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The impact of long-chain *n*-3 polyunsaturated fatty acids on human health

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A considerable literature has been published on the health benefits of fish, oil-rich fish and fish oils and their constituent long-chain (LC) *n*-3 PUFA. Evidence from epidemiological studies highlights the cardioprotective attributes of diets rich in fish, especially oil-rich fish. Data from intervention trials are consistent in suggesting that LC *n*-3 PUFA lower the risk of CVD, probably by the multiple mechanisms of lowering serum triacylglycerols, improving the LDL:HDL ratio, anti-arrhythmic effects on heart muscle, improved plaque stability, anti-thrombotic effects and reduced endothelial activation. Research indicates LC *n*-3 PUFA provision has an impact during development, and there is preliminary evidence that docosahexaenoic acid supplementation during pregnancy could optimise brain and retina development in the infant. LC *n*-3 PUFA are also postulated to ameliorate behavioural and mental health disturbances such as depression, schizophrenia, dementia and attention deficit hyperactivity disorder. However, despite some positive evidence in each of these areas, use of LC *n*-3 PUFA in these conditions remains at the experimental stage. In the case of immune function, there is little doubt that LC *n*-3 PUFA have a positive effect. Although intervention trials in rheumatoid arthritis show strong evidence of benefit, evidence for efficacy in other inflammatory conditions, including Crohn's disease, ulcerative colitis, psoriasis, lupus, multiple sclerosis, cystic fibrosis and asthma, is inconsistent or inadequate. More promising evidence in some conditions may come from studies which attempt to modify the fetal environment using LC *n*-3 PUFA supplementation during pregnancy.

Docosahexaenoic acid: Eicosapentaenoic acid: Cardiovascular disease: Cognitive function: Inflammatory disease: Immune function

Introduction

The purpose of the present review is to explore evidence relating to a range of health benefits associated with the consumption of fish and its constituent long-chain (LC) *n*-3 PUFA, namely EPA and docosahexaenoic acid (DHA). The link with CVD is well known, but other associations include the role of DHA in early brain development and in the maintenance of cognitive function in the elderly. The use of fish oil constituents to address mental ill-health will be discussed, as will the positive impact of LC *n*-3 PUFA on the immune system and autoimmune disorders. Although LC *n*-3 PUFA are worthy of medical practitioners' attention, there are still areas for which the evidence remains too scant to merit wider application in the sphere of public health nutrition. It is hoped that the exposure of such gaps will spur more research on, what could be, an extremely beneficial dietary component.

Biochemistry of long-chain *n*-3 PUFA

Fatty acid chain lengths vary from two to more than thirty carbon atoms, and they may incorporate one or more double bonds. In systematic chemical nomenclature the position of double bonds is given by numbering from the carboxyl group (see Fig. 1). An alternative shorthand notation for fatty acids numbers the carbon atoms from the terminal methyl group – i.e. the carbon furthest from the carboxyl group, known as the ω-carbon, and the position of the first

Abbreviations: AA, arachidonic acid; ADHD, attention deficit hyperactivity disorder; ALA, α-linolenic acid; COX, cyclo-oxygenase; DHA, docosahexaenoic acid; EFA, essential fatty acid; IFN, interferon; LA, linoleic acid; LC, long-chain; LT, leucotriene; MI, myocardial infarction; PG, prostaglandin.

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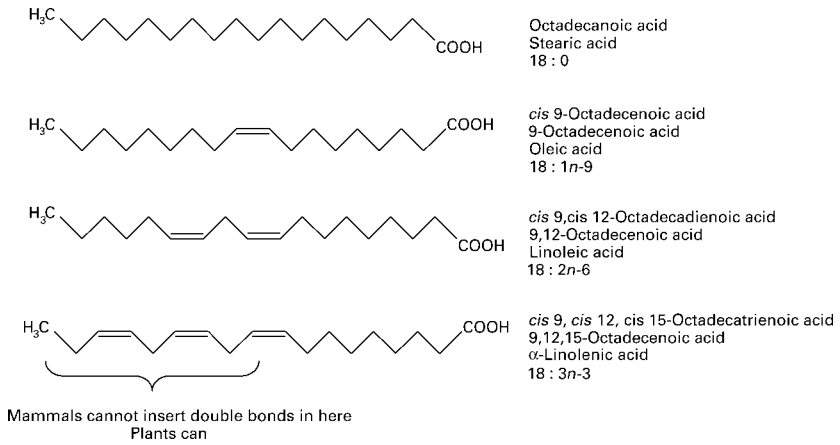

Fig. 1. Chemical composition of various fatty acids.

double bond from the methyl group is shown as ω-x or *n*-x, where x is the carbon number on which the double bond occurs. The essential fatty acids (EFA) fall into two groups: *n*-3 and *n*-6, sometimes referred to as omega-3 and omega-6.

This notation, rather than systematic chemical nomenclature, is useful because the actions of fatty acids within the human body are dependent on whether they are members of *n*-3 or *n*-6 families. Man can introduce additional double bonds between the carboxyl group and an existing double bond, but cannot interconvert *n*-3 and *n*-6 fatty acids. By contrast, plant enzymes normally introduce a new double bond between an existing double bond and the terminal methyl group. As shown in Fig. 2, the insertion of a double bond by plant enzymes between carbons 12 and 13 of oleic acid (counted from the carboxyl end; 18 : 1*n*-9) yields linoleic acid (LA; 18 : 2*n*-6). LA can be further desaturated by the insertion of a double bond between carbons 15 and 16 (counted from the carboxyl carbon) to yield α-linolenic acid

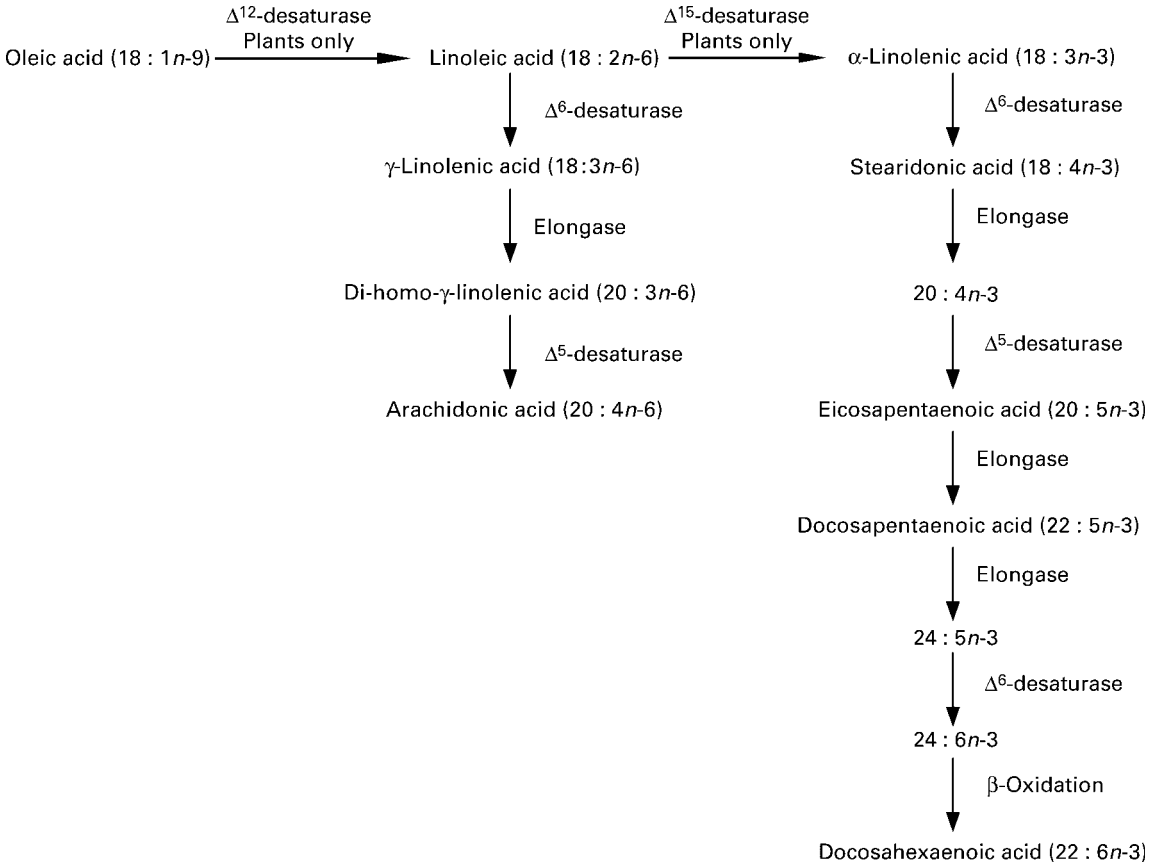

Fig. 2. Desaturation and elongation of *n*-3 and *n*-6 fatty acids.

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(ALA; 18 : 3*n*-3). LA and ALA are the parent compounds of the *n*-6 and *n*-3 families of fatty acids, respectively. Since mammals lack the Δ^{12}- and Δ^{15}-desaturases, they cannot synthesise LA and ALA, but require a dietary source of these two EFA (see Cunnane, 2000).

Although LA and ALA are not synthesised within mammals, they can be subjected to further desaturation and elongation as shown in Fig. 2. LA can be converted to γ-linolenic acid (18 : 3*n*-6), which can be elongated to dihomo-γ-linolenic acid (20 : 3*n*-6), further desaturated to yield arachidonic acid (AA; 20 : 4*n*-6). Using the same series of enzymes involved in the metabolism of *n*-6 PUFA, ALA can be converted to EPA (20 : 5*n*-3).

Further conversion of EPA to DHA (22 : 6*n*-3) involves chain elongation to yield docosapentaenoic acid (22 : 5*n*-3), then 24 : 5*n*-3, followed by desaturation at the Δ^6 position to form 24 : 6*n*-3 (Sprecher, 1999). Then, 24 : 6*n*-3 is translocated from the endoplasmic reticulum to peroxisomes where two carbons are removed by limited β-oxidation to yield DHA. EPA and docosapentaenoic acid can also be synthesised from DHA by retroconversion due to limited peroxisomal β-oxidation. In man, approximately 1 % of DHA may be retroconverted to EPA (Brossard *et al.* 1996).

It is evident from the pathway shown in Fig. 2 that there is competition between the *n*-6 and *n*-3 fatty acid families for metabolism, since the two pathways use the same enzymes. While the preferred substrate for Δ^6-desaturase is ALA, the relative excess of LA in the average Western diet (due to the fashion for vegetable oil margarines and related products) means that the pathway involving *n*-6 fatty acids prevails. Estimates based on stable-isotope tracer experiments indicate that overall conversion of ALA to DHA in man appears to be very low at 4 % to $<0{\cdot}05$ % (Pawlosky *et al.* 2001), although this may be about 9 % in women of reproductive age (Burdge & Wootton, 2002). This has led some commentators to question whether all human requirements for DHA, particularly those relating to brain development, can be met from the endogenous supply (Bourre *et al.* 1991).

Cardiovascular disease

Evidence collected over the last decade or so supports the beneficial effects of dietary LC *n*-3 PUFA, provided as supplements or via increased fish consumption, in the prevention and treatment of CVD. Others have provided detailed systematic reviews of the evidence (for example, Bucher *et al.* 2002; Hooper *et al.* 2003). Thus, this section will focus mainly on possible mechanisms of cardioprotection.

Dietary habits

Much of the epidemiological evidence in the literature relates to the inverse relationship between fish or LC *n*-3 PUFA consumption and risk of CVD. While there is much evidence that the habitual intake of fish offers protection against CVD (for example, Bulliyya, 2002), the benefits are affected by the type and preparation of fish consumed. Oil-rich fish provide more EPA and DHA, and confer greater cardioprotection, than white lean fish (Oomen *et al.* 2000). The extent of the cardiovascular benefit of fish consumption also appears to be affected by certain health characteristics within the population studied (for example, the presence of diabetes). A major American study, following a total of 84 688 subjects for 16 years (Hu *et al.* 2002), observed a dose-related effect between regular fish consumption and reduction in CHD events. The same authors (Hu *et al.* 2003) studied a sub-group of diabetic women and discovered an even stronger inverse relationship between fish intake and CHD, with a >60 % risk reduction for the highest fish intake and approximately 30 % reduction for women eating one to three fish meals per month. As noted by a recent Cochrane review, this may be because LC *n*-3 PUFA consistently lower blood triacylglycerol concentrations in patients with type 2 diabetes (Farmer *et al.* 2003). In contrast, Bjerregaard *et al.* (2000) found a weak positive association between a marine-based diet and blood glucose levels in Greenland Inuit, though the authors suggested that this new observation required confirmation.

The inverse relationship between fish intake and CHD is believed to be related to increasing LC *n*-3 PUFA intake with increasing fish (particularly oil-rich fish) intake. Dewailly *et al.* (2002) reported differences in LC *n*-3 PUFA concentrations between inland and coastal Canadian Cree Indians, a consequence of the availability of fresh fish in the latter population. Subsequently, Dewailly *et al.* (2003) were able to show that the fish intake of three ethnic groups (Inuit, Cree and Quebeckers) mirrored the respective plasma EPA and DHA concentrations. Overall, despite the high prevalence of CVD risk factors such as smoking habit and obesity, the Inuit, who had the highest fish intake, showed the lowest total CVD risk.

Dietary intervention

Clinical trials involving dietary supplementation have focused upon secondary prevention of CVD in survivors of acute myocardial infarction (MI). Large studies in this area have found that increasing LC *n*-3 PUFA intake either by supplementation or increased fish intake decreases CVD mortality risk. The GISSI-Prevenzione study (Anonymous, 1999), with over 11 000 subjects, found that LC *n*-3 PUFA supplementation (equivalent to 850 mg EPA and DHA/d) reduced the relative risk of death over 3·5 years by more than 10 % and of cardiovascular death by at least 17 %. Similarly positive results were found by the earlier DART study (Burr *et al.* 1989), which included over 2000 post-MI patients. Men advised to eat two fish meals per week experienced a 29 % reduction in total mortality over 2 years, which was ascribed to a reduction in CHD mortality. A later publication by this group (Burr *et al.* 2003), which attempted to repeat the fish intervention in 3000 men with stable angina, failed to find any benefits, although the reasons are unclear and do not accord with most literature in this field. A meta-analysis of eleven randomised controlled trials (Bucher *et al.* 2002) concluded that LC *n*-3 PUFA can reduce overall mortality and mortality due to MI and sudden death in patients with CHD, and a Cochrane review (Hooper *et al.* 2003) found a strong beneficial effect of LC *n*-3 PUFA in at-risk groups.

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Pathogenesis

Atheromatous disease of the vasculature is the result of partial or complete occlusion due to the deposition of material within the arterial intima leading to formation of plaque. CVD has a complex aetiology involving genetic and lifestyle factors, all of which contribute to the formation and stability of intimal plaque. The following brief outline highlights only those aspects of the pathogenesis which are thought to be related to LC *n*-3 PUFA protection. Changes to lipoprotein metabolism, blood pressure, immunological responses, endothelial function and blood clotting are all implicated in the progression of CVD to the point at which it becomes life-threatening. It is now generally accepted that the disease is an example of a chronic inflammatory condition (Libby *et al.* 2002).

The current model of atherosclerosis (developed originally by Ross, 1993, as the 'response to injury model') assumes a phenotypic change ('damage') to the endothelium. Such damage may be due to mechanical forces, as in hypertension, or due to biochemical abnormalities within the endothelial cells. Subsequently, there are alterations in the activity of the endothelium, notably the action of enzymes such as NO synthase and cyclo-oxygenase (COX), the latter changing the balance of the production of inflammatory and coagulant prostaglandins (PG) and thromboxanes. The localised release of inflammatory cytokines ('local hormones') such as TNF and interleukins (for example, IL-1) consequent to the endothelial dysfunction initiates the immunological response (for a review of cytokine involvement in CVD, see Young *et al.* 2002). Consequently, leucocytes adhere to the endothelial layer proximal to the 'damaged' region, marking the beginning of the atherosclerotic process.

Damage to the endothelium also allows blood-borne monocytes and LDL particles to pass into the arterial intima. Transmigration of monocytes from the bloodstream results from the cytokine-induced expression of adhesion molecules on the endothelium. Chemical oxidation of the LDL, probably within the intimal layer, stimulates further cytokine release and the modified LDL are engulfed by monocyte-derived macrophages, forming 'foam cells' (Glass & Witztum, 2001). The overgrowth of smooth muscle cells (also as a result of cytokine action), calcification and platelet aggregation on the luminal surface of the arterial wall complete the formation of the atheromatous plaque which eventually occludes the vessel, resulting in tissue ischaemia. Unstable plaques may rupture and bleed, exacerbating the inflammatory process and extending the size of the lesion (Plutzky, 1999).

In contrast to the pro-atherogenic LDL particles, HDL play a key role in reverse cholesterol transfer (i.e. removal of excess cholesterol) from tissues to the liver and are anti-inflammatory, modulating, for example, the effect of TNF and IL (see Cockerill & Reed, 1999 for a review, and Cockerill *et al.* 2001).

Mechanisms of cardioprotection

The beneficial effects of LC *n*-3 PUFA may arise from their intervention at any one (or more) of a number of steps in the pathogenesis of CVD (see Calder, 2004). Suggested mechanisms include:

- alterations in plasma lipoprotein profiles, particularly by reducing triacylglycerol concentrations and reducing the LDL:HDL ratio (Cobiac *et al.* 1991; Bjerregaard *et al.* 2000);
- reduced endothelial activation, i.e. reduction in expression of adhesion molecules and an increase in vascular NO production (Baro *et al.* 2003; Christon, 2003);
- anti-arrhythmic effects on heart muscle (Lemaitre *et al.* 2003);
- improved plaque stability (Thies *et al.* 2003);
- anti-thrombotic effects (Manzioris *et al.* 2000; Nordoy *et al.* 2003).

The relationship between LC *n*-3 PUFA and lipoprotein profiles has been well investigated. Studies into plasma lipoprotein profiles in fish-eaters compared with non-fish consumers (Bulliyya, 2002) or as a result of dietary LC *n*-3 PUFA supplementation (Baro *et al.* 2003) reveal overall improvements in HDL and reduction of LDL, indicating CVD risk reduction. Taken in isolation, however, such measures are difficult to interpret unequivocally. Furthermore, there is concern that the LDL produced in the context of a high LC *n*-3 PUFA intake may be more vulnerable to oxidation, given its increased PUFA content. Conflicting evidence exists regarding the 'oxidisability' of LDL following LC *n*-3 PUFA consumption (Higgins *et al.* 2001; Finnegan *et al.* 2003). However, it has been suggested that the benefits of LC *n*-3 PUFA may be offset to some extent by increased oxidation of LDL within the arterial intima and consequent increased inflammatory processes.

Although cholesterol has traditionally been viewed as the principal marker for CVD risk, high-dose supplementation with EPA and DHA in hyperlipidaemic subjects decreases plasma concentrations of triacylglycerols and VLDL as well (Chan *et al.* 2003; Park & Harris, 2003).

Much research effort has been directed towards understanding the effects of PUFA on endothelial homeostasis (de Caterina *et al.* 2000). Significant changes in favour of the more anti-coagulant and less potently inflammatory PG were produced following LC *n*-3 PUFA supplementation (Manzioris *et al.* 2000; Bagga *et al.* 2003), with implications for both the atherosclerotic and thrombotic processes. In addition, Cobiac *et al.* (1991) found improvements in thrombotic parameters (fibrinogen and thromboxane) following LC *n*-3 PUFA supplementation.

With respect to atherosclerosis, additional benefits in adults given fish oil supplements can arise from the reduced endothelial expression of adhesion molecules (Mayer *et al.* 2002; Thies *et al.* 2003). This would diminish the extravasation of leucocytes and so reduce atheroma formation. The reduction in adhesion molecule production is age-related, with potentially greater benefit accruing to older subjects (Miles *et al.* 2001), suggesting that dietary advice could be targeted at this group of individuals.

It has been suggested that LC *n*-3 PUFA affect the electrophysiological function of cardiac myocytes in such a way as to reduce their vulnerability to fibrillation (Charnock, 1999; for a review of anti-arrhythmogenesis operating via Na channel proteins, see Das, 2000).

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Supporting research includes a study by Lemaitre *et al.* (2003) which showed that higher LC *n*-3 PUFA in plasma phospholipids was associated with a lower risk of fatal, but not non-fatal, cardiac events. Note should be taken, however, that although Lemaitre *et al.* (2003) show a statistical significance, there was wide variability in the data.

Several authors have attempted to explain the mechanism of LC *n*-3 PUFA action on CVD by investigating aspects of plaque progression or stability. Neither von Schacky *et al.* (1999), using 6 g LC *n*-3 PUFA/d for 3 months followed by 3 g/d for 21 months, nor Angerer *et al.* (2002), using 1·65 g LC *n*-3 PUFA/d for 2 years, were able to show a significant effect of intervention on the progression of cardiac and carotid atherosclerosis. Thies *et al.* (2003), however, reported increased incorporation of LC *n*-3 PUFA into advanced carotid plaques when patients supplemented their diet with 1·6 g LC *n*-3 PUFA/d. Furthermore, this incorporation was associated with greater plaque stability (i.e. reduced vulnerability to rupture) and lower macrophage infiltration in the plaques. The morbidity of CVD is associated with plaque rupture rather than formation *per se* (Plutzky, 1999). Thus, the effect of LC *n*-3 PUFA on plaque stability could be an especially important benefit.

Atherosclerosis and thrombosis are multifactorial conditions, and evidence suggests that LC *n*-3 PUFA have multiple beneficial effects on CVD risk; the relative importance of any one possible mechanism is difficult to determine with certainty. The lack of a definitive mechanistic explanation does not diminish the strength of epidemiological evidence in support of cardioprotection due to fish or fish oil intake.

Long-chain *n*-3 PUFA through the life-cycle

Infant development

The role of DHA in the development and maintenance of the brain has been long recognised. Early animal studies revealed that the most abundant PUFA in rat brain lipid was DHA, while ALA and EPA were undetectable (Pullarkat & Reha, 1976). Crawford *et al.* (1976) compared thirty different mammalian species and found that, despite a wide variation in the fatty acid composition of the livers, that of brain tissue was remarkably constant, with DHA making up 25 % of grey matter phospholipid fatty acids (mainly as phosphatidyl ethanolamine and phosphatidyl serine). Studies of fetal cadavers linked the accumulation of DHA and AA with the rapid synthesis of brain tissue seen in the third trimester (Clandinin *et al.* 1982).

Although DHA is not considered to be an EFA, studies of the livers of premature infant cadavers suggest that it is unlikely that the fetus can make sufficient DHA to support brain development (Clandinin *et al.* 1981). A lack of DHA in the last trimester could impact on the differentiation and multiplication of brain cells, thus hampering the function of cerebral membranes and, ultimately, learning abilities (Bourre *et al.* 1991). The mechanism for this may relate to the important role of DHA in creating fluidity in neuronal membranes (Yehuda *et al.* 1999). Optimal fluidity is vital for a number of tasks including the regulation of neurotransmitters, the efficiency of enzyme activity, protein binding and nutrient transport (Bourre *et al.* 1991). Evidence from animal studies (Auestad & Innis, 2000) suggests that DHA deficiency can decrease brain DHA levels, increase the *n*-6:LC *n*-3 PUFA ratio in nerve membranes and result in poor functioning. DHA supplementation of deficient rats can, after several months, increase brain DHA levels and improve scores in tests of memory, learning ability and cognitive performance (Moriguchi *et al.* 2000).

DHA also has an important role to play in the development of visual function. Studies of mammalian retina revealed that DHA accounts for half of the total fatty acid content in the retina (Stinson *et al.* 1991). The functional importance of DHA may be related to the interaction of the photoactive protein rhodopsin with DHA-containing phospholipids (Gordon & Bazan, 1990; Niu *et al.* 2004). Phospholipid bilayers rich in DHA have a high fluidity and enhanced rates of fusion and permeability which are characteristics vital to the normal functioning of photoreceptor cells (Giusto *et al.* 2000).

Given that maternal DHA stores compensate for the limited ability of the fetus to synthesise DHA, it is likely that a poor LC *n*-3 PUFA status in the mother, perhaps due to multiple pregnancies or a lack of fish in the diet, could impact on fetal development (Otto *et al.* 1997). There may also be a disadvantage for the mothers themselves which will be described later. The converse is that enhancing DHA status during pregnancy could potentially benefit the fetus by providing an abundant source for brain development. Connor *et al.* (1996) reported that pregnant women who regularly consumed sardines or fish oil increased their plasma DHA levels and transferred additional DHA and other *n*-3 fatty acids to their fetuses. In a randomised, double-blind intervention trial, Montgomery *et al.* (2003) supplemented pregnant women with either DHA (200 mg/d) or oleic acid. Those in the DHA group preferentially transferred DHA to their fetuses and experienced less of a decline in their DHA status during the third trimester. Whether or not this impacts on intelligence of the infant is not fully confirmed and will be discussed later.

After birth, the fatty acid status of the mother can continue to impact on her newborn. While the maximum accretion of cerebral DHA occurs *in utero*, the infant's high requirements for AA and DHA continue into the first year of life. These are met by endogenous synthesis of AA and DHA and by supply from breast milk, a rich source of LC PUFA (Crawford *et al.* 1981). Breast milk composition reflects the habitual fatty acid intake of the mother, for example, the breast milk of vegans contains relatively low levels of DHA (Sanders, 1999), while mothers who regularly eat fish produce milk with a high DHA content (Jørgensen *et al.* 2001). The *n*-6:LC *n*-3 PUFA ratio of breast milk in Western countries has increased over the past decade (Sanders, 2000), probably due to low fish intakes coupled with an increased intake of margarines and vegetable oils (Makrides *et al.* 1995).

Potential benefits of long-chain n*-3 PUFA supplementation*

Given the importance of DHA in early brain and eye development, it seems logical that a poor maternal LC *n*-3 PUFA status would have an adverse effect on infant brain

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function and vision, while a high LC *n*-3 PUFA status would have a positive effect. While this appears to be the case in animal models (Wainwright, 2002), conclusive evidence in human subjects is lacking. Two stages of infant development tend to be researched when investigating the benefits of LC *n*-3 PUFA in this area: fetal and neonatal.

Fetal. Two studies used LC PUFA status at birth, mainly estimated from umbilical venous plasma phospholipids, as a proxy for prenatal fatty acid availability. Ghys *et al.* (2002) followed up their sample of 128 term infants after 4 years to determine whether cognitive development was correlated with prenatal DHA and AA availability. No relationship was found. In a similar study, Bakker *et al.* (2003) followed up a sample of term infants after 7 years and attempted to relate cognitive performance with prenatal DHA and AA. Again, no relationship was found. One problem with these studies was wide variation in the samples which may have obscured any biochemical relationships. In both studies, maternal intelligence, smoking, birth weight and duration of breast feeding were strongly related to child cognitive function.

Benefits of fish consumption during fetal development were reported by Daniels *et al.* (2004) who found that mothers with a fish consumption of four times per week during pregnancy had babies with higher developmental scores at 18 months compared with those who ate no fish. A positive effect for DHA was found by Helland *et al.* (2003) who conducted a randomised, double-blind study in pregnant women using a daily supplement of 10 ml cod liver oil as the intervention. Women took the oil, providing 1·2 g DHA and 0·8 g EPA/d, from 18 weeks gestation until 3 months after delivery. Maize oil was used as the placebo. Children were followed up after 4 years and performed better in tests of mental processing if they had been in the cod liver oil group. A weakness of this study was the high drop-out rate, which resulted in only ninety out of the original sample of 341 children being tested. However, the results are encouraging and suggest the need for similar studies.

Neonatal. Preterm, but not usually term, infants have a limited ability to synthesise DHA and AA from their precursor EFA. This increases their risk of LC *n*-3 PUFA deficiency during the period of rapid brain maturation in the first year of life. Breast-fed premature infants are protected by the high levels of LC *n*-3 and *n*-6 PUFA present in human milk. The restricted capacity for LC *n*-3 PUFA synthesis in preterm infants was not fully known in the early days of formula milk manufacture resulting in formulations which contained LA and ALA, but not DHA or AA. This led to concern that the fatty acid profile of baby milks was inappropriate for preterm infants, not just because of the lack of preformed DHA but also the high *n*-6:*n*-3 PUFA ratio which could inhibit the already limited supply of endogenous DHA. Clinical trials using different formulations of baby milk noted significant differences in visual acuity and intelligence between breast-fed and bottle-fed babies, which were minimised when the formula milks were supplemented with AA and DHA (SanGiovanni *et al.* 2000; O'Connor *et al.* 2001). The findings stimulated the call for standard formula milk to contain 0·2 % of total fatty acids as DHA with a higher amount for preterm infants (Koletzko *et al.* 2001). However, a recent Cochrane review has questioned the long-term benefits of supplementing preterm formula milks with LC *n*-3 PUFA (Simmer, 2003*a*). It concluded that, while a number of studies found DHA-related benefits in the first 4 months of life, there was insufficient high-quality evidence for the months beyond this. It is possible that, after 4 months, the preterm infant is able to synthesise sufficient AA and DHA to support normal development. The Cochrane review refuted the argument that DHA supplementation could lead to adverse effects on growth.

The evidence to support benefits for LC *n*-3 PUFA supplementation in term infants is inconsistent. Jørgensen *et al.* (2001) correlated maternal fish consumption during breast-feeding with infant development, finding that a higher consumption of fish related to better visual acuity in the infants. Birch *et al.* (2000, 2002) found developmental improvements when term infants were supplemented with LC PUFA but this was not seen by van Wezel-Meijler *et al.* (2002). Variation in assessment methods and supplementation levels may explain these discrepancies. Recently, Simmer (2003*b*) reviewed this area, finding that the evidence did not support a benefit for term infants in relation to visual or general development, although there was a possibility that information processing could be improved by giving LC *n*-3 PUFA.

Behavioural disturbances

It has been suggested that imbalances in fatty acid status could be linked to behavioural and learning disorders such as attention deficit hyperactivity disorder (ADHD), dyslexia, dyspraxia and autism (Richardson & Ross, 2000). Concerns about the rising prevalence of behavioural problems and the safety of medication used to treat them has prompted interest in certain dietary manipulations such as increasing the intake of LC *n*-3 PUFA.

ADHD is characterised by inattentive, impulsive and hyperactive behaviour and occurs mainly in children, although the condition is not unknown in adults. Richardson & Puri (2002) estimate that 2 % of British school-aged children and 4 % of American children are affected. The possibility of using LC *n*-3 PUFA in this condition arose when it was discovered that ADHD patients displayed lower plasma levels of EPA and DHA compared with normal children (Stevens *et al.* 1995). However, there is limited evidence from double-blind placebo-controlled trials. In a trial of DHA supplementation (345 mg/d for 16 weeks), Voigt *et al.* (2001) found no improvement in the symptoms of children with ADHD. Improvements in behaviour were seen when children with ADHD and learning difficulties were supplemented for 12 weeks with a daily dose of 186 mg EPA and 480 mg DHA (Richardson & Puri, 2002). It is possible that the discrepancy between the two studies arose due to the differences in supplementation level or the inclusion of EPA in the later trial.

Fatty acids have also been used to treat violence and uncooperative behaviour in adult offenders. Again, the interest was sparked by evidence showing links between behavioural problems and low LC *n*-3 PUFA status (Hibbeln, 1998), and with a higher plasma *n*-6:LC *n*-3

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PUFA ratio (Virkkunen *et al.* 1987). In a double-blind, placebo-controlled trial on young adult male prisoners, Gesch *et al.* (2002) used a combination of micronutrients, fish oil (providing 80 mg EPA + 44 mg DHA daily) and evening primrose oil. The supplemented group demonstrated fewer disciplinary offences compared with the placebo group. However, it is unclear which particular nutrient conferred the benefit.

Cognitive function in later life

Dementia is a common disorder among elderly individuals. An epidemiological study of 5386 older Dutch citizens identified a fish intake in excess of 20 g/d as a potential protective factor against cognitive decline, dementia and Alzheimer's disease (Kalmijn *et al.* 1997*b*). Another study in 476 men aged 69–89 years (Kalmijn *et al.* 1997*a*) linked a high LA intake to cognitive impairment. A prospective study of non-demented elderly found that, after 4 years of follow-up, those who consumed oily fish once per week had 60 % less risk of Alzheimer's disease than those who rarely or never ate oily fish (Morris *et al.* 2003). Total DHA, but not EPA, intake was associated with reduced risk of Alzheimer's disease.

Post-mortem studies show decreased levels of AA and DHA in the brains of Alzheimer's disease sufferers (for example, Prasad *et al.* 1998), while biochemical work has linked a low plasma LC *n*-3 PUFA status to dementia (Tully *et al.* 2003) and to cognitive impairment (Conquer *et al.* 2000). Longitudinal evidence to support this came from a survey of elderly French subjects (Heude *et al.* 2003) which measured erythrocyte membrane fatty acid composition and cognitive function in 246 elderly individuals and followed them up 4 years later. Those with a high plasma *n*-6:LC *n*-3 PUFA ratio at baseline were most likely to experience cognitive decline. The next stage to unravelling the relationship between LC *n*-3 PUFA and cognitive function in the elderly is a supplementation trial, but so far only one small pilot study has been published. Terano *et al.* (1999) supplemented ten elderly Japanese with 720 mg DHA/d for 12 months and compared them with an unsupplemented group. A significant improvement in dementia scores was found in the DHA group after 3 months, but not in controls. The potential usefulness of LC *n*-3 PUFA in this field now needs to be verified by a larger intervention study.

Mental ill-health

Depression

Clinical depression. Evidence for the role of *n*-3 fatty acids, particularly DHA, in the aetiology and treatment of depression and other mental disorders has been reviewed by a number of authors (Freeman, 2000; Mischoulon & Fava, 2000; Haag, 2003). It is hypothesised that a relationship exists between a poor LC *n*-3 PUFA status and an increased risk of depression. In a study of nine countries, Hibbeln (1998) demonstrated a significant correlation between high annual fish consumption and lower prevalence of major depression. Tanskanen *et al.* (2001) studied a sample of 3204 Finnish adults, finding a significant correlation between low fish consumption and depressive symptoms. While population comparisons are interesting, they are insufficiently controlled to establish a cause and effect relationship between LC *n*-3 PUFA and depression.

Evidence from supplementation trials is limited but promising. Elderly patients treated with DHA-rich phosphatidylserine demonstrated significant reductions in depressive symptoms compared with a placebo group (Cenacchi *et al.* 1993). In an 8-week randomised controlled trial, twenty-eight patients with major depression received either 9·6 g LC *n*-3 PUFA/d or placebo in addition to their usual treatment (Su *et al.* 2003). Those in the intervention group showed a significant reduction in their depressive symptoms. However, no significant effect on depressive symptoms was found by Marangell *et al.* (2003) when thirty-six patients were given 2 g DHA/d for 6 weeks in a randomised controlled trial, although the dose could have been too low to be effective. An intervention using 1 g DHA plus EPA/d as fish oil to treat clinical depression is underway in the UK (PJ Rogers, personal communication).

Uni- and bipolar affective disorders. In a double-blind placebo-controlled study using EPA, patients with unipolar depressive disorder experienced significant improvements in symptoms after 4 weeks of treatment (Nemets *et al.* 2002). An intervention in thirty patients with bipolar manic depression (Stoll *et al.* 1999) used a daily dose of 6·2 g EPA and 3·4 g DHA in a 4-month, double-blind, placebo-controlled trial. Significant improvements were seen in nearly every outcome measure particularly with respect to depressive symptoms.

Schizophrenia. A Cochrane review (Joy *et al.* 2003) reviewed five small studies which used EPA and DHA to treat the symptoms of schizophrenia. While some demonstrated benefits, the reviewers concluded that using LC *n*-3 PUFA as a treatment for this condition remained at the experimental stage.

Postnatal depression. There is evidence for associations between poor LC *n*-3 PUFA status and postnatal depression, and for minor reductions in maternal brain size during, and for 6 months after, pregnancy (Oatridge *et al.* 2002), perhaps indicating a temporary loss of maternal DHA. Useful evidence was provided by a prospective study which compared postpartum fatty acid profiles in women who did (*n* 10) and did not (*n* 38) develop postnatal depression (De Vriese *et al.* 2003). The authors found that the *n*-6:*n*-3 ratio was higher in the depressed mothers, concluding that LC *n*-3 PUFA supplementation should be given to women at risk from postnatal depression. However, the lack of controlled intervention trials relating to this condition hinders a full understanding of the benefits for pregnant women. The only clinical trial (Llorente *et al.* 2003), which supplemented mothers for 4 months after delivery with a relatively low 200 mg DHA/d, found no significant improvement in self-rated indices of depression, despite increases in plasma DHA status.

Immune function

Human immune and inflammatory cells tend to be rich in AA (Yaqoob *et al.* 2000), perhaps reflecting the relatively

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high *n*-6 PUFA content of most diets. In contrast to this, EPA is a fairly minor constituent of human immune and inflammatory cells, comprising an average of <1 % of total fatty acids (Yaqoob *et al.* 2000). Supplementing the human diet with fish oil results in incorporation of both EPA and DHA into blood immune and inflammatory cells (Healy *et al.* 2000; Yaqoob *et al.* 2000), largely at the expense of AA. Once dietary supplementation ceases, EPA is lost from the cells within 8 weeks, but DHA is better retained (Yaqoob *et al.* 2000). The balance between AA and LC *n*-3 PUFA within immune and inflammatory cells is thought to impact on immune function and on production of inflammatory mediators.

Implications of fatty acid composition of immune and inflammatory cells

Both AA and EPA are substrates for enzymes, such as COX and 5-LOX, which catalyse the generation of eicosanoids, including PG and leucotrienes (LT), which are involved in modulating the intensity and duration of inflammatory responses. For example, eicosanoids produced from AA can regulate leucocyte movement to sites of inflammatory activity (LTB_4) and production of reactive oxygen species and inflammatory cytokines such as TNF and IL (PGE_2 and LTB_4), and they can induce fever and pain (PGE_2). In addition, these mediators affect immune function by regulating lymphocyte proliferation, antibody production, natural killer cell activity and cytokine production (see Calder, 2003*a*).

The eicosanoids produced from EPA, including the '3' series of PG and thromboxanes, are considered to be less biologically potent than the analogues synthesised from AA, which include the '2' series of PG and thromboxanes (for example, Bagga *et al.* 2003). Therefore, it is thought that EPA gives rise to eicosanoids that move the balance toward a less inflammatory, and a less allergic, state than those produced from AA (see Miles *et al.* 2003).

Although increased intake of LC *n*-3 PUFA limits the availability of AA, resulting in decreased production of AA-derived eicosanoids. The LC *n*-3 PUFA EPA also competitively inhibits the oxygenation of AA by COX (Obata *et al.* 1999), and may suppress induction of COX-2 and 5-LOX gene expression (Curtis *et al.* 2002). It is the net result of these actions that has led to the idea that increasing intake of LC *n*-3 PUFA may have an anti-inflammatory effect.

Modulating immune and inflammatory activity with dietary n-*3 fatty acids*

There is a large literature from cell culture and animal feeding studies investigating the effects of LC *n*-3 PUFA on inflammation and immune function (for reviews, see Calder, 2003*a*; Harbige, 2003). A description of these studies is beyond the scope of the present review, which will focus upon results from human studies. These have revealed a number of pathways through which LC *n*-3 PUFA may regulate immune and inflammatory outcomes.

Effects on chemotaxis, respiratory burst, and adhesion. Supplementation with fish oil has been found to decrease neutrophil and monocyte chemotaxis (Schmidt *et al.* 1992; Sperling *et al.* 1993), neutrophil and monocyte respiratory burst (Fisher *et al.* 1990; Luostarinen & Saldeen, 1996) and neutrophil binding to endothelial cells (Lee *et al.* 1985). Amounts of fish oil consumed varied widely within these studies, providing between 2·3 and 14·5 g EPA plus DHA/d.

Effects on monocyte-derived inflammatory cytokines. Decreased production of the classic inflammatory cytokines TNF, IL-1 and IL-6 by mononuclear cells taken from subjects consuming $>2{\cdot}4$ g EPA plus DHA/d has been reported (Endres *et al.* 1989; Meydani *et al.* 1991; Caughey *et al.* 1996). In contrast to these findings, there are a number of studies reporting no effect of 0·55 to 3·4 g EPA plus DHA/d (see Calder, 2003*a*). Possible reasons for this may relate to the duration of the study, the age of the subjects studied, sample size, and differences in background diet. The dose of LC *n*-3 PUFA provided is likely to be important (Trebble *et al.* 2003; Wallace *et al.* 2003), although it cannot fully explain the discrepancy because some studies which found no effect on cytokine production provided as much as 3·2 g EPA plus DHA/d (for example, Yaqoob *et al.* 2000). A recent study has highlighted a further possible explanation, which is that polymorphisms in the promoter regions of the TNF-α and TNF-β genes play a role in conferring sensitivity of TNF-α production to fish oil intervention (Grimble *et al.* 2002).

Effects on lymphocyte proliferation. Research into the influence of LC *n*-3 PUFA on lymphocyte proliferation and production of cytokines by T lymphocytes suggests that these factors are difficult to influence using LC *n*-3 PUFA, except at rather high doses, although the effects appear to be stronger in older subjects. For example, supplementation with fish oil providing 2·4 g EPA plus DHA/d decreased the proliferation of lymphocytes and IL-2 production from older (aged 51 to 68 years) but not young (aged 21 to 33 years) women (Meydani *et al.* 1991), and inclusion of oil-rich fish providing 1·2 g EPA plus DHA/d in the diet of elderly subjects following a low-fat diet decreased lymphocyte proliferation and IL-2 production (Meydani *et al.* 1993). With respect to dosage, while 5·2 g EPA plus DHA/d was reported to decrease IL-2 and interferon (IFN)-γ production (Gallai *et al.* 1993) and decreased lymphocyte proliferation was found after men consumed 1·7 or 3·4 g EPA plus DHA/d (Molvig *et al.* 1991), there are several reports of no effect of 0·77 to 3·2 g EPA plus DHA/d on T lymphocyte proliferation or on production of various T cell-derived cytokines including IL-2 and IFN-γ (Yaqoob *et al.* 2000; Kew *et al.* 2003; Wallace *et al.* 2003).

Dietary long-chain n-*3 PUFA and inflammatory diseases*

Inappropriate immune activity and immune dysregulation are features of a range of degenerative human diseases. A common characteristic is recognition of a host antigen or of a normally-benign foreign antigen which results in activation of T lymphocytes, production of cytokines from activated T lymphocytes and of antibodies by B lymphocytes, and activation of monocyte macrophages. The latter release eicosanoids and inflammatory cytokines,

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so inducing a chronic inflammatory state and causing damage to host tissues. Generation of chemotactic agents, including eicosanoids such as LTB_4, is an element in the continuing inflammatory spiral. Aspirin and anti-inflammatory drugs are widely used for symptom relief in inflammatory diseases. Many of these act as COX inhibitors specifically targeting 2-series PG and thromboxanes. The discovery that LC *n*-3 PUFA can also act to decrease formation of these mediators prompted studies in animal models and clinical trials for a range of human diseases.

The efficacy of fish oil has been studied in several inflammatory diseases including rheumatoid arthritis, Crohn's disease, ulcerative colitis, psoriasis, lupus, multiple sclerosis, cystic fibrosis and asthma. Although there are clinical benefits reported from trials in each of these diseases (for example, see Belluzzi & Miglio, 1998; Rodgers, 1998; Ziboh, 1998; Beckles Willson *et al.* 2003), the only one for which there is really strong evidence of benefit is rheumatoid arthritis. This may be a reflection of the number of well-designed and well-conducted studies; there have been at least fourteen randomised, placebo-controlled, double-blind studies of fish oil in rheumatoid arthritis. These trials have been reviewed in some detail elsewhere (Geusens, 1998; Calder & Zurier, 2001), and so will be discussed only briefly. The dose of LC *n*-3 PUFA used was 1 to 7·1 g EPA plus DHA/d (mean dose 3·3 g/d) with a duration of 12 to 52 weeks. A variety of improvements in clinical outcome were reported, including reduced duration of morning stiffness, reduced number of tender or swollen joints, reduced joint pain, reduced time to fatigue, increased grip strength and decreased use of non-steroidal anti-inflammatory drugs. It has been concluded that the evidence for benefit from LC *n*-3 PUFA in rheumatoid arthritis is robust (Cleland & James, 2000).

There is currently considerable interest in the relative effects of *n*-3 and *n*-6 PUFA in asthma and other atopic diseases (see Prescott & Calder, 2004). The discussion centres on the roles of various eicosanoids produced from AA in mediating allergic inflammation and in programming T lymphocytes to a phenotype that predisposes to inflammation. AA-derived eicosanoids such as PGD_2, and LTC_4, D_4 and E_4 are produced by the cells that mediate pulmonary inflammation in asthma (for example, mast cells) and are believed to be the major mediators of asthmatic bronchoconstriction. Thus, it is considered that provision of LC *n*-3 PUFA to asthmatics might be beneficial because of the resulting decrease in AA-derived mediators. However, the situation is complicated by the fact that different eicosanoids have different effects, some antagonising others. Thus, interventions that aim to suppress PGE_2 production could be counterproductive, at least in some asthmatics. Nevertheless, a number of trials of fish oil in asthma and related atopic diseases have been performed (for a review, see Calder & Miles, 2000). Most of these reveal limited clinical impact, despite significant biochemical changes, although some have shown clinical improvements at least in some patient groups including children. A meta-analysis of fish oil in asthma concluded that there was no evidence of benefit (Woods *et al.* 2003), although this analysis did not separate studies in adults and children.

Perhaps intervention using LC *n*-3 PUFA once inflammatory diseases have already developed is the wrong approach and, instead, the focus should shift to prevention. PGE_2 regulates T lymphocyte differentiation promoting the development of the Th2-type phenotype that underlies sensitisation to environmental allergens. Thus it is possible that early exposure to LC *n*-3 PUFA may be protective towards allergy, asthma and related diseases. While some epidemiological evidence for this exists (Calder, 2003*b*) and there is a biologically plausible mechanism, well-designed placebo-controlled interventions are lacking. Because sensitisation to allergens occurs early in life, the characteristics of the maternal diet may be important in determining predisposition to inflammatory diseases. Therefore, studies addressing this question need to be performed early in life and perhaps in pregnant women. Several such studies are currently underway with early results available from two of them.

The first examined the role of postnatal fish oil supplementation (500 mg DHA-rich fish oil/d from weaning or from 6 months of age) in Australian children (*n* 616) at high risk of allergic disease (affected parents or siblings) (Mihrshahi *et al.* 2003). The only clinical follow-up data currently available are at 18 months, where fish oil supplementation was associated with a 9·8 % absolute reduction ($P = 0{\cdot}02$) in the prevalence of any wheeze and a 7·8 % absolute reduction ($P = 0{\cdot}04$) in prevalence of wheeze of more than 1 week. Further follow-up of this cohort will be of great interest.

The other study, also conducted in Australia, gave ninety-eight atopic women fish oil (providing 3·7 g EPA + DHA/d) or placebo from 20 weeks of gestation until delivery (Dunstan *et al.* 2003*a*,*b*). The average concentration of the Th2 cytokine IL-13 in umbilical cord plasma was 65 % lower in the fish oil group, and there was an inverse relationship between the concentration of IL-13 and the DHA content of neonatal erythrocytes (Dunstan *et al.* 2003*a*). Neonatal mononuclear cell responses to all allergens tested also showed a consistent trend for all cytokines studied (IL-5, IL-13, IL-10, IFN-γ) to be lower in the fish oil group (although this was statistically significant only for IL-10 in response to cat allergen) (Dunstan *et al.* 2003*b*). There were inverse relationships between the LC *n*-3 PUFA content of neonatal erythrocytes and cytokine responses (Dunstan *et al.* 2003*b*). These observations suggest that LC *n*-3 PUFA during pregnancy do have effects on neonatal immune function. Furthermore, infants in the fish oil group were consistently less likely to develop clinical features including food allergy, recurrent wheeze, persistent cough, diagnosed asthma, angioedema, or anaphylaxis, compared with the control group (Dunstan *et al.* 2003*b*). Although there was no difference in the frequency of atopic dermatitis at 1 year of age, infants in the fish oil group had significantly less severe disease ($P = 0{\cdot}045$), and were three times less likely to have a positive skin prick test to egg allergen at 1 year of age ($P = 0{\cdot}055$). Evidence of longer-term benefits of these interventions is awaited and should be taken into account when communicating the risks and benefits of fish oils to the public. At present in the UK, the Food Standards Agency (2004) advises against the consumption of certain types and

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amounts of fish during pregnancy and while breast-feeding due to concerns about possible excessive intakes of environmental toxins. Yet the perception that some fish are dangerous could lead to total avoidance, potentially depriving newborns of the protective effects of LC *n*-3 PUFA. Perhaps this advice needs to be updated, emphasising the importance of LC *n*-3 PUFA to fetal development, and suggesting safer dietary options, to ensure that the new research findings in the field of immune function are exploited.

Dietary intakes and recommendations

Intakes

Only limited data on LC *n*-3 PUFA by various populations and population sub-groups are available for a variety of reasons. Firstly, food composition data have only recently provided adequate information about the LC *n*-3 PUFA content of commonly eaten foods. Secondly, and probably more importantly, few studies use dietary assessment methods which are sufficiently precise, or of an ample duration, to give a reliable picture of the LC *n*-3 PUFA intakes of populations.

As Table 1 shows, intakes are expressed in different ways, hampering comparison. The most consistent data relate to the ratio between *n*-6 and *n*-3 PUFA, which seems to be increasing in Western countries, a fact that causes concern as it is thought that a high *n*-6:*n*-3 ratio may increase the risk of CVD (Lands, 2003). The mechanism for this most probably relates to the competition for metabolic pathways between *n*-6 and LC *n*-3 PUFA (Fig. 2). The ratio for Japan, at 4:1, is lower than that for the UK and considerably lower than that for the USA. Intakes of LC *n*-3 PUFA in the UK are low because most of the fish eaten is white rather than oil-rich, and amongst oil-rich fish consumers (only around one-third of the adult population) the average intake is one small portion per week (British Nutrition Foundation, 1999).

Recommendations

Information on recommendations (Table 2) is equally limited by the range of expressions used. Until recently the UK recommendation for intake of LC *n*-3 PUFA was 0·2 g/d (Department of Health, 1994). However, new advice to increase oil-rich fish consumption has superseded this. The Food Standards Agency, on advice from the Scientific Advisory Committee on Nutrition and Committee on Toxicity, now suggests that one to four oily-fish meals per week be consumed by the general UK population (Food Standards Agency, 2004; Scientific Advisory Committee on Nutrition/Committee on Toxicity, 2004). Advice for pregnant and lactating women remains at one to two portions per week due to concerns about possible contamination of some oily fish species. If translated into LC *n*-3 PUFA intakes, the new Food Standards Agency recommendations would result in a daily intake of 0·45 to 0·9 g (Ruxton *et al.* 2004). As this is considerably greater than the earlier recommendation, it would be helpful if dual advice on fish meals and on LC *n*-3 PUFA intakes were given. This would enable those who choose not to eat oil-rich fish to consume the recommended level of LC *n*-3 PUFA from other foods (for example, fortified products or supplements).

Much of the research showing therapeutic benefits of increased LC *n*-3 PUFA intake has used doses well above these recommendations, and significantly higher than could be realistically consumed through diet. It may be that supplementation, rather than dietary change, is required to attain the therapeutic effects of LC *n*-3 PUFA, for example, for individuals with an increased risk of CHD.

Vitamin E and peroxidation

Because of their larger number of double bonds, PUFA are at greater risk of peroxidation than less unsaturated fatty acids. This is a quality issue when considering foods with a high LC *n*-3 PUFA content, but a health issue when considering membranes with a high LC *n*-3 PUFA content, for example, brain and epithelial membranes. Vitamin E is a fat-soluble vitamin with antioxidant activity which is used *in vivo* to protect the PUFA in membrane lipids from peroxidation. Vitamin E requirement is closely related to the dietary intake of PUFA (Valk & Hornstra, 2000), and evidence from animal and human studies suggests that vitamin E requirement increases with the number of double bonds in the PUFA consumed (Meydani, 1992). Given the theoretical risk of peroxidation of LC *n*-3 PUFA incorporated within the body, experts have suggested supplementation of high LC *n*-3 PUFA diets with vitamin E (Weber *et al.* 1997). A review of the literature recommended that an appropriate ratio would be at least 0·6 mg α-tocopherol per g LC *n*-3 PUFA (Valk & Hornstra, 2000). This fits with an earlier review which suggested 0·4 to 0·8 mg vitamin E per g PUFA (Weber *et al.* 1997).

Food sources

Fish are the principal dietary source of LC *n*-3 PUFA and are classified as 'lean' (or white) fish, that store triacylglycerols in the liver (for example, cod), and 'oil-rich' fish, that store triacylglycerols in the flesh. The flesh of oil-rich fish is much richer in LC *n*-3 PUFA than that of lean fish. Oil-rich fish, such as mackerel, herring, salmon, trout and tuna

Table 1. Estimated daily intakes

Reference	Country	All *n*-3 PUFA	Long-chain *n*-3 PUFA	DHA	EPA	*n*6:*n*3 Ratio
Department of Health (1994)	UK		0·1 g (0·04 % energy)			
Gregory *et al.* (1990)	UK	0·72 % energy				6·9:1
Sugano & Hirahara (2000)	Japan					4:1
Simopoulos (2001)	USA			0·08 g	0·05 g	16·7:1

DHA, docosahexaenoic acid.

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Table 2. Daily recommendations (for adults unless stated otherwise)

Reference	Country	All *n*-3 PUFA	Long-chain *n*-3 PUFA	DHA	EPA	*n*6:*n*3 Ratio
Scientific Committee for Food of the European Community (1993)	General	0·5 % energy 0·5–1·0 g (children) 1–1·5 g (adults)				
Food and Agriculture Organization/World Health Organization (1998)	General	0·4–2·0 % energy				
Department of Health (1991)	UK					5:1
Department of Health (1994)	UK	>0·2 % energy	0·2 g (0·09 % energy)			
ISSFAL (1999)	UK		0·65 g (0·3 % energy)	>0·22 g >0·30 g*	>0·22 g	
Food Standards Agency (2004); Scientific Advisory Committee on Nutrition/Committee on Toxicity (2004)	UK		One to four portions oil-rich fish per week for men, boys and women past reproductive age One to two portions oil-rich fish per week for pregnant and lactating women, and girls. Translates as 0·45–0·9 g long-chain *n*-3 PUFA			
British Nutrition Foundation (1999)	General		1·1–1·4 g (0·5 % energy)			
Krauss *et al.* (2000)	General		One fatty fish meal per d or supplement equating to 0·9 g EPA + DHA			
Harris *et al.* (2003) American Heart Association guidelines	General		1 g to prevent sudden death in patients with CHD			

DHA, docosahexaenoic acid.
*The ISSFAL expert group recommended a greater amount of DHA for pregnant women.

contain an average 2 g LC *n*-3 PUFA/100 g, giving an intake of approximately 2·8 g per average portion (140 g), while lean fish, such as cod, haddock and plaice, contain an average 0·3 g LC *n*-3 PUFA/100 g (0·4 g per portion) (Scientific Advisory Committee on Nutrition/Committee on Toxicity, 2004). The LC *n*-3 PUFA content of different types of fish varies and depends on the dietary habits and metabolic characteristics of the fish, as well as the water temperature and season of the year. Tinned fish such as tuna may contain substantially less LC *n*-3 PUFA than fresh fish due to the canning process. It is not only the amount of LC *n*-3 PUFA that can vary between fish and fish oils, but the relative proportions of the individual LC *n*-3 PUFA can vary too. In a typical fish oil, EPA and DHA can comprise 20 to 30 % of the fatty acids. Fish oil can provide 200–300 mg EPA plus DHA/g. A volume of 5 ml (about 5 g) would provide about 1 to 1·5 g EPA plus DHA.

New techniques in food manufacturing have enabled the benefits of fish oils to be conferred on non-fish products. This is a useful development since the majority of Westerners rarely eat fish. Eggs can be fortified by feeding fish oils or their derivatives to poultry. A 10 % fish oil diet can increase the LC *n*-3 PUFA content of egg yolks 18-fold from 45 mg to 810 mg (Oh *et al.* 1994). However, as a high-fish-oil diet can affect flavour, a balance needs to be struck. Commercially available DHA-enriched eggs provide around 150 mg DHA per egg (Nu-Mega, personal communication). Animal diet manipulation can also be used to augment the LC *n*-3 PUFA content of meat; for example, the LC *n*-3 PUFA content of lamb muscle fat increased from 9·7 mg/g to 22·8 mg/g after lambs were fed diets supplemented with fish oil (Ponnampalam *et al.* 2002). The addition of fish oil to food products, such as baked goods, is limited by the alteration in taste which results. One way around this is to purify and microencapsulate the fish oils, then add the resulting flavourless powder to a range of food products. A growing number of products fortified in this way are now available in Europe and are considered to be both bioavailable and have the capacity to increase population intakes of LC *n*-3 PUFA (Higgins *et al.* 1999). An intervention study which gave fortified bread (providing 270 mg EPA and 195 mg DHA/d) to thirty-six volunteers with hyperlipidaemia found that plasma LC *n*-3 PUFA increased significantly after 4 weeks, while plasma levels of triacylglycerols decreased and HDL increased (Liu *et al.* 2001). This suggests the value of fortified foods, particularly in populations where oil-rich fish intakes are low.

Conclusion

Taking the evidence as a whole, and relying on the wisdom of systematic review, it is clear that LC *n*-3 PUFA have useful and consistent effects on existing CVD and on the risk of CHD in type 2 diabetes. Evidence for a clinical benefit of LC *n*-3 PUFA in rheumatoid arthritis is robust but this is not the case for other inflammatory conditions, for example, asthma, cystic fibrosis, Crohn's disease, where some studies show benefits but overall persuasive evidence is lacking. The growing literature on effects of LC *n*-3 PUFA on immune function modulation provides useful information on mechanisms, although interventions in adults and children give conflicting results. It may be that work on adapting the fetal environment using maternal LC *n*-3 PUFA supplementation could yield better data on the best way to use these nutrients to lower the risk of some conditions involving aberrant immunity.

With respect to mental health, it is clear from epidemiological and animal studies that DHA has an important role in brain development and maintenance of cognitive function. However, there is a need for more evidence from intervention studies, particularly relating to postnatal depression, child intelligence and ageing where LC *n*-3 PUFA could have far-reaching benefits. Evidence for clinical depression is slowly building and showing promising results for intakes of LC *n*-3 PUFA in the region of 1 g/d.

This leads onto the worrying gap between average population intakes and recommendations, although the latter are inconsistent and would benefit from a re-examination under the auspices of an international body. It may also be a useful idea to emphasise recommendations for different groups within the population as those with a high risk of MI seem to benefit from intakes which are high enough to be classed as therapeutic. Whichever recommendation is agreed, it is apparent that Western populations simply do not choose to eat enough fish and this may get worse in the light of concerns about fish contamination and recent changes to European Union fishing policy which could further increase prices. Alternatives to oil-rich fish are fish oil supplements and foods fortified with LC *n*-3 PUFA either during processing or animal rearing. To maximise the impact of these on LC *n*-3 PUFA intakes, nutrition education should highlight the range of options open to consumers.

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