarction. Whether this relationship is causal or coincidental is unclear. The status of PAI-1 as a risk factor for arterial and thrombotic disease has recently been reviewed (40). Whether an elevation of PAI-1 is of pathological importance or is merely a marker of disease remains to be established. The authors point out the need for large prospective studies to determine the importance of PAI-1 as a prognostic marker for disease. Preliminary prospective data from 231 apparently healthy individuals provide strong evidence against PAI-1 abnormalities as predictors of future myocardial infarction (152). Much attention has been focused on circulating levels of components of the fibrinolytic system in relation to disease. However, it has become clear that the essential reactions that govern clot lysis occur at a cellular level (5). The implication of these findings is that measurements of circulating components may barely reflect events at the molecular and cellular level. Most endothelial PAI-1 is secreted to the abluminal side and, complexed with vitronectin, in particular, it contributes to the proteolytic balance in the extracellular matrix. It is still unclear whether the PAI-1 level in the blood represents homeostatically regulated levels, maintained in order to control plasminogen activation-mediated proteolysis in the circulation. Alternatively, the circulating inhibitor could represent "spill-over" of inhibitors released from cells for the purpose of regulating local pericellular proteolysis. The relative contribution of plasma PAI-1 from endothelial cells, smooth muscle cells, hepatocytes and possibly activated platelets is not known, and neither is the origin of the elevated PAI-1 level after consumption of fish oil. There are suggestions that a possible source of increased PAI-1 is the vessel wall. The regulation of PAI-1 synthesis is complex and most studies have been carried out in vitro. Their relevance for the in vivo regulation is largely unknown.

Homocysteine. Recently an even moderately elevated homocysteine level in the plasma has been recognized as an important independent risk factor for CVD (32). Increased homocysteine may be the result of genetic defects in the metabolism of homocysteine, deficiences of vitamin B_6, fol-

ic acid or vitamin B_{12}, or a high intake of methionine (168). Recently there has been greatly increased interest in the very close relation between the metabolism of methionine and arginine (123,127). With S-adenosyl-methionine (SAM) as a key substance, alteration in one part of the system will, for example affect the synthesis of nitric oxide, creatine, polyamines and the free radical scavenger glutathione. An increase in homocysteine is believed to contribute to the pathogenesis of CVD, partly through free radical mechanisms (119). It is also associated with a decrease in the formation of cysteine and its products, e.g. glutathione. Increased homocysteine may lead to a reversal of the hydrolysis of S-adenosyl-homocysteine, with consumption of adenosine and inhibition of transmethylation reactions involving SAM. Adenosine has a great number of effects in metabolism and has been found to have a positive influence on the liver handling of glucose. Transmethylation has numerous important physiological functions; for example it increases membrane fluidity (127). Administration of pyridoxine and folic acid has recently been shown to lower the blood concentration of homocysteine (21).

Hypertension. Prospective studies have shown that high blood pressure is positively and independently associated with a risk of stroke, myocardial infarction, and mortality from all vascular causes (33). In the Framingham Study and the Multiple Risk Factor Intervention Trial (MRFIT) it was found that systolic blood pressure was more strongly related to CHD than diastolic blood pressure. However, this difference was age-dependent and under the age of 45 diastolic blood pressure was the stronger predictor. In the very recently published 25 years of follow-up of 50-year-old men, only blood pressure was an independent risk factor for CHD during the last 10 years in a multivariate analysis (193). In patients with malignant hypertension drug therapy has been found to have very strong benefits in the primary prevention of stroke and myocardial infarction. In mild-to-moderate hypertension, drug therapy reduced the incidence of stroke, but the question of benefits regarding CHD was somewhat controversial (33). Increasing attention has recently been directed towards non-pharmacologic interventions, with reduction in the intake of calories, sodium and alcohol (177). Non-pharmacological interventions are now recommended as a first step in the treatment of mild hypertension.

Diabetes. Diabetic patients are at increased risk for accelerated atherosclerosis with macrovascular complications. In addition there is a risk for diabetic microvascular disease, with thickened capillary basement membranes and microaneurysm formation in small capillaries and venules (130). Hyperinsulinaemia and insulin resistance may be present many years before the onset of non-insulin-dependent diabetes (NIDDM) and clearly play an important role in its aetiology. Hyperinsulinaemia and/or insulin resistance may be a key factor in the aetiology of associated glucose intolerance, hyperlipidaemia, hypertension, central obesity, and atherosclerosis. Data have consistently shown an excess risk of mortality in diabetic

persons of both sexes. Generally CVD accounts for the majority of these deaths.

Metabolic syndrome. The simultaneous occurrence of several risk factors for CVD is well known. A syndrome consisting of insulin resistance, hyperinsulinaemia, glucose intolerance, hypertension, dyslipidaemia, hypofibrinolysis and obesity has been described (151). It has been given several names, including metabolic syndrome, metabolic cardiovascular syndrome, syndrome X and insulin resistance syndrome. Insulin resistance in peripheral tissues with a secondary hyperinsulinaemia is believed to be a major underlying mechanism.

PATHOGENESIS OF ATHEROSCLEROSIS

Atherosclerosis is the main pathogenetic entity underlying ischaemic CVD. The pathogenesis of atherosclerosis is multifactorial and its details are still only partly understood (156). The initiating lesions occur very early in life. Recent findings even suggest that the pathogenesis of CVD begins in utero (11). From an intimal thickening, the lesions may progress to fatty streaks, transitional plaques, advanced fibrolipid plaques and complicated plaques (39). Atherosclerotic lesions are the consequence of what is initially a defensive and repair phenomenon. The vascular system is continually subject to insult of varying degrees and, if the injury-repair phenomenon is efficient, temporary arterial lesions may never lead to problems or clinical sequelae. However, if repair associated with an inflammatory process becomes excessive, then the excessive nature of the proliferative response associated with this repair becomes the disease entity (156). Several hypotheses for the pathogenesis have been proposed. These include initial changes/injuries in the endothelium, monoclonal proliferation of smooth muscle cells, changes in the oxidative status of LDL, viral infection, neurological changes and early changes in the vessel adventitia (39). There is general agreement that, at some point in the early progression of the lesion, adherence of white cells and platelets occur at the endothelial surface. Monocytes and lymfocytes enter the vessel wall, releasing growth factors, cytokines and other chemoattractant agents. Smooth muscle cells replicate as a result of the production of growth-promoting factors. These proliferated cells then generate connective tissue. Growth-regulatory factors, therefore, may play a critical role in determining whether or not proliferation and lesion progression will occur. All complex human atherosclerotic plaques contain lipid. This may be extracellular, occurring as a pool of cholesteryl ester and non-esterified and crystalline cholesterol, or it may be contained within lipid-laden macrophages known as foam cells. Lipid is largely taken up by macrophages as modified or oxidized LDL by scavenger receptors (178) and by bulk-phase endocytosis of aggregated lipoproteins. The lipid is deposited in the foam cells in the form of cholesteryl ester, converted from cholesterol by the enzyme acyl CoA: cholesterol acyltransferase (ACAT). Cholesterol is transported to the macrophage in the vessel wall mainly as LDL and re-

moved by HDL. The balance between these two lipoproteins is important determining the cholesteryl ester content of the plaque. The initiation of oxidation of LDL in vivo is still controversal. All three of the major cell types in the arterial wall, namely, monocyte-derived macrophages, smooth muscle cells, and endothelial cells, are capable of modifying LDL. At least three cellular mechanisms have been shown to operate in cell-induced oxidative modification of LDL: generation of free radicals in the form of superoxide anions, lipoxygenation by cellular lipoxygenase(s), and activity of phospholipase A_2 enzyme(s). The role of macrophage NO in the oxidative modification of LDL is controversal. It has been demonstrated in vitro that peroxynitrite is able to modify LDL oxidatively. Peroxynitrite is formed in the reaction between NO and superoxide radical. On the other hand, Jessup et al. (102) have proposed NO to protect the LDL particles against oxidation. Oxidized LDL may participate in the atherogenic process by many mechanisms. Besides being taken up by the scavengar receptor, it is chemotactic for circulating monocytes and it inhibits the motility of tissue macrophages. Most importantly, oxidized LDL, or soluble products from it, are highly cytotoxic and may contribute to a generalized cell death noted in the atherosclerotic plaque. Products of oxidized LDL, like 4-hydroxynonenal and malondialdehyde, may alter gene expression of intimal cells such as induction of monocyte chemotactic protein and colony-stimulating factors and/or alteration of the expression of Platelet Derived Growth Factor BB (PDGF-BB). Oxidized LDL has also been shown to be immunogenic, and can elicit autoantibody formation. Recently, oxidized LDL has been shown to alter the vasomotor properties of coronary arteries by inhibiting the release of nitric oxide and/or inducing the expression of the vasoconstrictor endothelin. Of great importance is that several lines of research have demonstrated that regression of atherosclerosis occurs and that different types of intervention can promote this change (124).

SUDDEN CARDIAC DEATH

Sudden cardiac death, i.e. death within one hour after the onset of symptoms, with or without myocardial infarction, is the most common cause of death. In most cases rupture of an atherosclerotic plaque with ensuing thrombosis is the precipitating event (56). Most ruptures are tiny, occurring at the periphery of the fibrous cap that covers the lipid-rich core, a point at which the cap is usually thinnest and most heavily infiltrated with macrophage foam cells. The dynamic interplay between the actual plaque vulnerability and external stresses, "triggers", probably determines the particular moment and point of rupture. A vulnerable plaque has a soft core of extracellular lipid, numerous macrophages in the periphery, few smooth muscle cells and a thin collagen cap. It has been hypothesized that release of proteolytic enzymes from the peripheral macrophages might contribute to the weakening of the collagen cap (56).

NITRIC OXIDE

Nitric oxide is an important molecular messenger, which is involved

in endothelial-derived relaxing activity in blood vessels, antithrombotic effects on platelets, neurotransmission and neuromodulation in the brain and periphery, and mediation of cytotoxic actions of macrophages (89,123). This latter function contributes to the defence against intracellular pathogens and tumour cells (90), at least in mice (68). There are at least three types of NO synthases, constitutive ones in endothelial cells and in nervous tissues and an inducible one in macrophages and many other cells (61). The NO synthases may be inhibited by inhibitors with varying degrees of specificity, e.g. N^G-monomethyl-L-arginine (L-NMMA) and L-canavanine. Vascular endothelium plays an important role in the modulation of vasomotor tonus by releasing various vasodilator substances, including endothelial-derived relaxing factors (EDRFs). one of which has been identified as NO, and prostacyclin (PgI_2). NO in the endothelium is released through shear stress and by vasodilators such as acetylcholine and bradykinin (123). Abnormal endothelium-dependent vascular relaxation has been demonstrated in patients with hypertension, atherosclerosis, hypercholesterolaemia and diabetes (31,143). The NO synthase in macrophages is induced by lipopolysaccharide (LPS), interferon-γ (IFN-γ) and combinations of many other cytokines, leading to production of high levels of NO for hours to days (61,123). Besides exhibiting cytotoxic effects against microorganisms and tumour cells, there are increasing numbers of reports on cytotoxic effects of nitric oxide on normal host cells, contributing to inflammatory and autoimmune states (106). NO has also been shown to release free iron from ferritin. The role of macrophage NO in atherogenesis is controversial. By combining with superoxide radical, forming peroxynitrite, NO has been claimed to contribute to the oxidative modification of LDL cholesterol (93). On the other hand, macrophage NO has also been reported to protect against oxidative modification (102). It has been reported that nitric oxide may exist in different redox-related forms. Very recently it has been shown that the free radical form of NO may damage and kill cells, while NO in the form of nitrosonium cation (NO^+) may protect cells (115). The balance between various redox forms of NO might thus explain some controversies. It is believed that these different effects of the various redox-related forms of NO can be utilized therapeutically.

FATTY ACIDS

Fatty acids are hydrocarbon chains with a methyl group in one end, the n end or omega (ω) end, and a carboxyl group in the other (171). The systematic numbering starts at the carboxyl end, whereas the most commonly used nomenclature starts at the methyl end. Carbon atoms numbers 2 and 3 from the carboxyl group are referred to as the α and β carbons, respectively. The fatty acids may be saturated, containing no double bonds, or unsaturated, containing one or more double bonds. The position of the double bond is represented by the symbol Δ followed by a number, i.e. Δ9 refers to a double bond between carbon atoms 9 and 10. Unsaturated fatty acid consists of monosaturates, polyunsaturates (PUFAs)

and eicosanoids. There are two classes of PUFAs, (n-3) and (n-6) PUFAs.

The parent substances for the (n-3) and the (n-6) series of PUFAs are α-linolenic acid (ALA; 18:3 n-3) and linoleic acid (LA; 18:2 n-6), respectively. Unlike members of the (n-9) family, they cannot be formed by animal cells and are therefore classified as essential fatty acids (EFAs) (14). The different series of unsaturated fatty acids are not interconvertible. The biosynthesis of other polyunsaturated fatty acids from ALA and LA involves alternating oxidative desaturations and chain elongations. The synthesis of (n-3) and (n-6) fatty acids is outlined in Figs. 1 and 2. The Δ6-desaturation has been considered to be the rate-limiting step in PUFA biosynthesis of both the (n-6) and (n-3) series, and the regulation of this reaction is of particular interest (22). The concept of separate Δ5 and Δ6-desaturase activities is now well confirmed (22). However, the existence of a separate Δ4-desaturase has again been questioned recently (187). There are few data supporting the commonly accepted hypothesis of an acyl-CoA-dependent Δ4-desaturase. An alternative pathway (Fig. 2) for the biosynthesis of DHA has been proposed, whereby DPA is elongated to 24:5 (n-3), which is desaturated by Δ6-desaturase to 24:6 (n-3), with subsequent partial β-oxidation to DHA (retroconversion). This further strengthens the importance of the Δ-6- desaturase. Cats and lions have been found to lack a Δ6-desaturase. The activities of the desaturases, which are mainly located in the endoplasmic reticulum, are modulated by nutritional and hormonal factors (22,72):

Modulation by nutrients: The Δ6-desaturase activity is increased by protein intake and decreased by many factors, e.g. linoleic and arachidonic acids, triolein, (n-3) PUFAs, trans-trans (18:2 n-6) fatty acid, cholesterol, ethanol, zinc and fasting. The Δ5-desaturase activity is increased by replacement with saturated fatty acids or triolein, by starch intake and by protein intake, and, is decreased by cholesterol and ethanol. Whether the activity is increased by fasting or by linoleic and arachidonic acids is still under discussion. PUFAs inhibit the desaturation of other fatty acids by competing at the Δ6 and Δ5-desaturase level. It has been well demonstrated that an increased intake of linoleic acid produces a decrease in the levels of EPA and DHA in the adipose tissue of rats receiving 1% of their energy as ALA. Conversely, an increased intake of ALA strongly decreases the content of (n-6) PUFAs in the adipose tissue.

Modulation by hormones: Δ5 and Δ6-desaturase activities are stimulated by insulin, but only Δ6-desaturase is stimulated by thyroxin. However, both hyper- and hypothyroidism lead to a decrease in Δ6-desaturase activity (72). Glucagon, epinephrine and glucocorticoids suppress Δ6-desaturase activity, and glucagon and epinephrine have the same effect on Δ5-desaturase activity.

Modulation by ageing: It is generally accepted that Δ6-desaturase activity declines with age. The onset of this decline varies from organ to organ.

Fig 1.

Metabolic pathways for the desaturation and elongation of (n-3) and (n-6) faltty acids.

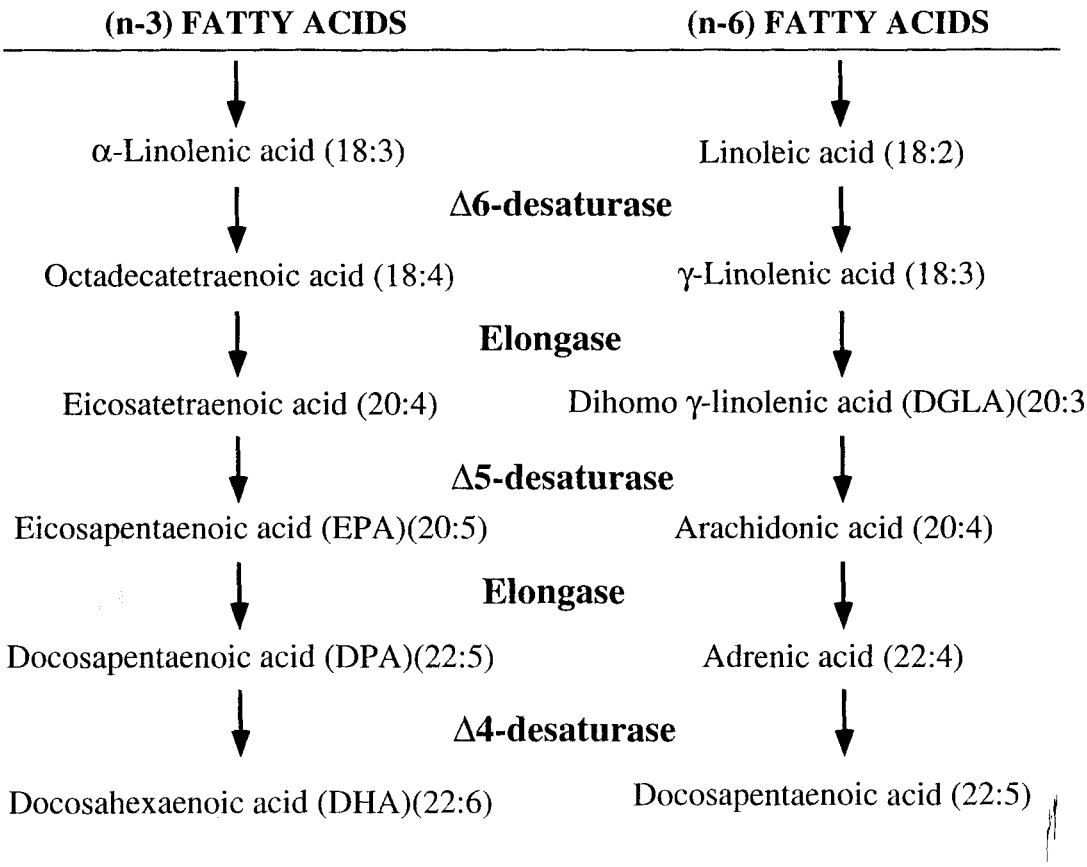
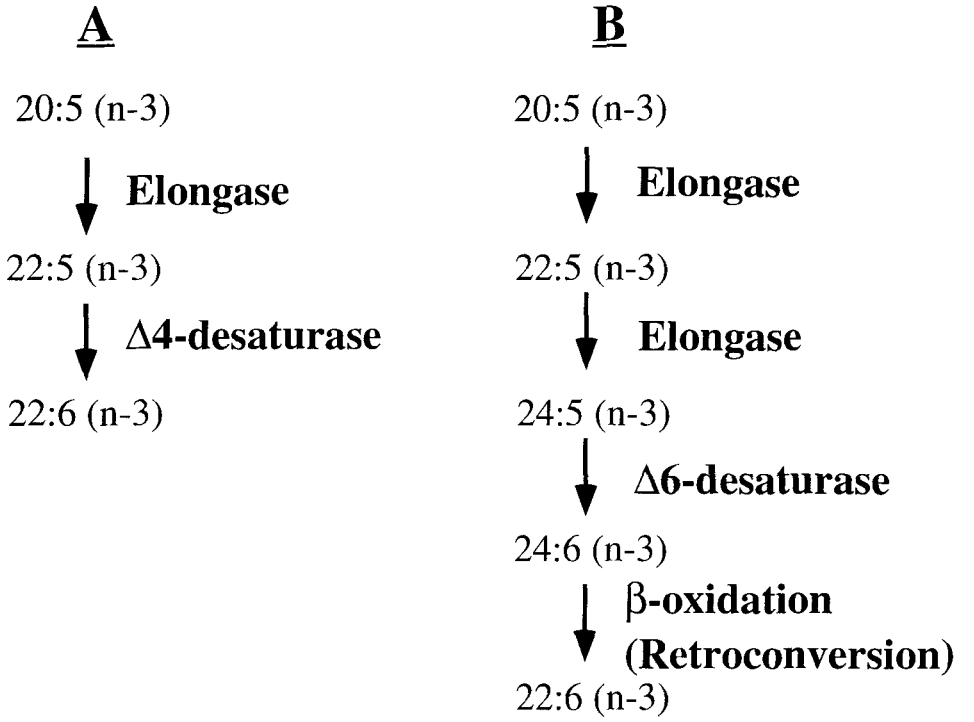


Fig. 2

Alternative pathways for the biosynthesis of docosahexaenoic acid (DHA)(22:6 n-3).



This has been used as an argument for short-circuiting the Δ6-desaturase by administering, for example, gamma-linolenic acid, EPA and DHA. On the other hand, the cellular accumulation of ageing pigment (lipofuchsin) has been reported to be due to the peroxidation of membrane PUFAs.

METABOLISM OF PUFAs

Most of the dietary long-chain PUFAs are transported in chylomicrons from the intestine to the liver. Once inside the hepatocytes, the activated fatty acids are either esterified directly into phospholipids and triglycerides or desaturated/chain-elongated and then esterified, or oxidized either in mitochondria or in peroxisomes (82). In addition to the mitochondrial fatty acid β-oxidation, by which fatty acids are completely degraded to acetyl-CoA without accumulation of any detectable intermediates, the existence of a partial oxidation system (retroconversion) of highly unsaturated fatty acids has now been generally accepted. It has been suggested that the balance between different PUFAs is regulated by chain elongation, desaturation and also retroconversion (174). The yin-yan balance between biosynthesis and retroconversion may probably differ from one tissue to another, explaining the high level of 22:4 (n-6) in adrenal and endothelial

cells, of 22:5 (n-6) in the testes and of 22:6 (n-3) in the central nervous system and in the retina (82). A relatively new and interesting factor in the regulation of the partitioning of intracellular fatty acids is Fatty Acid Binding Proteins (FABP). The cytosol of nearly all tissues studied appears to contain one or more proteins with a molecular weight of 12,000 to 14,000, that bind long-chain fatty acids or their CoA derivatives. These proteins represent approximately 5% of the total protein content in cytosol. The reactions incorporating PUFAs in the phospholipid fraction have a distinctly lower K_M values than competing pathways such as fatty acid oxidation and triglyceride synthesis. The intracellular partitioning of PUFAs is dependent on the chain length and degree of unsaturation of the substrate. In most investigations, regarding human and non-human primates, EPA competes with linoleic acid rather than arachidonic acid for selective incorporation into similar positions on phospholipids (72). This is in contrast to the findings in human platelets and various rat tissues. When ingested, fatty acids such as EPA and DHA are incorporated into the sn-2 position in cell membrane phospholipids, displacing arachidonic acid.

EICOSANOIDS

These compounds, derived from eicosa- (20-C) polyenoic acids, comprise the prostanoids, leukotrienes, lipoxines and hydroxy fatty acids (185). Prostanoids are cyclic derivatives formed by cyclooxygenase and include prostaglandins, thromboxane and prostacyclin. Three different eicosanoic fatty acids give rise to three groups of eicosanoids characterized by the number of double bonds in the side chains, e.g. PG_1, PG_2 and PG_3. Variations in the substituent groups attached to the rings give rise to different types in each series of prostaglandins and thromboxanes, labelled A, B, etc. The leukotrienes are formed via the lipoxygenase pathway rather than via cyclization of the fatty acid chain. First described in leucocytes, they are characterized by the presence of three conjugated double bonds. Arachidonic acid is the main PUFA that is oxygenated to various eicosanoids. In addition to arachidonic acid, dihomo-γ-linolenic acid and EPA are precursors for their own series of oxygenation products (69). Eicosanoids display pronounced and varied biological activities and are in fact among the most potent natural compounds ever found. A considerable part of the "essentiality" of EFAs might be explained by the formation and activities of these metabolites. Among their activities are effects on smooth muscle contractility, blood pressure, glandular excretion, hormone secretion, platelet aggregation, vessel tone, kidney function, cyclic nucleotide levels, chemotaxis, immune response, lipolysis, cell growth and differentiation, inflammatory response, etc. The PUFAs are usually esterified at the 2-position of glycerophospholipids. The first step in the eicosanoid biosynthesis is the release of the precursor fatty acid, usually by activation of phospholipase A_2. The free fatty acid, for example arachidonic acid, is subsequently oxygenated by the action of a fatty acid cyclooxygenase or various lipoxygenases. Dihomo-γ-linolenic acid and EPA may replace arachidonic acid in various membranes. They also compete for the cyclooxy-

genases and lipoxygenases. Eicosanoids produced from EPA, e.g. TxA_3 and LTB_5, have lower activity than corresponding eicosanoids formed from arachidonic acid. On the other hand the effect of PgI_3 is similar to that of PgI_2. EPA can in these ways modify (n-6) eicosanoid-mediated pathology (69).

LIPID PEROXIDATION

PUFAs in the lipid layers of biomembranes such as plasma membranes, microsomes, mitochondria and lipoprotein are readily attacked by oxidizing free radicals. The oxidative destruction of PUFAs, known as lipid peroxidation, is particularly damaging, as it proceeds as a self-perpetuating chain reaction. The general process of lipid peroxidation can be envisaged as in the scheme below, where LH is the target PUFA and $R^\bullet$ the initiating, oxidizing radical.

$LH + R^\bullet \longrightarrow L^\bullet + RH$ (1) $\qquad L^\bullet + O_2 \longrightarrow LOO^\bullet$ (2)

$LOO^\bullet + L'H \longrightarrow LOOH + L'^\bullet$ (3) $\qquad LOOH \longrightarrow LO^\bullet, LOO^\bullet$, aldehydes (4)

Oxidation of the PUFA generates a fatty acid radical ($L^\bullet$) that rapidly adds oxygen to form a fatty acid peroxyl radical ($LOO^\bullet$). The peroxyl radicals are the carriers of the chain reaction. They can oxidize further PUFA molecules and initiate new chains, producing lipid hydroperoxides (LOOH) that can break down to yet more radical species and to a wide range of compounds, notably aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal. These compounds can diffuse from the original site of attack and spread the damage to other parts of the cell. Lipid peroxidation has been implicated in a wide range of tissue injuries and diseases, including CVD (55). However, it must be remembered that production of free radicals in the cells is inevitable and they also have many physiological functions (44). The body has well elaborated defence mechanisms against the deleterious actions of free radicals. These are known as antioxidant defences and are of two main categories: those whose role is to prevent the generation of free radicals and those that intercept any that are generated. They exist in both the aqueous and membrane compartments of cells and can be enzymes or non-enzymes. Most free radical scavengers are not enzymes, however. In cell membranes, the best characterized and possibly the most important is α-tocopherol, the major member of the vitamin E family. This molecule is known as a "chain-break-ing antioxidant", as it functions to intercept lipid peroxyl radicals ($LOO^\bullet$) and so to terminate lipid peroxide chain reaction:

$LOO^\bullet$ + α-tocopherol-OH $\longrightarrow$ LOOH + α-tocopherol-$O^\bullet$ (5)

The resultant tocopheroxyl radical is relatively stable and, in normal circumstances, insufficiently reactive to initiate lipid peroxidation itself.

Other lipid-soluble chain-breaking antioxidants, such as ubiquinol (Q_{10}), are as yet insufficiently characterized for their physiological importance to be established. In the aqueous phase other compounds act as free radical scavengers. Ascorbic acid (vitamin C) is an important antioxidant both within cells and in the plasma. It has been shown to regenerate α-tocopherol from the tocopheroxyl radical, at least in vitro. Lipid peroxidation is usually assayed as thiobarbituric acid-reactive substances (TBARS). However, these assays are not specific for MDA as substances such as glucose and bilirubin may also interfere. Separation of MDA chromogens from other reaction products by fast performance liquid chromatography (FPLC) makes the method much more specific.

THE FOOD CHAIN FOR (n-3) FATTY ACIDS

There are relatively few sources of (n-3) fatty acids in human food. These PUFAs are derived from plants, seeds and phytoplankton. Seeds and plants are the principal sources of ALA. Vegetable oils rich in linoleic acid also contain small quantities of ALA. Canola oil (low erucic acid rapeseed oil), turnip rapeseed oil and, especially, linseed oil contain larger amounts of ALA. The very long chain fatty acids of the (n-3) family, EPA, DPA and DHA, are synthesized by algae and phytoplankton as a result of photosynthesis. These organisms are at the bottom of the marine food chain, so that all marine life may ultimately be enriched with these fatty acids, which may provide the required degree of unsaturation to allow cell membranes to remain fluid in cold water. EPA is usually the major (n-3) fatty acid in the plankton, whereas DHA is the major component of the fish tissues, showing that most fish species are capable of elongating and desaturating or selectively incorporating fatty acids in their lipids (34). There are great variations in the fat content and composition both between and within fish species, as a result of differences in their food, lipid-storing mechanisms, water temperature and other factors (2).

ESSENTIALITY OF (n-3) FATTY ACIDS

The parent substance of (n-3) fatty acids, ALA, cannot be synthesized in mammals. However, for a very long time ALA and the very long (n-3) polyunsaturated fatty acids were not considered to be essential, because they could not overcome all the symptoms of EFA deficiency and as their absence from the diet did not readily lead to a deficiency syndrome (183). Although the data are still limited, (n-3) fatty acids have now been established as essential nutrients in humans, and (n-3) fatty acid deficiency has been described both in adults and children (14). DHA has been established as important for the normal development and function of the brain and retina. It has been suggested that the precursor of DHA, ALA, may be an inadequate substitute for DHA, as ALA may not be converted in sufficient amounts to meet an infant's needs (134).

FISH OILS

The results of different clinical and experimental studies on (n-3)

fatty acids are often difficult to interpret. Sometimes lean or fatty fish has been used, in other cases cod liver oil and in still others fish oils of varying quality. Cod liver oil is produced from the liver of the cod. It usually contains about 30% of ω-3 fatty acid, mostly EPA and DHA. It also contains large amounts of vitamin A and D and for many years has been used to cure and prevent rickets and night blindness. Administration of high doses of cod liver oil may produce vitamin A and D toxicity. Cod liver oil often contains relatively high levels of dioxines and dibenzofuranes. Fish oil is produced from the flesh of different fishes, usually fatty fishes from the north Atlantic.

STABILITY OF FISH OILS

Unsaturated fatty acids rapidly undergo autooxidation at ambient and even subambient temperatures if not protected. The high content of EPA, DHA and other unsaturated fatty acids makes fish oil suspectible to oxidative deterioration. Concern has also been expressed over the possible inadvertent feeding of peroxides and aldehydes from autooxidized fish oils. The stability of fish oils should be controlled by assaying peroxide and anisidine values. In order to prevent rancidity of fish oils a mixture of α, γ and δ-tocopherols and spice oils like rosemary is often used. α-tocopherol is a relatively weak antioxidant in vitro and addition of α-tocopherol to fish oil under bulk storage conditions may increase the oxidative deterioration of the oil. However, for avoidance of lipid peroxidation in vivo α-tocopherol is most important. For fish oil capsules the capsule is considered to give some protection. Fish oils in unopened bottles is usually overlayered with nitrogen gas and should after opening be stored at +4° until consumed.

DIGESTION AND ABSORPTION OF FISH OILS

Long-chain polyunsaturated (n-3) fatty acids are digested, absorbed and transported in the same way as other long-chain fatty acids, with only minor variations (131). One difference in their digestion, however, is their resistance to lipolysis by pancreatic lipase. Low doses of fish oil administered as free fatty acids were more rapidly absorbed than fish oil given as triglycerides, and ethyl esters were absorbed most slowly (50). Later studies with use of larger amounts of (n-3) fatty acids and measurement of absorption directly by analysing chylomicrons indicated that the triglycerides of fish oil and the ethyl esters of fish oil were equally well absorbed when given to human subjects (138). The free fatty acids and monoglycerides produced are absorbed into the enterocytes of the small intestine and normally are incorporated into chylomicron particles in the smooth endoplasmic reticulum (131).

EARLY REPORTS ON EFFECTS OF FISH AND FISH OILS

Studies of Eskimo populations in northwest Greenland, where until recent years the economy has depended on whaling, sealing and fishing, have played a major role in raising the level of interest in dietary fish

oils. Anecdotal reports suggested that myocardial infarction was rare in this isolated population. Visitors to the region had also long been aware of the frequent, prolonged episodes of epistaxis among the Eskimos (9). Kromann et al. (108) reported on a 25-year study of mortality and morbidity among approximately 1,800 Eskimos in the remote Upernavik district carried out between 1950 and 1974. Instead of the anticipated 40 cases of myocardial infarction, they found only one. In another study, the death rate from myocardial infarction was estimated to be one-tenth of that of Danish men (47). Beginning in 1970, a series of Danish expeditions to the Umanak district of Greenland were led by Dyerberg and Bang (9, 47). Their initial studies focused on plasma lipids, and the Eskimos were found to have lower triglyceride and cholesterol levels than Danish controls. In later studies the diet was analysed in detail, and it was found that the Eskimos consumed about 7 g/day of (n-3) fatty acids; approximately 10 times the amount ingested by Danes (8). Dyerberg and Bang also performed haemostatic studies in the Eskimos, which revealed a mild but definite prolongation of the Ivy bleeding time, i.e. 8.1 min, compared with 4.8 in Danes. They also reported that incorporation of (n-3) fatty acids into platelets was associated with a decreased platelet aggregation response and a reduction in the platelet count. Together with Moncada and Vane, Dyerberg and Bang proposed a mechanism by which the (n-3) fatty acid EPA might alter the prostanoids generated by platelets and vessel walls, whereby thrombosis and possibly even atherosclerosis might be prevented. Their initial studies were supported by studies of Fischer and Weber (58), who demonstrated that: (1) the feeding of marine oils led to the formation of relatively inactive thromboxane A_3 (TxA_3) in human platelets and also reduced the formation of TxA_2; and (2) that intake of dietary fish oil led to the formation of prostaglandin I_3 (PgI_3) with biological activity comparable to that of prostacyclin (PgI_2) in humans. The Eskimo experiences have led to a series of epidemiological studies from other populations relating the fish content and (n-3) fatty acids in the diet to cardiovascular morbidity and mortality.

EFFECTS OF FISH OILS

EFFECTS ON BLOOD LIPIDS

Triglyceride-rich lipoproteins. The concentrations of serum triglycerides and of the endogenously derived triglyceride-rich lipoproteins VLDL and intermediate density lipoprotein (IDL), have in general been reported to be lowered by ingestion of fish oil (86,133,146). Fish oils have been effective in this respect in normal subjects and in patients with common phenotypes of hyperlipidaemia, in which the VLDL and, therefore, the triglyceride levels are raised. The minimal effective dose of (n-3) fatty acids appears to be slightly more than 1 g/day. At intakes of more than 2 g/day, the fall in VLDL averages 25% in normal subjects. The average reduction is greater in hypertriglyceridaemic subjects; it is approximately 50% in those with type 4 or 5 phenotype and approximately 40% in

those with type 2B. The degree of reduction is also related to the dosage but approaches a maximum at between 5 and 10 g (n-3) fatty acids/day. In patients with type 3 hyperlipidaemia, VLDL and chylomicron remnants are reduced and β-VLDL cleared (133). In more severe forms of hypertriglyceridaemia, such as type 5 hyperlipoproteinaemia, in which both VLDL and chylomicrons (or remnants) are present, excess (n-3) fatty acids can be highly effective. It has been shown that postprandial lipaemia is lessened after dietary fat intake if the background diet has been enriched with fish oil (192). The degree and duration of the lipaemia were significantly reduced when fish oil was part of the background diet. In humans fish or fish oils rich in EPA appear to be as effective as fish or fish oils rich in DHA. Dietary fish oil also modifies the hypertriglyceridaemia that is normally inducible by carbohydrate (86). The mechanism underlying the triglyceride-lowering effect of (n-3) fatty acids is mainly a decrease in the hepatic synthesis of triglycerides and VLDL particles (86,157). (n-3) fatty acids are poor substrates for triglyceride-synthesizing enzymes, and (n-3) fatty acids also inhibit esterification of other fatty acids. It has been demonstrated that the last step in the synthesis of triglycerides, acyl-CoA:diacylglycerol acyltransferase, is especially affected by EPA, whereas phosphatidate phosphohydrolase appears to be unaffected. There is also evidence from kinetic studies that fish oil increase the fractional catabolic rate of VLDL (171). After three weeks of feeding (n-3) fatty acids to rats, increased peroxisomal β-oxidation of fatty acids in the liver has also been observed (45). The postprandial plasma concentration of free fatty acids was markedly reduced in rats by feeding them fish oil. Intake of very-long-chain (n-3) fatty acids in humans has also been found to decrease the plasma concentration of free fatty acids (172).

Cholesterol-rich lipoproteins. The reports of fish oil effects on total cholesterol and LDL cholesterol are somewhat conflicting. This issue has been reviewed by Harris (86) and Sanders (158). The effects of fish oil on cholesterol-rich lipoproteins is dependent on the type of patients, the dose of fish oil and if (n-3) fatty acids replace saturated fatty acids or not. In normal patients and in patients with type IIa hyperlipidaemia the effects on total cholesterol and LDL cholesterol concentrations are small. Nagakawa et al. used purified EPA with no other dietary change. They found modest decreases in total cholesterol and LDL concentrations. In patients with combined hyperlipidaemia and type IIb hyperlipidaemia cholesterol concentration did not change, but LDL cholesterol concentration rose by 5-7% (86). In patients with isolated hypertriglyceridaemia total cholesterol decreased by 8% and LDL cholesterol increased by 30%. If saturated fatty acids are held constant during the trials, the LDL may rise but when saturated fatty acid were reduced, the LDL cholesterol tended to decrease (86). In general, a high dose of fish oil (10 g ω3) fatty acids daily may lower LDL whereas lower doses do not.

HDL-cholesterol. After consumption of fish oil unchanged or in-

creased levels of HDL cholesterol concentrations are usually reported (86, 158). In normal patients and in combined hyperlipidaemia and isolated hypertriglyceridaemia a slight increase in HDL cholesterol is often reported. The HDL_2 to HDL_3 ratio has been found to increase after consumption of fish oil (17).

Lipoprotein (a). As mentioned earlier is the plasma level of Lp (a) strongly genetically determined and there are only few reports on successful ways of decreasing it (28). Earlier observations of a positive effect of fish oil on Lp(a) were made after several months of consumption or in hypertriglyceridaemic patients (64,88).

EFFECTS ON CARBOHYDRATE METABOLISM

There has been much concern about the potential deterioriation of glucose homeostasis on consumption of fish oil. There are several reports on a glucose-increasing effect of fish oils, especially when given to patients with type II diabetes or other persons with decreased glucose tolerance (65,186). This has been used as a reason for not recommending fish oil to these categories. It has also been reported, however, that there is no glucose increase in type II diabetics (148). There is some evidence that fish oil enhances gluconeogenesis in the liver and increases insulin sensitivity (148).

EFFECTS ON BLOOD PRESSURE

There have been conflicting reports as to whether fish oil supplements are of value in the treatment of high blood pressure. From results of animal and clinical studies, it is theorized that the (n-3) fatty acids in fish oil have hypotensive properties through stimulation of the prostaglandins that control sodium and water excretion, cause vasodilation by inhibition of the vasoconstrictor thromboxane, regulate renin release, and decrease the response to vasopressor hormones (105). Stimulation of endothelial-dependent vasorelaxation by (n-3) fatty acids has also been suggested as an additional mechanism (20). There are a number of plausible explanations for the inconsistent findings. Many studies are based on small samples and may lack the statistical power to detect a modest effect. The blood pressure response to fish oil may vary depending on the (n-3) dose and length of treatment, or may occur only in certain types of patients, such as patients with hypertension or various CVD. In a large controlled double-blind study from Tromsø in Norway (25), 157 persons with mild untreated hypertension received 6 g fish oil or corn oil daily for 10 weeks. After confounding factors had been taken into account, the systolic pressure was lower in the fish oil group by 6.4 mm Hg and the diastolic by 2.8 mm Hg compared with the controls. In another double-blind cross-over study using 6 g fish oil for 12 weeks in mildly hypertensive men, no significant decrease was found (125).

EFFECTS ON HOMOCYSTEINE

There have been very few investigations on the effect of PUFAs on the level of plasma homocysteine, an independent risk factor for CVD. Hypercholesterolaemic men have elevated levels of homocysteine in the LDL and other lipoprotein fractions of the plasma (140). The plasma level of homocysteine in rabbits was decreased by feeding them a diet containing corn oil, suggesting that homocysteine accumulation is suppressed by PUFAs (120). Recently, administration of 12 g of fish oil daily for 3 weeks was found to decrease the serum homocysteine concentration by 48% (141).

EFFECTS ON INFLAMMATION

Epidemiological studies in populations of coastal Eskimo, Japanese and Dutch subjects have shown that a high intake of (n-3) fatty acids correlates with a low incidence not only of cardiovascular disease but also of inflammatory and autoimmune diseases such as rheumatoid arthritis, psoriasis, asthma, inflammatory bowel diseases, type I diabetes mellitus, thyrotoxicosis and multiple sclerosis (92,108). In many clinical studies a decrease in signs and symptoms of psoriasis, atopic dermatitis, rheumatoid arthritis and ulcerative colitis has been observed after intake of dietary fish oil supplements (107,171). Also, several experimental studies have shown a diminished inflammatory reaction to administration of (n-3) fatty acids. In mice given fish oil supplement, development of lupus nephritis and induction of amyloidosis were significantly inhibited (154), and in guinea pigs fed fish oil the survival after endotoxin administration was increased (118).

It is well accepted that the fatty acid composition of the diet can influence the fatty acid composition of cellular constituents. The members of the (n-3) and (n-6) PUFAs exhibit many complex interactions at several levels (175). Both (n-3) and (n-6) PUFAs have been found to have anti-inflammatory actions (107,142,171). However, it has also been proposed that many diseases, including inflammatory ones, in affluent countries are attributable to an imbalance between (n-6) and (n-3) fatty acids, with too great production of metabolites of arachidonic acid (AA) (20:4 n-6) (112). Recently the interaction of free fatty acids with cytoplasmic and nuclear steroid hormone receptors has received great attention (74,139). One type of these receptors is called peroxisome proliferator-activated receptors (PPARs).

EFFECTS ON PLASMA FIBRINOGEN

Some authors have reported that fish oils have a favourable effect on plasma fibrinogen (64,101), whereas others have found no such effect (62,163,167). In a later study Dyerberg et al. noted a dose-dependent decrease in fibrinogen when fish oil was given to 11 patients with primary hypertriglyceridaemia (48). The discrepancies between different studies may relate to the method of fibrinogen determination, as well as to the dose of fish oil administered. The mechanism underlying the fibrinogen-lowering effect of (n-3) fatty acids is not known, but it is believed to be

related to decreased production of fibrinogen in the hepatocytes, as part of a decreased acute phase protein production. (n-3) fatty acids are known to diminish the production of cytokines in leucocytes and macrophages. An effect through altered production of fibrin(ogen) degradation products is also possible (67).

EFFECTS ON FIBRINOLYSIS

There are only few reports on the influence of (n-3) fatty acids on fibrinolysis. The results are somewhat contradictory. In their Oslo study of cardiovascular disease, Andersen et al (4) found an increased fibrino-lytic potential after dietary intervention in healthy coronary high-risk individuals. Part of this intervention consisted of an increased consumption of fat fish. Barcelli et al. (10) administered 5 g of (n-3) fatty acids/day to nine volunteers and noted a significant increase in the vascular plasminogen activator and a decrease in its inhibitor. The methods were not, however, those that are used today, the observation time was only 2 weeks and there was no control group. Furthermore, of the nine participants, four were women, and it is known that fibrinolysis varies considerably during the menstrual cycle (169). Mehta et al. (121) noted a reduction in PAI-1 activity after administration of 6 g (n-3) fatty acids per day in the form of Max-EPA in eight patients with coronary artery disease and in four healthy volunteers. The changes were rather small and of the four volunteers, two actually showed a small increase in PAI-1. Measurements of tPA and PAI-1 were made in serum and are therefore less reliable. Takimoto et al. administered 8.2 g of fish oil daily to 14 healthy volunteers, seven men and seven women, for 30 days, and found a reduction in serum triglycerides but no significant change in tPA or PAI-1. Again, variations during the menstrual cycle may have had some influence. Schmidt et al. (163) gave 4.5 g of (n-3) fatty acids daily to 36 patients with angina pectoris for 12 weeks. The fibrinolytic activity at rest, determined by the fibrin plate method, showed a decrease after the intake of fish oil. PAI-1 activity did not change. tPA activity and antigen were not assayed. In 76 healthy volunteers, Emeis et al. (51) noted a 71% increase in plasma PAI-1 activity after daily intake of mackerel paste containing 4.7 g of EPA/DHA for 6 weeks. However, there was no change in euglobulin tissue plasminogen activator activity, suggesting that the fibrinolytic capacity of the plasma was not changed. Müllertz et al. (128) administered 1.7 g of EPA/DHA per day to 7 normal volunteers for 3 weeks, and observed a significant rise in PAI-1 antigen. However, they give no information about special measures to prevent platelet activation and three of the seven volunteers were women. Fumeron et al. (60) found that 36 healthy volunteers showed an increase in PAI-1 activity when eating fat-controlled diets. Administration of a single dose of 20 g (n-3) fatty acids in the evening to healthy volunteers increased the PAI-1 activity in the plasma by 62% next morning compared to the previous morning (129). At the same time the serum triglyceride level decreased by 33%. Hornstra (96), in an excellent review, has discussed the significance of fish and fish oil-enriched food for pre-

vention of ischaemic cardiovascular disease. In view of the divergent results, he suggests that the use of fish-enriched diets by people with low endogenous fibrinolytic activity should be discouraged until further research has been carried out.

EFFECTS ON PLATELETS

Fish oil supplementation has been reported to increase the long-chain (n-3) fatty acids in platelets, mainly at the expense of the (n-6) fatty acids (18,59). (n-3) fatty acids alter platelet eicosanoid formation, replacing arachidonate-derived TxA_2 with the biologically inert TxA_3. However, they are relatively inefficient approach to platelet inhibition and a pronounced effect on platelet reactivity is not always demonstrated (18).

EFFECTS OF FISH OILS ON THE PRODUCTION OF NITRIC OXIDE

Endothelium-dependent vasorelaxation has been found to be diminished in patients with atherosclerosis, hypertension and diabetes (31). (n-3) fatty acids have been shown to improve such vasorelaxation (20,111). Whether this is due to an increase in NO production, a decrease in superoxide with prolonged half-life of NO, or some other mechanism remains unclear. However, Bryant et al. (24) showed that the increased NO activity in the rat aorta after intake of fish oil is not due to increased NO synthesis but may be a result of decreased breakdown of NO, since the formation of superoxide radicals was decreased. It is thus possible that (n-3) fatty acids may increase the activity of the constitutive NO in the endothelial cells mainly by decreasing superoxide production. There are no published reports on the effect of (n-3) fatty acids on the inducible NO production.

OTHER EFFECTS OF FISH OILS

Fish oil consumption usually increases the bleeding time, but in many studies only a slight or no increase has been observed (96). The effects of different doses of fish oils on the prolongation of bleeding time were investigated by Saynor et al (160). With 1.8 g EPA daily there was no such prolongation. At 4 g daily the bleeding time increased and the platelet count decreased without any adverse effects.

(n-3) fatty acids have been found to reduce plasma viscosity and decrease erythrocyte deformability (53).

FISH OIL AND ATHEROSCLEROSIS

There is strong epidemiological evidence of a protective effect of (n-3) fatty acids on atherogenesis (9,108). Kromhaut et al (109) reported an inverse relationship between fish consumption and 20-year mortality from CHD in their epidemiological study of the town of Zuptfen, Netherlands. A recent study of coronary arteries from deceased Alaska Natives and non-natives showed that the extent of raised lesions increased with age in both groups, but the prevalence of raised lesions in native specimens was consistently lower than that in non-natives (135). A very recent meta-analys-

is of studies using fish oil after percutaneous transluminal coronary angioplasty (PTCA) has shown that these oils are effective in reducing restenosis (63). The optimal dose of (n-3) fatty acids after sucessful PTCA appeared to be 4 to 5 g/day .

Most animal studies support the concept of an antiatheromatous action of (n-3) fatty acids. In dogs fed a diet high in saturated fatty acids and cholesterol, supplementation with fish oil prevented the intimal hyperplasia that was induced on venous allografts inserted into their arteries (110). In a hyperlipidaemic swine model, dietary supplementation with cod liver oil reduced the development of coronary atherosclerosis without any significant changes in plasma lipid concentrations in the supplemented animals compared with the controls (191). In a primate model, dietary substitution with (n-3) fatty acids inhibited atherogenesis in the aorta and in the carotid and femoral arteries (38). With the rabbit atherogenesis model, fish oil feeding resulted in enhancement of cholesterol-induced atherogenesis (182).

FISH OIL AND ANTIARRHYTHMIC EFFECTS

There are many experimental studies on the protective effect of (n-3) fatty acids on the ventricular fibrillation so often induced by myocardial ischaemia and infarction (30). Studies with isolated papillary muscles from either rats or marmoset monkeys indicate much less susceptibility to catecholamine-induced arrhytmia in the muscles from animals fed fish oil supplements than from those on (n-6) or low-fat diets. Indomethacin abolishes these effects in vitro, suggesting a mechanism operating via the eicosanoids. Burr et al (26) studied the effects of dietary intervention in the secondary prevention of myocardial infarction. A modest intake of fatty fish two-to-three times per week (or 3 g fish oil daily) reduced all-cause mortality by 29% over a 2-year period, possibly by preventing sudden death from arrhytmia.

POTENTIALLY ADVERSE EFFECTS OF FISH AND FISH OILS

Concern has been raised about the potential toxicity or possible side effects of prolonged consumption of fish and fish oil by humans. For this reason, and pending further studies, the Food and Drug Administration (FDA) states that available data do not provide a basis upon which a health claim for (n-3) fatty acids against CHD can be authorized (57). This report has been criticized as being much too negative (113).

Fish caught in polluted coastal waters and fish oils produced from these fishes may contain appreciable amounts of toxic heavy metals, chlorohydrocarbons and dioxines. Several of these substances are carcinogenic. However, fish oil prepared by responsible producers has these toxic substances removed during preparation, and in this sense they are safer as a source of (n-3) fatty acids than some fish. In addition, most fish oils are produced from fatty fishes in the rather clean water of the north Atlantic.

When consuming cod liver oil, but not fish oil, there may be a risk of vitamin A and D toxicity.

In some studies fish oil administration has been found to cause slight gastrointestinal complaints such as oily belching after intake, and diarrhoea. However, in the reports from most studies no such complaints are mentioned.

Potentially, intake of fish oils could increase the body weight. Intake of 30 mL of fish oil could result in an increased energy intake of about 1050 KJ (248 Kcal) per day. This may represent an increase in total dietary intake by 10%, with a concomitant increase in fat energy percent from 30% to 36%. However, there are very few reports on weight gain after consumption of fish oil. This indicates that the increased energy intake from fish oil is balanced by a reduced dietary intake of other food items or by increased energy turnover.

Fish oils have been reported to produce an atherogenic lipid profile in certain groups (99). The effect of (n-3) fatty acids on total cholesterol, LDL cholesterol and apo-B are somewhat controversial, with reports on increased, unchanged and decreased values. It has been proposed that the effects of fish oils may be determined more by the changes in the intake of saturated fatty acids than by the fatty acids in the fish oil (86). In some subpopulations, e.g. persons with hyperlipidaemia and diabetes, fish oils appear to increase LDL cholesterol when the saturated fat is kept constant. It has also been suggested that after consumption of fish oil the LDL particles are less atherogenic (144). In view of the positive effects of (n-3) fatty acids on, for example serum triglycerides, VLDL cholesterol, fibrinogen, platelets, neutrophils and blood viscosity, the net effect of (n-3) fatty acids is clearly beneficial (171). There are few long-term studies on the effects of fish oils and it has been questioned if the initial decrease of triglycerides really sustain (162). However, a recent report demonstrated sustained triglyceride lowering after seven years (161).

(n-3) fatty acids have been claimed to deteriorate glucose control in diabetics and other groups with reduced glucose tolerance (65,96,186). This has been used as an argument against recommending fish oil to these categories. It has been reported that (n-3) fatty acids increase hepatic gluconeogenesis and glucose output. On the other hand an increased insulin sensitivity has been reported (189). There are reports that an increased content of vitamin E in the fish oil may counteract the increase in glucose (188, Luostarinen, personal communication 1993). Patients with non-insulin-dependent diabetes also, as a rule, exhibit other risk factors for cardiovascular disease, including increased serum lipids, platelet reactivity and blood pressure. Fish oil has a positive effect on several of these factors and the net effect of fish oil should be positive.

The effect of fish and fish oil on fibrinolysis is somewhat controversal. However, most studies show an impaired fibrinolytic capacity. Especially there is usually an increase in PAI-1 activity and antigen. Hamsten et al (83) found increased level of PAI-1 in young survivors of myocardial infarction. Whether an elevation of PAI-1 is of pathogenetic value or is merely a marker of disease remains to be established (40). Prelimi-

nary prospective data from 231 apparently healthy individuals provided strong evidence against PAI-1 abnormalities as predictors of future myocardial infarction (152). Of interest is that Eskimos, known to have a low incidence of IHD, show elevated plasma PAI-1 activity (164).

Other matters of concern are the risk of excessive bleeding and the possible increase in stroke on consumption of fatty fish or fish oil. Eskimos have been said to have frequent epistaxis and easy bruisability (47). Mild to moderate thrombocytopenia has also been found in the Eskimos. There are some reports that ingestion of high doses of salmon oil can produce thrombocytopenia (66). However, after the intake of more purified preparations of fish oils currently used, thrombocytopenia has not been reported. Fish consumption consistently produces an increase in bleeding time (184). Fish oil consumption usually increases the bleeding time, but in many studies only a slight or no increase has been observed. This appears to be largely a matter of the dose of fish oil taken (165). During consumption of fatty fish, other factors, e.g. selenium, may contribute to an increased bleeding time. Kromann et al. (108) reported an increased occurrence of stroke among Greenland Eskimos. It should be noted, however, that the type of CNS events (e.g. thrombotic, haemorrhagic) in these subjects has not been well characterized. In contrast, a decreased incidence of stroke was found in a Japanese fishing village (92). There is no evidence that bleeding is a predictor of a risk of haemorrhage and in humans there has been no report of clinical bleeding, even after operation in subjects who were taking fish oil supplements (41). Administration of EPA to spontaneosly hypertensive rats was recently reported to increase the incidence of cerebral haemorrhage (198).

In a rodent model (n-3) fatty acids promoted tumour growth regardless of how the tumour was initiated (1150). However, there are many reports on an anticarcinogenic effect of (n-3) fatty acids (104).

Concern has also been expressed over the possible inadvertent feeding of peroxides and aldehydes from autooxidized fish oils. The stability of fish oils should be controlled by assaying peroxide and anisidine values. In order to prevent rancidity of fish oils a mixture of α, γ and δ-tocopherols and spice oils like rosemary is often used. Administration of polyunsaturated fatty acids decreases the concentration of vitamin E in the body (15) and may lead to increased lipid peroxidation with enhanced formation of products such as malondialdehyde and 4-hydroxynonenal (55). This might lead to an increased oxidative modification of the LDL particles contributing to an accelerated atherogenesis. Susceptibility to oxidative damage of fatty acids increases proportionally to the degree of unsaturation of the fatty acids, and the (n-3) fatty acids are among the most unsaturated fatty acids in the diet. Theoretically, when these fatty acids are resident in LDL, they are more likely to be oxidized by the many oxygen free radicals produced within our bodies and thus render the LDL atherogen. Recently incubation of LDL particles with EPA, however, was found not to increase its susceptibility to oxidation (132). Oxidation of LDL particles may also be

prevented by supplementation of suitable lipid soluble antioxidants, mainly vitamin E.

(n-3) fatty acids have been found to decrease the production of leukotrienes, several cytokines, superoxide, and platelet activating factor (PAF) by monocytes and neutrophils (52). This is believed to have an anti-inflammatory effect, which might benefit individuals with autoimmune or similar diseases. However, they may have a potential to reduce the immune status and inflammatory responses unduly. To date there has been no evidence of increased infections or antibiotic resistance in individuals taking (n-3) fatty acid supplements (113). In a mouse model dietary fish oil increased the mortality in animals challenged with Salmonella typhimurium (29). However, in other mouse models fish oil increased the resistance to Klebsiella pneumoniae and Plasmodium berghei infections (16). Interestingly, supplementation with antioxidants has been shown to greatly reduce the (n-3)-induced killing of Plasmodiae, indicating involvement of lipid peroxides in the killing.

RESULTS

In order to facilitate comparisons, the effects of fish oil, or other oils, on different parameters have been compiled in tables. The following abbreviations have been used:

The oils used were soy bean oil (SBO), Eskimo-3 (E-3=SPFO), highly purified fish oil (HPFO) and a mixture of fish oil and evening primrose oil 50:50 (E-36).

The supplementations (Suppl.) were 0.3 IU of vitamin E per g oil (a), 1.5 IU of vitamin E per g oil (b), 80 mg of pyridoxine and 10 mg of folic acid daily (c) and 25 mg of pyridoxine and 0.4 mg of folic acid daily (d).

The length of the treatment (Time) and of the washout period (W.o.) in weeks is given.

The pretreatment values (Before) and the percentual change (Change) (%) are given, as is the degree of significance of the change before and after the treatment (*=$p<0.05$, **=$p<0.01$, ***=$p<0.001$).

Effects of fish oils and a mixture of fish oil and evening primrose oil on plasma phospholipid fatty acid composition. In one study (75), EPA increased by 490% and 300% and DHA increased by 47% and 35% after administration of 30 and 15 mL, respectively, of ESKIMO-3. The EPA/arachidonic acid ratio increased by 637% and 363% after administration of 30 and 15 mL, respectively, of ESKIMO-3. In another study (80), after intake of fish oil there was a considerable

increase in long-chain PUFAs of the (n-3) series (20:5 EPA and 22:6 DHA) and a concomitant decrease in (n-6) fatty acids (18:2, 20:3 and 20:4) ($p<0.001$ for all). The EPA/arachidonic acid ratio increased from 0.2 to 1.2 ($p<0.001$) and the EPA/DHA ratio from 0.4 to 1.2 ($p<0.001$). After intake of fish oil/evening primrose oil the decreases in (n-6) fatty acids and the changes in fatty acid quotients were considerably smaller. Oleic acid (18:1 n-9) significantly decreased by 14% ($p<0.001$) and 23% ($p<0.001$) after treatment with fish oil alone and fish oil/evening primrose oil, respectively. After fish oil, 20:3 (n-6) decreased by 57% ($p<0.001$) while the decrease after the oil mixture was only 8%.

Effects on serum triglycerides (mmol/L).
When fish oil was administered at a dose of 15 mL daily for 6 months there was a continous decrease in serum triglycerides (75). At the end of the 6-months period, the triglyceride level had decreased by 64% ($p<0.01$).

Reference	Oil	Suppl.	Time	W.o.	Before	Change
75	30 mL SBO	a	4	-	1.8±1.6	+3%
75	15 mL E-3	a	4	2	1.9±1.6	-32%
75	30 mL E-3	a	4	2	1.8±1.7	-43%*
76	30 mL E-3	a	4	2	2.0±1.9	-45%*
76	30 mL HPFO	a	4	2	3.0±1.9	-43%**
77 (80)	30 mL E-3	a	3	2	2.6±1.6	-21%
77 (80)	30 mL E-3	b	3	2	3.4±2.9	-48%**
78	30 mL E-3	b	4	5	1.7±0.8	-32%**
78	30 mL E-3	b,c	4	5	2.1±1.3	-48%**
79	30 mL E-3	b,d	4	5	1.8±0.7	-36%**
79	30 mL E-36	b,d	4	5	1.9±0.3	-29%*

In one study (77), the decrease in serum triglycerides was more pronounced with the vitamin E-rich oil as compared to the vitamin E-poor oil ($p<0.01$).

In the long-term study (80), triglycerides in serum were decreased ($p<0.05$) at all time points, and most of the decrease occurred during the first 3 months.

The rebound increase in serum triglycerides after 2 weeks of wash-out in one study (77) was 26%. The corresponding rebound increases after 5 weeks of wash-out were 10% and 8% respectively (78,79).

Effects on serum total cholesterol (mmol/L).
When fish oil was administered at a dose of 15 mL daily for 6 months there was a continous small decrease in serum total cholesterol (75). At the end of the 6-month period, the total cholesterol level had decreased by 8%

($p<0.001$).

Reference	Oil	Suppl.	Time	W.o.	Before	Change
75	30 mL SBO	a	4	-	5.0±0.9	+1%
75	15 mL E-3	a	4	2	5.1±1.0	-6%
75	30 mL E-3	a	4	2	5.2±0.9	-10%*
76	30 mL E-3	a	4	2	5.5±1.0	-6%*
76	30 mL HPFO	a	4	2	5.5±1.2	-3%
77	30 mL E-3	a	3	2	5.6±0.9	±0%
77	30 mL E-3	b	3	2	5.7±1.1	-1%
78	30 mL E-3	b	4	5	5.3±0.9	+1%
78	30 mL E-3	b,c	4	5	5.2±1.0	-4%
79	30 mL E-3	b,d	4	5	5.4±1.1	-2%
79	30 mL E-36	b,d	4	5	5.6±1.1	-5%

The differences in the decreases in serum total cholesterol between administration of 15 and 30 mL of fish oil in one study (75), and between administration of 30 mL of SPFO (ESKIMO-3) and HPFO in another study (76), are not statistically significant.

Effects on calculated serum LDL cholesterol (mmol/L).

Reference	Oil	Suppl.	Time	W.o.	Before	Change
76	30 mL E-3	a	4	2	3.5±0.9	-7%
76	30 mL HPFO	a	4	2	3.5±0.8	+3%
78	30 mL E-3	b	4	5	3.4±0.7	+7%
78	30 mL E-3	b,c	4	5	3.5±0.7	+2%
79	30 mL E-3	b,d	4	5	3.5±0.9	+7%
79	30 mL E-36	b,d	4	5	3.7±1.1	-1%

Effects on apolipoprotein B (g/L).

Reference	Oil	Suppl.	Time	W.o.	Before	Change
77	30 mL E-3	a	3	2	1.27±0.33	+2%
77	30 mL E-3	b	3	2	1.28±0.32	±0%

Effects on serum HDL cholesterol (mmol/L).

After treatment with 15 mL of fish oil daily for up to 6 months, the changes were not statistically significant (75).

Reference	Oil	Suppl.	Time	W.o.	Before	Change
75	30 mL SBO	a	4	-	1.2±0.3	+3%
75	15 mL E-3	a	4	2	1.3±0.4	+16%
75	30 mL E-3	a	4	2	1.2±0.4	+21%**
76	30 mL E-3	a	4	2	1.2±0.4	+17%**
76	30 mL HPFO	a	4	2	1.0±0.3	+4%
77	30 mL E-3	a	3	2	1.0±0.3	+11%**
77	30 mL E-3	b	3	2	1.0±0.3	+9%*
78	30 mL E-3	b	4	5	1.2±0.3	+12%*
78	30 mL E-3	b,c	4	5	1.1±0.2	+19%**
79	30 mL E-3	b,d	4	5	1.1±0.2	+6%
79	30 mL E-36	b,d	4	5	1.1±0.3	+5%

Effects on the atherogenic index.
After treatment with 15 mL of fish oil daily for up to 6 months in one study (75), the changes were not statistically significant.

Reference	Oil	Suppl.	Time	W.o.	Before	Change
75	30 mL SBO	a	4	-	4.1±2.3	-3%
75	15 mL E-3	a	4	2	4.2±2.0	-18%
75	30 mL E-3	a	4	2	4.0±2.3	-22%**
76	30 mL E-3	a	4	2	4.3±2.6	-22%*
76	30 mL HPFO	a	4	2	4.5±1.2	-9%
77	30 mL E-3	a	3	2	5.0±1.7	-11%**
77	30 mL E-3	b	3	2	5.2±2.2	-11%**
78	30 mL E-3	b	4	5	3.6±1.2	-12%*
78	30 mL E-3	b,c	4	5	3.8±1.3	-24%*
79	30 mL E-3	b,d	4	5	4.1±1.3	-6%
79	30 mL E-36	b,d	4	5	4.3±1.3	-11%*

Effects on lipoprotein (a) (mg/mL).

Reference	Oil	Suppl.	Time	W.o.	Before	Change
76	30 mL E-3	a	4	2	123±118	-19%*
76	30 mL HPFO	a	4	2	100±124	-4%
77 (80)	30 mL E-3	a	3	2	128±149	-2%
77 (80)	30 mL E-3	b	3	2	124±135	+3%

In a long-term study (80), the plasma concentration of Lp(a) was de-

creased ($p<0.05$) at 3 months and this decrease persisted over the 12 months of follow-up.

Effects on plasma glucose (mmol/l).

Reference	Oil	Suppl.	Time	W.o.	Before	Change
76	30 mL E-3	a	4	2	6.9±2.5	+4%
76	30 mL HPFO	a	4	2	7.1±2.9	+11%***
77	30 mL E-3	a	3	2	7.0±2.7	+9%**
77	30 mL E-3	b	3	2	6.9±3.0	+11%***
78	30 mL E-3	b	4	5	5.1±0.6	+10%*
78	30 mL E-3	b,c	4	5	5.1±0.5	+4%
79	30 mL E-3	b,d	4	5	5.9±0.7	+4%
79	30 mL E-36	b,d	4	5	5.9±0.6	±0%

Effects on serum insulin (μU/mL)

Paper	Oil	Suppl.	Time	W.o.	Before	Change
78	30 mL E-3	a	3	2	13.0±11.9	±0%
78	30 mL E-3	b	3	2	12.6±12.2	-7%
79	30 mL E-3	b,d	4	5	7.3±5.8	-4%
79	30 mL E-36	b,d	4	5	7.2±3.9	+2%

Effects on systolic blood pressure (mm Hg)

Reference	Oil	Suppl.	Time	W.o.	Before	Change
75	30 mL SBO	a	4	-	127±10	-1%
75	15 mL E-3	a	4	2	126±8	-5%*
75	30 mL E-3	a	4	2	127±9	-5%*

After consumption of 15 mL of fish oil for up to 6 months in one study (75), there was no significant reduction of the systolic blood pressure.

Effects on diastolic blood pressure (mm Hg)

Reference	Oil	Suppl.	Time	W.o.	Before	Change
75	30 mL SBO	a	4	-	80±13	-2%
75	15 mL E-3	a	4	2	80±12	-5%
75	30 mL E-3	a	4	2	79±12	-1%

After consumption of 15 mL fish oil daily for 6 months diastolic blood pressure had declined by 9% ($p<0.05$) (75).

Effects on bleeding time (min)

Reference	Oil	Suppl.	Time	W.o.	Before	Change
75	30 mL SBO	a	4	-	4.8±1.5	+38%
75	15 mL E-3	a	4	2	4.7±1.3	+8%
75	30 mL E-3	a	4	2	4.7±1.2	+10%

Effects on malondialdehyde in plasma (μmol/L)

Reference	Oil	Suppl.	Time	W.o.	Before	Change
76	30 mL E-3	a	4	2	0.6±0.2	+54%**
76	30 mL HPFO	a	4	2	0.4±0.1	+89%***
77	30 mL E-3	a	3	2	0.6±0.4	+122%*
77	30 mL E-3	b	3	2	0.7±0.3	+6%
78	30 mL E-3	b	4	5	0.5±0.2	+30%
78	30 mL E-3	b,c	4	5	0.4±0.2	+23%

Effects on 4-hydroxynonenal in plasma and in methanol-extracted plasma (μmol/L)

The concentration of 4-HNE in plasma and in methanol-extracted plasma was below the detection limit both before and after treatment (79).

Effects on vitamin E (mg/L)

Reference	Oil	Suppl.	Time	W.o.	Before	Change
77	30 mL E-3	a	3	2	13.6±3.6	-9%**
77	30 mL E-3	b	3	2	13.7±2.9	-1%

Effects on homocysteine in plasma (μmol/L)

Reference	Oil	Suppl.	Time	W.o.	Before	Change
78	30 mL E-3	b	4	5	3.8±1.1	+6%
78	30 mL E-3	b,c	4	5	4.6±1.4	-30%**
79	30 mL E-3	b,d	4	5	8.8±3.0	-4%
79	30 mL E-36	b,d	4	5	10.0±3.0	-10%*

It is to be noted that the assays of homocysteine in plasma in our two studies (78,79), were carried out with a similar method but in different laboratories. The reason for the lower values in the first study is not known. The decrease in plasma homocysteine after administraton of 30 mL of fish oil supplemented with 80 mg of pyridoxine and 10 mg of folic acid was significant compared with that after administration of fish oil alone ($p<0.001$) (78). In the other study (79), both oils were supplemented with

25 mg of pyridoxine and 0.4 mg of folic acid. There was no significant difference between the two groups.

Effects on pyridoxal 5′-phosphate in plasma (nmol(L)

Reference	Oil	Suppl.	Time	W.o.	Before	Change
78	30 mL E-3	b	4	5	76.8±50.2	-22%
78	30 mL E-3	b,c	4	5	62.7±34.8	+196%***

Effects on folic acid in serum (nmol/L)

Reference	Oil	Suppl.	Time	W.o.	Before	Change
78	30 mL E-3	b	4	5	24.5±16.2	-7%
78	30 mL E-3	b,c	4	5	16.5±10.1	+223%***

Effects on plasma fibrinogen (g/L)

Reference	Oil	Suppl.	Time	W.o.	Before	Change
75	30 mL SBO	a	4	-	3.7±0.9	-5%
75	15 mL E-3	a	4	2	3.6±0.8	+1%
75	30 mL E-3	a	4	2	3.7±0.8	-9%**
76	30 mL E-3	a	4	2	3.7±0.9	-10%**
76	30 mL HPFO	a	4	2	3.7±3.4	-7%*
77	30 mL E-3	a	3	2	3.1±0.4	-3%
77	30 mL E-3	b	3	2	3.3±2.9	-11%**
78	30 mL E-3	b	4	5	3.3±0.7	-6%
78	30 mL E-3	b,c	4	5	2.8±0.6	-15%**
79	30 mL E-3	b,d	4	5	2.9±1.0	-10%*
79	30 mL E-36	b,d	4	5	3.0±0.6	-8%*

After intake of 15 mL fish oil daily for 6 months there was a 23% decrease in plasma fibrinogen ($p<0.05$). After intake of 15 mL of fish oil daily up to 12 months, plasma fibrinogen levels were decreased by 17%, 20%, 17% and 17% at 3, 6, 9 and 12 months of fish oil intake ($p<0.05$ vs pretreatment values).

Effects on fibrinolytic parameters:

Effects on tPA activity

Reference	Oil	Subst.	Time	W.o.	Before	Change
78	30 mL E-3	b	4	5	0.66±0.46	-50%**

78	30 mL E-3	b,c	4	5	0.57±0.59	-26%

Effects on tPA antigen (ng/mL)

Reference	Oil	Suppl.	Time	W.o.	Before	Change
78	30 mL E-3	b	4	5	6.3±3.2	+8%
78	30 mL E-3	b,c	4	5	5.9±2.8	+4%
79	30 mL E-3	b,d	4	5	6.8±2.5	-1%
79	30 mL E-36	b,d	4	5	6.7±2.1	+3%
80	30 mL E-3	a	3	2	9.8±3.5	+4%
80	30 mL E-3	b	3	2	9.6±2.8	+7%

Over a 12 month period of consumption of 15 mL of fish oil plasma tPA antigen levels decreased 16% at 3 months, 17% at 6 months, 19% at 9 months and 35% at 12 months (all $p<0.05$) (80).

Effects on PAI-1 activity (AU/mL)

Reference	Oil	Suppl.	Time	W.o.	Before	Change
78	30 mL E-3	b	4	5	27.1±17.2	+35%
78	30 mL E-3	b,c	4	5	31.4±18.1	+34%
79	30 mL E-3	b,d	4	5	24.5±12.9	+50%*
79	30 mL E-36	b,d	4	5	25.3±11.5	+23%
80	30 mL E-3	a	3	2	46.3±18.1	+25%
80	30 mL E-3	b	3	2	49.8±29.2	+15%

Plasma PAI-1 activity over a 12 month period of consumption of 15 mL of fish oil increased 26% at 3 months, 28% at 6 months, 51% at 9 months and 78% at 12 months (80).

Effects on PAI-1 antigen (ng/mL)

Reference	Oil	Suppl.	Time	W.o.	Before	Change
78	30 mL E-3	b	4	5	39.4±32.5	+68%**
78	30 mL E-3	b,c	4	5	39.9±33.4	+43%
79	30 mL E-3	b,d	4	5	24.9±16.1	+49%*
79	30 mL E-36	b,d	4	5	29.7±20.1	+1%
80	30 mL E-3	a	3	2	51.2±27.0	+63%*
80	30 mL E-3	b	3	2	60.7±38.2	+18%

Over a 12 month period of consumption of 15 mL of fish oil plasma PAI-1 antigen increased in all subjects (mean increase of 32%, 37%, 93% and

105% at 3, 6, 9 and 12 months, respectively, all $p<0.05$ vs pretreatment values (80).

Effects of EPA and SOD on the production of nitrite in the stimulated J774,2 murine macrophage cells (μmol/L)

The mean nitrite concentration from six experiments, each using 1.5 x 10^6 cells, was calculated. Unstimulated macrophages produced 9.6 ± 1.8 μM of nitrite (Fig. 3, A). Stimulation of the macrophages with IFN-γ and LPS markedly increased the production of nitrite by 517% to 59.2 ± 5.8 μM ($p<0.001$) (Fig. 3, B). Addition of SOD (40 U/mL) to stimulated macrophages further increased the nitrite production to 71.2 ± 6.0 μM (Fig. 3, C). Compared to unstimulated cells this was an increase in nitrite concentration by 642% ($p<0.001$). When EPA/albumin was administered 6 h before the start of the stimulation, the nitrite concentration was 48.0 ± 4.9 μM (Fig. 3; D). Compared to stimulated cells without addition of EPA/albumin this was a decrease in nitrite concentration by 19% ($p<0.01$). Macrophages incubated with EPA/albumin at the start of the stimulation produced 37.8 ± 3.5 μM of nitrite (Fig. 3, E). Compared to stimulated cells without addition of EPA/-albumin this was a decrease in nitrate concentration by 38% ($p<0.001$). Administration of SOD, 20 μM and 40 μM, together with EPA/albumin, increased the concentration of nitrite (Fig. 3, F,G). Compared to stimulated cells in the presence of 40 μM SOD, stimulated cells in the presence of 40 μM SOD and EPA/albumin produced 22% less nitrite ($p<0.001$). Incubation with albumin alone did not influence the nitrite production (data not shown).

Stimulation of differentiated U937 cells with IFN-γ/LPS did not result in any production of NO. Variations of the concentrations of glutamine and arginine, supplementation with tetrahydrobiopterin and varying sequences of stimulation, different concentrations of the stimulants, and different durations of stimulation up to 96 h did not influence the results.

Effects of EPA and SOD on cell viability in the stimulated J774,2 murine macrophage cells

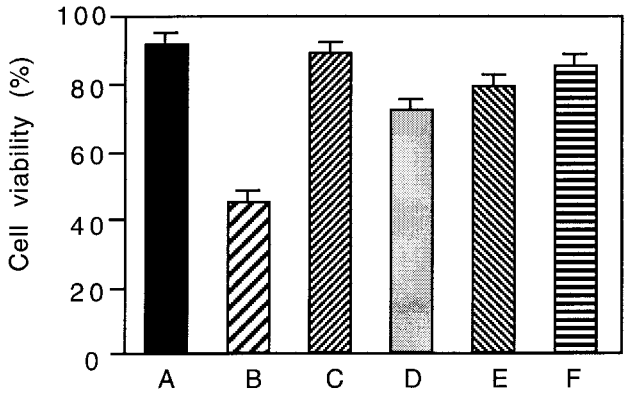
Unstimulated macrophages showed a viability of 91% with trypan blue exclusion (Fig. 4, A). After stimulation with IFN-γ and LPS for 40 h the viability decreased markedly to 44% ($p<0.001$) (Fig.4, B). Stimulation in the presence of the NO-synthase inhibitor L-canavanine (5 mM) increased the viability to 88% (Fig. 4, C). Cells incubated with 50 μM EPA/albumin at the start of the stimulation exhibited a viability of 71% (Fig. 4, D). Compared to stimulated cells in the absence of EPA/albumin this was an increased viability by 61% ($p<0.001$). After addition of SOD, 20 U/mL and 40 U/mL, the viability was increased to 77% and 83% respectively (Fig. 4, E,F).

Fig. 3

Nitrite production (μM/L) in J774,2 cells.

A. Unstimulated cells.
B. Stimulated cells (IFN-γ/LPS).
C. Stimulated cells + SOD (40 U/mL).
D. Stimulated cells + 50 μM EPA (6 h before IFN-γ).
E. Stimulated cells + 50 μM EPA.
F. Stimulated cells + 50 μM EPA + SOD (20 U/mL).
G. Stimulated cells + 50 μM EPA + SOD (40 U/mL).

Fig. 4



Viability (%) of J774,2 cells.

A. Unstimulated cells.
B. Stimulated cells (IFN-γ/LPS).
C. Stimulated cells + L-canavanine (5 mM).
D. Stimulated cells + 50 μM EPA.
E. Stimulated cells + 50 μM EPA + SOD (20 U/mL).
F. Stimulated cells + 50 μM EPA + SOD (40 U/mL).

DISCUSSION

The fish oils used in these studies were tolerated well by all subjects without any adverse effects during the experimental period. A few reported slight oily belching during the hour after intake of the fish oil. This was easily corrected by measures such as taking the fish oil together with certain foods. There were no unplanned drop-outs during the experiments.

ESKIMO-3, like most other fish oils, is available both in bottles and in small capsules. Reasons for using capsules has been to partially avoid an oily taste and to provide some protection aginst oxidation by the capsule material. However, the fish oil used in our experiments has been stabilized by addition of a mixture of tocopherols and of spices such as rosemary. The fish oil bottles were stored in a refrigerator at 2-6°C and the oil was provided in bottles containing 105 mL. In the studies using 30 mL the last portion was therefore consumed on the fourth day. The quality of the oil was registrated regularly by means of peroxide and anisidine values and it was found to be very stable. By adding taste substances such as lime, the oily taste has been strongly diminished. A capsule usually contains 0.5 g or 1.0 g of oil. In order to get 30 mL, about 28 one-gram capsules therefore have to be consumed. Difficulty in taking the capsules is fairly common, and we therefore chose to use fish oil in bottles.

Effects of fish oil on fatty acids in plasma phospholipids and blood lipids

After intake of fish oil for 4 weeks (75,76,79), there was a considerable increase in long-chain PUFAs of the (n-3) series (20:5 EPA and 22:6 DHA) in plasma phospholipids and a concomitant decrease in (n-6) fatty acids (18:2, 20:3 and 20:4). In one study (79), the EPA/arachidonic acid ratio increased from 0.2 to 1.2 ($p<0.001$) and the EPA/DHA ratio from 0.4 to 1.2 ($p<0.001$). After intake of fish oil/evening primrose oil for 4 weeks the decreases in (n-6) fatty acids and the changes in fatty acid quotients were considerable smaller. The members of the two different series of PUFAs exhibit many complex interactions at several levels. They have different affinities for enzymes regulating the production of prostanoids and leukotrienes. The different PUFAs also compete for the desaturase enzymes, with (n-3) fatty acids generally having greater affinities. Recently, the interaction of free fatty acids with intracellular steroid receptors has received great attention (139).

The effect of fish oil on serum triglycerides in our studies was very strong and the decrease ranged from 32% to 48% after intake of 15 or 30 mL fish oil daily for 3-4 weeks. The effect was dose-dependent. During consumption of 15 mL of fish oil daily for up to 12 months, the serum triglycerides were lower at all time points, and most of the decrease occurred during the first three months. The result of this long-term study (80) confirms the report by Saynor et al (161) that the triglyceride-lowering

effect of fish oil is sustained. The fish oil containing 1.5 IU vitamin E/g oil decreased serum triglycerides more than that containing 0.3 IU vitamin E/g oil (48% vs 21%, $p<0.01$) (77). In another study (79) fish oil and fish oil/-evening primrose oil decreased triglycerides by 36% and 29% respectively. These changes correspond well to the decrease in triglycerides seen in one of our studies (75), with use of 30 and 15 mL, respectively, of fish oil and the effect of fish oil/evening primrose oil on triglycerides is thus probably due to its content of (n-3) fatty acids. There was a positive correlation between plasma PAI-1 or antigen and serum triglycerides in the baseline state ($r=0.70$, $p<0.01$) (80). However, this relationship was no longer evident after fish oil treatment.

The effect of fish oil on serum total cholesterol in our trials was fairly weak. When in one study (75), fish oil was administered at a dose of 15 mL for 6 months, there was, however, a continuous decrease in cholesterol. At the end of the 6-month period, total cholesterol had decreased by 8% ($p<0.001$). When 30 mL of fish oil was given for 4 weeks there was a 10% decrease ($p<0.05$) (paper I). In another study (76), administration of 30 mL of fish oil (SPFO=Eskimo-3) decreased total cholesterol by 6% ($p<0.05$). In all other trials the changes were statistically non-significant. In the literature there are numerous experimental and clinical reports on unchanged, increased or decreased serum total cholesterol levels. According to Harris (86) the result is strongly dependent on the concomitant intake of saturated fat. When the intake of saturated fat is kept constant, no change in total cholesterol is to be expected. The type of patients and the duration of the treatment also have an impact.

The effect of fish oil on the calculated LDL cholesterol in our studies was also fairly small and statistically non-significant. The changes ranged from a decrease by 7% in one study (76) to an increase by 7% in two studies (78,79). In patients with hypertriglyceridaemia and type IIb hyperlipidaemia, fish oil fairly often produces a slight to moderate increase in LDL cholesterol. This seemingly proatherogenic lipid change has sometimes been used as an argument for not giving fish oil to these patients. However, there are reports linking smaller, more dense LDL particles with an increased risk of coronary heart disease (145). Recently, evidence has been presented that fish oil increases the size of the LDL particles in hypertriglyceridaemic patients (144). Even if in some patients fish oil increases the level of LDL cholesterol, this effect might be counteracted by the positive change in the LDL particle size.

The effect of administraton of 30 mL of fish oil daily for 3-4 weeks on HDL cholestrol was an increase ranging from 4% to 21% in the various studies. In one of our studies (76), consumption of 30 mL of SPFO (=ESKIMO-3) daily for 4 weeks resulted in a 17% ($p<0.01$) increase in HDL cholesterol, while consumption of 30 mL of HPFO daily only resulted in an increase by 4%. It is suggested that highly purified fish oil may not have better effects than semipurified fish oil concentrate, possibly because of loss of active component(s) during the purification procedure.

The effect of administration of 30 mL fish oil daily for 3-4 weeks on

the atherogenic index was a decrease ranging from 9% to 24% in the various studies. Again, consumption of 30 mL of SPFO daily for 4 weeks had a stronger effect than 30 mL of HPFO ($p<0.01$) (76). Intake of 30 mL fish oil supplemented with 80 mg pyridoxine and 10 mg folic acid daily for 4 weeks decreased the atherogenic index more than fish oil alone ($p<0.05$) (78). A combination of fish oil and evening primrose oil had a more favourable effect on the atherogenic index than fish oil alone ($p<0.05$) (79).

Effects of fish oil on carbohydrate metabolism.

There has been much concern about the potential deterioriation of glucose homeostasis on consumption of fish oil. There are several reports on a glucose-increasing effect of fish oils, especially when given to patients with type II diabetes or other persons with decreased glucose tolerance (65,186). This has been used as a reason for not recommending fish oil to these categories. It has also been found, however, that there is no glucose increase in type II diabetics (148). In one study (78), we found a tendency to a smaller increase in blood glucose when 80 mg pyridoxine and 10 mg folic acid were added to the fish oil. In another study (79), 25 mg pyridoxine and 0.4 mg folic acid were given together with both oils, resulting in only a minor (4%) increase in plasma glucose after administration of the fish oil alone, whereas intake of fish oil/evening primrose oil produced no change at all. Intake of fish oil led to a non-significant 4% decrease in serum insulin, and fish oil/evening primrose oil produced a corresponding 2% increase in insulin. Elevated insulin and increased insulin resistance have been shown to be a risk factor for ischaemic heart disease (179). There is some evidence that fish oil increases the gluconeogenesis in the liver and increases insulin sensitivity (148). Variations in insulin sensitivity have been found to be related to differences in the membrane content of long-chain polyunsaturated fatty acids (19). Thus the ratio of 20:4 to 20:3 in skeletal muscle tissue, an index for Δ5-desaturase activity, was directly correlated to insulin sensitivity (19). We have calculated this index from plasma phospholipid fatty acids. Interestingly, this index increased by 96% after intake of fish oil alone, but was unchanged after intake of the oil mixture, which might indicate an advantage of (n-3) fatty over (n-6) fatty acids regarding their effect on insulin sensitivity. Fish oil has positive effects on several other risk factors present in diabetics. Possibly supplementation with pyridoxine and folic acid and a higher concentration of vitamin E in the fish oil may reduce the risk of deterioriation of glucose homeostasis. The question as to whether fish oil should be given to type II diabetics has not yet been settled. There is a need for well-controlled long-term studies with small doses of (n-3) fatty acids to clarify these questions.

Effects of fish oil on lipid peroxidation

Administration of PUFAs decreases the concentration of vitamin E in the body, thereby increasing the risk of oxidative damage and lipid peroxidation. Lipid peroxides such as malondialdehyde and 4-hydroxynonenal are

believed to contribute to the oxidative modification of LDL cholesterol, with increased uptake in the vessel wall (103). In one study (77), we found that consumption of 30 mL of fish oil containing 0.3 IU vitamin E/g oil daily for 3 weeks decreased serum vitamin E concentration by 9% and increased plasma malondialdehyde by 122%. Ingestion of fish oil containing 1.5 IU vitamin E/g oil for 3 weeks prevented these changes. In anaother study (79), we assayed 4-hydroxynonenal in whole plasma and in methanol-extracted plasma, and the values remained below the detection limit. It has been suggested that 4-hydroxynonenal may be a potentially more important substance than malondialdehyde in the oxidative modification of LDL cholesterol (103); but it is more lipophilic than malondialdehyde and is therefore found in very low concentrations in plasma.

When administering fish oil it is of utmost importance firstly not to give a fish oil already containing lipid peroxides and aldehydes, and secondly that the fish oil should contain enough vitamin E to avoid a decrease in the serum vitamin E concentration and an increase in plasma malondialdehyde. We have found that for the fish oil usually used in our studies, ESKIMO-3, a vitamin E content of 1.5 IU/g oil seems to be optimal.

Effects of fish oil on plasma homocysteine

In one study (78), fish oil was found to have no effect on the plasma homocysteine concentrations. Fish oil supplemented with 80 mg of pyridoxine and 10 mg of folic acid reduced the plasma homocysteine level by 30%, an effect mainly due to the vitamins (78). In another study (79), interestingly, fish oil/evening primrose oil produced a 10% ($p<0.05$) decrease in the plasma homocysteine concentration. In 11 out of 12 subjects homocysteine decreased with this treatment. In the twelfth volunteer, the plasma homocysteine was greatly increased before treatment, probably because of some defect in the homocysteine metabolism. In this study both the fish oil and the fish oil/evening primrose oil mixture were supplemented with 25 mg of pyridoxine and 0.4 mg of folic acid. It is mainly the folic acid that is known to be able to reduce homocysteine. However, usually higher doses of folic acid is then required. It is therefore probable that the oil mixture is mainly responsible for the reduction in plasma homocysteine. Recently was found that administration of 12 g of fish oil daily for 3 weeks decreased serum homocysteine by 48% in hyperlipidaemic men (141). Hypercholesterolaemic men have been found to have elevated levels of homocysteine in the LDL and other lipoprotein fractions of plasma.

Effects of fish oil on bleeding time

In one study (75), there was no significant increase in bleeding time after consumption of 15 or 30 mL of fish oil for 4 weeks. However, in most studies an increased bleeding time has been observed after fish and fish oil consumption (171,184). The bleeding time is determined mainly by the number and reactivity of the platelets and their interaction with the vess-

el wall. During consumption of (n-3) fatty acids, the number of platelets usually declines slightly, and the platelet reactivity to agonists is often depressed (155), and fibrinogen, which contributes to the homeostatic plug as fibrin, is often reduced (75-80,101). It has recently been reported that inhalation of NO prolongs the bleeding time (100). (n-3) fatty acids have been shown to enhance blood vessel NO activity, possibly contributing to the increased bleeding time after consumption of fish and fish oil. An increased PAI-1 concentration is usually seen after ingestion of (n-3) fatty acids. This could be a compensatory mechanism for maintaining haemostasis, without which there would be an increased risk of bleeding.

Effects of fish oil on blood pressure

In one study (75), the systolic blood pressure was reduced by 5% ($p<0.05$) after intake of 15 and 30 mL of fish oil daily for 4 weeks. After consumption of 15 mL of fish oil there was a statistically significant decrease in diastolic blood pressure by 9% ($p<0.05$) after 6 months. Reports have been conflicting as to whether fish oil supplements are of value in the treatment of high blood pressure. In a very recent meta-analysis of 31 placebo-controlled trials, the effect of fish oil on blood pressure was examined (126). It was concluded that there is a dose-response effect of fish oil on blood pressure of -0.66/-0.35 mm Hg/gram (n-3) fatty acids for systolic and diastolic blood pressure respectively. The hypotensive effect may be strongest in hypertensive subjects and those with clinical atherosclerotic disease or hypercholesterolaemia. Even if the effect of fish oil on blood pressure is modest, its use may still have broader importance. First, in addition to the effect on blood pressure fish oil may also have effects on many other risk factors for CVD. Secondly, low doses of fish oil have no adverse effects and can also be used adjunctively with low doses of antihypertensive drugs, reducing the risk of adverse effects of these drugs.

Effects of fish oil on fibrinogen

In view of the strong relationship between increased fibrinogen and IHD (54), the consistent decrease in plasma fibrinogen levels in response to fish oil in most of our studies is of great interest. Intake of vitamin E-rich oil (1.5 IU/g oil) resulted in a significant (11%) reduction in plasma fibrinogen, whereas vitamin E-poor fish oil (0.3 IU/g oil) had no such effect ($p<0.01$ vs vitamin E-rich oil). In one study (78), the plasma fibrinogen decreased by 15% ($p<0.01$) after intake of the vitamin B-supplemented fish oil and by 6% (n.s.) after intake of the other fish oil. This effect differed significantly between the two treatments ($p<0.05$). Why the increased content of vitamin E and the supplementation with pyridoxine and folic acid resulted in a larger decrease in plasma fibrinogen is not known. However, vitamin E protects PUFAs from oxidation and pyridoxine has been reported to interact with the metabolism of unsaturated fatty acids (35). Our results confirm the observations of some investigators (48,101,64). Others, however, found no change in fibrinogen levels with dietary fish oil

intake (62,163,167). The discrepancies between different studies may be partly due to the method of fibrinogen determination used, as well as to the dose of fish oil given. We measured fibrinogen in plasma as clottable fibrinogen by the method of Nilsson and Olow (136). Knowledge about the determinants of the plasma level of fibrinogen in health and disease is as yet incomplete, as is our understanding of the mechanisms leading to the atherothrombogenic action of fibrinogen. Fibrinogen strongly affects blood coagulation, blood rheology and platelet aggregation; in addition fibrinogen and its metabolites have direct effects on the vascular wall. Finally, fibrinogen is a prominent acute phase protein. The consistent lowering of plasma fibrinogen in our studies might therefore contribute to the anti-atherothrombogenic action of fish oil.

Effects of fish oil on fibrinolytic parameters

In most of our studies administration of fish oil increased PAI-1 antigen. There was also a trend against an increase in PAI-1 activity. tPA antigen did not change after intake of fish oil for 3 weeks (81). After consumption of 15 mL fish oil up to 12 months (81) there was a moderate decrease in tPA-antigen. When consuming 30 mL of fish oil daily for 4 weeks (78), tPA activity increased by 50% ($p<0.01$), while the corresponding decrease with the vitamin B-supplemented fish oil was only 26%. The significance of an increased level of PAI-1 activity or antigen levels in general and after consumption of fish or fish oil is not known. In this regard, it is noteworthy that Eskimos who have a low incidence of IHD also have increased levels of PAI-1 activity (164). We suggest that the increase in PAI-1 may be a compensatory reaction to a tendency towards increased bleeding during (n-3) fatty acid consumption, without which primary haemostasis would be impaired.

Effects of EPA on nitric oxide production and cell viability

Endothelium-dependent vasorelaxation has been found to be reduced in patients with atherosclerosis, hypertension and diabetes (31). (n-3) fatty acids have been shown to improve endothelium-dependent vasorelaxation (20,111). Whether this is due to an increase in NO production, a decrease in superoxide with prolonged half-life of NO or some other mechanism remains unclear. However, Bryant et al. (24) showed that the increased NO activity in rat aorta after intake of fish oil is not due to increased NO synthesis but can be due to decreased breakdown of NO since the formation of superoxide radicals was decreased. It is thus possible that (n-3) fatty acids may increase the activity of the constitutive NO in the endothelial cells mainly by decreasing superoxide production. There are no published reports on the effect of (n-3) fatty acids on the inducible NO production. We have shown that addition of EPA to the murine macrophage cell line J774,2 decreases NO activity, assayed as nitrite, when the cells were stimulated with IFN-γ and LPS (81). EPA also improved the viability of the stimulated macrophages. The cell viability after stimulation for 40 hours without EPA was only 44%, while the viability of stimulated cells in the

presence of EPA was 71%. A decrease in NO activity can occur either through diminished production of NO or through increased inactivation of NO, mainly by increased production of superoxide radicals. As (n-3) fatty acids are known to decrease and not to increase superoxide formation, the effect of EPA in the present investigation should mainly be due to decreased NO production. Furthermore, it has been reported that although stimulated monocytes mainly produce superoxide, when maturated to macrophages they mainly produce NO (117). Many of the demonstrated actions of (n-3) fatty acids in macrophages are believed to be due to an altered production of eicosanoids, cytokines such as interleukin-1, platelet activating factor, and tumour necrosis factor. The role of NO production in macrophages in the pathogenesis of atherosclerosis is controversial. Hogg et al (93) have found that peroxynitrite, produced when NO reacts with superoxide, modifies LDL particles oxidatively. On the other hand, Jessup et al (102) have proposed that NO protects LDL particles against oxidative modification. Very recently it has been shown that NO in the free radical form of NO may damage and kill cells, while NO in the form of nitrosonium cation (NO^+) may protect cells (115). The production of NO in the stimulated J774,2 cells was inhibited by L-canavanine, an inhibitor of NO synthase. L-canavanine also diminished cell death of the stimulated cells. It is of very great interest that L-canavanine is also a known inducer of heat shock proteins (HSPs), also called stress proteins (194). These very conserved proteins are synthesized after various forms of stress such as heating, hypoxia, acidosis and after treatment with agents such as L-canavanine, ethanol, glutamine and cadmium. They are believed to be involved in the protection of cells from deleterious effects of various stresses. It is also urgent to investigate whether other inhibitors of NO synthase, e.g. L-MNNA, will induce heat shock proteins and whether EPA and other polyunsaturated fatty acids induce these proteins. We propose that the effects of EPA on NO activity and on cell viability may contribute to the antiatherogenic and inflammatory effects of (n-3) fatty acids.

(n-3) fatty acids and plaque vulnerability

The most common precipitating event in heart attacks and cardiac death is rupture of an atherosclerotic plaque. The rupture site is usually in the periphery of the plaque, where many macrophage foam cells are found (56). It has been hypothesized that release of proteolytic enzymes from the macrophages might contribute to the weakening of the collagen plaque cap. It has been demonstrated in vitro that macrophages from animals or cells supplemented with (n-3) fatty acids release less proteolytic enzymes when stimulated. If these in vitro findings are also applicable in vivo, plaque macrophages with a larger amount of (n-3) fatty acids will release a smaller amount of proteolytic enzymes, resulting in a potentially lower risk of plaque rupture. A vulnerable plaque also has a large soft core of extracellular lipid. This lipid is believed to come from killed foam cells. It has recently been reported that macrophages exposed to NO are killed, exhibiting a DNA fragmentation patten characteristic of apoptosis (3,159). In

one study (81), we demonstrated that addition of EPA to the macrophage cell line J774.2 decreased NO activity and improved viability.

Commments on the potentially adverse effects of fish oils in the present studies and in general

The fish oils used in the present studies were tolerated well by all subjects and no adverse effects were noted.

The possible negative effects of a rebound increase in, for example, triglycerides and fibrinogen after stopping intake of high doses of (n-3) fatty acids has not been stressed previously. In persons with increased risk factors for CVD the rebound change can potentially contribute to vascular complications. It is important to advice such persons to decrease the intake of the fish oil gradually, and also to give them life-style advice. In the present studies the participants were regularly informed about their laboratory values during the trials. After the completion of the trials they were carefully informed both orally and in writing about all results and they were given health advice. In the event of any urgency they were recommended to contact specialists.

The potentially adverse effects of fish oils have been discussed in a previous section. The fish oils used in our studies had a dioxin content below the detection level (less than 0.74 pg/kg). The oil had been stabilized against oxidation by natural antioxidants and in frequent assays the peroxide and anisidine values were low. This minimizes the risk of inadvertent feeding of peroxides and aldehydes from auto-oxidized fish oils. No increase in body weight was recorded after the various studies. This indicated that the increased energy intake from the fish oil was balanced by a reduced dietary intake of other food items or by increased energy turnover. Fish oils have sometimes been reported to produce an atherogenic lipid profile in certain groups. There were no statistically significant increases in serum total cholesterol or calculated serum LDL cholesterol in our studies. In two studies (75,76), 30 mL of ESKIMO-3 daily resulted in a significant decrease in total cholesterol after 4 weeks. When in one study (75), ESKIMO-3 was administered at a dose of 15 mL daily, the total cholesterol was decreased by 8% ($p<0.001$) after 6 months. In most of our studies there was a slight to moderate increase in plasma glucose. In one study (78), there was a tendency to a smaller rise in plasma glucose with the vitamin B-supplemented fish oil. Most of our studies showed an increase in PAI-1 activity and antigen. The significance of an increased level of plasma PAI-1 after consumption of fish oil is not clear. Increased levels of PAI-1 have been shown to be associated with IHD and peripheral vascular disease (84). It is unclear whether this relationship is causal or coincidental.

Eskimos, who have a low incidence of IHD, also have elevated levels of PAI-1 activity (164). We suggest that the increase in PAI-1 may be a compensatory reaction to a tendency towards increased bleeding during consumption of (n-3) fatty acids, without which primary haemostasis would be impaired. In most studies by other authors a slight to moderate increase in bleeding time has been found after intake of fish oil. In one of

our studies (75), there was no significant increase in bleeding time. Intake of 30 mL of fish oil containing 0.3 IU/g of vitamin E daily for 3 weeks (77), resulted in an increase in MDA by 122% ($p<0.05$) and a decrease in serum vitamin E by 9% ($p<0.01$). By intake of a fish oil containing 1.5 IU/g of vitamin E these changes were prevented.

Limitations of the studies

In most of the studies relatively small groups of subjects, usually 12-15 volunteers, were investigated. The use of larger groups would possibly have revealed more pronounced differences between the treatments.

In some studies premenopausal women participated. It has been demonstrated that some laboratory values, e.g. fibrinolytic parameters, can vary greatly during the menstruation cycle (169). The number of premenopausal women in the studies, however, was small. Most tests were performed with an interval of 4 weeks and most of the premenopausal women reported regular menstruation cycles of 4 weeks. The influence of menstrual variations in the total studies is therefore probably negligible.

No dietary records from the subjects were used. However, there are several reports on the limited value of most short-term dietary recalls (46). Furthermore, the participants were carefully instructed not to deliberately change their diets during the experimental period. They were also asked to record and report any significant changes in their diets and way of living.

Compliance was generally determined on the basis of information given by the subjects, who were told to report the amount of fish oil left after the treatment periods. In studies I, II and V assays of fatty acids in plasma phospholipids were regularly performed and these gave information about compliance. Determination of fatty acids in plasma phospholipids has been claimed to be the best measure of intake of (n-3) fatty acids (170). By using a group of stable, very well-motivated subjects, often directly or indirectly linked to the institute, the compliance was probably very good.

In some studies (75,76,77) the washout period was only 2 weeks, which is too short for normalization of the fatty acids in various compartments. After a washout period of 2 weeks following a period on fish oil there was still a slight but significant increase in both EPA and DHA, and a fairly large decrease in arachidonic acid in plasma phospholipids. The turnover in different membrane compartments, however, may be slower. A washout period of 5 weeks (78,79) gave very good restoration of the fatty acids in plasma phospholipids. No evidence of a relation between treatment and period was found in tests in these latter studies.

One study in which 13 subjects were given 15 mL of fish oil for 6 months (75) was uncontrolled. Also, the long-term study (80) was uncontrolled. Another study (75), in which 13 individuals were given 15 or 30 mL of fish oil for 4 weeks, was single-blind. In one study (76), two different groups of subjects were used. One group received SPFO or placebo oil and the other was given HPFO or placebo oil. Use of parallel study groups may have advantages over cross-over trials (91). However, it is important that

the study groups are well matched.

The balance between (n-6) and (n-3) fatty acids in human nutrition

The optimal ratio between the intake of (n-6) and (n-3) fatty acids remains to be established. The current recommended ratio varies between 10 and 3 (97,171,195). The interaction between these two series of PUFAs is complex and is still poorly understood. A better understanding of these relationships might lead to an optimal formulation of dietary allowances of fatty acids and possibly to an appropriate design of fatty acid mixtures for various therapeutic purposes.

There is much support for the view that overactivity of some arachidonic acid pathways greatly contributes to many chronic diseases in affluent countries (34,112,171). With the introduction of agriculture and with the industrial revolution, there was an increase in saturated fat and (n-6) fatty acids in the diets and a decrease in (n-3) fatty acids. These dietary changes together with smoking, hypertension, obesity, some forms of stress and increasing physical inactivity are believed to have contributed to the increase in many chronic diseases (112). It has also been proposed that the best preparation of fatty acids might be one containing a combination of EPA/DHA and gamma-linolenic acid (98). This bypasses the sensitive Δ6-desaturase, supplies important (n-3) fatty acids and inhibits the production of arachidonic acid. Gamma-linolenic acid is elongated to dihomo-gamma-linolenic acid, which is the precursor of anti-inflammatory prostaglandins of the 1 series (97,98).

α-linolenic acid-rich food, (fatty) fish or fish oil?

There is epidemiological, clinical and experimental support for the concept that (n-3) fatty acids have a protective effect against CVD, inflammatory diseases and possibly cancer. Through alternating desaturations and elongations, the body has the capacity to transform the parent compound of the (n-3) fatty acids, α-linolenic acid, to the very-long-chain (n-3) fatty acids EPA and DHA. However, Δ-6-desaturase, the rate-limiting enzyme in the formation of very-long-chain (n-3) fatty acid, has very low activity in humans (22). This results in a slow conversion of α-linolenic acid to EPA and DHA. Moreover, the Δ-6-desaturase activity is further inhibited with increasing age, by hyper- and hypothyroidism, by corticosteroids, in diabetes, by a high-cholesterol diet and by alcohol. In the neonatal period the precursor role of α-linolenic acid and also of β-oxidation may be important, especially in premature or low birth weight infants (36). With respect to their lipid-lowering effect, α-linolenic acid and EPA appear to have a similar effect, at least in younger people (173). This is different from the blood-pressure low-ering effect, which is most pronounced during diets rich in very-long-chain (n-3) fatty acids (173). It might be speculated that for the triglyceride-lowering potency the (n-3) position of the double bonds or the degree of unsaturation is the determining chemical

component, and for the blood pressure-lowering effect the chain length. An increased supply of very-long-chain (n-3) fatty acids should therefore be important. As a rule this should be provided by an increased intake of mainly fatty fish. There is epidemiological evidence that an average intake of only 30 g of fish daily will protect against CVD and mortality (109). This amount of fish contains only very small amounts of EPA. It has been proposed that even a very low intake of EPA will protect against CVD and mortality. It has also been speculated that fish contains other substances, yet to be defined, which also have a protective effect. In order to lower increased serum triglycerides and elevated blood pressure and to alleviate inflammatory diseases, a supply of larger amounts of very-long-chain (n-3) fatty acids is necessary. To receive these amounts of (n-3) fatty acids, a very high intake of fatty fish is required. In these situations administration of fish oil would be more practical. Moreover, a number of people for various reasons do not like eating fish. Fish from certain areas may also contain high levels of toxic heavy metals and dioxines. Fish oil may be taken together with low doses of anti-inflammatory drugs, reducing the risk of adverse drug effects. Another indication is in preterm and low birth weight children. Administration of very-long-chain (n-3) fatty acids to these children has been reported, for example, to improve visual acuity. Until recently no medical therapy has been shown to reduce the rate of restenosis following percutaneous transluminal coronary angioplasty (PTCA). A very recent meta-analysis of studies using fish oil after PTCA has shown that such oil is effective in reducing restenosis. The optimal dose of (n-3) fatty acids after sucessful PTCA appeared to be 4 to 5 g/day.

Future prospects of fish and fish oil consumption

There is strong evidence that fatty fish, at least two weekly portions (200-400 gr), or fish oil 0.5 gr/day, has a preventive effect on death due to cardiovascular disease and on total mortality (26,42). In this case the effect is probably not due to a change of well known risk factors such as blood lipids, fibrinogen or blood pressure, but may be due to a direct action on the heart (30). On the other hand, such a regimen does not appear to decrease the occurrence of myocardial infarction in which the abovementioned risk factors seem to be involved. For this, larger amounts of fish oil are necessary and intake of fish does not seem to be enough for such an effect. The dose required for an effect on different risk factors seems to vary and it also depends on the length of the treatment period. A dose of 5-10 ml/day seems to have no side effects and has been shown to have a good effect in long term studies (161). Larger doses seem to be required, for instance, if the fish oil is going to be used as the only treatment in hypertension. In this case the treatment could result in some side effects, although these would be less than in ordinary pharmacological treatment. It is very important that the vitamin E dose is high enough to prevent an increase in MDA and a decrease in the E-vitamin level in the blood. Furthermore, as a large dose of fish oil (eg. 30 ml/day) is very effective in decreasing triglycerides and fibrinogen, this treatment should not be stopped

abruptly, because of the risk of a rebound effect with an increased tendency to coagulation. The negative effect on blood glucose seen with the larger doses of fish oil may be prevented by addition of vitamins B and E and the effect of PAI-1 may be counteracted by addition of (n-6) fatty acids. However, the increase in PAI-1 might be beneficial by reducing the tendency to increased bleeding.

The question of which components in the natural fish oil have the beneficial effect is not settled. It is probable that other components besides the (n-3) fatty acids EPA and DHA are of importance. This is supported by the fact that more concentrated fish oils seem to have less effect, at least in some parameters (12).
Although natural fish oil seems to have great potential for the prevention and treatment of cardiovascular disease, much more research in this field is still necessary and seems to be a very urgent future task.

CONCLUSIONS

Ischaemic cardiovascular disease is still the most common cause of death in most Western societies. Some populations, such as Eskimos and coastland Japanese, are known to have a low incidence not only of cardiovascular disease but also of several inflammatory diseases. This is assumed to be partly due to a high consumption of diets rich in (n-3) fatty acids.

The effects of a new fluid fish oil containing (n-3) fatty acids and stabilized against oxidation on risk factors for cardiovascular disease were determined in a long-term study for up to 12 months and in several short-term double-blind cross-over studies.

Consumption of fish oil, 30 mL daily for 4 weeks, resulted in a very large increase in the plasma phospholipid eicosapentaenoic and docosahexaenoic acid concentrations and a 16-20% decrease in the concentration of arachidonic acid. Serum triglycerides decreased strongly in all studies. Fish oil with a higher content of vitamin E (1.5 IU/g oil) resulted in a greater decrease in serum triglyceride than a fish oil with only 0.3 IU vitamin E/g oil. HDL cholesterol was usually increased and the atherogenic index decreased after intake of fish oil. The effects on serum total cholesterol and calculated serum LDL cholesterol were small. Plasma glucose was slightly increased in most trials. This increase could be attenuated by supplementation with pyridoxine and folic acid. Consumption of fish oil with 0.3 IU vitamin E/g fish oil resulted in a 9% decrease in serum vitamin E and increased plasma malondialdehyde by 122%. After intake of fish oil with 1.5 IU vitamin E/g fish oil, serum vitamin E and plasma malondialdehyde remained normal. Plasma fibrinogen was reduced in most studies. In most studies there was an increase in plasminogen activator inhibitor-1 antigen after intake of fish oil. After consumption of 15 mL of fish oil

there was a progressive increase in plasminogen activator inhibitor-1 antigen (mean increase of 32%, 37%, 93% and 105% at 3, 6, 9 and 12 months, respectively, all $p<0.05$ vs pretreatment values). It is suggested that the increase in plasminogen activator inhibitor-1 may be a compensatory reaction, without which primary haemostasis would be impaired. The plasma level of lipoprotein (a) fell significantly after 3 months' administration of 15 mL fish oil daily. The initial positive correlation between triglycerides and plasminogen activator inhibitor-1 activity or antigen was reversed after intake of fish oil.

Further purification of the fish oil resulted in less effect on several parameters, e.g. HDL cholesterol, atherogenic index and lipoprotein (a), suggesting loss of active components during the purification procedure.

A combination of fish oil and evening primrose oil (1:1), while producing a relatively large decrease in serum triglycerides, also had a somewhat more favourable effect on the atherogenic index and caused no increase in plasminogen activator inhibitor-1 antigen. The interactions between (n-3) and (n-6) fatty acids warrant much further studies. This might aid in the optimal formulation of recommended dietary allowances of fatty acids and in the design of fatty acid mixtures for various therapeutic aims.

Addition of eicosapentaenoic acid to the murine macrophage cell line J774,2 decreased the production of NO, assayed as nitrite, when the cells were stimulated with interferon-γ and lipopolysaccharide. In addition this fatty acid improved the viability of stimulated cells. NO, but probably also peroxynitrite and hydroxyl radicals, formed in the reaction between NO and superoxide, contribute to the cell death.

The positive effect of consumption of fish oil on several risk factors for cardiovascular diseases supports the assumption that an increased intake of (n-3) fatty acids is of value in the prevention of these diseases.

REFERENCES

1. Abbey, M., Clifton, P., Kestin, M., Belling, B. & Nestel, P.: Effect of fish oil on lipoproteins, lecithin: cholesterol acyltransferase and lipid transfer protein activity in humans. Arteriosclerosis 10: 85-94, 1990.
2. Ackman, R.G.: Nutritional composition of fats in seafoods. Progr Food Nutr Sci 13: 161-241, 1989.
3. Albina, J.A., Cui, S., Mateo, R.B. & Reichner, J.S.: Nitric oxide-mediated apoptosis in murine peritoneal macrophages. J Immunol 150: 5080-5085, 1993.
4. Andersen, O., Nilsen, D.T., Lyberg Beckman, S., Holme, I. & Hjermann, I.: Increased fibrinolytic potential after diet intervention in healthy coronary high-risk individuals. Acta Med Scand 223: 499-506, 1988.
5. Andreasen, P.A., Georg, B., Lund, L.R., Riccio, A. & Stacey, S.N.: Plas-

minogen activator inhibitors: hormonally regulated serpins. Mol Cell Endocrin 68: 1-19, 1990.
6. Austen, M.A.: Plasma triglyceride and coronary heart disese. Arteriosclerosis Thromb 11: 2-14, 1991.
7. Austen, M.A.: Joint lipid risk factors and coronary heart disease. Circulation 85: 365-367, 1992.
8. Bang, H.O., Dyerberg, J. & Sinclair, H.M.: The composition of the Eskimo food in north western Greenland. Am J Clin Nutr 33: 2657-2661, 1980.
9. Bang, H.O. & Dyerberg, J.: Lipid metabolism and ischemic heart disease in Greenland Eskimos. In: Draper HH, ed. Advances in Nutrition Research, New York, Plenum. 3: 1-22, 1980.
10. Barcelli, U., Glas-Greenwalt, P. & Pollak, V.E.: Enhancing effect of dietary supplementation with n-3 fatty acids on plasma fibrinolysis in normal subjects. Thromb Res 39: 307-312, 1985.
11. Barker, D.J.P.: Fetal origins of coronary heart disese. Br Heart J 69: 195-196, 1993.
12. Bhatnagar, D., Mackness, M.I., Prais, H., Haynes, B., Morgan, J. & Durrington, P.N.: Effect of a new fish oil concentrate (K85) on plasma lipids and lipoproteins in patients with hypertriglyceridaemia. Congress abstracts. Oslo, Norway. Abstr. 127, 1992.
13. Beil, F.U., Terres, W., Orgass, M. & Greten, H.: Dietary fish oil lowers lipoprotein(a) in primary hypertriglyceridemia. Atherosclerosis 90: 95-97, 1991.
14. Bjerve, K.S.: n-3 fatty acid deficiency in man. J Intern Med 225 suppl. 1: 171-175, 1989.
15. Bjørneboe, A., Søyland, E., Bjørneboe, G-E., Rajka, G. & Drevon, C.A.: Effect of n-3 fatty acid supplement to patients with atopic dermatitis. J Intern Med 225, suppl. 1: 233-236, 1989.
16. Blok, W.L., Vogels, M.T.E., Curfs. J.H.A.J., Eling, W.M.C., Buurman, W.A. & van der Meer, J.W.M.: Dietary fish-oil supplementation in experimental gramnegative infection and in cerebral malaria in mice. J Infect Dis 165: 898-903, 1992.
17. Blonk, M.C., Bilo, H.J.G., Nauta, J.J.P., Popp-Snijders, C., Mulder, C. & Donker, A.J.M.: Dose-response effects of fsih-oil supplements in healthy volunteers. Am J Clin Nutr 52: 120-127, 1990.
18. Boberg, M., Vessby, B. & Selinus, I.: Effects of dietary supplementation with n-6 and n-3 long chain polyunsaturated fatty acids on serum lipoprot-eins and platelet function in hypertriglyceridemic patients. Acta Med Scand 220: 153-160, 1986.
19. Borkman, M., Storlien, L.H., Pan, D.A., Jenkins, A.B., Chisholm, D.J. & Campbell, L.V.: The relation between insulin sensitivity and the fatty acid composition of skeletal muscle phospholipids. N Engl J Med 328: 238-244, 1991.
20. Boulanger, C., Schini, V.B., Shimokawa, H., Lüscher, T.F. & Vanhoutte, P.M.: Effect of chronic exposure to cod liver oil and ω-3 unsaturated fatty acids on endothelium-dependent relaxations. In: Vanhoutte, P.M. & Douse-

Blazy P, eds. Fish and bloodvessel interactions. John Libbey Eurotext. Paris. 89-97, 1991.
21. Brattström, I.E., Israelsson, B., Jeppsson, J.O. & Hultberg, B.L.: Folic acid - an innocous means to reduce plasma homocysteine. Scand J Clin Invest 48: 215-221, 1988.
22. Brenner, R.R.: Factors influencing fatty acid chain elongation and desaturation. In: Vergroesen, A.J. & Crawford, M., eds. The role of fats in human nutrition. Academic Press. 45-79, 1988.
23. Brewer, H.B., Rader, D., Fojo, S. & Hoeg, J.M.: Frontiers in the analysis of HDL structure, function and metabolism. In: Carlson, L.A., ed. Disorders of HDL. Eldred Smith-Gordon. 51-58, 1990.
24. Bryant, J., Young, B.C., Mehta, P., Saldeen, T.G.P. & Mehta, J.: Dietary fish oil decreases superoxide radical generation without affecting nitric oxide synthase activity: a mechanism of vasorelaxation. J Am Coll Cardiol 21: 430 A (abstract 819-2), 1993.
25. Bønaa, K.H., Bjerve, K.S., Straume, B., Gram, I.T. & Thelle, D.: Effect of eicosapentaenoic and docosahexaenoic acids on blood pressure in hypertension. A population-based intervention trial from the Tromsø study. N Engl J Med 322: 795-801, 1990.
26. Burr, M.L., Gilbert, J.F. & Holliday, R.L.: Effects of changes in fat, fish and fibre intake on death and myocardial reinfarction: Diet and reinfarction trial (DART). Lancet II: 757-761, 1989.
27. Carlson, L.A., Böttiger, L. & Ahlfeldt, P.: Risk factors for myocardial infarction in the Stockholm prospective study: a 14-year follow-up focusing on the role of plasma triglycerides and cholesterol. Acta Med Scand 206: 351-360, 1979.
28. Carlson, L.A., Hamsten, A. & Asplund, A.: Pronounced lowering of serum levels of lipoprotein Lp (a) in hyperlipidemic subjects treated with nicotinic acid. J Intern Med 226: 271-276, 1989.
29. Chang, H.R., Dulloo, A.G., Vladoianu, I.R., Piguet, P.F., Arsenijevic, D., Girardier, L. & Pechère, J.C.: Fish oil decreases natural resistance of mice to infection with Salmonella typhimurium. Metabolism 41: 1-2, 1992.
30. Charnock, J.S.: Antiarrhytmic effects of fish oils. In: Simopoulos, A.P., Kifer, R.R., Martin, R.E. & Barlow, S.M., eds. Health effects of ω-3 polyunsaturated fatty acids in seafoods. World Rev Nutr Diet, Karger, Basel. 66: 278-291, 1991.
31. Chester, A.H., O'Neil, G.S.,, Moncada, S., Tadjkarimi, S. & Yacoub, M.H.: Low basal and stimulated release of nitric oxide in atherosclerotic epicardial coronary arteries. Lancet 336: 897-900, 1990.
32. Clarke, R., Daly, L., Robinson, K., Naughten, E., Cahalane, S., Fowler, B. & Graham, I.: Hyperhomocysteinemia: An independent risk factor for vascular disease. N Engl J Med 324: 1149-1155, 1991.
33. Collins, R., Peto, R. & MacMahon, S.: Blood pressure, stroke, and coronary heart disease: part 2, short-term reductions in blood pressure: overview of randomised drug trials in their epidemiologic context. Lancet 355: 827-838, 1990.

34. Crawford, M.A., Doyle, W., Drury, P., Chebremeskel, K., Harbige, L., Leyton, J. & Williams, G.: The food chain for n-6 and n-3 fatty acids with special reference to animal products. In: Galli C, Simopoulos AP, eds. Dietary ω3 and ω6 fatty acids. Plenum Press, New York. 5-19, 1989.
35. Cunnane, S.C., Manku, M.S. & Horrobin, D.F.: Effect of vitamin B-6 deficiency on essential fatty acid metabolism. In: Reynolds, R.D. & Leklem, J.E., eds. Vitamin B-6: Its role in health and disease, New York: Alan R. Liss Inc. 447-451, 1985.
36. Cunnane, S.C.: What is the nutritional and clinical significance of α-linolenic acid in humans? In: Sinclair, A. & Gibson, R., eds. Essential fatty acids and eicosanoids. American Oil Chemists'Society, Champaign, Illinois. 379-382, 1992.
37. Davey Smith, G., Song, F. & Sheldon, T.A.: Cholesterol lowering and mortality: the importance of considering initial level of risk. Brit Med J 306: 1367-1373, 1993.
38. Davies, H.R., Bridenstine, R.T., Vesselinovitch, D. & Wissler, R.W.: Fish oil inhibits development of atherosclerosis in rhesus monkeys. Atherosclerosis 7: 441-449, 1987.
39. Davies, M.J. & Woolf, N.: Atherosclerosis: what is it and why does it occur? Br Heart J 69, suppl.: S3-S11,1993.
40. Dawson, S. & Henney, A.: The status of PAI-1 as a risk factor for arterial and thrombotic disease: A review. Atherosclerosis 95: 105-117, 1992.
41. Dehmer, G.J., Pompa, J.J. & Van de Berg, E.K.: Reduction in the rate of early restenosis after coronary angioplasty by a diet supplemented with n-3 fatty acids. N Engl J Med 319: 733-740, 1988.
42. Dolecek, T.A. & Grandits, G.: Dietary polyunsaturated fatty acid and mort-ality in the multiple risk factor intervention trial (MRFIT). In: Simopoulos, A.P., Kiferm, R.R., Martin, R.E. & Barlow, S.M., eds. Health effects of ω-3 polyunsaturated fatty acids in seafoods. World Rev Nutr Diet, Karger, Basel. 66: 205-217, 1991.
43. Dolecek, T.A., Shaten, J. & Stamler, J.: Dietary ω-fatty acids and mortality in the Multiple Risk Factor Intervention Trial. Omega-3 fatty acids. Metabolism and biological effects. Congress abstracts. Oslo, Norway. Abstr. 9, 1992.
44. Dormandy, T.L.: In praise of peroxidation. Lancet II: 1126-1128, 1988.
45. Drevon, C.A.: Marine oils and their effects. Nutr Rev 50: 38-45, 1992.
46. Dwyer, J.T.: Assessment of dietary intake. In: Shils, M.E. & Young, V.R.: Modern nutrition in health and disease. 7th ed. Lea & Febiger, Philadelphia. 887-905, 1988.
47. Dyerberg, J. & Bjerregaard, P.: Mortality from ischaemic heart disease and cerebrovascular disease in Greenland. In: Lands, W.E.M., ed. Proceedings of the AOCS short course on polyunsaturated fatty acids and eicosanoids. Biloxi, Miss., American Oil Chemists Society. 2-8, 1987.
48. Dyerberg, J. & Schmidt, E.B.: Modulation of atherothrombogenesis by n-3 polyunsaturated fatty acids. In: Sinclair, A. & Gibson, R., eds. Essential

fatty acids and eicosanoids. American Oil Chemists'Society, Champaign, Ill. 293-296, 1992.
49. Edelberg, J.M., Gonzales-Gronow, M. & Pizzo, S.V.: Lipoprotein (a) inhibition of plasminogen activation by tissue-type plasminogen activator. Thromb Res 57: 155-162, 1990.
50. El Boustani, S., Colette, C., Monnier, L., Descomps, B., Crastes de Paulet, A.C. & Mendy, F.: Enteral absorption in man of eicosapentaenoic acid in different chemical forms. Lipids 22: 711-714, 1987.
51. Emeis, J.J., Van Houvlingen, A.C. & Van Den Hoogen, C.M.: A moderate fish intake increases plasminogen activator inhibitor type-1 in human volunteers. Blood 74: 233-237, 1989.
52. Endres, S., Ghorbani, R., Kelley, V.E., Georgilis, K., Lonnemann, G., Klempner, M.S. Weber, P.C., Schaefer, E.J., Woolff, S.M. & Dinarello, C.A.: The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. N Engl J Med 320: 265-271 1989.
53. Ernst, E.: Effects of n-3 fatty acids on blod rheology. J Intern Med 225: 129-132, 1989.
54. Ernst, E.: The role of fibrinogen as a cardiovascular risk factor. Atherosclerosis 100: 1-12, 1993.
55. Esterbauer, H., Wäg, G. & Puhl, H.: Lipid peroxidation and its role in atherosclerosis. Brit Med Bull 49: 566-576, 1993.
56. Falk, E.: Why do plaques rupture? Circulation 86, suppl. III: III-39-II-42, 1992.
57. Federal Register.: Food labeling: Health claims and label statement: Omega-3 fatty acids and coronary heart disease. 56 no. 229: 60663-60689, 1991.
58. Fischer, S., Weber, P.C.: Prostaglandin I_3 is formed in vivo in man after dietary eicosapentaenoic acid. Nature 307: 165-168, 1984.
59. Fitzgerald, G.A., Braden, G., Fitzgerald, D.J. & Knapp, H.R.: Fish oils in cardiovascular disease. J Intern Med 225, suppl. 1: 25-29, 1989.
60. Fumeron, F., Brigant, L., Ollivier, V., de Prost, D., Driss, F., Darcet, P., Bard, J.M., Parra, H.J., Furchart, J.C. & Apfelbaum, M.: n-3 polyunsaturated fatty acids raise low-density lipoproteins, high density lipoprotein 2, and plasminogen-activator inhibitor in healthy young men. Am J Clin Nutr 54: 118-122, 1991.
61. Förstermann, U., Schmidt, H., Pollock, J.S., Sheng, H., Mitchell, J.A., Warner, T.D., Nakane, M. & Murad, F.: Isoforms of nitric oxide synthase. Characterization and purification from different cell types. Biochem Pharmacol 42: 1849-1857, 1991.
62. Gans, R.O.B., Bilo, H.J.G., Schouten, J.A. & Rawerda, J.A.: Fish oil and fibrinogen. Brit Med J 297: 978-979, 1988.
63. Gapinski, J.P., Van Ruiswyk, J.V., Heudebert, G.R. & Schectman, G.S.: Preventing restenosis with fish oils following coronary angioplasty. A meta-analysis. Arch Intern Med 153: 1595-1601, 1993.
64. Gavish, D., Azrolan, N., Cundey, K. & Breslow, J.L.: Fish oil reduces

plasma Lp(a) levels and affects postprandial association of apo(a) with triglyceride rich lipoproteins. Clin Res: abstract 250, 1990;
65. Glauber, H., Wallance, P., Griver, K. & Brechtel, G.: Adverse effects of omega-3 fatty acids in noninsulin-dependent diabetes mellitus. Ann Intern Med 108: 663-668, 1988.
66. Goodnight, S.H., Fischer, M., Fitzgerald, G.A. & Levine, P.H.: Assessment of the therapeutic use of dietary fish oil in atherosclerotic vascular disease and thrombosis. Chest 95: 19S-24S, 1989.
67. Goodnight, S.H.: Fish oil: effects on atherogenesis and thrombosis. Curr Opin Lipidol 1: 334-340, 1990.
68. Granger, D.L.: Macrophage production of nitrogen oxides in host defence against microorganisms. Res Immunol 142: 570-572, 1991.
69. Granström, E.: Omega-3 polyunsaturated fatty acids: Biochemical actions. In: Somogyi, J.C. & Hötzel, D., eds. Marine foods. Bibl Nutr Dieta. Basel, Karger. 87-94, 1990.
70. Grayston, J.T.: Chlamydia in atherosclerosis. Circulation 87: 408-409, 1993.
71. Grundy, S.M. & Vega, G.L.: Two different views of the relationship of hypertriglyceridemia to coronary heart disease. Implication for treatment. Arch Intern Med 152: 28-34, 1992.
72. Grønn, M.: Metabolism of n-3 fatty acids. Studies of β-oxidation and desaturation in isolated hepatocytes and human fibroblasts. Thesis. Oslo 1992.
73. Gustafson, A., Elmfeldt, D., Wilhelmsen, L. & Tibblin, G.: Serum lipids and lipoproteins in men after myocardial infarction compared with representative population sample. Circulation 46: 709-716, 1972.
74. Göttlicher, M., Widmark, E., Li, Q. & Gustafsson, J.Å.: Fatty acids activate a chimera of the clofibric acid-activated receptor and the glucocorticoid receptor. Proc Natl Acad Sci 89: 4653-4657, 1992.
75. Haglund, O., Wallin, R., Luostarinen, R. & Saldeen, T.: Effects of a new fluid fish oil concentrate, ESKIMO-3, on triglycerides, cholesterol, fibrinogen and blood pressure. J Intern Med 227: 347-353, 1990.
76. Haglund, O., Luostarinen, R., Wallin, R. & Saldeen, T.: Effects of fish oil on triglycerides, cholesterol, lipoprotein (a), atherogenic index and fibrinogen. Influence of degree of purification of the oil. Nutr Res 12: 455-468, 1992.
77. Haglund, O., Luostarinen, R., Wallin, R., Wibell, L. & Saldeen, T.: The effects of fish oil on triglycerides, cholesterol, fibrinogen and malondialdehyde in humans supplemented with vitamin E. J Nutr 121:165-169, 1991.
78. Haglund, O., Hamfelt, A., Hambraeus, L. & Saldeen, T.:Effects of fish oil supplemented with pyridoxine and folic acid on homocysteine, atherogenic index, fibrinogen and plasminogen activator inhibitor-1 in man. Nutr Res 1993, in press.
79. Haglund, O., Wallin, R., Wretling, S., Hultberg, B. & Saldeen T.: Effects

of fish oil alone and combined with long chain (n-6) fatty acids on some coronary risk factors in man. J Nutr Biochem 1993, in press.
80. Haglund, O., Mehta, J.L. & Saldeen, T.: Effects of fish oil on some parameters of fibrinolysis and lipoprotein (a) in man. Am J Cardiol 1993, in press.
81. Haglund, O., Dutertre, Y. & Saldeen, T.: Eicosapentaenoic acid decreases nitric oxide activity and increases cell viability in a stimulated macrophage cell line. Possible implications for the involvement of (n-3) fatty acids in the protection against cardiovascular and inflammatory diseases. Manuscript.
82. Hagve, T.A., Christensen, E., Gronn, M. & Christophersen, B.O.: Regulation of the metabolism of polyunsaturated fatty acids. Scand J Clin Lab Invest 48, suppl. 191: 33-46, 1988.
83. Hamsten, A., Wiman, B., de Faire, U. & Blombäck, M.: Increased plasma level of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. N Engl J Med 313: 1557-1563, 1985.
84. Hamsten, A.: Hypertriglyceridemia, triglyceride-rich lipoproteins and coronary heart disease. In: Baillière's Clinical Endocrinology And Metabolism: Lipid and Lipoprotein Disorders. 4: 895-922, 1990.
85. Hamsten, A.: The hemostatic system and coronary heart disease. Thromb Res 70: 1-38, 1993.
86. Harris, W.S.: Fish oils and plasma lipid and lipoprotein metabolism in humans: a critical review. J Lipid Res 30: 785-807, 1989.
87. Heller, R.F., Chinn, S., Tunstall-Pedoe, H.D. & Rose, G.: How well can we predict coronary heart disease? Br Med J 288: 1409-1411; 1984.
88. Herrmann, W., Biermann, J., Lindhofer, H.G. & Kostner, G.: Beeinflussung des atherogenen Risikofaktors Lp(a) durch supplementäre Fischölaufnahme bei Patienten mit moderatem physischen Training. Med Klin 84: 429-433, 1989.
89. Hibbs, J.B., Taintor, R.R., Vavrin, Z. & Rachlin, E.M.: Nitric oxide: a cytotoxic activated macrophage effector molecule. Biochem Biophys Res Commun 157: 87-94, 1988.
90. Hibbs, J.B.: Synthesis of nitric oxide from L-arginine: a recently discovered pathway induced by cytokines with antitumour and antimicrobial activity. Res Immunol 142: 565-569, 1991.
91. Hills, M. & Armitage, P.: The two-period cross-over clinical trial. Br J Pharmac 8: 7-20, 1979.
92. Hirai, A., Terano, T., Tamura, Y. & Yoshida, S.: Eicosapentaenoic acid and adult diseases in Japan: epidemiological and clinical aspects. J Intern Med 225, suppl. 1: 69-75, 1989.
93. Hogg, B., Darley-Usmar, V.M., Graham, A. & Moncada, S.: Peroxynitrite and atherosclerosis. Biochem Soc Trans 21: 358-362, 1993.
94. Holme, I.: An analysis of randomized trials evaluating the effect of cholesterol reduction on total mortality and coronary heart disease incidence. Circulation 82: 1916-1924, 1990.
95. Hopkins, P.N. & Williams, R.R.: A survey of 246 suggested coronary risk factors. Atherosclerosis 40: 1-52, 1981.

96. Hornstra, G.: The significance of fish and fish oil enriched food for prevention and therapy of ischemic cardiovascular disease. In: The role of fats in human nutrition, 2nd ed. Vergroessen, A.J. & Crawford, M., eds. Academic Press, London. 152-235, 1989.
97. Horrobin, D.F. & Manku, M.S.: Clinical biochemistry of essential fatty acids. In: Horrobin, D.F., ed. Omega-6 essential fatty acids. Pathophysiology and roles in clinical medicine. New York: Wiley-Liss. 21-53, 1990.
98. Huang, Y.S. & Nassar, B.A.: Modulation of tissue fatty acid composition, prostaglandin production and cholesterol levels by dietary manipulation of n-3 and n-6 essential fatty acid metabolites. In: Horrobin, D.F., ed. Omega-6 essential fatty acids. Pathophysiology and roles in clinical medicine. New York: Wiley-Liss. 21-53, 1990.
99. Hughes, G.S., Ringer, T.V., Watts, K.C., DeLoof, M.J., Framcom, S.F. & Spillers, C.R.: Fish oil produces an atherogenic lipid profile in hypertensive men. Atherosclerosis 84: 229-237, 1990.
100. Högman, M., Frostell, C., Arnberg, H. & Hedenstierna, G.: Bleeding time prolongation and NO inhalation. Lancet 341: 1664, 1993.
101. Høstmark, A.T., Bjerkedal, T., Kierulf, P., Flaten, H. & Ulshagen, K.: Fish oil and plasma fibrinogen. Br Med J 297: 180-181, 1988.
102. Jessup, W. & Dean, R.: Autoinhibition of murine macrophage-mediated oxidation of low-density lipoprotein by nitric oxide synthesis. Atherosclerosis 101: 145-155, 1993.
103. Jürgens, G., El-Saadani, M., Streigl, G. & Esterbauer, H.: LDL modified by endogenous lipid peroxidation products. In: Steinberg, D., ed. Modified lipoproteins. 8th Internatonal symposium on atherosclerosis. Venice. 13-20, 1988.
104. Karmali, R.A.: n-3 fatty acids and cancer. J Intern Med 225, suppl. 1: 197-200, 1989.
105. Knapp, H.R.: Hypotensive effects of w-3 fatty acids: Mechanistic aspects. In: Health effects of ω-3 polyunsaturated fatty acids in seafoods. World Rev Nutr Diet, Karger, Basel. 66: 313-328, 1991.
106. Kolb, H. & Kolb-Bachofen, V.: Nitric oxide: a pathogenetic factor in autoimmunity. Immunol Today 13: 157-160, 1992.
107. Kremer, J.M., Jubiz, W. & Michalek, A.: Fish-oil supplementation in active rheumatoid arthritis. Ann Intern Med 106: 497-503, 1987.
108. Kromann, N. & Green, A.: Epidemiological studies in the Upernavik District, Greenland: incidence of some chronic diseases 1959-1974. Acta Med Scand 208: 401-406, 1980.
109. Kromhaut, D., Bosschieter, E.B. & de Lezenne Coulander, C.: The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N Engl J Med 312: 1205-1209, 1985.
110. Landymore, R.W., MacAulay, M., Sheridan, B. & Cameron, C.: Comparison of cod-liver oil and aspirin-dipyridamole for the prevention of intimal hyperplasia in autologous vein grafts. Ann Thorac Surg 41: 54-57, 1986.
111. Lawson, D.L., Mehta, J.L., Saldeen, K., Mehta, P. & Saldeen, T.G.P.: Omega-3 polyunsaturated fatty acids augment endothelium-dependent

vasorelaxation by enhanced release of EDRF and vasodilator prostaglandins. Eicosanoids 4: 217-223, 1991.
112. Leaf, A. & Weber, P.C.: A new era for science in nutrition. Am J Clin Nutr 45: 1048-1053, 1987.
113. Leaf, A.: Health claims: Omega-3 fatty acids and cardiovascular disease. Nutr Rev 50: 150-154, 1992.
114. Lee, T.H., Hoover, R.L., Williams, J.D., Sperling, R.I., Ravalese III, J., Spur, B.W., Robinson, D.R., Corey, E.J., Lewis, R.A. & Austen, K.F.: Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. N Engl J Med 312: 1217-1224, 1985.
115. Lipton, S.A., Choi, Y.B., Pan, Z.H., Lei, S.Z., Chen, H.S.V., Sucher, N.J., Loscalzo, J., Singel, D.J. & Stamler, J.S.: A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. Nature 364: 626-632, 1993.
116. Loscalzo, J.: Lipoprotein (a). A unique risk factor for atherothrombotic disease. Arteriosclerosis 10: 672-679, 1990.
117. Martin, J.H.J. & Edwards, S.W.: Changes in the mechanisms of monocyte/macrophage-mediated cytotoxity during culture. Reactive oxygen intermediates are involved in monocyte-mediated cytotoxity, wheras reactive nitrogen intermediates are employed by macrophages in tumor cell killing. J Immunol150: 3478-3486, 1993.
118. Mascioli, E.A., Yoshie, I., Trimbo, S., Leader, L., Bistrian, B.R. & Blackburn GL. Endotoxin challenge after menhaden oil diet; effects on survival of guinea pigs. Am J Clin Nutr 49: 277-282, 1989.
119. McCully, K.S.: Homocysteine theory of arteriosclerosis; development and current status. Atherosclerosis Rev 11: 157-246, 1983.
120. McCully, K.S., Olszewski, A.J. & Vezeridis, M.P.: Homocysteine metabolism in atherogenesis: effect of the homocysteine thiolactonyl derivatives, thioretinaco and thioretinamide. Atherosclerosis 83: 197-206, 1990.
121. Mehta, J., Lawson, D. & Saldeen, T.: Reduction in plasminogen activator inhibitor-1 (PAI-1) with omega-3 polyunsaturated fatty acid (PUFA) intake. Am Heart J 116: 1201-1206, 1988.
122. Mensink, .RP.: Effect of the individual saturated fatty acids on serum lipids and lipoprotein concentrations. Am J Clin Nutr 57, suppl.: 711S-714S, 1993.
123. Moncada, A., Palmer, R.M.J. & Higgs, E.A.: Nitric oxide: Physiology, pathophysiology, and pharmacology. Pharmacol Rev 43: 109-142, 1991.
124. Moncada, S., Martin, J.F. & Higgs, A.: Symposium on regression of atherosclerosis. Eur J Clin Invest 23: 385-398, 1993.
125. Morris, M.C., Taylor, J.O., Stampfer, M.J., Rosner, B. & Sacks, F.M.: The effect of fish oil on blood pressure in mild hypertensive subjects: a randomized cross-over trial. Am J Clin Nutr 57: 59-64, 1993.
126. Morris, M.C., Sacks, F. & Rosner, B.: Does fish oil lower blood pressue? A meta-analysis of controlled trials. Circulation 88: 523-533, 1993.
127. Mudd, S.H., Levy, H.L. & Skovby, F.: Disorders of transsulfuration. In:

Scriver, C.R., Beaudet, A.L., Sly, W.S. & Valle, D., eds. The metabolic basis of inherited disease. Alan Liss, Inc, New York. 482-519, 1989.
128. Müllertz, A., Hølmer, G. & Grøndahl-Hansen, J.: Increased concentration of plasminogen activator inhibitor type-1 in plasma after intake of fish oil. Fibrinolysis 4, suppl. 2: 86-88, 1990.
129. Møller, J.M., Svaneborg, N., Lervang, H.H., Varming, K., Madsen, P., Dyerberg, J. & Schmidt, E.B.: The acute effect of a single very high dose of n-3 fatty acids on coagulation and fibrinolysis. Thromb Res 67: 569-577, 1992.
130. Nathan, D.M.: Long-term complications of diabetes mellitus. N Engl J Med 328: 1676-1685, 1993.
131. Nelson, G.J. & Ackman, R.G.: Absorption and transport of fat in mammals with emphasis on n-3 polyunsaturated fatty acids. Lipids 23: 1005-1014, 1988.
132. Nenseter, M.S., Rustan, A.C., Lund-Katz, S., Søyland, E., Mælandsmo, G., Phillips, M.C., Drevon, C.A.: Effect of dietary supplementation with n-3 polyunsaturated fatty acid on physical properties and metabolism of low-density lipoprotein in humans. Arteriosclerosis Thromb 12: 369-379, 1992.
133. Nestel, P.J.: Effect of fish oils on plasma lipids, atherosclerosis and the heart. In: Sinclair, A. & Gibson, R., eds. Essential fatty acids and eicosanoids. AOCS, Champaign, Ill. 248-251, 1993.
134. Nettleton, J.A.: Are n-3 fatty acids essential nutrients for fetal and infant development? J Am Diet Ass 93: 58-64, 1993.
135. Newman, W.P., Middaugh, J.P., Propst, M.T., Rogers, D.R.: Atherosclerosis in Alaska Natives and non-natives. Lancet 341: 1056-1057, 1993.
136. Nilsson, I.M. & Olow, B.: Determination of fibrinogen and fibrinolytic activity. Throb Diath Haemorrh 8: 297-310, 1962.
137. Nilsson, I.M., Ljungner, H. & Tengborn, L.: Two different mechanisms in patients with venous thrombosis and defective fibrinolysis: low concentration of plasminogen activator or increased concentration of plasminogen activator inhibitor. Brit Med J 290: 1453-1456, 1985.
138. Nordøy, A., Barstad L,, Connor. W. & Hatcher. L.: Absorption of the n-3 eicosapentaenoic and docosahexaenoic acids as ethyl esters and triglycerides by humans. Am J Clin Nutr 53: 1185-1190, 1991.
139. Nunez, E.A.: Free fatty acids as modulators of the steroid hormone message. Prost Leuk Essent Fatty Acids 48: 63-70, 1993.
140. Olszewski, A.J. & McCully, K.S.: Homocysteine content of lipoproteins in hypercholesterolemia. Atherosclerosis 88: 61-68, 1991.
141. Olszewski, A.J. & McCully, K.S.: Fish oil decreases serum homocysteine in hyperlipemic men. Coron Art Dis 4: 53-60, 1993.
142. Palmblad, J. & Gyllenhammar, H.: Effect of dietary lipids on immunity and inflammation. APMIS 96: 571-583, 1988.
143. Panza, J.A., Quyyumi, A.A., Brush, J.E. & Epstein, S.E.: Abnormal endothelium-dependent relaxation in patients with essential hypertension. N Engl J Med 323: 22-27, 1990.
144. Parks, E.J., Tinker, L.F., Behr, S.R., Scheeman, B.O., Davis, P.A.: Fish oil

supplementation changes LDL particle size in hypertriglyceridemics. Faseb J 7: abstract A870, 1993.
145. Parks, J.S. & Bullock, B.C.: Effect of fish oil versus lard diets on the chemical and physical properties of low density lipoproteins of nonhuman primates. J Clin Invest 28: 173-182, 1987.
146. Phillipson, B.E., Rothrock, D.W., Connor, W.E., Harris, W.S. & Illingworth, D.R.: Reduction of plasma lipids, lipoproteins, and apoproteins by dietary fish oils in patients with hypertriglyceridemia. N Engl J Med 312: 1210-1216. 1985.
147. Pike, M.C.: Antiinflammatory effects of dietary lipid modification. J Rheumatol 16: 718-720, 1989.
148. Popp-Snijders, C., Schouten, J.A., Heine, R.J., van der Meer, J. & van der Veen, E.A.: Dietary supplementation of omega-3 polyunsaturated fatty acids improves insulin sensitivity in non-insulin-dependent diabetes. Diabetes Res. 4: 141-147, 1987.
149. Prins, M.H. & Hirsh, J.A.: A critical review of the relationship between impaired fibrinolysis and myocardial infarction. Am Heart J 122: 545-551, 1991.
150. Pritchard, G.A., Jones, D.L. & Mansel, R.E.: Lipids in breast carcinogenesis. Br J Surg 76: 1069-1073, 1989.
151. Reaven, G.: Role of insulin resistance in human disease. Diabetes 37: 1595-1607, 1988.
152. Ridker, P.M., Vaughan, D.E., Stampfer, M.J., Manson, J.E., Hennekens, C.H.: A prospective study of plasminogen activator inhibitor and the risk of future myocardial infarction. Circulation 86, suppl.: I-325, 1992.
153. Ridker, P.M., Vaughan, D.E., Stampfer, M.J., Manson, J.E., Hennekens, C.H.: Endogenous tissue-type plasminogen activator and risk of myocardial infarction. Lancet 341: 1165-1168, 1993.
154. Robinson, D.R., Xu, L.L., Tateno, S., Guo, M., Colvin, R.B.: Suppression of autoimmune disease by dietary n-3 fatty acids. J Lipid Res 34: 1435-1444, 1993.
155. Rodgers, R.P.C. & Levin, J.: A critical reappraisal of the bleeding time. Semin Thromb Hemostas 16: 1-20, 1990.
156. Ross, R.: The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 362: 801-809, 1992.
157. Rustan, A.C., Nossen, J.Ø., Christiansen, E.N. & Drevon, C.A.: Eicosapentaenoic acid reduces hepatic synthesis and secretion of triacylglycerol by decreasing the activity of acyl-coenzyme A:1,2-diacylglycerol acyltransferase. J Lipid Res 29: 1417-1426, 1988.
158. Sanders, T.A.B.: Influence of ω3 fatty acids on blood lipids. In: Simopoulos, A.P., Kifer, R.R., Martin, R.E. & Barlow, S.M., eds. Health effects of ω3 polyunsaturated fatty acids in seafoods. World Rev Nutr Diet 66: 358-366, 1991.
159. Sarih, M., Souvannavong, V. & Adam, A.: Nitric oxide synthase induces macrophage death by apoptosis. Biochem Biophys Res Commun 191: 503-508, 1993.
160. Saynor, R., Verel, D. & Gillott, T.: The long term effect of dietary

supplementation with fish lipid concentrate on serum lipids, bleeding time, platelets and angina. Atherosclerosis 50: 3-10, 1984.
161. Saynor, R. & Gillott, T.: Changes in blood lipids and fibrinogen with a note of safety in a long term study on the effects of n-3 fatty acids in subjects receiving fish oil supplements and followed for seven years. Lipids 27: 533-538, 1992.
162. Schectman, G., Kaul, S., Cherayil, G.D., Lee, M. & Kissebah, A.: Can the hypotriglyceridemic effect of fish oil concentrate be sustained? Ann Intern Med 110: 346-352, 1989.
163. Schmidt, E.B., Kristensen, S.D. & Dyerberg, J.: The effect of fish oil on lipids, coagulation and fibrinolysis in patients with angina pectoris. Artery 15: 316-329, 1988.
164. Schmidt, E.B., Sørensen, P.J., Ernst, S.D., Kristensen, S.D., Pederson, J.O. & Dyerberg, J.: Studies on coagulation and fibrinolysis in Greenland eskimos. Thromb Res 56: 553-558, 1989.
165. Schmidt, E.B., Varming, K., Ernst, E., Madsen, F.P. & Dyerberg, J.: Dose-response studies on the effect of n-3 polyunsaturated fatty acids on lipids and haemostasis. Thromb Haemost 63: 1-5, 1990.
166. Schmidt, E.B., Klausen, I.C. & Kristensen, S.D.:The effect of n-3 polyunsaturated fatty acids on Lp(a). Clin Chim Acta 198:271-278, 1991.
167. Schmidt, E.B., Lervang, H.H., Varming, K., Madsen, P. & Dyerberg, J.: Long-term supplementation with n-3 fatty acids. I: Effect on blood lipids, haemostasis and blood pressure. Scand J Clin Lab Invest 52: 221-228, 1992.
168. Selhub, J. & Miller, J.W.: The pathogenesis of homocysteinemia: interuption of the coordinate regulation by S-adenosylmethionine of the remethylation and transsulfuration of homocysteine. Am J Clin Nutr 55: 131-138, 1992.
169. Siegbahn, A., Odlind, V. & Hedner, U.: Coagulation and fibrinolysis during the normal menstrual cycle. Upsala J Med Sci 94: 137-153, 1989.
170. Silverman, D.I., Reis, G.J., Sacks, F.M., Boucher, T.M. & Pasternak, R.C.: Usefulness of plasma phospholipid n-3 fatty acid levels in prediciting dietary fish intake in patients with coronary artery disease. Am J Cardiol 66: 860-862, 1990.
171. Simopoulos, A.P.: Omega-3 fatty acids in health and disease and in growth and development. Am J Clin Nutr 54: 438-463, 1991.
172. Singer, P., Wirth, M. & Berger, I.: A possible contribution of decrease in free fatty acids to low triglyceride levels after diets supplemented with n-6 and n-3 polyunsaturated fatty acids. Atherosclerosis 83: 167-175, 1990.
173. Singer, P., Wirth, M., Berger, I., Heinrich, B., Gödicke, W., Voigt, S., Taube, C., Jaross, W. & Gehrisch, S.: Long-chain ω-3 fatty acids are the most effective polyunsaturated fatty acids for dietary prevention and treatment of cardiovascular risk factors. In: Simopoulos, A.P., ed. Nutrients in the control of metabolic diseases. World Rev Nutr Diet. Karger, Basel. 69: 74-112, 1992.

174. Sprecher, H.: Biochemistry of essential fatty acids. Progr Lipid Res 20: 13-22, 1981.
175. Sprecher, H.: Interactions between the metabolism of n-3 and n-6 fatty acids. J Intern Med 225 (suppl. 1): 5-10, 1989.
176. Stamler, J., Wentforth, D., Neaton, J.D.: Is relationship between serum cholesterol and risk of premature death from coronary heart disease continous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). JAMA 256: 2823-2828, 1986.
177. Stamler, R., Grimm, R.H. jr & Dyer, A.R.: Cardiac status after four years in a trial on nutritional therapy for high blood pressure. Arch Intern Med 149: 661-665, 1989.
178. Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C., Witzum, J.L.: Beyond cholesterol: modification of low-density lipoprotein that increase its atherogenecity. N Engl J Med 320: 915-924, 1989.
179. Stout, R.W.: Insulin as a mitogenic factor: role in the pathogenesis of cardiovascular disease. Am J Med 90: 62S-65S, 1991.
180. Sönnerborg, A., Carlin, G., Åkerlund, B., Jarstrand, C.: Increased production of malondialdehyde in patients with HIV infection. Scand J Infect Dis 20: 287-290, 1988.
181. The international committee for the evaluation of hypertriglyceridemia as a vascular risk factor.: The hypertriglyceridemias: Risk and management. Am J Cardiol 68: 1A-42A, 1991.
182. Thiery, J. & Seidel, D.: Fish oil feeding results in an enhancement of cholesterol induced atherosclerosis in rabbits. Atherosclerosis 63: 53-56, 1987.
183. Tinoco, J.: Dietary requirements and functions of α-linolenic acid in animals. Prog Lipid Res 21: 1-45, 1982.
184. Thorngren, M. & Gustafson, A.: Effects of a 11-week increase in dietary eicosapentaenoic acid on bleeding time, lipids, and platelet aggregation. Lancet II: 1190-1193, 1981.
185. Vance, D.E. & Vance, D.E., eds. Biochemistry of lipids, lipoproteins and membranes. Elsevier, 1991.
186. Vessby, B.: n-3 fatty acids and glucose control in diabetes mellitus. J Intern Med 225, suppl. 1: 207-210, 1989.
187. Voss, A., Reinhart, M., Sankarappaa, S. & Sprecher, H.: The metabolism of 7,10,13,16,19-docosapentaenoic acid to 4,7,10,13,16,19-docosahexaenoic acid in rat liver is independent of a Δ4-desaturase. J Biol Chem 266: 19995-20000, 1991.
188. Wahle, K.W.J. & Brown, J.E.: Plasma lipid peroxidation and vitamin E supplementation during high intakes of fish-oil fatty acids. Fat Sci Technol 92: 326-330, 1990.
189. Waldhäusl, W., Ratheiser, K., Komjati, M., Nowotny, P., Pirich, K. & Vierhapper, H.: Increase of insulin sensitivity and improvement of intravenous glucose tolerance by fish oil in healthy man. In: Chandra, R.K., ed. Health effects of fish and fish oils. ARTS Biomedical Publishers & Distributors, St John's, Newfoundland. 171-187, 1989.
190. Wallin, R., Saldeen, T.: Oxidation of n-3 fatty acids. Omega-3 fatty

acids. Metabolism and biological effects. Congress abstracts. Oslo, Norway. Abstr. 119, 1992.
191. Weiner, B.H,. Ockene, I.S. & Levine, P.H.: Inhibition of atherosclerosis by cod-liver oil in a hyperlipidemic swine model. N Engl J Med 315: 841-846, 1986.
192. Weintraub, M.S., Zechner, R., Brown, A., Eisenberg, S. & Breslow, J.L.: Dietary polyunsaturated fats of the omega-6 and omega-3 series reduce postprandial lipoprotein levels. J Clin Invest 82: 1884-1893, 1988.
193. Welin, L., Eriksson, H., Larsson, B., Swärdsudd, K., Wilhelmsen, L. & Tibblin, G.: Risk factors for coronary heart disease during 25 years of follow-up. The study of men born in 1913. Cardiology 82: 223-228, 1993.
194. Welch, W.J.: Mammalian stress response: cell physiology, structure/ function of stress proteins, and implications for medicine and disease. Physiol Rev 72: 1063-1081, 1992.
195. WHO: Diet, nutrition, and the prevention of chronic diseases. WHO Technical Report Series No 797. Geneva. 1990.
196. Wilhelmsen, L., Swärdsudd, K., Korsan-Bengtsen, K., Larsson, B., Welin, C. & Tibblin, G.: Fibrinogen as a risk factor for stroke and myocardial infarction. N Engl J Med 311: 501-505, 1984.
197. Wiman, B. & Hamsten, A.: The fibrinolytic system and its role in the etiology of thromboembolic disease. Semin Thromb Hemostas 16: 207-216, 1990.
198. Yasugi, T., Tochihara, T., Fujioka, T., Saito, E., Kanno, H., Ueno, T., Matsumoto, T., Takahashi, Y., Watanabe, H., Yamada, T. & Saito, N.: Eicosapentaenoic acid, docosahexaenoic acid and cerebral hemorrhage: EPA and DHA loading study in spontaneously hypertensive rats. In: Sinclair, A. & Gibson, R., eds. Essential fatty acids and eicosanoids. American Oil Chemists' Society, Champaign, Illinois. 339-344, 1992.

Correspondence to:

Dr. Olle Haglund
Department of Forensic Medicine
Dag Hammarskjölds väg 17
S-752 37 UPPSALA
Sweden