Marine oils: metabolic effects and role in human nutrition

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Marine oils are rich sources of eicosapentaenoic acid (20:5*n*-3;EPA) and docosahexaenoic acid (22:6*n*-3;DHA). When consumed in small amounts they may contribute towards meeting a requirement for *n*-3 fatty acids and in larger amounts exert pharmacological effects and modify the requirements for other nutrients. Some fish oils, such as herring and mackerel, also contain substantial quantities of cetoleic (22:1*n*-11) and gadoleic (20:1*n*-11). These C_{20-22} monounsaturated fatty acids are oxidized by peroxysomal oxidation similar to erucic acid.

ROLE OF MARINE OILS IN MEETING ESSENTIAL FATTY ACID REQUIREMENTS

The administration of EPA and DHA to rats fed on fat-free diets restores growth but does not correct the dermal abnormalities or those related to an inability to produce active eicosanoids. Small amounts of dietary EPA and DHA can spare linoleic acid (18:2*n*-6) for its unique functions and, thus, can decrease the requirement for linoleic acid. The major argument for the essentiality of *n*-3 fatty acids is related to the high levels of DHA found in human brain and retina. If DHA is physiologically indispensable then the essentiality of α-linolenic acid (18:3*n*-3) hinges on the extent to which it can be converted to DHA. Studies in strict vegetarians, whose diets are devoid of EPA and DHA, and infants fed on artificial formulas show that man can convert α-linolenic acid to EPA but the capacity to make DHA is limited. Preformed dietary DHA appears to be more important in determining the levels in tissues than DHA derived from α-linolenic acid.

ROLE OF DOCOSAHEXAENOIC ACID IN THE VISUAL PROCESS

DHA appears to perform an important physiological function in the retina, especially in the rod-outer segment. Elongated homologues of DHA notably 32:6*n*-3 have been found in retinal phosphatidyl cholines. Abnormal electroretinogram (ERG) recordings were first reported in rats deprived of *n*-3 fatty acids. Neuringer *et al.* (1988) reported a delay in the maturation of the ERG response in monkeys deprived of *n*-3 fatty acids, in particular a decrease in the amplitude of the a-wave and an increased latency in response to light stimulation. The amount of DHA in retinal phospholipids was greatly diminished and had been replaced by docosapentaenoic acid (22:5*n*-6). Supplementing the diet with fish oil increased the level of DHA but surprisingly did not correct the ERG recording (Neuringer *et al.* 1988). This could be interpreted as meaning that an inadequate supply of DHA during a vulnerable phase of development leads to irreversible damage.

Yamamoto *et al.* (1987) found that the learning performance in a brightness-discrimination learning ability test was inferior in animals deprived of *n*-3 fatty acids. Neuringer *et al.* (1988) reported impaired visual acuity and mild polydipsia in animals

deprived of *n*-3 fatty acids. The brain lipids of these animals showed the characteristic reduction in DHA in phospholipids and its replacement with 22:5*n*-6. Mild polydipsia has also been reported by the same workers in rhesus monkeys exposed to both prenatal and postnatal deprivation of *n*-3 fatty acids (Reisbick *et al.* 1992).

Implications for infant nutrition

The development of brain and retina occurs *in utero* and in infancy. It is relevant, therefore, to consider the metabolism of *n*-3 fatty acids in human infants. Most DHA accumulates in the foetus between the 26th and 40th week of gestation. Infants born before week 32 of gestation have low brain concentrations of DHA (and limited hepatic stores). Maternal diet influences the fetal stores of *n*-3 fatty acids and infants born to vegetarian mothers have lower proportions of DHA in their blood and tissue lipids (Sanders & Reddy, 1992). Preterm infants fed on formula devoid of DHA have lower levels of DHA in their erythrocyte phospholipids compared with those fed on breast-milk even though their intakes of linoleic and α-linolenic acids were similar. Levels of DHA in the blood phospholipids of the infants could only be attained by the addition of DHA to preterm formula in an amount equivalent to that present in breast-milk (Liu *et al.* 1987; Uauy *et al.* 1990): it was estimated that the amount of DHA required was 11 mg/kg body weight per d, in order to maintain the proportion of DHA in plasma and erythrocyte phospholipids the same as that in breast-fed infants.

Term infants fed on breast-milk substitutes have approximately half the level of DHA in their erythrocyte phospholipids compared with omnivore breast-fed infants. This is also the case in infants receiving formulas containing similar amounts of linoleic and α-linolenic acids to those found in breast-milk (Clark *et al.* 1992). Farquarson *et al.* (1992) obtained brains from infants who had died from sudden infant death and found about 10% less DHA in the cerebral phospholipids of formula-fed infants compared with breast-fed infants. The higher proportion of DHA in the brain lipids of breast-fed infants has been attributed to the presence of preformed DHA in breast-milk.

Human milk contains significant but variable amounts of DHA. For example, the proportion of DHA is lower (0·2 *v.* 0·6%) in strict vegetarians than in omnivores (Sanders & Reddy, 1992). The proportion of DHA in breast-milk is increased following the consumption of fish oil (Harris *et al.* 1984) and the proportion of DHA is highest in Eskimos where it accounts for 1·4% of the total fatty acids (Innis & Kuhnlein, 1984). Thus, the range of DHA in breast-milk is 0·2–1·4% of the total fatty acids or 0·1–0·7% of the dietary energy.

Uauy *et al.* (1990) compared visual function in four groups of preterm infants given either breast-milk or formula supplemented with DHA, supplied as fish oil, or formula supplemented with α-linolenic acid, or unsupplemented formula. Visual function was significantly impaired in infants deprived of *n*-3 fatty acids. Compared with the breast-fed infants, the visual function was similar in the formula-fed infants given the DHA. Visual function in the group given α-linolenic acid was not significantly different from that of breast-fed infants or that of the unsupplemented group; their indices of visual function were intermediate between the two groups. Lucas *et al.* (1992) found that children who had been fed previously on a preterm formula devoid of DHA performed less well on intelligence tests aged 7–8 years compared with those who had received breast-milk. Whether these findings apply to term infants is a matter for further investigation.

However, commercial formulas devoid of DHA have been in use as breast-milk substitutes for many years without any obvious ill-effects on physical and mental development. Yet it does not exclude the possibility that there may be subtle functional differences. Recent reports (for example, British Nutrition Foundation, 1992) have taken the view that the fatty acid composition of breast-milk substitutes should emulate that of breast-milk and contain both *n*-6 and *n*-3 fatty acids. Consequently, some manufacturers are adding fish oil as a source of DHA to breast-milk substitutes.

ROLE OF *n*-3 POLYUNSATURATED FATTY ACIDS AS PHARMACOLOGICAL AGENTS

High intakes of marine oil containing EPA and DHA display several pharmacological properties: inhibition of inflammation, anti-thrombotic effects, altered lipoprotein metabolism, inhibition of atherosclerosis, inhibition of tumour growth. Not all the effects are beneficial. For example, fish oil may cause a deterioration in glucose tolerance in non-insulin-dependent diabetics (Vessby & Boberg, 1990). Fish oil supplements have not been shown to be of benefit in asthma and may exacerbate symptoms, especially in patients who are sensitive to aspirin, probably EPA is rediverted down the lipoxygenase (*EC* 1.13.11.12) pathway to form leukotriene C_5 and D_5 which are potent broncho-constrictors.

Many of the effects resulting from the consumption of fish oils can be explained by changes in the balance between *n*-6 and *n*-3 fatty acid in membranes. This may affect the physical properties of the membrane as well as the activity of membrane-bound enzymes. It can also be argued that the EPA plays an important role in modulating the production of proinflammatory and prothrombotic eicosanoids derived from arachidonic acid. The partial replacement of arachidonic acid with EPA in membrane lipids decreases the production of active eicosanoids in two ways: (1) by leading to the generation of less active eicosanoids derived from EPA, (2) by inhibiting the formation of eicosanoids from arachidonic acid (Sanders, 1988). DHA contributes to this effect by acting as an inhibitor of cyclooxygenase.

n-3 FATTY ACIDS AND REPRODUCTIVE FUNCTION

The consumption of considerable amounts of menhaden oil (50 ml/d) for 1 month by healthy male volunteers did not influence sperm count or motility but was accompanied by a reduction in concentrations of prostaglandins in PGE_2 and PGF_2 and an increase in PGE_3 and PGF_3 (Knapp, 1990). Decreased prostaglandin PGE_2 production by uterus and delayed parturition occur in animals fed on fish oil. It is also known that increased rates of prostaglandin PGE_2 production are associated with premature birth and that the onset of labour can be delayed by drugs that inhibit the formation of PGE_2. Prolonged gestation and increased birth weight have been observed among the population of the Faro Isles, who have a high intake of fish. Consequently it was argued that the consumption of EPA and DHA prolonged the duration of pregnancy. Fish oil supplements have subsequently been found to prolong gestation in women (Olsen *et al.* 1992) without any obvious adverse effects on the outcome of pregnancy. It is of interest to note that a shorter duration of pregnancy has been reported in vegetarians (Reddy *et al.* 1993) whose diets are devoid of EPA and DHA.

INFLUENCES OF *n*-3 FATTY ACIDS ON THE IMMUNE SYSTEM AND THE INFLAMMATORY RESPONSE

Greenland Eskimos on their traditional diet, which is rich in marine oils, have a low incidence of inflammatory disorders. The consumption of fish oils containing EPA and DHA, besides decreasing the production of inflammatory eicosanoids, also decreases the production of interleukin-1 and other cytokines (Endres *et al.* 1989). The metabolic response to interleukin-1 may also be attenuated by dietary *n*-3 fatty acids provided as fish oil (Cooper *et al.* 1992). Moderate intakes (2–4% of dietary energy) of EPA and DHA do not appear to be immunosuppressive in animal models (Trocki *et al.* 1987; Hinds & Sanders, 1993), although high intakes (>10% energy) do suppress the Host *v.* Graft response (Hinds & Sanders, 1993) and the development of autoimmune disease (Robinson, 1991). Animal studies have generally found that diets rich in *n*-3 fatty acids decrease the inflammation in experimental arthritis (Cathcart & Gonnerman, 1991). Anti-inflammatory effects of EPA appear to occur at lower doses than the immunosuppressive effects. For example, the injection of carrageenin into the foot paw of the rat or the implantation of a sponge soaked in carrageenin in the abdominal cavity elicits an inflammatory response which is decreased by relatively small amounts of dietary EPA (Terano *et al.* 1986).

Several clinical trials have evaluated the influence of fish oil supplements (typically providing 3–6 g *n*-3 fatty acids/d) in several autoimmune diseases. One small study has examined the influence of fish oil supplementation on patients with multiple sclerosis (Bates *et al.* 1989). Although there was a tendency for fewer relapses, this was not statistically significant. Three clinical trials (Clark *et al.* 1989; Westberg & Tarkowski, 1990; Walton *et al.* 1991) on systemic lupus erythematosus have been conducted. The first trial, which was an open study, showed no benefit whereas the latter two studies, which were larger, better controlled and used higher doses, showed mild to moderate symptomatic improvement. Hawthorne *et al.* (1992) reported mild clinical benefit and decreased requirement for corticosteroids in a double-blind controlled trial of fish oil in colitis.

Mild to moderate symptomatic improvement has been reported in trials with patients suffering rheumatoid arthritis (Kremer *et al.* 1985, 1987, 1990; Cleland *et al.* 1988; Skoldstam *et al.* 1992). Supplements of cod-liver oil were not found to be of benefit in patients with severe osteoarthritis (Stammers *et al.* 1992). Fish oil supplements have been found to decrease the need for non-steroidal anti-inflammatory medication (Belch *et al.* 1988; Skoldstam *et al.* 1992).

A mild improvement in psoriasis and psoriatic arthritis has been observed in the majority of studies in subjects given fish oil supplements containing EPA and DHA (Ziboh *et al.* 1986; Maurice *et al.* 1987; Bittiner *et al.* 1988; Lassus *et al.* 1990).

Over all it can be seen that EPA and DHA have mild anti-inflammatory effects. However, there are still many problems to be solved before dietary supplements of this nature can be recommended as standard treatment. Further studies need to be carried out to investigate the effects on cartilage and bone erosion in view of the well-known adverse effects of non-steroidal anti-inflammatory drugs.

n-3 FATTY ACIDS AND CANCER

The majority of studies have shown a significant reduction in the growth of transplantable mammary tumours (both human and rodent) when the animals were fed on a diet

rich in *n*-3 fatty acids (Cave, 1991). It is possible that part of this effect is mediated by way of the effect of lipid peroxides, especially as many chemotherapeutic agents generate free radicals that are cytotoxic. A diet containing 100 g MaxEPA (Seven Seas Ltd, Hull)/kg fed to nude mice increased sensitivity of transplanted human mammary tumours to two anti-neoplastic agents, mitomycin C and doxorubicin (Borgeson *et al.* 1989). Tisdale & Dhesi (1990) found that fish oil containing EPA and DHA at a level of 25–50% total energy in a colonic tumour model had a greater anti-tumour effect than conventional cytotoxic drugs without the attendant toxicity, thus promising a more specific anti-tumour effect. The anti-tumour effect of the fish oil has been attributed to its content of EPA rather than DHA (Tisdale & Beck, 1991). EPA also was found to have a powerful anti-cachectic effect.

Colo-rectal polyps are associated with increased risk of developing colon cancer. Anti *et al.* (1992) investigated the effects of fish oil supplementation on rectal mucosal proliferation in a double-blind, placebo-controlled study of twenty patients with sporadic adenomatous colo-rectal polyps. In the group of ten patients that received fish oil containing EPA (4·1 g/d) and DHA (3·6 g/d) for 12 weeks, the mean percentage of replicative S-phase cells in the upper part of colonic crypts (considered a reliable marker of colon cancer risk) significantly dropped from the baseline level after only 2 weeks of treatment and remained lower throughout the study period; no change in upper-crypt labelling was observed in the ten placebo patients. Rectal mucosal EPA content increased in fish oil-treated patients, whereas arachidonic acid levels decreased. In this short-term trial, fish oil appeared to exert a rapid effect that may protect high-risk subjects from colon cancer.

So far only one clinical evaluation of *n*-3 fatty acids on human cancer has been made. In this study twelve patients with breast cancer who had received substantial therapy previously were given MaxEPA capsules providing 3·6 g EPA and 2·4 g DHA (Holroyde *et al.* 1988). A measurable clinical response was observed in two patients and no side effects were observed. This result would warrant further evaluation using pure fatty acids or their derivatives enabling higher doses to be achieved.

INFLUENCE ON RISK FACTORS FOR CARDIOVASCULAR DISEASE

Blood lipids

It is now fairly well established that elevated levels of certain lipoproteins increase the risk of coronary atherosclerosis, in particular high levels of very-low-density lipoprotein (VLDL), intermediate-density lipoproteins (IDL), low-density lipoprotein (LDL), apolipoprotein B (apoB) and lipoprotein(a) Lp(a). On the other hand, low levels of high-density lipoprotein (HDL) and apoAI are associated with increased risk. A reduction in VLDL and LDL concentrations by aggressive lipid-lowering drug therapy halts progression and may even lead to regression of coronary atherosclerosis. There is a strong correlation between the degree of prevention of progression/regression and change in LDL-cholesterol concentrations.

Normolipidaemic subjects. Both EPA and DHA, but not α-linolenic acid, markedly lower plasma triacylglycerols and VLDL concentrations (Harris, 1989; Sanders *et al.* 1989). Total or LDL-cholesterol concentrations are not usually influenced by moderate intakes (about 3 g *n*-3 fatty acids/day) of fish oil rich in EPA and DHA. However, Fumeron *et al.* (1991) claimed that as little as 6 g MaxEPA given as a supplement to

subjects consuming a high intake of saturated fatty acids (20·9% energy) and cholesterol (about 600 mg/d) raised LDL-cholesterol by about 6% and HDL_2-cholesterol by 12%. Very high intakes of fish oil (24 g C_{20-22} *n*-3/d) do lower the concentration of both LDL-cholesterol and LDL-apoB (Harris, 1989).

Moderate intakes of EPA and DHA, either as fish oil or ethyl esters, increase HDL_2-cholesterol but high intakes decrease HDL-cholesterol, as seen with very high intakes of linoleic acid (Harris, 1989; Sanders *et al.* 1989). This is accompanied by a decrease in both cholesterol transfer protein and lecithin-cholesterol acyltransferase (*EC* 2.3.1.43) activities (Abbey *et al.* 1990) and these changes might explain the increase in HDL_2:HDL_3. The increase in the average HDL particle size probably reflects a reduced cholesteryl ester acceptor capacity within the smaller pool of VLDL, as well as the decline in lipid transfer activity in plasma involving transfer protein itself, LDL and HDL. It might also explain the decrease in cholesterol:apoB in LDL observed in some studies (Nenseter *et al.* 1992).

Fish oil leads to less postprandial lipaemia compared with olive oil (Sanders *et al.* 1989) probably as a result of decreased VLDL production. Prolonged treatment with *n*-3 fatty acids leads to a marked increase in chylomicron clearance (Harris, 1989). Brown & Roberts (1991) showed that as little as 5 g MaxEPA, providing 1·8 g *n*-3 fatty acids/d, significantly decreased postprandial lipaemia in healthy subjects.

Hyperlipidaemic subjects. Numerous studies have examined the influence of *n*-3 fatty acids in patients with hyperlipoproteinaemias. In many studies *n*-3 fatty acids, usually provided as fish oils, have been compared with supplements of maize oil or olive oil. The observed responses to *n*-3 fatty acids differ according to World Health Organization lipoprotein phenotypes and have been reviewed elsewhere (Sanders, 1991).

Patients with type IIa hyperlipoproteinaemia have raised LDL concentrations usually due to decreased removal of LDL by hepatic apoB receptors. Although fish oil supplements providing up to 5 g *n*-3 fatty acids/d lower plasma triacylglycerols and VLDL concentrations, they have little effect on total or LDL-cholesterol concentrations in these patients or in patients with mild to moderate hypercholesterolaemia. Higher doses of EPA and DHA have modest LDL-cholesterol-lowering effects (Davidson *et al.* 1991; Friday *et al.* 1991) in patients with familial hypercholesterolaemia, especially if the intake of saturated fatty acids is low.

Patients with type IIb phenotype have raised VLDL and LDL concentrations. In these patients, fish oil supplements or diets providing EPA and DHA lead to a reduction in VLDL-triacylglycerols and -cholesterol but no significant change in LDL-cholesterol concentrations. HDL-cholesterol concentrations tend to increase with fish oil supplements, and increases in LDL have been noted in some, but not all, patients.

Patients with the type III phenotype have elevated plasma concentrations of IDL which has a late pre-beta electrophoretic mobility. Fish oil supplements have been found to normalize the electrophoretic profile in most patients and this is accompanied by a reduction in plasma triacylglycerols and cholesterol (Molgaard *et al.* 1990; Dallongeville *et al.* 1991; Davidson *et al.* 1991).

Patients with the type IV phenotype have raised concentrations of VLDL and low concentrations of HDL. Fish oil supplements (but not maize oil, safflower oil, olive oil or evening primrose oil) have a marked VLDL and triacylglycerol-lowering effect in these patients. Total cholesterol concentrations fall or remain unchanged. LDL-cholesterol or LDL-apoB concentrations, which tend to be low in these patients, usually rise even with

low intakes. This increase in LDL-cholesterol is also seen with ester concentrates low in cholesterol (Harris *et al.* 1988). HDL-cholesterol concentrations either remain unchanged or increase. Fat tolerance is improved in hypertriacylglycerolaemic patients, as it is in normal subjects, following treatment with fish oil. The response of patients with the type V phenotype is similar to that of those with type IV and the reduction in plasma triacylglycerol can be quite dramatic even with relatively small amounts of certain fish oil supplements (10–20 ml/d).

Lp(a) concentrations are not reduced either by conventional cholesterol-lowering drug therapy or modification of the total fat, cholesterol and saturated fat intakes. An early report claimed that patients with elevated levels of Lp(a) showed a significant reduction in this risk factor with a fish oil supplement. This seemed plausible as similar observations have been made for nicotinic acid, another triacylglycerol-lowering agent. However, a subsequent study could not confirm that fish oil decreased Lp(a) concentrations (Gries *et al.* 1990).

Lipid-lowering mechanism

There have been few LDL kinetic studies carried out with *n*-3 fatty acids and all have been with high intakes of fish oil. LDL-apoB synthesis is markedly inhibited but the reduction in LDL pool size is smaller than would be predicted from the decrease in LDL synthesis. Moreover, despite the reduced LDL pool size there is no increase in fractional catabolic rate of LDL. This might imply down regulation of the LDL receptors by fish oils. Lindsey *et al.* (1992) reported that LDL from humans supplemented with *n*-3 fatty acids depressed both LDL-receptor activity and LDL-receptor mRNA abundance in HepG2 cells. Sorci-Thomas *et al.* (1992) showed that DHA decreased LDL receptor activity in HepG2 cells in culture.

The consumption of fish oils containing EPA and DHA does not affect the activity of lipoprotein lipase (*EC* 3.1.1.34) or hepatic triacylglycerol lipase (*EC* 3.1.1.3). Consequently an increased rate of removal of VLDL-triacylglycerols from plasma cannot explain the triacylglycerol-lowering effect of fish oil. Singer *et al.* (1990) claim that fish oil decreases free fatty acid release in response to adrenergic stimulation. This would in turn decrease the availability of substrate for hepatic triacylglycerol synthesis. However, EPA and DHA have also been shown to directly inhibit both triacylglycerol synthesis and apoB synthesis and secretion in cell cultures and in perfused liver. VLDL turnover studies in man show that fish oils decrease hepatic triacylglycerol synthesis (Sanders, 1991). We demonstrated that fish oil supplementation resulted in an increased proportion of small VLDL particles in circulation. These small particles are known to be more readily converted to LDL than the larger triacylglycerol-rich ones and this would explain the increase in fractional catabolic rate of VLDL reported by Harris *et al.* (1990). Huff & Telford (1989) also showed that the conversion of VLDL to LDL was enhanced in mini-pigs fed on fish oil. These findings explain why fish oil supplements increase LDL levels in some patients with type IV and type V hyperlipoproteinaemias. This is a general phenomenon seen with most forms of triacylglycerol-lowering therapy including energy restriction. Small cholesterol-rich VLDL particles may also be more effective at decreasing the expression of LDL receptors.

Atherosclerosis

EPA and DHA decrease the plasma concentration of several atherogenic lipoproteins (VLDL, IDL, chylomicron remnants) and at moderate doses may increase HDL_2-:HDL_3-cholesterol. On the other hand, they lead to an increase in LDL levels in some patients. EPA and DHA have also been shown to be incorporated into the lipids of the advanced atherosclerotic plaque in man (Rapp *et al.* 1991). This observation, coupled with the knowledge that EPA and DHA are particularly prone to oxidation, might lead the reader to conclude that they might increase the risk of atherosclerosis. However, atherosclerosis was reported to be rare among North American Inuits on their traditional diet of fish, seal and caribou. This no longer appears to be the case, as Hart Hansen *et al.* (1990) were able to demonstrate atherosclerosis in native Greenlanders using ultrasound. However, few of these native Greenlanders still strictly adhere to the traditional diet (de Knijff *et al.* 1992) and have higher concentrations of LDL-cholesterol compared with a reference Dutch population.

Fish oil inhibits the development of atherosclerosis in pigs, dogs and primates (Shimokawa & van Houte, 1988; Parks *et al.* 1990), even in the presence of hypercholesterolaemia, but not in the cholesterol-fed or Watanabe heritable hyperlipidaemic rabbit. LDL from fish oil-fed primates are smaller and show decreased binding to arterial proteoglycans compared with LDL from lard-fed animals (Edwards *et al.* 1991). Animals fed on fish oils also appear to be insensitive to the toxic effects of oxidized LDL (Lehr *et al.* 1991). It seems likely, therefore, that the protective effect of high intakes of EPA and DHA is mediated by mechanisms independent of plasma LDL concentrations and oxidative modification of LDL. Fish oil, however, appears to be unable to cause regression of atheroma in the vervet monkey (*Cercopithecus aethiops*) (Fincham *et al.* 1991).

Cod-liver oil supplements were found to markedly reduce intimal thickening in autogenous vein grafts implanted as arterial bypasses in cholesterol-fed dogs. MaxEPA, a fish-oil concentrate low in vitamins A and D, was later shown to have the same effect. Sarris *et al.* (1989) showed that MaxEPA with or without aspirin was more effective than aspirin alone in reducing intimal thickening. In the pig, luminal encroachment of coronary arteries, which was induced by arterial injury and a high-cholesterol diet, was inhibited by cod-liver oil (Shimokawa & van Houte, 1988). EPA and DHA have also been shown to inhibit the production of mitogenic factors by endothelial cells *in vitro* (Fox & DiCorletto, 1988). It appears that oxidation products of EPA and DHA are responsible for this inhibitory effect because the addition of antioxidants to the system abrogates the effect.

Percutaneous transluminal coronary angioplasty (PTCA) is a surgical procedure used to relieve myocardial ischaemia. It can lead to marked symptomatic improvement but restenosis occurs in about 30% of patients. As yet there are no means of preventing restenosis. Restenosis is believed to result from intimal hyperplasia, a phenomenon that appears to be inhibited by fish oils. Experiments carried out in swine show that cod-liver oil (1 g/kg body weight) prevented restenosis following angioplasty and decreased platelet adhesion to the damaged vascular endothelium (Lam *et al.* 1992). Several controlled clinical trials have examined the effects of fish oil supplementation on restenosis following coronary angioplasty in humans. Although some studies showed benefit from fish oil supplementation, others show no benefit. Most studies have compared the influence of fish oil in addition to aspirin. Consequently any prostaglandin-

mediated effects that might be exerted by fish oil may well be masked by the aspirin. The studies used different doses of *n*-3 fatty acids and not all the studies initiated fish oil supplementation at least 2 weeks before carrying out the PTCA. O'Connor *et al.* (1992) have carried out a meta-analysis on seven of the published reports and report a typical odds ratio of restenosis of 0·71 (95% CI 0·54–0·91, $P=0·016$). The studies carried out lacked sufficient statistical power to detect small changes in restenosis rate. For a 20% reduction in the restenosis rate where the usual rate is 30% would require a sample in excess of 1000 patients to have a 90% power of detecting a significant difference at $P=0·05$. Further studies are in progress to evaluate whether fish oil supplementation is of benefit.

INFLUENCE ON HAEMOSTATIC FACTORS

Blood pressure

Supplements of EPA and DHA, but not α-linolenic acid, lead to modest reductions in blood pressure in both normotensive and mildly hypertensive individuals (Bønna *et al.* 1990; Kestin *et al.* 1990). The blood-pressure-lowering effect of *n*-3 fatty acids is more apparent in subjects with mild or moderate hypertension than in normotensive individuals and appears to persist as long as intake levels are maintained. In general, however, reductions of more than 3–5 mm Hg in systolic and diastolic pressure cannot be expected with intakes in excess of 2–3 g *n*-3 fatty acids/d unless other measures such as dietary Na restriction are also employed (Cobiac *et al.* 1991).

The mechanism for the blood-pressure-lowering effect appears to be due to changes in vascular resistance rather than cardiac output. Prostacyclin PGI_2 and PGI_3 have vasodilatory properties and total prostacyclin urinary excretion increases at least during the initial period of fish oil supplementation. However, Knapp & Fitzgerald (1989) found that prostacyclin metabolite excretion returned to baseline after several weeks of increased intake of fish oil while blood pressure reduction was sustained. Furthermore, prostacyclin metabolite excretion remained stable as blood pressure recovered after withdrawal of the dietary supplement.

Shimokawa & van Houte (1988) have shown that cod-liver oil leads to an increase in the production of endothelial-derived relaxing factor (EDRF). EPA was found to increase an EDRF-like fraction that was not nitric oxide (NO) by endothelial cells grown in culture (van Houte *et al.* 1991). Further work is needed to elucidate the mechanisms by which *n*-3 fatty acids decrease blood pressure.

Bleeding, coagulation and fibrinolysis

A consistent observation has been that moderate intakes of oily fish or fish oil supplements lead to prolongation of template bleeding time. There is, however, no evidence that post-operative blood loss is increased following administration of fish oil (DeCaterina *et al.* 1990). Prolongation of bleeding time is normally only seen with intakes of EPA and DHA greater than 2–3 g/d, about 1% of the dietary energy intake.

Several factors control bleeding time and these include platelet count and function, packed cell volume, blood pressure and vascular reactivity. The increase in bleeding time with fish oil is not accompanied by any significant change in platelet count and packed cell volume. At least one study has reported an increase in capillary blood flow

(Bruckner *et al.* 1987). Platelet adhesiveness to the vascular endothelium may also be a major determinant of bleeding time. Changes in whole blood viscosity and erythrocyte deformability may also contribute to the increase in bleeding time (Sanders, 1988). Juan & Sametz (1989) found that guanethidine, an adrenergic neurone-blocking agent, prolonged bleeding time strongly in control animals, but not with fish oil-treated rats, even when treated with indomethacin. This would suggest that altered vascular sensitivity explains the prolongation of bleeding time.

The consumption of moderate amounts of EPA and DHA (1–3% dietary energy) is accompanied by a more marked decrease in thromboxane TxA_2 production by platelets than in prostacyclin production, whereas higher intakes would be expected to inhibit prostacyclin production as well. It has been argued, therefore, that the relative imbalance between thromboxane and prostacyclin production with moderate intakes of EPA and DHA might explain the observed prolongation of bleeding time (Leaf & Weber, 1988).

Results with regard to measurements of platelet aggregation by optical densitometry have yielded inconsistent results, although there is a trend for partial inhibition of platelet aggregation induced by low doses of collagen (Fumeron *et al.* 1991; British Nutrition Foundation, 1992). However, the degree of inhibition of platelet aggregation *ex vivo* by fish oil is mild compared with the effects induced by aspirin. It is also a far less potent inhibitor of thromboxane production. The fact that bleeding time is prolonged to a greater extent by a combination of aspirin and fish oil than by either alone (Harris *et al.* 1990) implies that inhibition of thromboxane production is not the mode of action of fish oil. Lei & Steiner (1991) have shown that fish oil containing EPA and DHA decreases platelet adhesion *in vitro* to a greater extent than platelet aggregation.

The coagulation pathway comprises a series of linked proteolytic reactions which culminate in the generation of thrombin activity. High plasma concentrations of fibrinogen and factor VII coagulant activity (VIIc) as determined by bioassay are strong predictors of fatal coronary heart disease in middle-aged men. Most studies have found fibrinogen concentration to be unchanged by dietary fish oil supplementation, although a small number have observed a reduction and one study found an increase (British Nutrition Foundation, 1992). Several studies have failed to demonstrate any effect of fish oils on factor VII coagulant activity. However, two studies using assays and patient groups which were different from those used in the earlier studies, have shown an increase in VIIc activity in subjects receiving fish oil concentrates.

The dissolution of fibrin in a blood clot is mediated by the enzyme plasmin (*EC* 3.4.21.7). Plasmin is formed locally from plasminogen by the proteolytic action of tissue plasminogen activator (tPA). tPA is neutralized rapidly by plasminogen-activator inhibitor (PAI-1) which is secreted by endothelial cells. Most sudies have failed to observe any effect of fish oils on tPA, although some have reported an increase or a reduction (British Nutrition Foundation, 1992). Dietary fish oil has been reported to increase, reduce or have no effect on PAI-1 activity. The reasons for this discrepancy are not clear, but it may be related to differences in the methods used to measure PAI-1. A recent study suggests that PAI activity but not PAI-1 concentrations were decreased in subjects given aspirin (40 mg/d) in combination with 16 g MaxEPA/d (Iacoviello *et al.* 1992). Although the trend towards decreased PAI activity in some studies might be regarded as an adverse effect, it may reflect a homeostatic compensation for a decreased procoagulant activity. Further studies are needed to clarify the effect of specific *n*-3 fatty acids on fibrinolytic activity.

McLennan *et al.* (1992) have shown that susceptibility to cardiac arrhythmias is influenced by fat intake. Animals fed on sheep fat show a high incidence of cardiac arrhythmias compared with those fed on tuna oil, which is high in DHA. However, the amounts of fish oil used in these studies have been relatively high and, therefore, extrapolation to human studies using low amounts of fish oil may not be relevant.

INTERACTION WITH ANTIOXIDANT NUTRIENTS

Yellow fat disease is a naturally occurring disorder in animals living on diets containing oxidized fats usually of marine origin. The term steatitis is used to describe the inflammatory changes observed in adipose tissue. Adipose tissue and other soft tissues accumulate a yellow/brown pigment called lipofuscin that results from the reaction between polyunsaturated fatty acid peroxides and ethanolamine phosphoglycerides which has been induced in domestic animals by feeding fish oils. Pigs appear to be sensitive to yellow fat disease induced by feeding fish oil. The severity of the steatitis is correlated with the peroxide value of the oil. It can be prevented to some extent by adding extra vitamin E or synthetic antioxidants to the diet. Fish oil is not the only oil that can cause yellow fat disease. Linseed oil also can cause it. It has been suggested that a low value for *n*-6:*n*-3 polyunsaturated fatty acid exacerbates the disease (Bjister & Vles, 1984).

Several studies have noted falls in plasma vitamin E concentrations following fish oil supplementation (Bjorneboe *et al.* 1988; Sanders & Hinds, 1992); this may occur even though additional vitamin E and antioxidants are added to the oil. A small but statistically significant fall in packed cell volume has also been noted in some studies using fish oil supplements (Sanders & Hinds, 1992) and in a study where the subjects consumed fish paste (Van Houwelingen *et al.* 1987).

It has been estimated that approximately 0·4 mg vitamin E/g linoleic acid is required in order to guard against lipid peroxidation. Danse & Verschuren (1978) showed that 0·4 mg vitamin E/g polyunsaturated fatty acid in cod-liver oil did not protect against yellow fat disease. Further studies are needed to ascertain the increased requirement for vitamin E in relation to the intake of *n*-3 fatty acids. Preliminary data (Pollard & Sanders, 1993) suggest that 3–4 mg vitamin E/g *n*-3 fatty acids is adequate.

CONCLUSION

Small amounts of fish oil may play an important role in meeting requirements for *n*-3 fatty acids. Larger amounts exert pharmacological effects that may be of value in the management of certain diseases. Care, however, must be taken to guard against excessive lipid peroxidation. Further studies are needed to assess the increased requirement for antioxidant nutrients caused by the consumption of fish oils.

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