



Omega-3 DHA and its **Importance in Human Nutrition**

A Systematic Review of the Literature

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EXECUTIVE SUMMARY

Health authorities around the world, including the UK Food Standards Agency and the US Food and Drug Administration, advise that most people should aim to increase their intake of long chain omega-3 polyunsaturates by eating more oily fish. A wealth of research conducted over the last thirty years has demonstrated beyond doubt that dietary omega-3 fatty acids are essential for healthy heart, eye and brain function. Ironically, as scientific knowledge of the health benefits has increased amongst scientists, average population consumption of oily fish has declined in Western countries as a result of evolving dietary preferences and opportunities. Furthermore, the task of reversing this negative dietary trend has been made more difficult in recent months by public concern about fish as a possible source of toxins such as mercury.

Pacific tuna is the richest natural source of omega-3 DHA and innovative food manufacturers are addressing the shortfall in dietary omega-3s by using tuna oil to fortify everyday foods, thereby increasing the range of healthy food choices available to consumers. A combination of careful sourcing and refining ensures that the tuna oil used is free from environmental contaminants while recent developments in microencapsulation technology enable manufacturers to incorporate it into a wide variety of food products without compromising flavour and stability.

In support of the rationale for developing such products, this systematic review assesses and summarises the substantial body of scientific evidence relating to the health benefits of long-chain omega-3 polyunsaturates and DHA in particular. The following conclusions are presented.

- a. **DHA in brain and retina structure:** The evidence shows that DHA is present in high concentrations in vertebrate grey matter and retina tissue, which points to an important role for DHA in these tissues.
- b. **DHA biosynthesis in humans:** The evidence is clear that the physiological activity of DHA is not the same as short chain omega-3s and its biosynthesis is relatively inefficient. It is concluded therefore that if DHA is helpful in the maintenance of healthy heart, eye and brain function, then it will be most helpful in the form of DHA rather than its precursors such as ALA from vegetable sources.
- c. **DHA dietary consumption:** There is a wide gap between recommended intakes of n-3 fatty acids and estimated levels in the Western diet.
- d. **DHA at the molecular level:** It is now generally accepted that DHA performs a special role in the function of retinal and neural membranes and it seems likely that this is a consequence of the unique characteristics it provides to membrane structure. While the level of DHA in membranes has been shown to influence sensory receptors and specific enzyme activities, the clearest link between the molecular level and behaviour has been indicated by its effect on dopamine neurotransmission. Although the significance of this mechanism remains to be comprehensively demonstrated in humans, it is already evident that diets low in n-3 and/or high in n-6 could cause reduced neural membrane DHA levels in the frontal cortex, resulting in impaired dopamine neurotransmission and adverse effects on aspects of behaviour and learning that depend on attention, motivation and emotional stability.
- e. **DHA dietary deficiency and animal studies:** Numerous studies have demonstrated beyond doubt that dietary n-3 fatty acids are essential for healthy eye and brain structure and function in mammals. DHA is the most important n-3 building block of retinal and neural structure and its availability to perform that role is influenced dramatically by its

availability in the diet. Diets deficient in n-3s reduce eye and brain levels of DHA and cause sub-optimal visual and cognitive function as well as abnormal behaviour.

- f. **The unique heart health benefits of DHA:** It is now generally accepted that the long chain n-3 fatty acids are vital nutrients for the maintenance of heart health and that low intake of DHA and EPA is a modifiable risk factor for coronary artery disease. Knowledge of the cellular and molecular processes of atherosclerosis is increasing and it is now clear there are several interwoven mechanisms by which DHA and EPA might have beneficial effects on endothelial dysfunction, inflammatory processes and lipid imbalance. Although it has been suggested that DHA provides specific benefits in controlling inflammatory mechanisms and improving blood lipid profile, the evidence is inconsistent and these possibilities require further research. However, when the evidence for other physiological mechanisms is considered in detail it seems likely that DHA does have some unique beneficial actions, which are not shared by EPA. There are strong indications that DHA in particular helps to maintain vascular endothelial function with important benefits in control of high blood pressure. In addition DHA acts on myocardial cells to reduce heart rate. Since EPA is not readily converted in vivo to DHA, consumption of DHA-rich oils rather than EPA-rich oils is most likely to provide the specific benefits of DHA.

- g. **DHA and obesity:** Obesity is recognized as one of the most important public health concerns of our time. Much more than a store of energy, abdominal fat has profound effects on health mediated by altered gene function, inflammatory cytokines and adipose hormones. The long chain n-3 polyunsaturates have been found to influence all these mediators and to produce particular benefits with respect to endothelial function, inflammatory processes, blood lipid profiles and, in certain circumstances, insulin sensitivity. For obese and overweight individuals, the likely result of increased intake of DHA and EPA is therefore reduced risk of hypertension, type-2 diabetes, atherosclerosis and cardiovascular disease. It is also clear that DHA and EPA may have different physiological effects. While EPA may be more effective in reducing elevated blood triglycerides, and both EPA and DHA may be involved in controlling the adverse effects of inflammation, recent evidence indicates that DHA has the predominant role in improving endothelial dysfunction, reducing high blood pressure and reducing heart rate abnormalities. In addition the link between endothelial dysfunction and insulin resistance also suggests that DHA could be more effective than EPA in reducing the risk of type-2 diabetes although conclusive evidence is lacking. As yet there is no evidence that DHA and EPA can directly promote weight loss in humans as they can in rodent models of obesity. However, the wealth of research data now available clearly indicates that increased fish intake, fish oil supplementation and more specifically increased DHA intake represent a powerful additional approach to weight reduction and exercise programmes in the treatment of excess abdominal fat and prevention of its deadly consequences.

- h. **DHA and pregnancy:** Supply of DHA to the developing foetus is dependent on maternal concentrations, which may be limiting but can be enhanced by increased dietary intake. Enhanced DHA intake may therefore be of particular value to pregnant women.

- i. **DHA and lactation:** The DHA content of human breast milk depends on diet and can be increased with supplementation. Investigations suggest that such supplementation could be of benefit to both the infant and the mother.

- j. **DHA and infants:** The balance of evidence suggests that dietary DHA is essential for healthy eye and brain development and function in the premature infant and also in the full term infant. The balance of evidence also suggests that fortification of infant formula with DHA is likely to support healthy eye and brain development in preterm and term infants.
- k. **DHA and behavioural conditions:** The epidemiological evidence that DHA-deficiency is a cause of violent and impulsive behaviour is supportive but not conclusive. Also, the available studies of blood fatty acid levels demonstrate lower DHA levels in individuals with ADHD. Data from supplementation studies are inconsistent but there are sufficient positive results to strengthen the view that DHA deficiency may be associated with adverse behavioural consequences in humans.
- l. **DHA and depression:** Epidemiological and tissue studies indicate a possible causal link between low intake of long chain n-3 fatty acids and depression. Results of supplementation trials have been mixed although there is some evidence that long chain n-3 fatty acids, particularly EPA and DHA may provide effective treatment for depression.
- m. **DHA and dementia:** Most recent studies provide relatively strong evidence that the incidence of dementia is higher in individuals who consume lower levels of DHA. Similarly, most recent studies show decreased tissue DHA in individuals with Alzheimer's disease. Although it may be unreasonable to expect relatively short-term supplementation to reverse physiological changes resulting from long-term dietary deficiency, one study did indeed demonstrate improvement. Taken together, the evidence strongly suggests that DHA can help to maintain brain function in the elderly.
- n. **DHA and age-related macular degeneration:** Although the evidence is still developing, most published epidemiological studies, including the largest, conclude that increased long chain n-3 intake reduces the risk of AMD. However supporting data from tissue studies and supplementation trials are lacking.

The evidence supporting the benefits of increased dietary consumption of long chain omega-3 fatty acids, and particularly DHA, continues to grow. On the basis of this systematic review presented here, two important facts are clear:

1. DHA is an essential part of optimum nutrition throughout life and most of us do not consume enough.
2. Increasing dietary consumption of DHA provides health benefits that are supported by scientific and medical research and the leading expert authorities.

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CHAPTER ONE - INTRODUCTION

1.1 Overview

The following systematic review of the published scientific literature relates to the validity of the claim that:

DHA is an essential part of optimum nutrition throughout life

The systematic review assesses the evidence that this is justified on the basis that:

- DHA is an essential constituent of eye and brain tissue throughout life.
- Endogenous synthesis of omega-3 DHA from dietary alpha linolenic acid (ALA) is inefficient and likely to be inadequate.
- Average intake of DHA in developed countries is inadequate.
- Low DHA intake is implicated in some of the most significant threats to human health and well-being.

The term 'DHA' means the omega-3 fatty acid docosahexaenoic acid (DHA). If DHA is an essential nutrient throughout life, the target group would be the whole population including infants, children, pregnant women, lactating mothers and the elderly. In fact, the evidence demonstrates that DHA intake is of particular importance for all these groups. Human studies show that endogenous synthesis of DHA from alpha-linolenic acid (ALA) is inefficient and nutritional data demonstrate that the intake of omega-3 fatty acids in the developed world is generally lower than is desirable relative to the intake of omega-6 fatty acids. Although this imbalance could be remedied at least partially by increased consumption of oily fish and the use of supplements, modern dietary regimes and preferences reflect a decline in the consumption of oily fish and an irregular consumption of supplements by the general population. Alternatively, consumption of novel foods containing enhanced levels of long chain omega-3 fatty acids in the context of a balanced diet could make a useful contribution to addressing this public health issue.

Relevant scientific evidence has been accumulating and diversifying for over 30 years and most rapidly over the last decade. Consequently, this review is wide in scope and includes over 300 published papers. While individual pieces of evidence come from many different sources their combined weight is substantial and persuasive. It is concluded that the above claim, when applied to the population as a whole, is likely to be true and that the scientific evidence in support of associated claims outweighs opposing evidence or opinion. An earlier version of this literature review was adapted for publication in 2004 (Ruxton, Reed, Simpson and Millington 2004).

1.2 Selection of articles and approach to their review

The Medline database was searched up to September 2004 using the terms docosahexaenoic acid and fatty acid. English-language, peer-reviewed papers relating to DHA and the following subjects were selected for review: biosynthesis, dietary levels, dietary recommendations, brain, nervous system, eye, retina, cardiovascular, obesity, diabetes mellitus, insulin resistance, pregnancy, lactation, infants, infant formula, dyslexia, dyspraxia, attention deficit hyperactivity disorder, aggression, stress, suicide, violence, impulsive behaviour, Alzheimer's disease, dementia, elderly, ageing, cognitive function, depression, bipolar disorder, age-related macular degeneration, antioxidants and vitamin E. Bibliographies from primary sources and reviews were also used to identify relevant papers.

1.3 Fatty acid structure and nomenclature

Unsaturated fatty acids are characterised by their chain lengths and the number of double bonds in the molecule. Docosahexaenoic acid (DHA; 22:6 omega-3 or n-3) is an omega-3 fatty acid with 22 carbon atoms and six double bonds. The term 'omega-3' indicates that, counting from the methyl (CH₃) end of the molecule, the first double bond is located between the third and fourth carbons. Counting from the carboxyl (COOH) end of the molecule the six double bonds are located after carbons 4, 7, 10, 13, 16 and 19. For this reason, the molecule is also referred to as 4, 7, 10, 13, 16, 19 – 22:6 (Sprecher, Luthria, Mohammed et al 1995, Sprecher 1999). In omega-6 fatty acids such as arachidonic acid (AA; 20:4 omega-6 or n-6) the first double bond is located after the sixth carbon atom counting from the methyl end. AA is also referred to as 5, 8, 11, 14 – 20:4 (Sprecher, Luthria, Mohammed et al 1995, Sprecher 1999).

The main fatty acids mentioned in this review are listed in Table 1.1.

Table 1.1 Fatty Acid Nomenclature

Fatty Acid	Abbreviation	Structure	Family
Docosahexaenoic	DHA	22:6	n-3
Eicosapentaenoic	EPA	20:5	n-3
Arachidonic	AA	20:4	n-6
Alpha linolenic	ALA	18:3	n-3
Linoleic	LA	18:2	n-6

Other abbreviations used are as follows:

PUFA: polyunsaturated fatty acid

LCPUFA (or LCP): long chain polyunsaturated fatty acid

EFA: essential fatty acid

1.4 DHA in brain and retina structure

A fundamental indication of the importance of DHA in healthy eye and brain function would be a high concentration of this fatty acid relative to others in these tissues. The first studies to establish that this is indeed the case were some of the earliest in the field of essential fatty acids.

The essentiality of dietary fat was first demonstrated conclusively in 1929 by Burr and Burr (Holman 1968) who in the following year demonstrated the importance of LA and introduced the term 'essential fatty acid' (Burr and Burr 1930). With improved technology for isolation of pure fatty acids, it became clear that the actions of LA were not equivalent to those of ALA (Holman, Johnson and Hatch 1982). DHA was found to be present in high concentration in human nervous tissue. O'Brien, Fillerup and Mead (1964) analysed the fatty acids in brain grey matter of three human subjects and reported high levels of DHA in the phospholipids

phosphatidylethanolamine and phosphatidylserine. Similar results were produced by O'Brien and Sampson (1965) and Svennerholm (1968).

Early studies of rat and bovine brain also demonstrated the high content of DHA in mammalian brain tissue, particularly in grey matter rather than white matter or myelin (Kishimoto, Agranoff, Radin et al 1969, Breckenridge, Gombos and Morgan 1971, 1972, Sinclair and Crawford 1972, Breckenridge, Morgan, Zanetta et al 1973). Synaptic plasma membranes were found to be particularly rich in DHA (Cotman, Blank, Moehl et al 1969). Pullerkat and Reha (1976) confirmed that the most abundant polyunsaturated fatty acid in rat brain lipids was DHA while ALA and EPA were undetectable. Crawford, Casperd and Sinclair (1976) compared 30 different mammalian species and found that despite a wide variation in fatty acid composition of livers, that of brains was remarkably constant and the most abundant long chain fatty acid in phosphatidylethanolamine in grey matter in all species was DHA. Studies of bovine brains revealed that DHA is almost exclusively confined to the 2-position of membrane phospholipids (Yabuuchi and O'Brien 1968).

Early studies of mammalian retina revealed even higher concentrations of DHA, for example Anderson and Sperling (1971) examined bovine retina and reported that 'the levels of DHA in rod outer segment phospholipids are among the highest yet reported for membrane phospholipids'. Phosphatidyl serine and phosphatidylethanolamine were found, in retinas of six mammalian species including human, to contain high levels of DHA together with the saturated fatty acid stearic acid. As reported for rat brain phospholipids (Yabuuchi and O'Brien 1968) DHA was usually found in the 2-position of phosphatidylethanolamine and phosphatidylserine with saturated fatty acids in the 1-position (Anderson and Sperling 1971, Deese, Dratz, Dahlquist et al 1981, Fliesler and Anderson 1983, Stinson, Wiegand and Anderson 1991a, b, Wiegand and Anderson 1983). Experiments with DHA – deficient rats demonstrated the importance of DHA for normal retina function (Benolken, Anderson and Wheeler 1973, Wheeler, Benolken and Anderson 1975, Futterman, Downer and Hendrickson 1971). Results from animal studies are discussed in more detail in Chapter 4.

Later studies have confirmed that, over a wide range of species, the phospholipid and fatty acid composition of vertebrate retinal membranes is remarkably uniform (Fliesler and Anderson 1983). Phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylserine (PS) account for 85-90% of the phospholipids and in PE and PS, DHA accounts for half of the total fatty acid content (Stinson, Wiegand and Anderson 1991a). The functional importance of DHA in the retina may be related to the interaction of the photoactive protein rhodopsin with particular DHA-containing phospholipids (Gordon and Bazan 1990). It has also been found that phospholipid bilayers rich in DHA have high fluidity and enhanced rates of fusion and permeability, which are characteristics important in the normal functioning of photoreceptor cells (Giusto, Pasquare, Salvador et al 2000). Studies of DHA at the molecular level are discussed further in Chapter 3.

Numerous post-mortem studies have demonstrated that human brain cells are rich in DHA (Table 1.2).

Table 1.2 Studies demonstrating that DHA is a major building block of brain grey matter

Alling and Svennerholm (1969)
Byard, Makrides, Need, Neumann and Gibson (1995)
Carver, Benford, Han and Cantor (2001).
Farquarson, Cockburn, Patrick, Jamieson and Logan (1992)
Farquarson, Jamieson, Abbasi, Patrick, Logan and Cockburn (1995)
Gershbein, Baburao, Pedroso, Rao, Arrellano and Logan (1985)
Jamieson, Farquarson, Logan, Howatson, Patrick, Weaver and Cockburn (1999)
Makrides, Neumann, Byard, Simmer and Gibson (1994)
O'Brien and Sampson (1965)
O'Brien, Fillerup and Mead (1964)
Sun (1973)
Svennerholm (1968)
White, Galli and Paoletti (1971)

Post-mortem studies on premature infants who had died soon after birth, demonstrated that rapid synthesis of brain tissue and accretion of DHA and AA occurs during the third trimester of human development (Clandinin, Chappell, Leong et al 1980, Clandinin, Chappell and Heim 1982). Also DHA reserves in the liver were found to be insufficient to meet the requirement of the developing brain in premature infants (Clandinin, Chappell, Heim et al 1981a). This led to the suggestion that pre-term infants should be fed with human milk or formulas with a fatty acid balance similar to human milk i.e. containing DHA and AA (Clandinin, Chappell, Heim et al 1981b, Farquharson, Jamieson, Abbasi et al 1995).

Subsequent post-mortem studies confirmed that preterm infants are especially at risk of dietary fatty acid imbalance (Martinez 1992) while the neurodevelopment of term infants would also benefit from adequate DHA supply (Farquharson, Cockburn, Patrick et al 1992, Jamieson, Farquharson, Logan et al 1999). Full term, breast-fed infants were found to have higher brain concentrations of DHA compared to those fed with formula not containing DHA (Makrides, Neumann, Byard et al 1994, Byard, Makrides, Need et al 1995).

1.5 Conclusion

The evidence shows that DHA is present at relatively high concentrations in vertebrate grey matter and retina tissue, which is suggestive of an important role for this fatty acid in these tissues.

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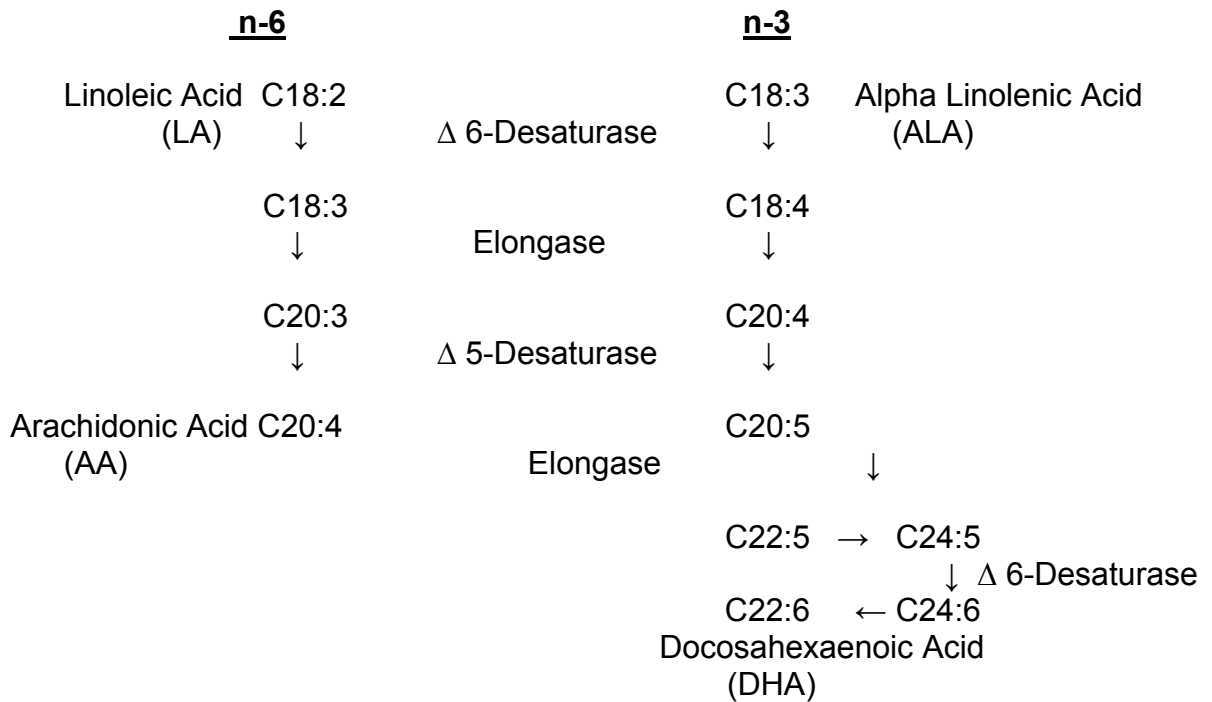
CHAPTER TWO - DHA BIOSYNTHESIS

2.1 DHA biosynthesis

DHA is synthesised from the n-3 precursor essential fatty acid, alpha-linolenic acid (ALA; 18:3 n-3) while n-6 long chain polyunsaturated fatty acids such as AA are synthesised from the precursor essential fatty acid linoleic acid (LA; 18:2 n-6). ALA and LA are essential to the human diet because neither are synthesised endogenously by vertebrates and the n-3 and n-6 families cannot be interconverted.

The n-3 and n-6 biosynthetic pathways (Figure 2.1) involve alternating steps of desaturation and elongation and are rate limited especially by the enzyme delta-6-desaturase, which, within the n-3 family, converts ALA to 18:4 n-3 as well as 24:5 n-3 to 24:6 n-3 (Crawford 2000, Sprecher 1999). Biosynthesis within the n-3 and n-6 families uses the same set of enzymes and there is competition between the two families for these enzymes. While delta-6 desaturase has a preference for ALA over LA, ALA tends to be at a competitive disadvantage because of high dietary intake of LA (Budowski 1988). Relative excess of LA in the Western diet tends to inhibit endogenous conversion of ALA to EPA and DHA (Gerster 1998).

Figure 2.1. n-3 and n-6 biosynthetic pathways



It was discovered recently that the conversion of EPA to DHA involves more than single desaturation and elongation steps. In fact, two chain elongations and a desaturation are followed by partial beta-oxidation in peroxisomes (Sprecher, Luthria, Mohammed et al 1995). Compared to the earlier understanding, as indicated by Crawford (2000) 'this circuitous route involves more metabolic time, an additional rate-limiting delta-6 desaturation, translocation of 24:6n-3 to peroxisomes for the final oxidation step, and then export to the reticuloendothelial

system'. The DHA produced by this route is then used in the synthesis of membrane phospholipids (Cockburn 1997). Studies with labelled ALA and DHA demonstrate that preformed DHA, rather than that synthesised de novo from ALA is preferentially incorporated into neural cell membranes while ALA is more important to the brain as a substrate for cholesterol synthesis than for brain DHA (Crawford 2000).

2.2 DHA biosynthesis in humans

It has been recognised since the 1970s that human capacity to convert ALA to EPA and DHA may be limited (Sanders and Younger 1980, Singer, Berger, Wirth et al 1986) and subsequent studies have confirmed this (Emken, Adlof and Gulley 1994). In studies of vegans who consume no EPA or DHA, erythrocyte levels of DHA were less than half as high as in omnivores, and supplementation of the vegan diet with ALA-rich linseed oil did not significantly increase DHA levels (Sanders and Younger 1981, Agren, Tormala, Nenonen et al 1995).

In a comparison of linseed oil and fish oil supplementation in healthy volunteers, high doses of linseed oil increased platelet EPA levels but much less markedly than lower doses of fish oil while linseed oil supplementation did not increase platelet DHA levels (Sanders and Roshanai 1983). Although four ALA supplementation studies found increased blood levels of DHA (Beitz, Mest and Forster 1981; Kestin, Clifton, Belling 1990; Layne, Goh, Jumpson et al 1996; Ezaki, Takahashi, Shigematsu et al 1999); the majority have confirmed that ingestion of ALA increases blood EPA but not DHA (Table 2.1).

Table 2.1 Studies demonstrating that ALA is not readily converted to DHA

ALA supplementation studies

Allman, Pena and Pang (1995)
Chan, McDonald, Gerrard et al (1993)
Cunnane, Hamadeh, Liede et al (1995)
Fokkema, Brouwer, Hasperhoven et al (2000)
Freese, Mutanen, Valsta et al (1994)
Kelley, Nelson, Love et al (1993)
Kwon, Snook, Wardlaw et al (1991)
Lasserre, Mendy, Spielmann et al (1985)
Li, Sinclair, Wilson et al (1999)
Mantzioris, James, Gibson et al (1994)
Mutanen, Freese, Valsta et al (1992)
Renaud, Godsey, Dumont et al (1986)
Seppanen-Laakso, Vanhanen, Laakso et al (1992)
Seppanen-Laakso, Vanhanen, Laakso et al (1993)
Sanders and Roshanai (1983)
Sanders and Younger (1981)
Singer, Berger, Wirth et al (1986)
Svahn, Feldl, Raiha et al (2002)
Tarpila, Aro, Salminen et al (2002)
Valsta, Salminen, Aro et al (1996)
Weaver, Corner, Bruce et al (1990)
Wensing, Mensink and Hornstra (1999)

Labelled ALA supplementation studies

Burdge, Finnegan, Minihane et al (2003)
Burdge, Jones and Wootton (2002)
Burdge and Wootton (2002)
Emken, Adlof and Gulley (1994)
Emken, Adlof, Rakoff et al (1990)
Pawlosky, Hibbeln, Novotny et al (2001)
Vermunt, Mensink, Simonis et al (2000)

In 22 out of 26 supplementation studies, ALA as linseed oil did not significantly increase blood levels of DHA. In seven studies that administered a single high dose of radio-labelled ALA, the conversion of ALA to DHA was usually detected but at low levels of efficiency compared with conversion to other fatty acids or carbon dioxide. The results demonstrate that ALA is converted only inefficiently to EPA and even less efficiently, if at all, to DHA.

Comparisons of the various physiological consequences of supplementation with linseed oil and fish oil have also demonstrated that they are not equivalent in their actions (Harris 1997, Knapp 1997). Kestin, Clifton, Belling et al (1990) found that supplementation with fish oil caused a decrease in plasma cholesterol, triglycerides and systolic blood pressure but there were no significant effects of the diet supplemented with ALA. Kelley, Nelson, Love et al (1993) found that linseed oil supplementation increased EPA but not DHA levels in plasma and monocytes, and had no significant effect on plasma triglycerides or lipoprotein levels. Layne, Goh, Jumpson et al (1996) found that supplementation with linseed oil produced small changes in lipoprotein levels of EPA and DHA but did not alter plasma triglycerides level. By contrast, fish oil supplementation increased EPA and DHA levels and reduced plasma triglycerides level. In a comparison of the effects of linseed oil and fish oil in well-controlled type II diabetics, McManus, Clandinin, Jumpson et al (1996) found that fish oil reduced triglycerides levels while linseed oil did not.

Both full term and pre-term infants are capable of synthesising AA and DHA from their 18-carbon precursors (Carnielli, Wattimena, Luijendijk et al 1996, Sauerwald, Hachey, Jensen et al 1996, 1997, Uauy, Mena, Wegher et al 2000) although the amount of DHA produced from ALA may be inadequate (Salem, Wegher, Mena et al 1996, Woods, Ward and Salem 1996). In a primate study, Su, Bernardo, Mirmiran et al (1999) concluded that the contribution of endogenous synthesis of DHA from ALA did not match that of diets with preformed DHA.

In 2002, the UK Food Standards Agency convened a group of expert scientists to review current research investigating whether ALA was as beneficial to cardiovascular health as EPA and DHA (Wootton, Finnegan, Williams et al 2002). The group concluded that the evidence suggests little, if any, benefit of ALA and that the effects observed with fish oil supplementation are not replicated by ALA supplementation.

2.3 Conclusion

The evidence seems clear that the physiological activity of DHA is not the same as its shorter chain precursors and its biosynthesis is relatively inefficient. It is concluded therefore that if DHA is found to be helpful in the maintenance of healthy eye and brain function, then it will be most helpful in the form of DHA rather than its precursors.

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CHAPTER THREE - MOLECULAR LEVEL

3.1 DHA at the molecular level

In the eyes and brains of all vertebrates, DHA is found at high concentrations within retinal and neural membranes in the form of phospholipids such as phosphatidylethanolamine and phosphatidylserine. The highest concentrations of DHA are found in the membranes of nerve synapses and in the membranes of the outer segments of rod photoreceptors (Lauritzen, Hansen, Jorgensen and Michaelsen 2001). It is generally accepted that this highly specific tissue distribution of DHA in eyes and brains points to a special function for the molecule that other fatty acids either perform less well or cannot perform at all (Salem and Niebylski 1995). Thus the retina and nervous system have extremely low or undetectable levels of the other n-3 fatty acids that can be obtained from the diet, namely ALA and EPA, and it seems likely that DHA itself, rather than these precursors, is necessary to support optimal function of the eye and brain (Mitchell, Gawrisch, Litman and Salem 1998).

Evidence for the unique functional status of DHA at the molecular level has accumulated over several decades, especially in recent years with the development of new techniques and an improved understanding of the fundamental molecular mechanisms of cell membranes. It is possible that DHA, when released from membrane phospholipids by the action of phospholipase A2, could have important biochemical functions as a ligand for certain receptors involved in cell signalling (Xu, Sanchez, Sali and Heintz 1996; de Urquiza, Liu, Sjoberg, Zetterstrom, Griffiths, Sjoval and Perlmann 2000) or as a precursor of active metabolites (Bazan 2003; Hong, Gronert, Devchand, Moussignac and Serhan 2003) as is well known for arachidonic acid and its eicosanoid metabolites. However, evidence of the significance of similar actions for DHA is limited and, while AA is physiologically most important as a messenger in cell signalling pathways, the primary role of DHA seems to be as a component of membranes rather than the free fatty acid (Lauritzen, Hansen, Jorgensen and Michaelsen 2001; Mitchell, Gawrisch, Litman and Salem 1998).

3.2 DHA and membrane properties

It is now well recognised that biological membranes are complex, dynamic structures with fundamental roles in physiological function (Clandinin, Jumpsen and Suh 1994; Mitchell, Gawrisch, Litman and Salem 1998; Kurlak and Stephenson 1999; Li, Chen and Anderson 2001). In the membranes of neurons and retinal cells, the DHA in phospholipids confers specific structural qualities that not even docosapentaenoic acid (22:5, n-6) can replicate despite having the same chain length as DHA and only one fewer double bonds (Mitchell, Salem and Niebylski 1995; Eldho, Feller, Tristram-Nagle, Polozov and Gawrisch 2003; Stillwell and Wassall 2003). Evidence comes from in vitro studies of artificial membranes which demonstrate that the interaction of DHA-rich phospholipids and cholesterol results in the sorting of phospholipids into highly organised complexes called rafts or microdomains (Simons and Ikonen 1997; Shaikh, Brown and London 1998; Huster, Arnold and Gawrisch 1998; Rietveld and Simons 1998; Petrache, Salmon and Brown 2001; Huber, Rajamoorthi, Kurze, Beyer and Brown 2002; Brzustowicz, Cherezov, Caffrey, Stillwell and Wassall 2002; Niu and Litman 2002; Armstrong, Brzustowicz, Wassall, Janski and Stillwell 2003; Cherezov, Caffrey, Stillwell and Wassall 2003). The interaction of proteins and lipids is a fundamental relationship in membrane function and studies indicate that microdomains provide platforms that support the actions of specific proteins. Such investigations have led many researchers to suggest that it is by virtue of membrane structural properties that DHA performs a vital role in eye and brain function. The types of protein that may be influenced by DHA in membranes include sensory receptors, ion channels and enzymes.

3.3 DHA and sensory receptors

The influence on protein function of DHA in membranes has been investigated most thoroughly in respect to G-protein coupled signalling in the retina (Kurlak and Stephenson 1999). The visual transduction system converts photons of light to neural impulses through the action of a membrane-bound G-protein called rhodopsin (Litman and Mitchell 1996). Interaction between a photon and rhodopsin causes a change in shape of the protein that is most rapid and efficient when the surrounding membrane is rich in DHA-containing phospholipids (Brown 1994; Koenig, Strey and Gawrisch 1997; Mitchell, Niu and Litman 2001; Niu, Mitchell and Litman 2001; Saiz and Klein 2001; Litman, Niu, Polozova and Mitchell 2001; Mitchell, Niu and Litman 2003). It therefore seems possible that decreased retinal DHA levels caused by n-3 deficient diets could cause the visual deficits observed in experimental animals as a result of decreased efficiency in visual signalling pathways.

G-protein coupled receptors modulate many physiological and behavioural signalling pathways via changes in receptor activation and inactivation states (Tan, Brady, Nickols, Wang and Limbird 2004). The completion of the human genome project has identified approximately 720 genes that belong to the G-protein coupled receptor family and about half of these genes are thought to encode sensory receptors (Wise, Jupe and Rees 2004). Since DHA may affect the efficiency with which rhodopsin adopts the activated state upon stimulation through its role in promoting membrane flexibility and reactivity, it seems likely that DHA may also affect the activation of G-protein coupled receptors other than rhodopsin, such as those involved in the transmission of nerve impulses through other sensory neurons (Lauritzen, Hansen, Jorgensen and Michaelsen 2001).

3.4 DHA and ion channels

Electrical signals in nerve cells and other excitable tissues such as heart muscle are generated by currents of ions passing through specific membrane ion channels in a highly organised way. In vitro experiments have demonstrated that several fatty acids in the free form, rather than incorporated in phospholipids, have the ability to influence the passage of ions through ion channels and therefore the transmission of electrical impulses (Ordway, Singer and Walsh 1991, Honore, Barhanin, Attali, Lesage, Lesage and Lazdunski 1994; Poling, Karanian, Salem and Vicini 1995; Poling, Vicini, Rogawski and Salem 1996). Leaf and co-workers found that polyunsaturated fatty acids are able to correct cardiac arrhythmias by blocking cardiac sodium channels, the major class of ion channel that determines cardiac excitability (Kang and Leaf 1996; Leaf 2001). Further studies demonstrated that a similar mechanism operates in neural tissue since sodium and calcium channels in rat hippocampus CA1 neurons were also modified by PUFA (Vreugdenhil, Bruell, Voskuyl, Kang, Leaf and Wadman 1996). It was suggested that these effects may reduce neuronal excitability and may exert anticonvulsive effects in vivo. More recently, a study conducted on rat hippocampus neurons found that DHA could attenuate epileptic activity by frequency-dependent blockade of sodium channels leading to dampening of the repetitive firing of neurons (Young, Gean, Chiou and Shen 2000). DHA was also found to inhibit potassium channels in rat olfactory receptor neurons (Seebungkert and Lynch 2002). It should be noted however that this phenomenon is not restricted to DHA. AA and other fatty acids in the free form may have a direct inhibitory effect on ion channels in vitro (Kang and Leaf 1995) and it is therefore not possible to explain the unique benefits of DHA by reference to ion channels alone.

It is possible that free PUFAs including DHA could have a direct effect on ion channels in vivo since membranes undergo a continual remodelling process involving release of fatty acids from phospholipids by the action of phospholipases such as phospholipase A2 (Poling, Vicini, Rogawski and Salem 1996; Jones, Arai and Rapoport 1997; Farooqu and Horrocks 2001; Li, Chen and Anderson 2001; Rapoport 2003). It is also possible that metabolites of PUFAs, rather than PUFAs themselves could influence ion channel function and while this has been demonstrated for AA metabolites (Ordway, Singer and Walsh 1991) there is little current evidence that DHA metabolites function in this way in vivo (Mitchell, Gawrisch, Litman and Salem 1998).

3.5 DHA and enzymes

In addition to ion channels themselves, maintenance of the correct ionic gradient across neuronal membranes also depends on the activity of particular enzymes. For example, calcium-ATPase has the essential function of keeping intracellular calcium levels within a particular range as required for normal neurotransmission. DHA was found to inhibit calcium-ATPase in synaptic membranes and it has been suggested that modulation of this enzyme could contribute to the possible mechanisms by which omega-3 fatty acids exert their beneficial effects (Kearns and Haag 2002). The authors also noted that DHA exerted its effect on calcium-ATPase while bound to a phospholipid in the membrane and not as a free fatty acid. Sodium, potassium-ATPase is another enzyme with a vital role in maintaining ionic homeostasis in neurons and many other cell types through its action as a sodium pump (Dunbar and Caplan 2001, Scheiner-Bobis 2002). Supplementation of rat diets with DHA was found to increase DHA levels in synaptic membranes and influence sodium, potassium-ATPase activity (Bowen and Clandinin 2002). Recent studies have also found a new family of membrane-bound protein regulators of sodium pump function, named FXYD (Crambert and Geering 2003). The evidence suggests that sodium, potassium-ATPase interacts with a particular FXYD to maintain ion gradients essential for neuronal excitability and future studies might reveal membrane effects of DHA that influence FXYD action. It has been noted in studies of simple life forms such as leeches that modulation of potassium and calcium channels is involved in the molecular mechanism for learning (Brunelli, Garcia-Gil, Mozzachiodi, Scuri and Zaccardi 1997).

The protein kinases represent another group of enzymes with important roles in learning and the formation of long-term memories (Micheau and Riedel 1999). It is known that DHA and several other PUFAs may influence protein kinase C (PKC) activity although the significance of this effect in vivo is not known (Hardy, Ferrante, Robinson, Johnson, Poulos, Clark and Muray 1994; Kim, Weeber, Sweatt, Stoll and Marangell 2001). DHA also stimulates diacylglycerol kinase activity in rat brain membranes (Vaidyanathan, Rao and Sastry 1994). Although nearly all of the possible products of phospholipid cleavage (i.e. phospholipid headgroups, lysophospholipids, diacylglycerols, phosphatidic acid and fatty acids) have been implicated in PKC activation (Merrill and Schroeder 1993), it is possible that DHA in membranes has a more fundamental role in regulating its activity. It is possible that DHA-enhanced partitioning of the enzyme into specific membrane domains has the effect of exposing PKC more effectively to activators such as diacylglycerols. In studies of artificial membranes, it was found that DHA acyl chains produced the highest level of PKC activity compared to less saturated fatty acids (Giorgione, Epanand, Buda and Farkas 1995).

Stillwell and Wassall (2003) note that there is a long and growing list of proteins that are affected by DHA including phospholipases A2 and C. Activation of enzymes and other proteins may involve promotion of gene expression and research is currently being directed to understanding the effects of n-3 supplemented diets at the genetic level (Kitajka, Puskas, Zvara,

Hackler, Barcelo-Coblijn, Yeo and Farkas 2001; Puskas, Kitajka, Nyakas, Barcelo-Coblijn and Farkas 2003). While changes in gene expression in rat brains were associated with n-3 supplementation, ALA-rich oil produced similar effects to fish oil and the significance of these observations in terms of brain function is not yet understood.

3.6 DHA and neurotransmission

The importance of DHA in brain function may be related in part to its involvement in the action of neurotransmitters such as acetylcholine, serotonin, noradrenalin and especially dopamine (Owens and Innis 1999, 2000; Itokazu, Ikegaya, Nishikawa and Matsuki 2000, Chalon, Vancassel, Zimmer, Guilloteau and Durand 2001). In early experiments, increased neurotransmitter activity was associated with increased incorporation of DHA from the bloodstream into synaptic membranes (Jones, Arai and Rapoport 1997). In rodents maintained on a diet deficient in n-3 fatty acids, the level of DHA in cerebral membranes was reduced with resultant adverse effects on dopamine neurotransmission (Kodas, Vancassel, Lejeune, Guilloteau and Chalon 2002).

This area of research has been developed in detail by researchers at the French Laboratoire de Biophysique Medicale et Pharmaceutique who have found changes in the activities of dopamine pathways specific to particular areas of the brain that are likely to influence behaviour (Chalon, Delion-Vancassel, Belzung, Guilloteau, Leguisquet, Besnard and Durand 1998, Chalon, Vancassel, Zimmer, Guilloteau and Durand 2001, Zimmer, Vancassel, Cantagrel, Breton, Delamanche, Guilloteau, Durand and Chalon 2002). Earlier studies by the same institute found that DHA could modulate adrenoreceptors and thereby influence melatonin production in the rat pineal gland (Gazzah, Gharib, Delton, Moliere, Durand, Christon, Lagarde and Sarda 1993; Delton-Vandenbroucke, Sarda, Moliere, Lagarde and Gharib 1996; Zaouali-Ajina, Gharib, Durand, Gazzah, Claustrat, Gharib and Sarda 1999). Japanese researchers also found that DHA exerts different effects on neurotransmission in different parts of the hippocampus emphasising that a full explanation of structure-function relationships in neurons will involve an understanding of the functions and interactions of particular brain regions (Itokazu, Ikegaya, Nishikawa and Matsuki 2000). Nevertheless, the French group has revealed potential links between n-3 fatty acids, neuron structure and behaviour. Synapses of the frontal cortex had decreased numbers of vesicles, especially in the nucleus accumbens, an area known to be important in the control of attention, motivation and emotion (Delion, Chalon, Guilloteau, Besnard and Durand 1996; Zimmer, Hembert, Durand, Breton, Guilloteau, Besnard and Chalon 1998; Zimmer, Delpal, Guilloteau, Aioun, Durand and Chalon 2000; Zimmer, Delion-Vancassel, Durand, Guilloteau, Badard, Besnard and Chalon 2000). Resultant behavioural deficits involved reduced motivation or response to reward rather than direct effects on learning ability. Also, greater reactivity to external stimuli in dopamine-deficient animals could cause reduced attention and therefore delayed acquisition of learning tasks (Wainwright, Xing, Mutsaers, McCutcheon and Kyle 1997, Wainwright 2002). It is not yet possible to relate these results to humans but mouse models of attention deficit hyperactivity disorder (ADHD) have been developed (Paule, Rowland, Ferguson, Chelonis, Tannock, Swanson and Castellanos 2000) and studies are underway of the possible relationship between dietary DHA and AA, and the behavioural symptoms of ADHD (Clements, Girard, Xing and Wainwright 2003).

It is clear that dietary omega-3 may influence animal behaviour by influencing neurotransmitter function. Modifications of the physical properties of neuronal membranes by changing their DHA content may affect the function of receptors for neurotransmitters and other proteins that influence neurotransmission as has been found for ion channels and membrane transporter enzymes (Bazan 2003; Haag 2003). Also, the speed with which synaptic membranes release

neurotransmitters from synaptic vesicles may depend on the physical properties of the membranes as determined by their DHA content (Zimmer, Delpal, Guilloteau, Aioun, Durand and Chalon 2000; Stillwell and Wassall 2003).

3.7 Conclusion

It is now generally accepted that DHA performs a special role in the function of retinal and neural membranes and it seems likely that this is a consequence of the unique characteristics it provides to membrane structure. While the level of DHA in membranes has been shown to influence sensory receptors and specific enzyme activities, the clearest link between the molecular level and behaviour has been indicated by its effect on dopamine neurotransmission. Although the significance of this mechanism remains to be demonstrated in humans, it is possible that diets low in n-3 and/or high in n-6 could cause reduced neural membrane DHA levels in the frontal cortex, resulting in impaired dopamine neurotransmission and adverse effects on aspects of behaviour and learning that depend on attention, motivation and emotional stability.

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CHAPTER FOUR - ANIMAL STUDIES

4.1 DHA in brain and retina function: studies on non-human mammals

The role of DHA in eye and brain function has been the subject of hundreds of experiments on mammals over the last thirty years. These studies have focussed on rodents because of their short life cycle and relative ease of management, but cats, pigs and primates have also been used to investigate the possibility that DHA deficiency causes impaired visual or brain function, as indicated by deficits in learning or cognitive performance. Several procedures have been developed to induce DHA deficiency in brain and retinal tissues but the method most commonly used with rodents is to feed n-3 deficient diets to the dams prior to breeding as well as during gestation and lactation, and to the offspring until the time of testing (Wainwright, Huang, Coscina et al 1994).

It is generally accepted that rodent brain and retina cells retain DHA tenaciously. However, the degree of retention may have been overestimated in early experiments because commonly used fat, protein and carbohydrate sources for laboratory animals contained low but significant levels of n-3 fatty acids (Greiner, Moriguchi, Slotnick et al 2001). A recent study in rats found that in the second generation, a diet truly deficient in n-3 fatty acids produced an 82% decrease in brain DHA compared to n-3 adequate controls (Greiner, Moriguchi, Slotnick et al 2001). The process can be accelerated by an artificial rearing procedure whereby infant rats are removed from their mothers before normal weaning and fed synthetic formula. Using this technique, total brain DHA was reduced by more than 50% in one generation and more than 90% over two generations (Ward, Woods, Reyzer et al 1996). It has also been observed in rodents that a diet rich in saturated fat as well as low in n-3 fatty acids causes a more rapid and severe decline in brain DHA level (Wainwright, Huang, Bulman-Fleming et al 1994).

The phenomenon of tenaciously retained brain DHA is less evident in primates, which appear to be relatively more sensitive to dietary n-3 deficiency (Connor, Neuringer and Lin 1990). Also, DHA levels in brain and synaptic membranes were significantly lower in piglets fed a low n-3 diet for as little as 2-3 weeks after birth compared to piglets fed formulas containing DHA (De la Presa Owens and Innis 1999, Hrboticky, MacKinnon, Puteman and Innis 1989, Hrboticky, MacKinnon and Innis 1990). The overall picture is that in rodents, primates and other mammals, numerous studies have shown that n-3 deficient diets cause reduced brain and retina levels of DHA with compensatory increases in a fatty acid of the n-6 series, namely docosapentaenoic acid (22:5 n-6). In addition to severe dietary restriction of n-3s, experiments have measured the effects of supplementing the diet with n-3 and various n-3 to n-6 ratios (Table 4.1). The general conclusion is that brain and retina fatty acid composition reflects dietary availability (Wainwright 2000).

Table 4.1 Studies demonstrating that brain and retina levels of DHA in mammals are determined by dietary availability of n-3 fatty acids.

Abedin, Lien, Vingrys et al(1999)
Alling, Bruce, Karlsson et al (1972)
Bourre, Durand, Pascal et al (1989)
Bourre, Pascal, Durand et al (1984)
Bourre, Youyou, Durand et al (1987)
Cocchi, Pignatti, Carpigiani et al (1984)
Connor, Neuringer and Lin (1990)
Favreliere, Barrier, Durand et al (1998)
Galli, Trzeciak and Paoletti (1971)
Hargreaves and Clandinin (1988)
Houmayoun, Duran, Pascal et al(1988)
Hrboticky, MacKinnon and Innis (1990)
Hrboticky, MacKinnon, Puterman et al (1989)
Jumpsen, Lien, Goh et al (1997)
Kurvinen, Kuksis, Sinclair et al (2000)
Lin, Anderson, Connor et al (1994)
Lin, Connor, Anderson et al (1990)
Mohrhauer and Holman (1963)
Moriguchi, Loewke, Garrison et al (2001)
Philbrick, Mahadevappa, Ackman et al (1987)
Tarozzi, Barzanti, Biagi et al (1984)
Tinoco, Babcock, Hincenbergs et al (1978)
Ward, Woods, Reyzer et al (1996)
Watanabe, Doshi and Hamazaki (2003)
Weisinger, Vingrys and Sinclair (1995)
Youyou, Durand, Pascal et al (1986)

Since the mid 1970s (Lamptey and Walker 1976) a substantial body of work has been conducted on mammals and there is now no doubt that the reduced brain and retina levels of DHA caused by experimental deficits in dietary n-3 fatty acids are likely to cause abnormalities in visual function and various measures of brain performance (Table 4.2). The functional consequences of reduced brain and retinal DHA include abnormal electroretinograms, diminished visual acuity, impaired recovery after dark adaptation, reduced brightness and olfactory discrimination, reduced performance in maze tests and other spatial learning tasks and abnormal shock avoidance behaviour.

Table 4.2 Studies demonstrating that low n-3 intake results in reduced eye and brain function

Bourre, Francois, Youyou et al(1989)
Bush, Malnoe, Reme et al (1994)
Carrie, Clements, De Javel et al (1999)
Carrie, Smirnova, Clement et al (2002)
Catalan, Moriguchi, Slotnick et al (2002)
Champoux, Hibbeln, Shannon et al (2002)
Connor, Neuringer, Barstad et al (1984)
Coscina, Yehuda, Dixon et al (1986)
Diau, Loew, Wijendron et al (2003)
Enslin, Milan and Malnoe (1991)
Frances, Coudereau, Sandouk et al (1996)
Frances, Monier and Bourre (1995)
Frances, Monier, Clement et al (1996)
Gamoh, Hashimoto, Hossain et al (2001)
Greiner, Moriguchi, Hutton et al (1999)
Greiner, Moriguchi, Slotnick et al (2001)
Ikemoto, Ohishi, Sato et al (2001)
Jeffrey, Mitchell, Gibson et al (2002)
Kameyama, Ohara, Nakashima et al (1996)
Lamprey and Walker (1976)
Lim and Suzuki (2000a)
Lim and Suzuki (2000b)
Lim and Suzuki (2001)
Lim and Suzuki (2002)
Mills, Ward and Young (1988)
Morgan, Oppenheimer and Winick (1981)
Moriguchi, Greiner and Salem (2000)
Moriguchi and Salem (2003)
Nakashima, Yuasa, Hukamizu et al (1993)
Neuringer, Connor, Lin et al (1986)
Neuringer, Connor, Van Petten et al (1984)
Ng and Innis (2003)
Okaniwa, Yuasa, Yamamoto et al (1996)
Pawlosky, Denkins, Ward et al (1997)
Reisbick, Neuringer, Connor et al (1991)
Reisbick, Neuringer, Hasnain et al (1990)
Reisbick, Neuringer, Hasnain et al (1994)
Salem, Moriguchi, Greiner et al (2001)
Shirai and Suzuki (2004)
Stockard, Saste, Benford, Barness, Austad and Carver (2000)
Sugimoto, Taga, Nishiga, Fujiwara, Konishi, Tanaka and Kamei (2002)
Suzuki, Park, Tamura and Ando (1998)
Tsukada, Kakiuchi, Fukumoto et al (2000)

Watanabe, Kato, Aonuma et al (1987)
Wainwright, Xing, Girard et al (1998)
Wainwright, Xing, Mutsaers et al (1997)
Weisinger, Vingrys and Sinclair (1996a)
Weisinger, Vingrys and Sinclair (1996b)
Wheeler and Benolken (1974)
Wheeler, Benolken and Anderson (1975)
Yamamoto, Hashimoto, Takemoto et al(1988)
Yamamoto, Okaniwa, Mori (1991)
Yamamoto, Saitoh, Moriuchi et al (1987)

Although some rodent studies failed to demonstrate significantly reduced performance in certain tests of learning ability (Table 4.3), it is possible that aspects of the experimental design could explain the discrepancies. For example, the experiments conducted by Wainwright's group were conducted during the dark cycle of mice, which is when they were likely to be most active (Wainwright, Huang, Coscina et al 1994). The authors note that the circadian differences in activity could interact with treatments to affect outcomes in different studies. Wainwright (1992) and Salem's group (Greiner, Moriguchi, Hutton et al 1999, Greiner, Moriguchi, Slotnick et al 2001) discuss other possible sources of error in such experiments.

Table 4.3 Studies that failed to demonstrate a significant effect of reduced DHA levels on eye and brain performance measures

Leat, Curtis, Millichamp et al (1986)
Wainwright, Huang, Bulman-Fleming et al (1994)
Wainwright, Huang, Bulman-Fleming et al (1991)
Wainwright, Huang, Coscina et al (1994)
Wainwright, Xing, Ward, Huang, Bobik, Austed and Montalto (1999)

While the weight of evidence demonstrates a crucial role for DHA in eye and brain function, it is not yet possible to describe the precise nature of this role because of the limitations of the tests used. For example, reduced performance in a spatial learning task could be due to reduced sensory or motor capacity or reduced motivation or arousal as well as cognitive deficits in acquiring learning tasks. However, most recent experiments have addressed these problems and results suggest that learning and motivational deficits rather than sensory or motor deficits were the most likely cause of reduced performance (Catalan, Moriguchi, Slotnick et al 2002, Greiner, Moriguchi, Slotnick et al 2001, Moriguchi and Salem 2003).

A few papers suggest possible negative effects on rodent development of very high levels of n-3 fatty acid supplementation (Haubner, Stockard, Saste et al 2002, Stockard, Saste, Benford et al 2000, Wainwright, Jalali, Mutsaers et al 1999) but it seems likely that adverse effects on growth and auditory function were due to severely reduced levels of membrane arachidonic acid brought about by the excessive n-3 levels (Wainwright, Xing, Mutsaers et al 1997).

An important question with all animal experiments is the extent to which they provide insights into human health and nutrition (Innis 2000, Carlson 2000). Such studies are more likely to provide evidence of relevance to humans if test animals and humans share physiological structures and mechanisms that DHA deficiency or supplementation can be shown to affect (Yehuda, Rabinovitz and Mostofsky 1999). Over the last few years, several DHA-related animal

studies have been focussed on possible mechanisms of this sort (Table 4.4). It is now accepted that the electrical activity of nerve cells described as brain waves depends on the structure of cell membranes since the ion channels they contain control the transport of ions into and out of the cell. It also seems likely that neuronal membranes perform most efficiently in their transmission of brain waves when their structural components such as cholesterol, arachidonic acid and DHA are present in optimal proportions (Yehuda, Rabinovitz and Mostofsky 1999). The evidence shows that reduced DHA intake causes clear physiological effects that are likely to influence behaviour and performance. In addition to structural changes in neural membranes, these effects include changes in gene expression, reduced cell size in the hippocampus, altered cholinergic, serotonergic and dopaminergic neurotransmission, alterations in cannabinoid receptors and changes in pineal function.

Table 4.4 Studies investigating possible physiological mechanisms for behavioural and cognitive deficits resulting from reduced brain DHA.

Ahmad, Murthy, Greiner et al (2002)
Aid, Vancassel, Pumes-Ballihaut et al (2003)
Barcelo-Coblijn, Hogenes, Kitajka et al (2003)
Barcelo-Coblijn, Kitajka, Puskas et al (2003)
Berger, Crozier, Bisogno et al (2001)
Chalon, Delion-Vancassel, Belzung et al (1998)
De la Presa Owens and Innis (1999)
Delion, Chalon, Guilloteau et al (1996)
Delion, Chalon, Herault et al (1994)
Favreliere, Perault, Huguet et al (2003)
Favreliere, Stadelmann-Ingrand, Huguet et al (2000)
Hamilton, Greiner, Salem et al (2000)
Kitajka, Puskas, Zvara et al (2002)
Murthy, Hamilton, Greiner et al (2002)
Puskas, Kitajka, Nyakas et al (2003)
Takeuchi, Fukumoto and Harada (2002)
Takeuchi, Iwanaga and Harada (2003)
Watanabe, Doshi and Hamazaki (2003)
Ximenes da Silva, Lavialle, Gendrot et al (2002)
Yehuda, Rabinovitz and Mostofsky (1999)
Yoshida, Miyazaki, Takeshita et al (1997)
Yoshida, Yasuda, Kawazato et al (1997)
Zhang, Hamilton, Salem et al (1998)
Zimmer, Vancassel, Cantagrel et al (2002)

It is evident from Table 4.4 that the study of physiological mechanisms relevant to eye and brain function is accelerating and therefore seems likely to generate new insights and explanations for the acknowledged vital role of DHA. Although extrapolation from animal studies to humans is of limited value on its own, it does point the way to the kinds of human trials that are most likely to yield useful results. The physiological mechanisms discovered in animals may also lead to the identification of useful biological markers in humans.

4.2 Conclusion

Numerous studies have demonstrated beyond doubt that dietary n-3 fatty acids are essential for healthy eye and brain structure and function in mammals. DHA is the most important n-3 building block of retinal and neural structure and its availability to perform that role is influenced dramatically by its availability in the diet. Diets deficient in n-3s reduce eye and brain levels of DHA and cause sub-optimal visual and cognitive function as well as abnormal behaviour.

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CHAPTER FIVE - THE UNIQUE HEART HEALTH BENEFITS OF DHA

5.1 Introduction

Population studies, clinical trials and meta-analyses have brought expert scientific opinion to the point where it is no longer doubted that generally increased consumption of the long chain n-3 fatty acids, particularly EPA and DHA, helps to maintain heart health and reduces the risk of developing coronary artery disease (Kris-Etherton, Harris and Appel 2002; Lefevre, Kris-Etherton, Zhao and Tracy 2004; Bucher, Hengstler, Schindler and Meier 2002; Marchioli, Schweiger, Tavazzi and Valagussa 2001; Nordoy, Marchioli, Arnesen, Videbaek 2001; Bucher, Hengstler, Schindler and Meier 2002). This conclusion, coupled with the recognition that fish intake in Western diets is declining, has prompted numerous expert committees and national governments to recommend increased consumption of long chain n-3 polyunsaturates as discussed in Chapter 14.

5.2 Coronary artery disease

Coronary artery disease, in which blood supply to the heart is obstructed, is the most common form of heart disease and the single most important cause of premature death in the developed world (Nguyen and McLoughlin 2002; Shah 2003). In the UK, one in four men and one in five women die from the disease (Haslett, Chilvers, Hunter and Boon 1999). Disease of the coronary arteries is almost always due to atherosclerosis and its complications, particularly thrombosis. Atherosclerosis is a disease of the arterial wall in which fatty streaks develop as circulating monocytes migrate into the sub-endothelial space, take up oxidized low-density lipoprotein (LDL) from the plasma, and become lipid-laden foam cells. Fatty streaks can resolve or progress to form lesions called plaques. If plaque development progresses, smooth muscle cells migrate into and proliferate within the plaque and as it grows it reduces the lumen of the vessel. A mature plaque has a core of lipid surrounded by smooth muscle cells and is separated from the lumen by a cap of fibrous tissue. Plaques may rupture, allowing blood to enter and disrupt the arterial wall, precipitating thrombosis and local vasospasm. Plaque rupture leads to rapid growth of the lesion, which may then block the coronary artery entirely causing a heart attack.

Risk factors for coronary artery disease have been identified including the non-modifiable risks of increasing age, male gender and family history. However, several approaches to risk reduction arise from the identification of modifiable risks (Table 5.1).

Table 5.1 Modifiable risk factors for coronary artery disease

Smoking
Hypertension
Lipid disorders
 - high LDL cholesterol level
 - low HDL cholesterol level
 - high triglyceride level
Diabetes mellitus
Insufficient physical exercise

Obesity

Excessive alcohol intake

Diets deficient in polyunsaturated fatty acids, and fruit and vegetables

Although these risk factors are well established and quoted in medical text books (e.g. Haslett, Chilvers, Hunter and Boon 1999), it is recognized that they cannot account for all individuals who develop coronary heart disease and other potential determinants of pathogenesis are emerging from a deeper understanding of the cellular and molecular processes of atherosclerosis including endothelial dysfunction and inflammatory processes as well as lipid disorders (Gonzalez and Selwyn 2003; Nguyen and McLaughlin 2002). Increased understanding of these physiological mechanisms has also shed light on the beneficial actions of EPA and DHA in maintaining heart health and in addition, revealed ways in which EPA and DHA have different effects. Recent research challenges the view that EPA is the most important long chain n-3 for heart health and suggests that DHA has unique beneficial actions.

5.3 DHA and endothelial dysfunction

One of the earliest events in the development of atherosclerosis is defective function of the vascular endothelium, the layer of cells lining blood vessels that performs several important roles including maintenance of blood circulation and fluidity, regulation of vascular tone, modulation of monocyte and platelet adhesion and migration of monocytes across the endothelium (Briner and Luscher 1994; Triggle, Hollenberg, Anderson, Ding, Jiang, Ceroni, Wiehler, Ellis, Andrews, McGuire and Pannirselvam 2003; Brown and Hu 2001; Valgimigli, Merli, Malagutti, Soukhomovskaia, Cicchitelli, Macri and Ferrari 2003). In endothelial dysfunction, cell-surface molecules involved in adhesion such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) are up-regulated in the process known as endothelial activation, which involves increased production of inflammatory cytokines, growth factors and proatherogenic molecules such as monocyte chemoattractant protein-1 (MCP-1) and macrophage colony-stimulating factor (M-CSF). As a result, platelets and monocytes stick more easily to the endothelium and vascular permeability is increased, allowing monocytes to migrate into the sub-endothelial space where they develop into macrophages and take up oxidized LDL to form foam cells. The inflammatory cytokines produced by macrophages including interleukin-1 (IL-1) and interleukin-6 (IL-6) also promote smooth muscle cell proliferation. During continued inflammation, inflammatory cells release enzymes such as metalloproteinases, which cause further damage to the arterial wall, sometimes progressing to rupture of plaques, thrombus formation and blockage of the artery (Ross 1999; Katsuda and Kaji 2003).

Most of these intricacies of endothelial function and inflammation have been investigated in relation to the observed cardiovascular benefits of n-3 fatty acids (Brown and Hu 2001; Hirafuji, Machida, Hamaue and Minami 2003; Chin and Dart 1995). Fish oil improved endothelial function in three supplementation studies (Goodfellow, Bellamy, Ramsey, Jones and Lewis 2000; Abe, El-Masri, Kimball, Pownall, Reilly, Osmundsen, Smith and Ballantyne 1998; Fleischhauer, Yan and Fischell 1993) and another study of the effects of fish oil in hypercholesterolaemic individuals found that vascular tone was significantly improved by supplementation for three months (Goode, Garcia and Heagerty 1997). Although two supplementation studies by a Norwegian group did not find reduced adhesion molecule levels with fish oil supplementation (Johansen, Seljeflot, Hostmark and Arnesen 1999; Seljeflot, Arnesen, Brude, Nenseter, Drevon and Hjermann 1998) these studies did show that consumption of fish oil beneficially affected blood clotting factors associated with endothelial function.

In vitro studies have revealed how endothelial activation involves interactions between monocytes and a variety of adhesion molecules such as VCAM-1 and ICAM-1, which are influenced in their level of activity by cytokines and numerous other molecules. It was found that, when added to cultured endothelial cells before activation with cytokines, DHA significantly more than EPA or n-6 fatty acids inhibited molecular events associated with endothelial activation including expression of VCAM-1 and ICAM-1 (Caterina, Bernini, Carluccio, Liao and Libby 1998). Three other in vitro studies also indicated that DHA in particular has beneficial effects on endothelial function by reducing monocyte adhesion (Caterina, Cybulsky, Clinton, Gimbrone and Libby 1994; Weber, Erl, Pietsch, Danesch and Weber 1995; Khalfoun, Thibault, Bardos and Lebranchu 1996).

Dietary supplementation with fish oil prolongs bleeding time and may decrease risk of thrombosis (Lefevre, Kris-Etherton, Zhao and Tracy 2004). Rodent studies found that fish oil and DHA in particular has antithrombotic effects probably due to inhibition of platelet action and reduction of blood viscosity (Kimura, Tamayama, Minami, Hata and Saito 1998; Andriamampandry, Leray, Freund, Cazenave and Gachet 1999). Another rat study concluded that EPA and DHA have similar antithrombotic effects (Nieuwenhuys and Hornstra 1998). However, a recent study in hypertensive type 2 diabetics found that DHA was a more effective anti-thrombotic agent than EPA (Woodman, Mori, Burke, Puddey, Watts and Beilin 2003).

In vitro studies found that DHA more than EPA or AA increased nitric oxide (NO) production by endothelial cells leading to improved endothelial relaxation (Hirafuji, Machida, Tsunoda, Miyamoto and Minami 2002). There is also indirect evidence in humans based on the urinary excretion of NO metabolites that DHA may enhance endothelial function compared to EPA (Harris, Rambjor, Windsor and Diederich 1997). Recent reviews concluded that although the mechanisms are not yet fully understood, it seems likely that DHA has more potent and beneficial effects in relation to endothelial function than EPA (Brown and Hu 2001; Hirafuji, Machida, Hamaue and Minami 2003).

5.4 DHA and hypertension

The functions of vascular endothelium are likely to influence one of the most important modifiable risk factors of cardiovascular disease, namely hypertension. Several studies in experimental animals and humans with hypertension have demonstrated a moderate reduction of blood pressure following supplementation with fish oils (Appel, Miller, Seidler and Whelton 1993; Morris, Sacks and Rosner 1993; Kenny and Egan 1994; Knapp 1996; Bao, Mori, Burke, Puddey and Beilin 1998; Abeywardena and Head 2001; Hirafuji, Machida, Hamaue and Minami 2003). It was found that blood pressure was likely to be reduced significantly in patients with elevated rather than normal levels if they received at least 3g long chain n-3 fatty acids per day. These results are supported by a population study conducted in south India in which 1000 healthy adults in fish-consuming and non-fish consuming communities were compared. Mean blood pressures and pulse rates were significantly lower in older men and women who were fish consumers (Bulliyya, Reddy and Reddama 1999).

A link between n-3 deficiency and hypertension was revealed in a recent study in which n-3 deficient rats gave birth to offspring that went on to develop significantly elevated blood pressures (Armitage, Pearce, Sinclair, Vingrys, Weisinger and Weisinger 2003). DHA was found to be more effective than EPA at retarding the development of hypertension in spontaneously hypertensive rats (McLennan, Howe, Abeywardena, Muggli, Raederstorff, Mano, Rayner and Head 1996). Other rat studies demonstrated that dietary DHA increased levels of DHA in blood

vessel tissue, reduced vascular wall thickness and reduced systolic blood pressure (Engler, Engler, Kroetz, Boswell, Neeley and Krassner 1999; Engler, Engler, Pierson, Molteni and Molteni 2003). A recent study in humans found that DHA but not EPA reduced ambulatory blood pressure and heart rate in mildly hyperlipidaemic men (Mori, Bao, Burke, Puddey and Beilin 1999). Evidence for the involvement of vascular function in these differential effects was found in studies of vascular reactivity in hyperlipidaemic, overweight men (Mori, Watts, Burke, Hilme, Puddey and Beilin 2000). Relative to placebo, DHA but not EPA enhanced vasodilator mechanisms and reduced constrictor responses in forearm blood vessels. It was concluded that this effect might contribute to the blood pressure lowering effect observed with DHA but not EPA.

5.5 DHA and the electrical function of myocardial cells

In addition to demonstrating that DHA and not EPA reduced blood pressure in humans, the study by Mori et al (1999) found that DHA but not EPA reduced heart rate. Another randomized double-blind placebo-controlled supplementation trial in healthy, non-smoking men also compared the effects of EPA and DHA on blood pressure and heart rate. Although blood pressure did not change during the intervention as expected in normotensive men, mean heart rate declined significantly in the DHA group and increased significantly in the EPA group (Grimsgaard, Bonna, Hansen and Myhre 1998). Earlier studies also indicated that fish oil consumption could reduce heart rate (Bonna, Bjerve, Straume, Gram and Thelle 1990; Vandongen, Mori, Burke, Beilin, Morris and Ritchie 1993) and led to the suggestion that long chain n-3 fatty acids might have beneficial effects on the electrical function of myocardial cells.

Animal experiments have demonstrated that n-3 fatty acids are readily incorporated into myocardial cells and have potent antiarrhythmic actions (McLennan, Bridle, Abeywardena and Charnock 1993; Leaf and Kang 1997; Pepe and McLennan 1996). Leaf, Kang and colleagues hypothesized that when PUFAs are incorporated into myocardial cells, they act to provide electrical stability by modulating conductance of ion channels (Kang and Leaf 2000; Leaf, Xiao, Kang and Billman 2003). However the effect is not unique to DHA and EPA has also shown antiarrhythmic effects in studies of isolated myocardial cells (Hallaq, Sellmayer, Smith and Leaf 1990) and animal models (Billman, Kang and Leaf 1999). By contrast, McLennan et al (1996) showed that DHA but not EPA prevented ischaemic-induced cardiac arrhythmias in rat studies and concluded that DHA may be the principal active component conferring cardiac protection.

5.6 DHA and inflammatory markers in coronary heart disease

Patients with coronary heart disease produce elevated levels of inflammatory markers such as C-reactive protein (CRP) as well as ICAM-1, IL-6 and tumour necrosis factor (TNF) (Ross 1999). It has been shown that CRP levels in particular may be useful in predicting coronary heart disease and other vascular diseases such as stroke (Backes, Howard and Moriarty 2004; Ridker and Cooke 2004; Libby and Ridker 2004; Hirschfield and Pepys 2003). In the Physicians' Health Study, 14,916 apparently healthy men were screened for CRP as well as HDL cholesterol and total cholesterol (Ridker, Cushman, Stampfer, Tracy and Hennekens 1997). After more than eight years of follow-up it was found that base-line CRP levels accurately predicted risk of heart attacks and stroke. Baseline levels of total cholesterol and HDL were also correlated with myocardial events but CRP and lipid parameters provided a significantly better method of predicting risk than lipids alone (Ridker, Glynn and Hennekens 1998).

Dietary fish oil supplementation has been shown to suppress the synthesis of the pro-inflammatory cytokines IL-1 and TNF (Endres, Ghorbani, Kelley, Georgilis, Lonnemann, van der

Meer, Cannon, Rogers, Klempner and Weber 1989) and this suppression may lead to the inhibition of CRP expression in the liver. In a double-blind placebo-controlled trial on post-menopausal women, it was found that dietary supplementation with a combination of safflower oil and fish oil for five weeks (7g per day of each) more efficiently reduced CRP and IL-6 than 14 g per day of either oil alone (Ciubotaru, Lee and Wander 2003). It is notable that the highest levels of plasma EPA were achieved in the high fish oil group but the highest levels of plasma DHA were obtained with the combined oil supplement. In a study of 405 healthy men and 454 healthy women, intake of both EPA and DHA was inversely associated with plasma levels of TNF and CRP (Pischon, Hankinson, Hotamisligil, Rifai, Willett and Rimm 2003). However, treatment with a combination of n-6 and n-3 fatty acids was associated with the lowest levels of inflammation. Another study in men with clinical suspicion of coronary artery disease found that the DHA content of granulocyte membranes was inversely correlated with CRP concentrations but there was no correlation between the EPA content of granulocytes and CRP concentrations (Masden, Skou, Hansen, Fog, Christensen, Toft and Schmidt 2001). Differential effects of EPA and DHA may help to explain why several recent studies of fish oil supplementation have failed to produce significant reductions in inflammatory markers such as CRP and IL-6 (Chan, Watts, Barrett, Beilin and Mori 2002; Grundt, Nilsen, Mansoos, Hetland and Nordoy 2003; Madsen, Christensen, Blom and Schmidt 2003; Vega-Lopez, Kaul, Devaraj, Cai, German and Jialal 2004). If fish oil supplementation results in blood levels of EPA higher than those of DHA, the particular beneficial effects of the latter, including reduction of inflammatory markers, may be obscured. However, one study of treated hypertensive type 2 diabetics compared the effects of EPA and DHA separately and found no significant effect of either on CRP, IL-6 or TNF (Mori, Woodman, Burke, Puddey, Croft and Beilin 2003).

5.7 DHA and blood lipids

As well as endothelial and inflammatory changes, it is well known that the development of atherosclerosis involves disturbed lipid profiles including blood triglycerides, total cholesterol, low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol (Nguyen and McLaughlin 2002; Weber and Raederstorff 2000). In a meta-analysis involving over 57,000 subjects, elevated triglycerides were associated with an increased cardiovascular risk of 76% in women and 32% in men (Austin, Hokanson and Edwards 1998). The association between elevated cholesterol levels and coronary heart disease was first noted in the 1930s (Nguyen and McLaughlin 2002). Numerous studies since then have established that high total cholesterol, high LDL cholesterol and low HDL cholesterol are independent cardiovascular risk factors (Carson 2003; Carneiro, Costa and Borges 2004). Many other studies have investigated the effect of fish oil supplementation on blood lipids and usually found that triglyceride levels are reduced (Connor, De Francesco and Connor 1993; Harris 1996; 1999; Pilot, Blanche, Boulet, Fortin, Dubreuil, Marcoux, Davignon and Lussier-Cacan 2003). For example, in a double-blind placebo-controlled cross over study in men with mild hypertriglyceridaemia, a supplement of 6g fish oil caused a significant decrease in triglyceride levels (Leigh-Firbank, Minihane, Leake, Wright, Murphy, Griffin and Williams 2002). However, multivariate analysis indicated that it was EPA rather than DHA that was responsible for the effect.

A similar conclusion was reached by a supplementation in which the independent effects of EPA and DHA were compared (Rambjor, Walen, Windsor and Harris 1996). By contrast, another double-blind placebo-controlled supplementation study found that triglyceride levels were reduced by both EPA and DHA (Grimsgaard, Bonna, Hansen and Nordoy 1997). Similarly, a double-blind placebo-controlled trial on overweight, non-smoking and mildly hypertensive men found that both DHA and EPA reduced triglyceride levels (Mori, Burke, Puddey, Watts, O'Neal, Best and Beilin 2000). More recently, a trial on 42 normolipidaemic adults found that DHA

supplementation (4.9 g per day for four weeks) reduced triglyceride level significantly but a similar dose of EPA did not (Buckley, Shewring, Turner, Yaqoob and Minihabe 2004). An apparently negative effect of DHA was to increase LDL cholesterol levels by 8%. However, this was associated with an overall increase in LDL particle size, a change the authors believed could contribute to a reduction in atherogenic risk through reduced oxidation of LDL particles. A similar increase in LDL particle size and increase in plasma LDL cholesterol concentration was found in a fish oil supplementation trial on treated hypertensive subjects (Suzukawa, Abbey, Howe and Nestel 1995).

5.8 An important metabolic difference between EPA and DHA

Several studies investigating the independent cardiovascular effects of DHA and EPA have found that on consumption of DHA, some is retroconverted to EPA but on consumption of EPA, none is elongated to DHA (Rambjor, Walen, Windsor and Harris 1996; Grimsgaard, Bonna, Hansen and Nordoy 1997; Nelson, Schmidt, Bartolini, Kelley and Kyle 1997; Mori, Bao, Burke, Puddey and Beilin 1999; Mori, Watts, Burke, Hilme, Puddey and Beilin 2000; Mori, Burke, Puddey, Watts, O'Neal, Best and Beilin 2000; Stark and Holub 2004). It is clear therefore that the specific benefits of DHA cannot be obtained by consuming EPA. Furthermore it is possible that when supplied in combination where EPA predominates, as in EPA-rich fish oil, EPA could actually interfere with the beneficial actions of DHA.

5.9 Conclusion

It is now generally accepted that the long chain n-3 fatty acids are vital nutrients for the maintenance of heart health and that low intake of DHA and EPA is a modifiable risk factor for coronary artery disease. Knowledge of the cellular and molecular processes of atherosclerosis is increasing and it is now clear there are several interwoven mechanisms by which DHA and EPA might have beneficial effects on endothelial dysfunction, inflammatory processes and lipid imbalance. Although it has been suggested that DHA provides specific benefits in controlling inflammatory mechanisms and improving blood lipid profile, the evidence is inconsistent and these possibilities require further research. However, when the evidence for other physiological mechanisms is considered in detail it seems likely that DHA does have some unique beneficial actions, which are not shared by EPA. There are strong indications that DHA in particular helps to maintain vascular endothelial function with important benefits in control of high blood pressure. In addition DHA acts on myocardial cells to reduce heart rate. Since EPA is not readily converted in vivo to DHA, consumption of DHA-rich oils rather than EPA-rich oils is most likely to provide the specific benefits of DHA.

Omega-3 DHA
and its
Importance in Human Nutrition

CHAPTER SIX - DHA AND OBESITY

6.1 Introduction

According to the World Health Organization there are more than one billion overweight adults globally and at least 300 million of them are obese (WHO 2004). Overweight is defined as a Body Mass Index (BMI) of greater than or equal to 25 and obesity is defined as a BMI of greater than or equal to 30 where BMI expresses weight in kg divided by (height in m)² (Montague 2003). The incidence of obesity and overweight has increased dramatically over the last 30 years especially in developed countries (Swinburn, Caterson, Seidell and James 2004) and the upward trend shows no sign of slowing down (Flegal, Carroll, Ogden and Johnson 2002; Montague 2003). An International Obesity Task Force (IOTF) has been formed as part of the International Association for the Study of Obesity (IASO) and its chairman stated recently that 'Obesity constitutes one of the most important medical and public health problems of our time' (James 2004). In the USA, 64% of adults and nine million children are already either overweight or obese (James, Thomas, Cavan and Kerr 2004; Ogden, Carroll and Flegal 2003).

Obesity in adults is associated with excess mortality and excess risk of coronary heart disease, high blood pressure, abnormal lipid profile, type 2 diabetes, gall bladder disease, osteoarthritis and certain cancers including those of the prostate, breast and uterus (Felson 2004; Nammi, Koka, Chinnala and Boini 2004; Mathus-Vliegen, Van Ierland-Van Leeuwen and Terpstra 2004; Ogden, Carroll and Flegal 2003; Adderley-Kelly, Williams-Stephens 2003; Pi-Sunyer FX 1999). While popular use of fish oil dietary supplements may be motivated by a desire to prevent cardiovascular disease, treat arthritis or prevent cancer (Harrison, Holt, Pattison and Elton 2004; Harel, Riggs, Vaz, White and Menzies 2001), recent research indicates a major role for long chain n-3 polyunsaturates in the prevention and treatment of the metabolic consequences of obesity. These complications represent some of the most important modifiable risk factors for coronary heart disease, namely type 2 diabetes, abnormal lipid profile and hypertension. Although the physiological mechanisms involved are not fully understood, evidence is emerging for the interwoven involvement of inflammatory processes, endothelial dysfunction and insulin resistance in the development and progression of obesity-related diseases and it is in relation to such mechanisms that long chain n-3 polyunsaturates may exert their most beneficial effects. Recent evidence also suggests that dietary n-3 polyunsaturates may deliver some of their benefits because they cause positive changes at an even more fundamental level of metabolism - that is at the level of gene transcription.

6.2 Obesity and its metabolic complications

Recent studies indicate that the metabolic complications of obesity are related to excess fat located in the abdomen rather than elsewhere on the body such as the gluteofemoral or subcutaneous regions (Lebovitz 2003; Despres, Lemieux and Prud'homme 2001; Jensen 1998). This specifically abdominal obesity is now included in a cluster of medical conditions named the 'metabolic syndrome' which is also characterized by insulin resistance, high blood cholesterol, high blood triglycerides and hypertension (Grundey 2002). The syndrome was first defined in the Third Report of the US National Cholesterol Education Programme Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults, released in 2001 (LaRosa and Gotto 2004; Lipy 2003). It has been estimated that 47 million Americans exhibit the metabolic syndrome (CDC 2004).

Abdominal obesity is positively associated with the risk of developing insulin resistance and considerable research effort is aimed at understanding this connection (Reaven, Abbasi and McLaughlin 2004; Cruz, Wegensberg, Huang, Ball, Shaibi and Goran 2004; Turkoglu, Duman, Gunay, Cagatay, Ozcan and Buyukdevrim 2003; Caprio 2002; Garvey and Hermayer 1998; Carey, Walters, Colditz, Solomon, Willett, Rosner, Speizer and Manson 1997). A recent meta-analysis of clinical studies reported that obesity can increase the risk of diabetes by greater than ninety fold (Anderson, Kendall and Jenkins 2003). It is now understood that abdominal adipose tissue is much more than a store of energy. It is involved in many other activities via the synthesis and secretion of a range of biologically active molecules including hormones such as adiponectin and leptin (Guerre-Millo 2004; Matsuda and Shimomura 2004) and pro-inflammatory cytokines (Faraj, Lu and Cianflone 2004; Havel 2004; Jazet, Pijl and Meinders 2003; Lebovitz 2003; Sowers 2003). Both leptin and adiponectin have positive effects in obesity in that they exert an insulin-sensitizing effect and insulin resistance has been linked to leptin resistance and decreased plasma adiponectin (Guerre-Millo 2004).

However, there is also evidence that normally positive effects can become negative when the hormone is present in excess. For example, unusually high leptin levels are correlated with insulin resistance and other markers of the metabolic syndrome including abnormal lipid profile and hypertension (Ren 2004). Research on such hormonal activities is at an early stage and although the mechanisms are largely unknown it seems that they are important in coordinating the balance between fat utilization by oxidation and fat storage. There are also indications that leptin provides a link between abdominal fat and central nervous circuits that lead to reduced appetite (Ren 2004). Although such connections are potentially of great significance, there is more evidence for the role of inflammatory cytokines in obesity and it is clear they are important modulators of obesity-related disease (Browning 2003).

6.3 Inflammation and insulin resistance

While inflammation is a vital defense mechanism initiated in response to infection and trauma, and involving pro-inflammatory cytokines such as interleukin (IL)-1 beta, IL-6 and tumour necrosis factor – alpha (TNF-alpha), these molecules are also key factors in the aetiology of inflammatory diseases. It is now increasingly recognized that obesity may be regarded as a state of abnormal inflammatory response (Lyon, Law and Hsueh 2003; Aldahi and Hamdy 2003) and obese individuals have been found to have elevated levels of TNF-alpha in plasma, adipose and muscle tissues (Hotamisligil 2003; Tsigos, Kyrou, Chala, Tsapogas, Stavridis, Raptis and Katsilambros 1999; Saghizadeh, Ong, Garvey, Henry and Kern 1996; Kern, Saghizadeh, Ong, Bosch, Deem and Simsolo 1995; Hotamisligil and Spiegelman 1994). During acute infection or injury, IL-6 and TNF-alpha are released from the site of tissue injury and promote an acute-phase response. This mechanism is also characterized by production of a range of molecules including C-reactive protein (CRP) and sialic acid such molecules are now used routinely in research studies as markers of inflammation (Browning 2003). For example, it has been shown that elevated CRP is associated with all the obesity-related metabolic pathologies including coronary heart disease, myocardial infarction, hypertension, insulin resistance, type-2 diabetes and abnormal blood lipid profiles (Ridker and Cooke 2004; Chae, Lee, Rifai and Ridker 2001; Danesh 1999; Yudkin, Stehouwer, Emeis and Coppel 1999; Ross 1999).

It is now clear from numerous studies that chronic low-grade inflammation characterized by elevated circulating inflammatory markers is associated with insulin insensitivity and the development of diabetes (Dandona, Aljada and Bandyopadhyay 2004; Crook 2004; Fernandez-real and Ricart 2003; Xu, Barnes, Yang, Tan, Yang, Chou, Sole, Nichols, Ross, Tartaglia and

Chen 2003; Hotamisligil 2003; Dandona and Aljada 2002; Grimble 2002, Corry and Tuck 2001). For example in a study on healthy Asian and European men and women, on the link between CRP, body fat distribution and insulin insensitivity it was concluded that abdominal obesity promotes chronic inflammation, thereby contributing to the metabolic syndrome (Forouhi, Sattar and McKeigue 2001). Similarly, a Brazilian study found that markers of inflammation correlated with components of the metabolic syndrome including insulin resistance and abdominal obesity (Duncan and Schmidt 2001) while a study on normal weight, overweight and obese Italian women found independent relationships between abdominal fat accumulation, insulin resistance and plasma CRP levels (Pannacciulli, Cantatore, Minenna, Bellacicco, Gioirgino and De Pergola 2001). In a large US study involving over 27,000 women, it was found that 188 women developed type-2 diabetes during the 4-year follow-up period. Compared to age-matched controls, elevated levels of CRP and IL-6 predicted the development of type-2 diabetes (Pradhan, Manson, Rifai, Buring and Ridker 2001). When the acute phase response was studied in a group of Caucasian subjects it was found that serum sialic acid and IL-6 were at relatively high levels in individuals with type-2 diabetes and metabolic syndrome (Pickup, Mattock, Chusney and Burt 1997). In non-diabetic individuals taking part in the US Insulin Resistance Atherosclerosis Study involving over 1000 subjects, strong associations were found between CRP, body fat and insulin resistance (Festa, D'Agostino, Howard, Mykkanen, Tracy and Haffner 2000). In a comparison of overweight and normal subjects, it was found that high levels of inflammation as determined by plasma content of CRP, were correlated with relatively low insulin sensitivity in overweight subjects (Fernandez-Real, Broch, Vendrell and Ricart 2003).

Studies on the metabolic syndrome in rodent models of obesity as well as in humans have implicated TNF-alpha as an important factor in the development of insulin resistance (Hotamisligil, Shargill and Spiegelman 1993; Grimble 2002). Several mechanisms have been suggested to account for the negative actions of TNF-alpha including down-regulation of genes that are required for normal insulin action and direct effects on insulin signaling and induction (Moller 2000).

6.4 Control of gene expression

A potentially important point of influence for dietary factors such as n-3 polyunsaturates in treatment of the metabolic syndrome is at the level of gene expression. Polyunsaturated fatty acids including n-6 as well as n-3 long chain polyunsaturates and their oxidized derivatives have been shown to regulate lipid metabolism by modulating gene expression in several ways including changes to transcription, processing of messenger RNA and post-translational protein modification (Clarke 2004). According to Clarke (2004), the liver appears to use polyunsaturated fatty acids as a nutrient sensor to determine whether fatty acids are to be stored or oxidized. A key mechanism involves regulation by polyunsaturates of the activity or abundance of several transcription factors including peroxisome proliferator activated receptor (PPAR) alpha, beta and gamma, liver X receptors (LXR) alpha and beta, hepatic nuclear factor-4 (HNF-4) alpha and sterol regulatory element binding proteins (SREBP) 1 and 2 (Jump 2002). These transcription factors are essential mediators in the liver metabolism of carbohydrates, fatty acids, triglycerides, cholesterol and bile acid. As an example of the action of cytokines in modulating transcription factors, TNF-alpha inhibits PPAR-gamma, an important insulin-sensitizing nuclear receptor, which plays a key role in the regulation of glucose and lipid metabolism (Moller and Berger 2003; Bocher, Pineda-Torra, Fruchart and Staels 2002).

In addition to affecting metabolism in the liver, long chain polyunsaturated fatty acids are also involved at the level of gene activation in processes of inflammation, which may indicate fundamental connections between inflammatory processes and metabolism. It is now believed that an important trigger mechanism of inflammation involves a transcription factor called NF-kappa-B (Aggarwal, Takada, Shishodia, Gutierrez, Oommen, Ichikawa, Baba and Kumar 2004). Stimulation of cells by any process that causes inflammation activates NF-kappa-B, which is translocated into the nucleus where it switches on the genes that control the transcription of other genes for inflammatory proteins such as TNF-alpha (Lebovitz 2003; Sonnenberg, Krakower and Kissebah 2004). TNF-alpha and other cytokines modulate transcription factors involved in metabolic processes in the liver. Furthermore, members of the PPAR family of transcription factors are also expressed in vascular endothelial cells where they exhibit anti-inflammatory and antiatherogenic properties (Marx, Duez, Fruchart and Staels 2004).

6.5 Endothelial dysfunction

In addition to increased risk of insulin resistance and type-2 diabetes, it has been found that abdominal obesity is also likely to be associated with endothelial dysfunction (Caballero 2003; Hak, Pols, Stehouwer, Meijer, Kiliaan, Hofman, Breteler and Witteman 2001). Obesity, insulin resistance and endothelial dysfunction are all found in groups at increased risk of type-2 diabetes such as those with hypertension and abnormal lipid profile. Some recent clinical studies demonstrated that treatment of obesity and/or insulin resistance ameliorates endothelial dysfunction as well as the inflammatory response (Caballero 2003). Similarly, studies in type-2 diabetics show that endothelial dysfunction is linked to insulin resistance and improved metabolic control in diabetic patients is associated with restoration of endothelial function (Guerci, Bohme, Kearney-Schwartz, Zannad and Drouin 2001). In one study of a population characterized by high incidence of obesity and insulin resistance it was found that markers of inflammation increased with abdominal obesity but also markers of endothelial dysfunction increased in proportion to insulin resistance and inflammation (Vozarova, Weyer, Lindsay, Pratley, Bogardus and Tataranni 2002). In another study, obese, insulin-resistant individuals were also found to have endothelial dysfunction and the effect of insulin on enhancement of endothelium-dependent vasodilation was abnormally reduced in these individuals (Steinberg, Chaker, Leaming, Johnson, Brechtel and Baron 1996). Insulin resistance has been found to result in vascular smooth muscle proliferation as well as impaired nitric oxide-mediated vasodilation, both of which contribute to the development of atherosclerosis (Reusch 2002).

As discussed in Section 5.3, defective function of the vascular endothelium is one of the first events in the development of atherosclerosis and involves increased production of inflammatory cytokines, cell adhesion molecules and other substances that promote atherogenesis. Nitric oxide (NO) has been identified as an essential mediator in the maintenance of normal vascular function while two of the key molecules involved in the relationship between obesity, inflammation and insulin resistance, namely TNF-alpha and NF-kappa-B, are also key molecules in the relationship between obesity and endothelial dysfunction (Aldhahi and Hamdy 2003; Park, Park, Kang and Kang 2003; Collins, Read, Neish, Whitley, Thanos and Maniatis 1995; Read, Whitley, Williams and Collins 1994).

6.6 Abnormal lipid profile

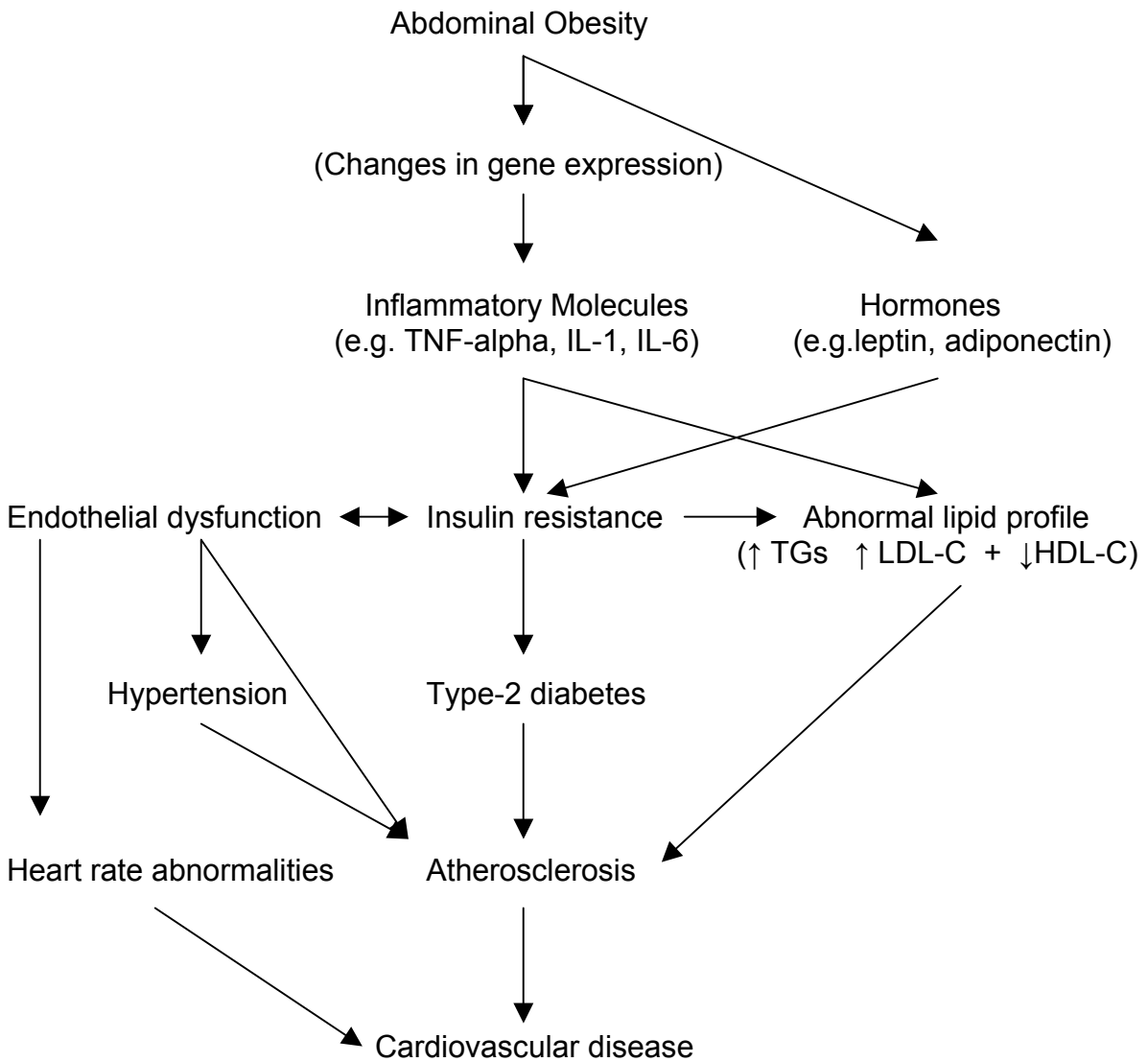
Abdominal obesity and insulin resistance are frequently associated with high plasma levels of triglycerides, LDL-cholesterol (LDL-C) and low plasma levels of HDL-cholesterol (HDL-C) (Nieves, Cnop, Retzlaff, Walden, Brunzell, Knopp and Kahn 2003; Carmena, Ascaso and Real

2001; Howard 1999). Insulin resistance is thought to lead to overproduction of very low density lipoprotein (VLDL) cholesterol through decreased lipoprotein lipase activity, increased production of apolipoprotein B-100 and decreased clearance of remnant particles (Yagi and Mabuchi 2000). Increased assembly and secretion of VLDL particles leads to increased plasma levels of triglyceride which in turn results in a reduction in HDL level and the generation of small, dense LDL (Ruotolo and Howard 2002; Ginsberg and Huang 2000). It is important to note that elevated triglyceride levels are the cause of the changes in HDL and LDL (Taskinen 1995). In vitro studies with endothelial cells found that LDL induces expression of vascular cell adhesion molecule-1, which is involved in endothelial activation and therefore in the development of atherosclerosis (Lin, Zhu, Liao, Kobari, Groszek and Stemerman 1996). It has also been found that the blood of patients with diabetes and abnormal lipid profiles is more prone to clotting than normal because of alterations in levels of several clotting factors, which again may indicate a relationship between abnormal lipid profiles and abnormal endothelial function (Frohlich and Steiner 2000).

6.7 Overall relationships

Although it is not yet fully understood how abdominal obesity is related to type-2 diabetes, cardiovascular disease, and hypertension it seems likely that the main physiological mechanisms involved are inflammation, insulin resistance, endothelial dysfunction and abnormal lipid profiles. Considerable progress has been made in identifying the key molecules and processes and it is possible to outline a summary of likely interactions (Figure 6.1).

Fig 6.1 The consequences of abdominal obesity



6.8 Potential benefits of omega-3s and DHA in particular

Epidemiological studies conducted over 30 years ago and subsequent investigations have demonstrated relatively low incidence of heart disease in populations, which traditionally consume high levels of long chain n-3 polyunsaturates in the form of whale meat, seal meat and fish (Sagild, Littauer, Jespersen and Andersen 1966; Kromann and Green 1980, Bang, Dyerberg and Sinclair 1980; Feskens, Bowles and Kromhout 1991). As discussed in Section 5.1, it is now generally accepted that for most Western populations, increased consumption of long chain n-3 polyunsaturates would help to maintain heart health and reduce the risk of developing cardiovascular disease (Calder 2004). Some of the earliest population studies also

found that high intake of long chain n-3 fatty acids was correlated with low incidence of glucose intolerance and diabetes mellitus (Sagild, Littauer, Jespersen and Andersen 1966; Kromann and Green 1980; Feskens, Bowles and Kromhout 1991). Possible explanations for such metabolic benefits have involved several physiological mechanisms but most recently attention has focused on the interaction of inflammatory processes, endothelial dysfunction, abnormal lipid profile and insulin resistance.

As described in Chapter 5, dietary long chain n-3 fatty acids may help to moderate the inflammation response and therefore reduce the adverse consequences indicated in Figure 6.1 (Browning 2003). Increased consumption of EPA in particular and reduced n-6 intake is believed to produce a less inflammatory lipid profile because EPA inhibits arachidonic acid metabolism and formation of inflammatory eicosanoids such as prostaglandin E2 and leukotriene B4 (James, Gibson and Cleland 2000). Although there is some negative evidence (Mori, Woodman, Burke, Puddey, Croft and Beilin 2003; Blok, Deslypere, Demacker, van der Ven-Jongekrijg, Hectors, van der Meer and Katan 1997) dietary fish oil supplementation has usually also been shown to suppress the synthesis of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-alpha, the major cytokines implicated in the chronic inflammation now associated with abdominal obesity (Endres, Ghorbani, Kelley, Georgilis, Lonnenann, van der Meer, Cannon, Rogers, Klempner and Weber 1989; Meydani, Lichtenstein, Cornwall, Meydani, Goldin, Rasmussen, Dinarello and Schaefer 1993; Gallai, Sarchielli, Trequattrini, Floridi, Firenze, Alberti, Di Benedetto and Stragliotto 1995; Mantzioris, Cleland, Gibson, Neumann, Demasi and James 2000; Grimble, Howell, O'Reilly, Turner, Markovic, Hirrell, East and Calder 2002; Trebble, Arden, Stroud, Wootton, Burdige, Miles, Ballinger, Thompson and Calder 2003). However, it is not known whether EPA or DHA has the most important role in suppression of cytokine production (James, Gibson and Cleland 2000). While increased consumption of the short-chain n-3 alpha linolenic acid was shown to increase EPA levels and reduce IL-6 and TNF-alpha levels in mononuclear cells of healthy subjects (Caughey, Mantzioris, Gibson, Cleland and James 1996), DHA has also been shown to reduce production of IL-1 and TNF-alpha (Kelley, Taylor, Nelson, Schmidt, Ferretti, Erickson, Yu, Chandra and Mackey 1999). Another potential anti-inflammatory mechanism is the inhibition of NF-kappa-B by long chain n-3 polyunsaturates (Calder 2002). Limited in vitro studies suggest that both EPA and DHA are effective (Lee, Zhao, Youn, Weatherill, Tapping, Feng, Lee, Fitzgerald and Hwang 2004; Lee, Plakidas, Lee, Heikkinen, Chanmugam, Bray and Hwang 2003; Komatsu, Isihara, Murata, Saito and Shinohara 2003).

The relationship between abdominal obesity, inflammation and insulin resistance has been investigated in relation to possible improvement of insulin sensitivity using fish oil (Browning 2003). In a dietary intervention trial on overweight women, consumption of fish oil (1.3 g EPA and 2.9 g DHA per day) for 12 weeks resulted in increased insulin sensitivity in women identified as having increased inflammatory status according to serum sialic acid content (Browning, Krebs, O'Connell, Mishra and Jebb 2002). However, there were no changes with fish oil treatment in the low inflammatory status group. Earlier animal and human studies of fish oil supplementation produced variable results on insulin sensitivity. In one positive study, rats fed high fat diets developed insulin resistance while those fed fish oil in addition did not (Storlien, Kraegen, Chisholm, Ford, Bruce and Pascoe 1987). Regarding human studies, a recent meta-analysis concluded that fish oil has no adverse effect on glycaemic control in subjects with type-2 diabetes (Friedberg, Heine, Janssen and Grobbee 1998) although no consistent positive effect was reported. Browning (2000) suggests that some previous studies may not have shown significant improvements in insulin sensitivity with n-3 polyunsaturates because it is only individuals with an inflammatory phenotype that are likely to respond.

Another possibility is that DHA and EPA have different effects in this respect and the outcome might therefore depend on the extent to which treatment alters levels of these fatty acids. In one mouse model of obesity, it was found that single and repeated doses of DHA alone produced useful hypoglycaemic effects (Shimura, Miura, Usami, Ishihara, Tanigawa, Ishida and Seino 1997). Similarly, in a rat model of diabetes, dietary DHA caused a slight reduction in glycaemia (Ovide-Bordeaux and Grynberg 2004). However neither EPA nor DHA improved insulin resistance induced by a high fructose diet in a study of hyperinsulaemic rats (Rousseau, Helies-Toussaint, Moreau, Raederstorff and Grynberg 2003). Evidence for specific benefits of DHA in improvement of insulin resistance in humans is lacking.

Fish oil supplementation has been shown to lower plasma triglycerides and it is generally accepted to be an effective treatment for abnormal lipid profiles related to obesity (Section 5.7). Some studies indicate that it is EPA rather than DHA that is responsible for this effect although the evidence is inconsistent.

As discussed in Section 5.3, there is more evidence for specific benefits of DHA in relation to endothelial function and blood clotting behaviour, which are often impaired by obesity-related insulin resistance. For example, a recent study in hypertensive type-2 diabetics found that DHA was a more effective anti-thrombotic agent than EPA (Woodman, Mori, Burke, Puddey, Barden, Watts and Beilin 2003). In vitro studies using human umbilical vein endothelial cells found that incorporation of DHA but not EPA into cell phospholipids inhibited TNF-alpha induced expression of the adhesion molecule VCAM-1 and subsequent monocyte adhesion (Weber, Erl, Pietsch, Danesch and Weber 1995). Specific benefits of DHA in improvement of endothelial function related to insulin insensitivity may include control of high blood pressure (Section 5.4). The vasorelaxant properties of DHA were found to contribute to the blood pressure lowering effect of dietary fish oil in hypertensive rats (Engler and Engler 2000). In recent human studies, DHA but not EPA reduced ambulatory blood pressure and heart rate in hyperlipidaemic overweight men at least partly by enhancing endothelial function in blood vessels (Mori, Bao, Burke, Puddey and Beilin 1999; Mori, Watts, Burke, Hilme, Puddey and Beilin 2000) and DHA may have more beneficial effects than EPA in modulation of vascular smooth muscle cell functions (Hirafuji, Machida, Hamaue and Minami 2003).

As discussed in section 5.5, several studies have shown that fish oil supplementation reduces heart rate in healthy subjects as well as hypertensives and more recent investigations found that this effect was due to DHA and not EPA. Since such specific benefits of DHA and not EPA have also been found in relation to improved endothelial dysfunction and control of hypertension, it is possible that these abnormalities are linked at a functional level. For example, nitric oxide (NO) is involved in the maintenance of normal endothelial function and fish oil stimulates NO production (Calder 2004; Abeywardena and Head 2001). NO has also been shown by recent human studies to have an important role in the control of heart rate perhaps via a direct effect on pacemaker activity of the sino-atrial node (Chowdhary, Marsh, Coote and Townend 2004; Chowdhary, Harrington, Bonser, Coote and Townend 2002).

6.9 Regulation of gene expression by omega-3 polyunsaturates

Polyunsaturated fatty acids are known to regulate gene expression in several tissues including brain, liver, heart and adipose tissue (Ntambi and Bene 2001). Of particular importance in fat build-up and metabolism it has been found that n-3 polyunsaturates and their metabolites modify the expression and development of SREBP and PPAR transcription factors. Down-regulation of SREBPs leads to suppressed expression of lipogenic genes while up-regulation of

PPARs promotes expression of genes for proteins involved in lipolysis and fatty acid oxidation (Price, Nelson and Clarke 2000; Clarke 2001; Kersten 2001; Clarke, Gasperikova, Nelson, Lapillonne and Heird 2002). Rodent and in vitro studies have indicated that such alterations in gene expression in liver and adipose tissue contribute to the physiological activities of n-3 polyunsaturates with the result that body fat accumulation is inhibited and glucose metabolism is improved (Belzung, Raclot and Groscolas 1993; Oudart, Groscolas, Calgari, Nibbelink, Leray, Le Maho and Malan 1997; Xu, Nakamura, Cho and Clarke 1999; Takahashi and Ide 2000; Moon, Latasa, Griffin and Sul 2002). It has also been shown that dietary long chain n-3 polyunsaturates can decrease expression of genes for fat-related hormones such as leptin by mechanisms associated with reduced SREBP-1 gene expression (Reseland, Haugen, Hollung, Solvoll, Halvorsen, Ingeborg, Nenseter, Christiansen and Drevon 2001). As noted above, excessive leptin production in obesity may be associated with diseases related to the metabolic syndrome (Ren 2004).

There is some evidence that DHA and EPA have different effects at the level of gene expression, for example one rat study found a significant inhibitory effect of DHA but not EPA on expression of adipose genes (Raclot, Groscolas, Langin and Ferre 1997). Also a recent in vitro study using fat cells from rats found that DHA induced expression of the gene for phosphoenolpyruvate carboxikinase, a key enzyme in fat metabolism (Duplus, Glorian, Tordjman, Berge and Forest 2002). Although evidence in humans is lacking, it seems likely that a full understanding of the specific benefits of DHA in prevention and treatment of obesity will require knowledge of its effects at the level of gene regulation.

6.10 Weight reduction

Since excess abdominal fat is the cause of profound metabolic disturbances, it is not surprising that body fat reduction, through increased exercise and decreased calorific intake, is generally recommended to reduce the metabolic consequences of obesity summarized in Figure 6.1 (Wagh and Stone 2004; Carroll and Dudfield 2004). Few studies have yet considered the possible additional benefits of combining an exercise and weight reduction regime with increased intake of DHA and EPA. It seems likely from the evidence discussed in this chapter and in Chapter 5 that such a dietary modification could be beneficial in reducing elevated blood pressure, reducing blood triglyceride levels and, at least in subjects with an inflammatory genotype (Browning 2000), reducing insulin resistance.

One study on overweight hypertensive subjects found that incorporating a daily fish meal into a weight-loss diet was more effective than either measure alone at improving insulin sensitivity and abnormal lipid profile (Mori, Bao, Burke, Puddey, Watts and Beilin 1999). By contrast, another study on 51 moderately obese, moderately hypertensive subjects reported that supplementation with unspecified n-3 fatty acids had minimal additional effect compared to weight loss alone (Kriketos, Robertson, Sharp, Drougas, Reed, Storlien and Hill 2001). However, the authors indicate that the study may have had too few subjects to distinguish statistically significant differences between supplementation groups. An earlier study on 219 Norwegian subjects found that exercise and a weight-loss diet including additional fish meals, and particularly a combination of the two, were effective in improving insulin sensitivity, blood pressure and blood lipid profile (Anderssen, Hjermann, Urdal, Torjesen and Holme 1996). Although some of the benefits of fish meals may be associated with nutritional components other than DHA and EPA, for example with increased protein intake (Burke, Hodgson, Beilin, Giangiuloi, Rogers and Puddey 2001) their well known physiological effects signal the predominant role of long chain n-3 polyunsaturates (Calder 2004).

It seems likely that dietary supplementation with DHA and EPA can help to ameliorate the metabolic consequences of abdominal obesity especially as part of a weight reduction programme involving reduced calorie intake and increased exercise. It is also possible that increased DHA and EPA intake could have a direct effect on weight loss. Although evidence in humans is lacking, several rodent studies have demonstrated that long chain n-3 polyunsaturates limit enlargement of fat cells and fat content of adipose tissue (Raclot, Groscolas, Langin and Ferre 1997; Belzung, Raclot and Groscolas 1993; Hill, Peters, Lin, Yakubu, Greene and Swift 1993; Parrish, Pathy, Parkes and Angel 1991). In a study of this antiobesity effect in mice, it was found that while fish oil feeding decreased fat mass in a dose-dependent manner, at the same time, PPAR-alpha expression was up-regulated and SREBP-1 expression was down-regulated (Nakatani, Kim, Kaburagi, Yasuda and Ezaki 2003). Similarly, a recent rat study concluded that fish oil reduced body fat by down-regulating lipogenic genes in the liver (Jang, Hwang, Chae, Lee, Kim, Kang, Hwang, Lim, Huh and Cho 2003). In another mouse study, supplementation with saturated fat for seven weeks caused an increase in fat mass, which was completely reversed by changing the diet to include n-3 polyunsaturates for four weeks. However, changing to a low fat diet was less effective (Wang, Storlien and Huang 2002). Similarly, rats fed high fat diets rich in fish oil did not increase white adipose tissue mass compared to a low fat diet while those fed linoleic acid-rich safflower oil had significantly higher white adipose tissue weight compared to those on the low fat diet (Takahashi and Ide 2000). In a feeding study on obese mice, those with the highest DHA levels in plasma, as a result of combined perilla oil (a source of ALA) and fish oil supplementation, were found to lose more fat and have lower levels of blood glucose than animals supplemented with perilla oil alone (Hun, Hasegawa, Kawabata, Kato, Shimokawa and Kagawa 1999).

6.11 Conclusion

Obesity is recognized as one of the most important public health concerns of our time. Much more than a store of energy, abdominal fat has profound effects on health mediated by altered gene function, inflammatory cytokines and adipose hormones. The long chain n-3 polyunsaturates have been found to influence all these mediators and to produce particular benefits with respect to endothelial function, inflammatory processes, blood lipid profiles and, in certain circumstances, insulin sensitivity. For obese and overweight individuals, the likely result of increased intake of DHA and EPA is therefore reduced risk of hypertension, type-2 diabetes, atherosclerosis and cardiovascular disease. It is also clear that DHA and EPA may have different physiological effects. While EPA may be more effective in reducing elevated blood triglycerides, and both EPA and DHA may be involved in controlling the adverse effects of inflammation, recent evidence indicates that DHA has the predominant role in improving endothelial dysfunction, reducing high blood pressure and reducing heart rate abnormalities. In addition the link between endothelial dysfunction and insulin resistance also suggests that DHA could be more effective than EPA in reducing the risk of type-2 diabetes although conclusive evidence is lacking. As yet there is no evidence that DHA and EPA can directly promote weight loss in humans as they can in rodent models of obesity. However, the wealth of research data now available clearly indicates that increased fish intake, fish oil supplementation and more specifically increased DHA intake represent a powerful additional approach to weight reduction and exercise programmes in the treatment of excess abdominal fat and prevention of its deadly consequences.

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CHAPTER SEVEN - DHA AND PREGNANCY

7.1 DHA in pregnancy

The DHA requirement of the pregnant women and the developing foetus is high especially during the last trimester because of the rapid synthesis of foetal brain tissue (Al et al 2000, Hornstra 2000). The EFA status of the pregnant woman declines during pregnancy and maternal LCPUFA status affects the LCPUFA status of the newborn infant (Otto, Houwelingen, Antal et al 1997). Studies of multiple births demonstrated that the EFA status of infants born after a multiple pregnancy is lower than that of infants born after a singleton pregnancy, which supports the view that the maternal EFA supply to the foetus is limiting (Foreman-Van, Drongelen, Zeijdner, Houwelingen et al 1996; McFadyen, Farquarson and Cockburn 2003).

The foetus depends primarily on placental transfer and although there is a preferential materno-foetal transfer of LCPUFA compared with the transfer of precursor fatty acids (Berghaus, Demmelmair and Koletzko 1998) the maternal concentration of individual fatty acids can have large effects on PUFA delivery to the foetus (Haggerty, Ashton, Joynson et al 1999). Increasing intake of n-3 fatty acids during pregnancy can enhance maternal DHA status. Consumption of sardines or fish oil was found to increase DHA levels in plasma and erythrocytes of pregnant women while DHA and other n-3 fatty acids were transferred into the foetus (Connor, Lowensohn and Hatcher 1996). Levels of n-3 fatty acids in plasma and erythrocyte phospholipids of pregnant women were found to reflect n-3 intake (Olsen, Hansen, Sandstrom et al 1995; Montgomery, Speake, Cameron et al 2003; Sanjurjo, Ruiz-Sanz, Jimeno et al 2004) and in line with this result it was found that vegetarians give birth to infants with less DHA in their plasma and cord artery phospholipids (Sanders and Reddy 1992, Reddy, Sanders and Obeid 1994).

As well as improving the DHA status of the developing foetus it was suggested, following a series of early studies, that increased n-3 intake by the pregnant women was related to increased birth weight (Crawford, Doyle, Drury et al 1989, Olsen, Olsen and Frische 1990, Olsen, Grandjean, Weihe et al 1993) although a more recent study did not find this relationship (Rump, Mensink, Kester et al 2001). Early studies indicated that increased n-3 intake by pregnant women resulted in longer pregnancies (Olsen, Sorensen, Secher et al 1992) and a recent US study on 291 pregnant women also found that DHA supplementation during the third trimester caused a significant increase in the duration of gestation (Smuts, Huang, Mundy et al 2003). A study involving 8729 pregnant Danish women found that low fish consumption was a strong risk factor for preterm delivery and low birth weight (Olsen and Secher 2002) and a recent review of the literature concluded that that supplementation with DHA might be useful in prolonging the duration of gestation in high-risk pregnancies (Allen and Harris 2001).

Recent studies also point to a relationship between LCPUFA status and later infant development. The amount of DHA in human milk was found to be positively correlated with visual and language development in breast fed infants (Innis 2003) while a recent Norwegian study found that the children of women supplemented with cod liver oil when they were pregnant had significantly improved mental processing score at four years of age (Helland, Smith, Saarem et al 2003). Similarly a Scottish study found an association between the DHA status of term infants and retinal sensitivity (Malcolm, Hamilton, McCulloch et al 2003; Malcolm, McCulloch, Montgomery et al 2003).

7.2 Conclusion

Supply of DHA to the developing foetus is dependent on maternal concentrations, which may be limiting but can be enhanced by increased dietary intake. Enhanced DHA intake may therefore be of particular value to pregnant women.

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CHAPTER EIGHT - DHA AND LACTATION

8.1 DHA in breast milk

It has long been recognised that human milk contains DHA and that it is essential for the neural and visual development of the infant (Crawford, Hassam and Stevens 1981, Gibson and Kneebone 1981). The fatty acid profile of human milk is influenced by the length of gestation and lactation period (Beijers and Schaafsma 1996) and preterm milk is richer in DHA than term milk (Luukkainen, Salo and Nikkari 1994). Recent studies on in vivo conversion of ALA to DHA found that 9% of ALA consumed was converted to DHA in women of reproductive age (Burdge and Wootton 2002) while in a study on healthy young men, the same researchers recorded an ALA to DHA conversion figure of zero (Burdge et al 2003). It was suggested that the difference may reflect adaptation in women to meet the DHA demands of foetus and neonate. However in one recent study, ALA supplementation did not increase DHA content in breast milk (Francois et al 2003), and Genzel-Boroviczeny, Wahle and Koletzko (1997) found that the milk of mothers of preterm infants might not contain sufficient DHA and AA to meet the needs of the preterm baby.

The fatty acid content of human milk also depends on diet, for example, the breast milk of vegans contains relatively low levels of DHA (Sanders, Ellis, Path et al 1978, Sanders 1999) while differences in n-3 intake lead to wide variation in the DHA content of breast milk (Jensen 1999, Rodriguez-Palmero, Koletzko, Kunz et al 1999, Jorgensen, Lauritzen and Michaelsen 1999, Sauerwold, Demmelmair, Fidler et al 2000, Scopesi, Ciangherotti, Lantieri et al 2001). Similarly, a study of fatty acid composition of human colostrums demonstrated wide variation related to diet (Fidler and Koletzko 2000).

Supplementation with fish oil increases breast milk DHA concentration (Harris, Connor and Lindsey 1984, Henderson, Jensen, Lammi-Keefe et al 1992, Jensen, Lammi-Keefe, Henderson et al 1992, Makrides, Neumann and Gibson 1996b, Francois, Connor, Wander et al 1998, Koletzko, Sauerwold, Keicher et al 2003). Experiments also demonstrate that increased breast milk DHA levels cause a dose-dependent increase in infant plasma and erythrocyte phospholipid DHA (Gibson, Neumann and Makrides 1997, Jensen, Maude, Anderson et al 2000).

As with experiments on formula feeding of term infants, investigations of the functional consequences of increasing DHA levels in breast milk have produced inconsistent results (Makrides and Gibson 2000) although most recent studies suggest benefits. Gibson, Neumann and Makrides (1997) did not find an effect of maternal DHA intake on visual acuity of breast fed infants although they did find a transient dose dependency on one measure of mental development. Jorgensen, Lauritzen and Michaelsen (1999) reported an observational study in which they found a relationship between breast milk DHA levels and a measure of visual acuity. Also in a later study, Jorgensen, Hernell, Hughes et al (2001) found that infant red blood cell phosphatidylethanolamine DHA level was significantly related to visual acuity at two months and twelve months of age while certain discrimination abilities were also related to blood DHA levels. It was concluded that DHA may influence the development of visual acuity and neural pathways associated with the developmental progression of language acquisition in term breast fed infants.

8.2 Conclusion

The DHA content of human breast milk depends on diet and can be increased with DHA supplementation. Limited investigations suggest that such supplementation could be of benefit to both the infant and the mother.

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CHAPTER NINE - DHA AND INFANTS

9.1 DHA and human infants

The importance of polyunsaturated fatty acids, especially DHA, in human eye and brain function has been most thoroughly researched with respect to infant nutrition (Gibson, Chen and Makrides 2001, San Giovanni, Parra-Cabrera, Colditz et al 2000) and numerous reviews of trials on full term and preterm infants have been published. Reviews of preterm studies are virtually unanimous in concluding that the available evidence supports a role for preformed dietary DHA in the visual and neural function of preterm infants (Hoffman, Birch, Birch et al 1993, Crawford 1993, Uauy-Dagach, Mena and Hoffman 1994, Crawford, Costeloe, Ghebremeskel et al 1997, 1998, Uauy and Mena 1999, Uauy and Hoffman 2000)

A recent paper by Lapillonne and Carlson (2001) mentions early work on preterm infants, which seemed to indicate that those fed EPA and DHA had reduced growth achievement compared to controls (Carlson, Cooke, Werkman et al 1992). To examine this issue, the review considers 32 randomised trials, 13 in preterm infants and 19 in term infants. It was concluded that 'from the data published to date, it seems clear that long-chain n-3 fatty acids can reduce growth achievement in preterm and term infants under some experimental conditions. However, the effect of n-3 PUFA supplementation on the growth of preterm and term infants appears to be minimal and of questionable clinical and/or physiologic relevance'. Tolley and Carlson (2000) discuss experimental design in studies of DHA and visual acuity development.

Table 9.1 Trials related to LCPUFA nutrition of preterm infants

Carlson, Rhodes and Ferguson (1986)
Uauy, Birch, Birch et al (1990)
Birch, Birch, Hoffman et al (1992)
Lucas, Morley, Cole et al (1992)
Birch, Birch, Hoffman et al (1993)
Hoffman, Birch, Birch et al (1993)
Carlson, Werkman, Rhodes et al (1993)
Carlson, Werkman, Peeples et al (1994a)
Carlson, Werkman, Peeples et al (1994b)
Uauy, Hoffman, Birch et al (1994)
Carlson, Werkman and Tolley (1996)
Faldella, Govoni, Alessandroni et al (1996)
Uauy, Peirano, Hoffman et al (1996)
Werkman and Carlson (1996)
Clandinin, Van Aerde, Parrott et al (1997)
Bougle, Denise, Vimard et al (1999)
Clandinin, Van Aerde, Parrott et al (1999)
Hoffman, Birch, Birch et al (1999)
Koletzko, Knoppke, Von Schenck et al (1999)
Vanderhoof, Gross, Hegyi et al (1999)
Woltil, Van Beusekom, Schaafsma et al (1999)
O'Connor, Hall, Adamkin et al (2001)
Innis, Adamkin, Hall et al (2002)

While most studies indicated a clear benefit (Table 9.1), the study by Bougle, Denise, Vimard et al (1999) was unusual in that it found no significant difference in measures of visual, auditory and nerve function between three groups of preterm infants fed breast milk or formula containing only 18 carbon polyunsaturated fatty acids (PUFA) or long chain PUFA (including DHA and AA). The study by Innis, Adamkin, Hall et al (2002) also failed to demonstrate improvements in visual acuity by DHA and AA supplementation although weight gain was enhanced.

The importance of DHA in nutrition of full term infants has not been so clearly established, despite more numerous investigations. Some of the earliest studies of infant nutrition compared attainment score in breast fed infants with those fed with formula devoid of LCPUFAs and found that breast feeding resulted in higher scores (Rodgers 1978, Taylor and Wadsworth 1984, Morrow-Tlucak, Haude and Ernhart 1988, Temboury, Otero, Polanco et al 1994) and it was speculated that the difference could be due to lower DHA levels in formula-fed infants. More recent studies have confirmed these findings (Khedr et al 2004, Williams et al 2001). Infants fed formula lacking LCPUFAs had significantly lower levels of DHA and AA in red blood cells and plasma compared to breast-fed infants (Ponder, Innis, Benson et al 1992, Decsi, Thiel and Koletzko 1995). Post-mortem studies demonstrated deficiencies in infant brain, retina and blood DHA of formula-fed compared with breast-fed infants (Farquharson, Cockburn, Patrick et al 1993, Makrides, Neumann, Byard et al 1994).

Subsequent feeding trials demonstrated that blood LCPUFA levels could be increased to the levels found in breast-fed infants by supplementing infant formula with LCPUFA (Kohn, Sawatzki, Van Biervliet et al 1994, Makrides, Neumann, Simmer et al 1995, Innis, Auestad and Siegman 1996, Lapillonne, Brossard, Claris et al 2000, Maurage, Guesnet, Pinault et al 1998, Connor, Zhu, Anderson et al 2000). Feeding trials have also investigated the effect on infant development, particularly in relation to visual acuity, of DHA and AA supplementation. More trials have demonstrated beneficial effects (Table 9.2) than those that did not show a benefit (Table 9.3).

Table 9.2 Papers on full term Infants showing a positive effect of LCPUFAs

- Makrides, Simmer, Goggin et al (1993)
- Innis, Nelson, Rioux et al (1994)
- Agostoni, Trojan, Bellu et al (1995)
- Makrides, Neumann, Simmer et al (1995)
- Carlson, Ford, Werkman et al (1996)
- Agostoni, Trojan, Bellu et al (1997)
- Birch, Hoffman, Uauy et al (1998)
- Courage, McCloy, Herzberg et al (1998)
- Willatts, Forsyth, Di Modugno et al (1998b)
- Birch, Garfield, Hoffman et al (2000)
- Hoffman, Birch, Birch et al (2000)
- Hoffman, Birch, Castaneda et al (2003)

Table 9.3 Papers on full term infants showing no significant effect of LCPUFAs

Auestad, Montalto, Hall et al (1997)
Innis, Akrabawi, Diersen-Schade et al (1997)
Jorgensen, Holmer, Lund et al (1998)
Scott, Janowsky, Carroll et al (1998)
Lucas, Stafford, Morley et al (1999)
Makrides, Neumann, Simmer et al (2000)
Auestad, Halter, Hall et al (2001)
Auestad, Scott, Janowsky et al (2003)

Despite the negative papers (Table 9.3) the most recent reviews have concluded that the balance of evidence indicates LCPUFA nutrition is important in neural and visual development. SanGiovanni, Berkey, Dwyer et al (2000) concluded that 'dietary n-3 intake is associated with performance on visual resolution acuity tasks at 2 and possibly 4 months of age in healthy full term infants'. Uauy, Hoffman, Peirano et al (2001) concluded that the 'studies summarised in this review provide evidence supporting the view that essential fatty acid supply affects visual development of preterm and term infants'. A review by Uauy, Mena and Rojas (2000) concluded that 'Recent clinical trials convincingly support LCPUFA supplementation of preterm infant formulations and possibly term formula to mimic human milk compositions'. Gibson and Makrides (2000) conclude that 'because the LCPUFAs of breast milk appear to be dependent on maternal dietary LCPUFAs it seems prudent to ensure that breast milk from mothers who include some fish in their diets is used to guide dietary recommendations for infants'. Larque, Demmelmair and Koletzko (2002) concluded that 'LCPUFA are conditionally essential substrates during early life that are related to the quality of growth and development. Therefore a dietary supply during pregnancy, lactation and early childhood that avoids the occurrence of LCPUFA depletion is desirable'. Other reviews related to LCPUFA nutrition of full term infants are listed in Table 9.4.

Table 9.4 Reviews on full term infants

Clandinin, Chappell and Van Aerde (1989)
Koletzko (1992)
Uauy, Birch, Birch et al (1992)
Uauy-Dagach and Mena (1995)
Bendich and Brock (1997)
Makrides, Neumann and Gibson (1996a)
Gordon (1997)
Heird, Prager and Anderson (1997)
Gibson and Makrides (1998)
Carlson and Neuringer (1999)
Heird (1999)
Innis, Sprecher, Hachey et al (1999)
Kurlak and Stephenson (1999)

Cunnane, Francescutti, Brenna et al (2000)
Neuringer (2000)
Gibson, Chen and Makrides (2001)
Jensen and Heird (2002)
Uauy, Hoffman, Mena et al (2003)

In addition to direct studies on LCPUFA supplementation a number of trials have indicated that the ratio of linoleic acid to ALA in formula feeds can influence the AA and DHA status of infants (Gibson, Makrides, Neumann et al 1994, Jenson, Chen, Fraley et al 1996, Makrides, Neumann, Jeffrey et al 2000). Another potential confounding factor in infant feeding trials is the LCPUFA status of infants at birth i.e. before supplementation begins (Guesnet, Pugo-Gunsam, Maurage et al 1999) while Makrides, Neumann and Gibson (2001) reported that a variety of perinatal characteristics including birth weight, gender and number of smokers in the household could influence the effects of diet on visual acuity measurements.

Studies of later infant development have produced mixed results. An investigation on 128 full-term neonates found no relationship between levels of DHA in cord blood and cognitive development at four years of age (Ghys, Bakker, Hornstra et al 2002) or at seven years of age (Bakker, Ghys, Kester et al 2003). By contrast, a trial on 65 healthy full-term infants found that supplementation with LCPUFA during weeks 7-52 had significantly better visual function than infants that did not receive supplementation. Better visual function was correlated with higher plasma DHA levels (Birch, Hoffman, Castaneda et al 2002). Another recent study found evidence of greater central nervous system maturity among infants born to mothers with higher plasma levels of DHA as demonstrated by infant sleeping patterns (Cheruka, Montgomery, Farkar et al 2003).

An authoritative report was published in 2001 following a workshop in Munich aimed at reviewing the available scientific information on LCPUFA and perinatal development (Koletzko, Agostoni, Carlson et al 2001). The workshop concluded that 'for healthy infants we recommend and strongly support breast feeding as the preferred method of feeding, which supplies preformed LCPUFA. Infant formulas for term infants should contain at least 0.2% of total fatty acids as DHA and 0.35% as AA. Since preterm infants are born with much less body DHA and AA we suggest that preterm infant formulas should include at least 0.35% DHA and 0.4% AA'.

9.2 Conclusion

The balance of evidence suggests that dietary DHA is essential for healthy eye and brain development and function in the premature infant and also in the full term infant. The balance of evidence also suggests that fortification of infant formula with DHA is likely to support healthy eye and brain development in preterm and term infants.

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CHAPTER TEN - DHA AND BEHAVIOURAL DISORDERS

10.1. Human diseases that may involve DHA deficiency

Chapters 10 to 13 review papers on attention deficit hyperactivity disorder, dyslexia, depression, bipolar disorder, stress, violent and impulsive behaviour, dementia and age-related macular degeneration. For each condition, reviews and papers are considered in terms of their relevance to the claim that omega-3 DHA helps to maintain healthy eye and brain function. Evidence is considered from epidemiological studies on the relationship of each condition to DHA, n-3, fish or fish oil consumption. If the claim is true, the incidence of these eye and brain diseases may be expected to be higher where consumption of DHA is lower. Also, if the claim is true, studies on tissue levels of DHA in these diseases may be expected to indicate lower than normal DHA levels. Finally, if the claim is true, supplementation with DHA may be expected to improve disease parameters. It should be noted however that the supplementation trials discussed were designed to assess treatment of disease states while the claim relates to the maintenance of health in the whole population. If a disease state exists as a result of long-term dietary deficiency of DHA, the resultant physiological changes may or may not be reversible. For this reason, the ability of DHA supplementation to improve disease parameters supports the claim but failure to improve disease parameters does not necessarily contradict it.

10.2 Behavioural disorders, impulsivity and violent behaviour

Attention deficit hyperactivity disorder (ADHD) is characterised by inattentive, impulsive and hyperactive behaviour occurring in children but some aspects of the condition may persist into adulthood (Richardson and Puri 2000, Richardson and Ross 2000, Arnold 2001). ADHD is a significant and increasing problem. It is estimated that it affects about 2% of school-aged children in the UK and 4% of school-aged children in the USA (Richardson and Puri 2000) and the use of medication to treat ADHD has increased dramatically in the last 10 years. Results of one study suggest that fish consumption may be associated with violent and impulsive behaviour (Hibbeln 2001). This cross-national survey of seafood consumption in 26 countries found that those with higher rates of seafood consumption tended to have lower rates of mortality due to homicide. The authors point out, however, there were many potentially confounding factors in this study and the hypothesis that fish consumption may help to reduce impulsive and violent behaviour should be tested in double-blind, placebo-controlled trials.

In a recent study of the adult form of ADHD, erythrocyte membranes and serum phospholipids of affected individuals contained significantly lower levels of DHA than controls (Young, Maharaj and Conquer 2004). In another study, patients with ADHD had significantly elevated levels of exhaled ethane, a measure of endogenous fatty acid oxidation (Ross, McKenzie, Glen et al 2003). The authors suggested that this could indicate a biochemical abnormality involving a higher level of omega-3 loss by oxidation in patients with ADHD. Boys aged 6-12 years with ADHD were found to have significantly lower plasma levels of AA, EPA and DHA compared to normal controls (Stevens, Zentall, Deck et al 1995). In a further study of boys of the same age, significantly greater scores indicating behaviour problems, temper tantrums and sleep problems were reported in subjects with lower plasma total n-3 fatty acid concentrations (Stevens, Zentall, Abate et al 1996). However, a double-blind placebo controlled trial of DHA supplementation (345 mg/day for 4 months) in children with ADHD found that DHA treatment did not decrease ADHD symptoms compared with placebo (Voigt, Llorente, Jensen et al 2001). The authors state that lack of response to DHA supplementation did not necessarily mean that a low brain content of DHA is not involved in the aetiology of ADHD. It is possible that in the population studied, a benefit of DHA was not produced because other essential nutrients were also lacking. However,

a recent study using fish oil supplementation also failed to produce a benefit in children with ADHD (Hirayama, Hamazaki and Terasawa 2004).

It has been suggested in recent reviews that ADHD may be linked to some other behavioural and neurological disorders, namely dyslexia, dyspraxia and autism, by an involvement of fatty acid metabolism (Richardson and Ross 2000; Bell, Sargent, Tocher et al 2000) and some studies of violent, impulsive and antisocial behaviour have also made this connection. Such behaviour has been linked to tissue deficiencies of n-3 fatty acids (Corrigan, Gray, Strathdee et al 1994; Stevens, Zentall, Deck et al 1995; Stevens, Zentall, Abate et al 1996; Hibbeln, Umhau, Linnoila et al 1998; Burgess, Stevens, Zhang et al 2000) and other nutrients including vitamins and minerals (Schoenthaler, Amos, Doraz et al 1997, Walsh, Isaacson, Rehman et al 1997). Virkkunen, Horrobin, Jenkins et al (1987) found that in a group of violent and impulsive offenders, plasma DHA was significantly lower than controls while n-6 fatty acids were significantly elevated. A study of 3581 urban young adults in the USA found that consumption of any fish rich in n-3 fatty acids compared to no consumption was associated with a lower hostility score (Iribarren, Markovitz, Jacobs et al 2004). The authors suggested that high intake of DHA may be related to lower likelihood of hostility in young adulthood.

In a double-blind, placebo-controlled trial on young adult male prisoners, dietary supplementation with vitamins and minerals, as well as fish oil (80 mg per day EPA and 44 mg per day DHA) and evening primrose oil, resulted in 26% fewer disciplinary offences in the supplemented group compared to placebo and 35% fewer disciplinary offences in the supplemented group compared to the baseline frequency (Gesch, Hammond, Hampson et al 2002). A recent double-blind placebo-controlled trial investigated the effects of dietary supplementation for 12 weeks with tuna oil (186 mg per day EPA, 480 mg per day DHA) and evening primrose oil in children with specific learning difficulties such as dyslexia (Richardson and Puri 2002). It was found that supplementation produced significant benefits. It has also been suggested that DHA in particular might be useful in treatment of dyslexia and dyspraxia as well as ADHD (Stordy 1995, 1997, 2000). Dyspraxia is a condition involving reduced motor skills manifesting as excessive clumsiness and there is a close link between dyspraxia and dyslexia (Stordy 1997). Stordy (1995) reported that, in a preliminary study, supplementation for one month with 480 mg per day DHA significantly improved an aspect of vision called dark adaptation in five dyslexic children. In a later open study of 15 children with dyspraxia, supplementation with the same dose of tuna oil and evening primrose oil as used in the study by Richardson and Puri (2002), produced significant improvements in scores for manual dexterity, ball skills and static and dynamic balance.

The studies described above, of impulsive and violent behaviour amongst prisoners and its possible association with PUFA status (Virkkunen, Horrobin, Jenkins et al 1986, Gesch, Hammond, Hampson et al 2002) may be compared to a series of studies of aggression in Japanese students. Hamazaki, Sawazaki, Itomura et al (1996) conducted a double-blind, placebo-controlled trial of fish oil supplementation (1.5-1.8 g DHA per day) and after three months of treatment, aggression scores were significantly lower in the DHA group compared to placebo. However, the reason for the difference was that aggression scores in the placebo group had increased while those in the DHA group did not change significantly. The difference was accounted for by the fact that the final assessment in the trial occurred just before academic examinations, which it was suggested had caused psychological stress. A similar trial was conducted on different students who did not face such stress and no significant change in hostility was recorded in the DHA or placebo group (Hamazaki, Sawazaki, Nagao et al 1998). The authors concluded that DHA administration could help to control aggression only at times of psychological stress (Hamazaki, Sawazaki, Itomura et al 2001). Hibbeln, Umhau, George et al

(1997) pointed out that an apparent prevention of increased aggression is surprising because baseline intake of n-3 PUFA in the study population was relatively high. In a third double-blind, placebo- controlled trial on students. Plasma catecholamines were measured during a two-month period of continuous psychological stress due to university examinations (Sawazaki, Hamazaki, Yazawa et al 1999). In the DHA group, who took 1.5g DHA per day during the examination period, noradrenaline levels were significantly reduced. The authors interpreted this change as indicating that subjects in the DHA group adapted to stress more favourably than controls and that DHA may help to reduce the risk of stress-related diseases in individuals under long-lasting psychological stress (Hamazaki, Sawazaki, Nagasawa et al 1999, Hamazaki, Itomura, Sawazaki et al 2000).

In another study by the same group, Thai subjects aged 50-60 years, from a university and surrounding villages, were studied in a double-blind placebo-controlled trial in which the treatment was the same DHA supplement as used in the previous trials (Hamazaki, Thienprasert, Kheovichai et al 2002). DHA administration reduced aggression scores amongst university employees but not amongst village-dwellers. The authors speculated that the difference was caused by a larger placebo effect amongst villagers or a lower sensitivity amongst villagers to the psychological stressor (a video of stressful events) used in the study.

10.3 Conclusion

The epidemiological evidence that DHA-deficiency is a cause of violent and impulsive behaviour is supportive but not conclusive. Also, the few available studies of plasma fatty acids demonstrate lower DHA levels in individuals with ADHD. Data from supplementation studies are inconsistent but there are sufficient positive results to strengthen the view that DHA deficiency may be associated with adverse behavioural consequences.

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CHAPTER ELEVEN - DHA AND DEPRESSION

11.1. Depression

Evidence for the role of DHA in the aetiology of depression has been reviewed by Brunner, Parhofer, Schwandt et al (2002), Freeman (2000), Maidment (2000), Mischoulon and Fava (2000), Fugh-Berman and Cott (1999), Stoll, Locke, Marangell et al (1999), Horrobin and Bennett (1999), Hibbeln and Salem (1995) and Reggers, Castronovo and Ansseau (2003).

Epidemiological studies show that the risk of suffering major depression is probably increasing (Bland 1997). An analysis of results from nine epidemiological surveys and three family studies, involving 43,000 individuals in six geographical areas (North America, Puerto Rico, Western Europe, the Middle East, Asia and the Pacific Rim) showed an overall increase in the rates of major depression over time over all countries (Cross-National Collaborative Group 1992). It is hypothesized that an apparent increase over the last 100 years is related to a declining intake of n-3 fatty acids over the same period (Smith 1991, Hibbeln and Salem 1995, Hibbeln, Umhau, George et al 1997, McGrath, Hanna, Greene et al 2003). In a study of nine countries, Hibbeln (1998) demonstrated a significant correlation between high annual fish consumption and lower prevalence of major depression. Comparing Japan, which has relatively high average fish consumption, with the USA, several studies of the prevalence of depression in elderly populations have demonstrated lower incidence of depression in Japan and lowest incidence where fish intake is highest (Klerman and Weissman 1989, Hasegawa 1985, Makiya 1978, Blazer and Williams 1980, O'Hara, Kohout and Wallace 1985, Sarai 1979, Zung 1967, Krouse and Liang 1992).

In a sample of 3,204 Finnish adults, a significant correlation was found between low fish consumption and depressive symptoms (Tanskanen, Hibbeln, Tuomilehto et al 2001). It has also been suggested that a relative lack of seasonal affective disorder found in the populations of Iceland and Japan could be explained by their high levels of fish intake (Cott and Hibbeln 2001). A survey of 4644 New Zealand adults found that fish consumption was significantly associated with higher self-reported mental health status (Silvers and Scott 2002). However, a recent study of 29,133 men in Finland found no association between dietary intake of n-3 fatty acids and low mood level (Hakkarainen, Partonen, Haukka et al 2004). Similarly an Australian study of 755 women found no correlation between n-3 intake and prevalence of depression (Jacka, Pasco, Henry et al 2004).

Most studies of lipid composition of tissues in depressed individuals indicate reduced PUFA levels. In several studies, severity of depression was found to be correlated positively with AA:EPA ratio in plasma and erythrocyte phospholipids and a lower level of n-3 PUFA (Maes, Smith, Christophe et al 1996, Maes and Smith 1998, Adams, Lawson, Sanigorski et al 1996, Maes, Christophe, Delanghe et al 1999, Edwards, Peet, Shay et al 1998, Peet, Murphy, Shay et al 1998, Tiemeier, van Tuijl, Hofman et al 2003, Chiu, Huang, Su et al 2003). A Greek study of 139 adults found that mildly depressed subjects had a 34% lower level of DHA in adipose tissue than non-depressed subjects (Mamalakis, Tornaritis and Kafatos 2002). However, results of two early studies did not support this association. Ellis and Sanders (1977) and Fehily, Bowey, Ellis et al (1981) found that plasma DHA and EPA levels were higher in depressed patients than in control subjects. Reasons for this discrepancy are not clear but Hibbeln and Salem (1995) stated that the results of the earlier studies are difficult to interpret because of the diagnostic heterogeneity of the patients, lack of dietary assessment and use of medications that can affect blood lipids. A Belgian study of 23 healthy volunteers recorded significant seasonal rhythms in serum phospholipid levels of DHA, EPA and AA, which were significantly correlated with the number of violent suicide deaths in Belgium (De Vriese, Christophe and Maes 2004).

Evidence from supplementation trials, although limited, tends to support the association of depression and reduced n-3 PUFA status. Treatment of elderly patients with DHA-rich phosphatidylserine prepared from bovine cerebral cortex, produced significant reductions in depressive symptoms compared to placebo (Cenacchi, Bertoldini, Farina et al 1993, Maggioni, Picotti, Bondiolotti et al 1990). Puri, Counsell, Richardson et al (2002) report a single case in which a 21-year-old patient with depression was treated with 4g per day of ethyl ester of EPA, which produced a rapid, dramatic and sustained improvement in symptoms. In a double-blind placebo-controlled study of 20 patients with unipolar depressive disorder, ethyl ester of EPA (10 patients) or placebo (10 patients) was added to their current antidepressant treatment for four weeks (Nemets, Stahl and Belmaker 2002). Highly significant benefits of the addition of EPA compared with placebo were found by week three of treatment. Although a different condition, bipolar affective disorder (manic depression) involves depressive symptoms. In a four-month, double-blind, placebo-controlled pilot study on 30 out-patients with bipolar affective disorder, supplementation with n-3 fatty acid ethyl esters (supplying 6.2g EPA and 3.4g DHA per day) produced significant improvements in nearly every outcome measure particularly with respect to depressive symptoms (Stoll, Severus, Freeman et al 1999). Similarly, a Chinese clinical trial in 28 patients with major depression found that supplementation with 6.6 g per day of n-3 PUFA for eight weeks significantly reduced depression scores compared to placebo (Su, Huang, Chiu et al 2003). However a USA supplementation study found that 2g per day of DHA for six weeks did not cause a significant effect compared to placebo (Marangell, Martinez, Zboyan et al 2003).

Depression is associated with alcoholism (Merikangas and Gelertner 1990, Winobur 1990, Schmidt 1986) and chronic alcohol intoxication has been found to reduce DHA levels in red blood cells, platelets and neuronal membranes (Hibbeln and Salem 1995). It has been postulated therefore, that DHA supplementation may improve symptoms of depression in alcoholics (Hibbeln and Salem 1995).

Considering the possible link between low tissue DHA level and depression, together with the drain on maternal DHA imposed by pregnancy and lactation, it is not surprising that the phenomenon of post-partum depression has been postulated to be related to reduced tissue DHA levels (Hibbeln 2002). In a study of 112 women it was found that lower DHA level in plasma phospholipids was associated with increased risk of developing depressive symptoms (Otto, de Groot and Hornstra 2003). In an Australian study of 380 women, a 1% increase in plasma DHA was associated with a 59% reduction in reporting of depressive symptoms (Makrides, Crowther, Gibson et al 2003). However, recent fish oil or DHA supplementation trials on women prone to postpartum depression were not successful in reducing the incidence of the condition (Marangell, Martinez, Zboyan 2002, Chong and Puryear 2004, Llorente, Jensen, Voigt et al 2003).

As discussed in Chapter 5, the link between n-3 PUFA intake and heart health is now well established. It has also been suggested that low n-3 intakes contribute to both depression and coronary artery disease (Hibbeln and Salem 1995, Severus, Ahrens and Stoll 1999). If this is correct then depression and coronary artery disease should be positively correlated and a consistent positive correlation has indeed been found. Depression is reported to be the strongest psychological predictor of coronary heart disease (Booth-Kewley and Friedman 1987, Frasure-Smith, Lesperance and Talajic 1995). A factor that could connect both conditions with a beneficial effect of n-3 PUFAs is heart rate (HR) variability (Severus, Ahrens and Stoll 1999), which is under nervous control. Low HR variability may increase the risk of fatal ventricular fibrillation and in a study of patients with coronary artery disease, HR variability was found to be lower in depressed patients than non-depressed patients (Carney, Saunders, Freedland et al

1995). A Canadian case control study of serum PUFA levels and major depression in patients recovering from acute coronary syndromes found that depressed patients had significantly lower serum concentrations of total n-3s and DHA than controls (Frasure-Smith, Lesperance and Julien 2004).

11.2. Conclusion

The epidemiological evidence and results of tissue studies, particularly of most recent studies, are indicative of a relationship between low intake of long chain n-3 fatty acids and depression. Results of supplementation trials have been mixed although there is some evidence that long chain n-3 fatty acids, particularly EPA and DHA may provide effective treatment for depression.

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CHAPTER TWELVE - DHA AND DEMENTIA

12.1. Alzheimer's disease and cognitive decline

Dementia is a common disorder among elderly people. Its prevalence increases with age to more than 30% among those aged 85 years and over (Ott, Breteler, van Harskamp et al 1995). The two most important subtypes of dementia are vascular dementia and Alzheimer's disease (Kalmijn 2000). A review by Grant (1997) summarises recent findings that African-Americans in Indianapolis, USA and on Japanese living in the state of Washington and Honolulu, USA, have significantly higher prevalence rates of Alzheimer's disease than individuals still living in their ethnic homelands. These findings suggest that the prevalence of Alzheimer's disease is more strongly influenced by diet, environment and/or lifestyle than by genetics.

An early large-scale study, which examined a possible relationship between the incidence of dementia and diet was conducted in California on 272 meat eaters and vegetarians aged 65 years and over, matched for age, sex and post code, and on 2984 unmatched meat eaters and vegetarians aged 65 years and over (Giem, Beeson and Fraser 1993). The matched subjects who ate meat were more than twice as likely to become demented as their vegetarian counterparts. However, in the unmatched cohort, there was no significant difference in the incidence of dementia in the vegetarian versus meat-eating subjects. The reason for these conflicting results was not clear but they illustrate the difficulty of this kind of research. The study provided no evidence for an effect of high fish consumption since the comparison was between eaters of any meat including fish, and vegetarians who avoided all meat including fish.

Several later studies have identified a significant benefit of fish consumption. A USA study conducted on 815 individuals aged 65 to 94 years who were initially unaffected by Alzheimer's disease found that during a seven year follow-up period, 131 individuals developed the disease (Morris, Evans, Bienias et al 2003). Participants who consumed fish once or more per week had 60% less risk of developing Alzheimer's disease compared with those who rarely or never ate fish. DHA intake was significantly associated with reduced risk but EPA intake was not. A prospective study conducted in a suburb of Rotterdam produced data on diet, incidence of dementia and confounding factors in 5,386 citizens aged 55 years or older (Kalmijn, Launer, Ott et al 1997). Subjects with a fish consumption of more than 20 grams per day had a reduced risk of cognitive impairment, cognitive decline, dementia and especially of Alzheimer's disease. However, in a six-year follow up study, low n-3 PUFA intake was not associated with increased risk of dementia (Engelhart, Geerlings, Ruitenberg et al 2003). Another Dutch study conducted in Zutphen investigated the development of cognitive impairment and cognitive decline in relation to food intake in 476 men aged 69-89 years (Kalmijn, Feskens, Launer et al 1997). High linoleic acid intake was associated with cognitive impairment after adjustment for age, education, cigarette smoking, alcohol consumption and energy intake while high fish consumption tended to be inversely associated with cognitive impairment and cognitive decline. However, the most recent Dutch study of 1,613 subjects aged 45 to 70 years found that fatty fish, EPA and DHA consumption were associated with a significantly reduced risk of impaired cognitive function (Kalmijn, van Boxtel, Ocke et al 2004).

Grant (2000) conducted a meta-analysis of epidemiological studies of Alzheimer's disease in individuals aged 65 and over in 11 countries and reported that fat and total calorific intake were highly correlated with prevalence rates of Alzheimer's disease. In addition, fish consumption was found to reduce the prevalence of Alzheimer's disease in the seven European and North American countries included in the study.

Early post-mortem studies of fatty acids from various regions of the brains of dementia sufferers and normal subjects produced inconsistent results (Brooksbank and Martinez 1989, Skinner,

Watt, Besson et al 1993). One of the earliest dementia studies involving analysis of fatty acids in tissues identified a linear decrease in grey matter PUFA (mainly AA and DHA) with increasing age in 28 normal subjects aged 52 to 83 years but did not observe any such systematic change in brains of 11 patients with senile dementia aged 73 to 91 years (Bowen, Smith and Davison 1973). However, most recent studies have tended to show decreased levels of AA and DHA in Alzheimer's disease (Soderberg, Edlund, Kristensson et al 1991, Guan, Soderberg, Sindelar et al 1994; Prasad, Lovell, Yatin et al 1998).

Plasma phospholipid fatty acid concentrations were measured in 735 Norwegian individuals aged 12-89 years (Bjerve, Fougner, Midthjell et al 1989). The absolute concentrations of AA, EPA and DHA increased from the third to the fifth decade of life then remained fairly constant into the ninth decade. The authors speculated that in this sample, younger people might have tended to eat less fish than older people. More recently, Conquer, Tierney, Zecevic et al (2000) found lower levels of plasma phospholipid DHA in patients with Alzheimer's disease and other dementias. They also found a significantly decreased level of plasma DHA in a group of elderly individuals who were more cognitively impaired but did not have dementia. The authors hypothesised that decreased fish consumption prior to disease onset may be at least partly responsible for the lower levels of DHA and the initiation or potentiation of cognitive decline. A recent French study of 246 individuals aged 63-74 years found that higher levels of erythrocyte total n-3 fatty acids were associated with a lower risk of cognitive decline (Heude, Ducimetiere and Berr 2003). Similarly, in an Irish study, 148 cognitively impaired individuals aged about 75 years were found to have significantly lower levels of serum cholesteryl ester DHA compared with 36 similarly aged individuals who were not cognitively impaired (Tully, Roche, Doyle et al 2003). By contrast, a Canadian study examined the association between plasma n-3 PUFA levels and incidence of dementia in a group of individuals aged 65 years or older (Laurin, Verreault, Lindsay et al 2003) and, contrary to previous studies, higher DHA concentrations were found in dementia cases.

Few studies have investigated whether fatty acid supplementation reduces the occurrence or symptoms of dementia. This is perhaps not surprising since if the process of cognitive decline represents physiological change that is, at least partly, explained by the dietary habits of a lifetime, relatively short-term treatment with n-3 fatty acids perhaps should not be expected to reverse such change. In one study of 45 elderly subjects aged 60 to 92 years in a Spanish nursing home, a milk formula containing 1% fish oil (supplying 400 mg per day PUFA) was given to each subject for 15 months (Rodriguez-Palmero, Lopez-Sabater, Castello-Bargallo et al 1997). The study demonstrated that supplementation significantly increased DHA and EPA levels in plasma and erythrocytes of elderly subjects. Similarly, a supplementation study conducted in France on 10 healthy elderly subjects aged 70-83 years found that a tuna oil supplement supplying 150 mg per day DHA and 30 mg per day EPA for 42 days produced a significant increase in platelet phosphatidylethanolamine DHA (Vericel, Calzada, Chapuy et al 1999). In a Japanese pilot study conducted in a home for the elderly, 20 subjects of average age of 83 years with mild to moderate dementia, 10 individuals were supplied with 720 mg DHA for 12 months while 10 individuals in the control group received no supplementation (Terano, Fujishiro, Ban et al 1999). A significant improvement in dementia scores was found in the DHA treated group but not in controls after three months.

12.2. Conclusion

Most recent studies provide relatively strong evidence that the incidence of dementia is higher in individuals who consume lower levels of DHA. Similarly, most recent studies

show decreased tissue DHA in individuals with Alzheimer's disease. Although it may be unreasonable to expect relatively short-term supplementation to reverse physiological changes resulting from long-term dietary deficiency, one study did indeed demonstrate improvement. Taken together, the evidence strongly supports the claim that DHA can help to maintain brain function in the elderly.

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CHAPTER THIRTEEN - DHA AND AGE-RELATED MACULAR DEGENERATION

13.1. Age-related macular degeneration

Age-related macular degeneration (AMD) is the leading cause of irreversible visual impairment and blindness in developed countries, where 25% of individuals over 75 years of age have some sign of AMD (Seddon, Rosner, Sperduto et al 2001, La Cour, Kiilgaard and Nissen 2002). A recent Japanese study concluded that AMD is less common among Japanese people than among white people in Western countries (Oshima, Ishibashi, Murata et al 2001). A study published in 1996 found that, after standardisation to control for changes in the age structure of the population over time, the incidence of AMD increased by 30-40% between 1950 and 1990 (Evans and Wormald 1996). Consumption of n-3 fatty acids has declined over the same period although it is not clear that this is a causative factor.

Some studies have investigated a possible link between dietary n-3 and AMD but early findings were negative. The Beaver Dam Eye Study conducted in Wisconsin, USA on 2152 individuals reported that high intake of saturated fat and cholesterol was associated with increased risk of early AMD but no association was found between AMD and seafood consumption (Mares-Perlman, Brady, Klein et al 1995). In a larger study involving 11, 448 individuals, the same group found no significant relationship between dietary fat or particular fatty acids and AMD (Heuberger, Mares-Perlman, Klein et al 2001). It has been suggested that consumption of fish in this population may have been too infrequent to identify differences (Smith, Mitchell and Leeder 2000).

By contrast, more recent studies suggest that increased intake of n-3 fatty acids and fish can indeed reduce the risk of AMD (Constable 2004, Mitchell, Smith, Cumming et al 2003). In a case control study on 349 individuals aged 55-80 years with AMD and 504 controls without AMD matched by age, sex and geography, it was found that higher intake of n-3 fatty acids was associated with a lower risk of AMD among individuals consuming diets low in linoleic acid (Seddon, Rosner, Sperduto et al 2001). A cross-sectional study of an Australian urban population of 3654 people aged 49 years or older found that a higher frequency of fish consumption was associated with decreased odds of late AMD. Those who had fish once per week were half as likely to have late AMD compared with those who had fish less than once per month (Smith, Mitchell and Leeder 2000). In a large prospective study of health professionals in the USA, including 42,743 women and 29,746 men aged 50 years or over, 567 individuals developed AMD (Cho, Hung, Willett et al 2001). Consumption of four servings of fish per week was associated with a 35% lower risk of AMD compared with three servings or less per month. In a recent study conducted in the USA, of 261 individuals aged 60 years and older with early signs of AMD, those with higher intakes of fish and nuts had significantly reduced risk of progression to advanced AMD (Seddon, Cote and Rosner 2003).

So far, only one paper has been published on fatty acid levels in tissues of individuals with AMD. In a matched control study of 65 elderly patients aged 66-87 years and 65 control subjects matched for age and sex, no significant differences were found in levels of DHA or any other fatty acid in plasma and red blood cells (Sanders, Haines, Wormald et al 1993). Again it is possible that consumption of fish in the sample population did not vary sufficiently to identify it as a factor influencing AMD (Smith, Mitchell and Leeder 2000).

Although supplementation studies would test the hypothesis that long chain n-3 fatty acids can protect against AMD, no papers on such trials were found in this survey of the literature.

13.2. Conclusion

Although the evidence is preliminary, most published epidemiological studies, including the largest, conclude that increased long chain n-3 intake reduces the risk of AMD. However supporting data from tissue studies and supplementation trials are lacking.

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CHAPTER FOURTEEN - DHA AND N-3 INTAKE

14.1 Dietary intake of n-3 and n-6 fatty acids

In 2004, the UK Food Standards Authority sought advice from the Scientific Advisory Committee on Nutrition (SACN) on the health benefits of fish consumption. In their final report, SACN noted that 'most people in the UK consume very little fish. For example during the period of the National Diet and Nutrition Survey (2000/2001) 74% of the participants did not consume oily fish and 82% did not consume other fish'.

Further evidence of low and declining fish intake in countries with western diets can be seen in the fatty acid levels found in breast milk. A study of the fatty acid composition of breast milk from Australian women revealed that between 1981 and 1994 DHA level declined from 0.32% to 0.21% and during the same period, linoleic acid level increased from 11% to 14% (Makrides, Simmer, Neumann et al 1995). The authors speculated that these changes in fatty acid profile reflected a change in diet towards increased consumption of vegetable oils rich in linoleic acid. Similarly, in the UK there is evidence that the proportion of linoleic acid in breast milk lipids has increased from 7-10% to about 14% and while the proportion of ALA may also have increased due to increased use of rapeseed oil, Sanders (2000) reports that the proportion of DHA in breast milk has declined over the last 30 years. A recent Canadian study concluded that the intake of DHA among pregnant women was generally low (Innis and Elias 2003). A further cause of change was the marked decline in consumption of oily fish, which has been taking place over the last century, although a slight reversal of this trend has been reported in recent years (Taylor, Gibney and Morgan 1979, BNF 1999, Sanders 2000, Endevelt and Shahar 2004). As discussed by Simopoulos (1991), intake of linoleic acid in corn oil and sunflower oil increased in western countries from around the beginning of the twentieth century with the development of the modern vegetable oil industry.

Also, while meat is a potential source of n-3 fatty acids, the n-3 content of farmed animal products is low compared to products from related wild animals because modern intensive agriculture tends to rely on feeds such as grain, which are high in n-6 but low in n-3 fatty acids. Examples of this have been demonstrated in studies of meat (Crawford 1968) eggs (Simopoulos and Salem 1989) and even farmed fish (Van Vliet and Katan 1990). A recent French study confirmed that the lipid composition of farmed fish is largely dependent on the fatty acid composition of their feed (Cahu, Salen and de Lorgeril 2004).

Some researchers suggest that the change in dietary n-6 to n-3 ratio has happened over a longer timescale and estimate from anthropological and palaeontological studies that about 10,000 years ago, before the advent of agriculture, the Palaeolithic hunter-gatherer had a diet providing a ratio of n-6 to n-3 of approximately 1:1 (Simopoulos 1999, Eaton, Eaton, Sinclair et al 1998). It is suggested that a return to this ratio is desirable because it is part of the dietary pattern to which the human species is adapted.

Estimates of the n-6 to n-3 ratio in the modern diet are more numerous and show consistently that, in western countries, n-6 intake substantially exceeds n-3 intake (Table 14.1).

Table 14.1 Estimates of dietary n-6:n-3 intake

Country	n-6: n-3 ratio	Reference
UK	5.7:1	MAFF (1997) quoted in BNF (1999).
UK	6.9:1	Gregory, Foster, Tyler et al (1990) quoted in BNF (1999)
USA	9.8:1	Kris-Etherton, Taylor, Yu-Poth et al (2000)
USA	16.7:1	Simopoulos (2001)
Japan	4:1	Sugano and Hirohara (2000)
Japan	4-4.5:1	Okita, Yoshida, Yamamoto et al (1995)

Although published estimates indicate that the n-6:n-3 ratio in Japan is substantially lower than in the UK and USA, the Japanese figure in 1985 (3.9:1) was higher than it was in 1960 (2.9:1) because of a trend towards lower fish consumption (Lands, Hamazaki, Yamazaki et al 1990). A study of the American diet based on annual food use data, found that in 1985, 96% of total n-3 was the short chain ALA rather than the long chain fatty acids EPA and DHA (Raper, Cronin and Exler 1992). In the same study, average per capita DHA intake was estimated at 78 mg per day. A later study found that, among American adults and children, the average intake of EPA plus DHA was 100 mg per day (Jonnalagadda, Egan, Heimbach et al 1995). An Australian study conducted between 1989 and 1991 found that dietary intakes of long chain n-3 fatty acids, including EPA and DHA combined were less than 100 mg per day (Sinclair, O’Dea and Johnson 1994). Another small Australian study on 83 healthy adults found that median intake of EPA and DHA was 180 mg per day (Ollis, Meyer and Howe 1999). In a recent American study of 14-15 year olds, their reported intake of long chain n-3 fatty acids (DHA, EPA and docosapentaenoic acid) was estimated at about 40 mg per day (Harel, Riggs, Vaz et al 2000).

14.2. Dietary recommendations

A number of authoritative organisations have made recommendations on dietary intake of fatty acids and although the levels vary, they are unanimous in stating that n-6 intake should be reduced and n-3 intake should be increased (Holub 1998, Roche 1999). Table 14.2 summarises recommendations that have been made on LCPUFA intake.

Table 14.2 Summary of official recommendations for adult Intakes of long chain n-3 polyunsaturated fatty acids

Source	Date	Recommendations
National Nutrition Council, Norway	1989	n-3 0.5% of daily energy: 1000-2000 mg per day
NATO Workshop on n-3 and n-6	1989	n-3 0.27% of daily energy: 800 mg per day EPA plus DHA
Scientific Review Committee of Canada	1990	n-3 at least 0.5% of daily energy
British Nutrition Task Force	1992	EPA: 0.2-0.5% and DHA 0.5% of daily energy
Scientific Committee for Food of the European Community	1993	n-3 0.5% of daily energy
FAO/WHO Expert Committee on Fats and Oils in Human Nutrition	1994	Consider pre-formed DHA in pregnancy
Committee on Medical Aspects of Food Policy	1994	EPA plus DHA: 200 mg per day; or a minimum of 2 portions of fish a week, one of which should be oily
Ad hoc Expert Workshop held in USA, organized by ISSFAL	2000	EPA plus DHA: 0.3% of daily energy, or 650 mg per day
Eurodiet – Nutrition and Diet for Healthy Lifestyle Conference, University of Crete	2000	EPA plus DHA: 200 mg per day
AFSSA, CNERNA and CNRS, France	2001	Long chain polyunsaturates: 500 mg per day; DHA: 120 mg per day
US National Academy of Science Institute of Medicine	2002	EPA plus DHA: 130-260 mg per day
American Heart Association	2002	Consume fish (preferably oily) twice per week
International Society for the Study of Fatty Acids and Lipids	2004	EPA plus DHA: 500 mg per day
UK Scientific Advisory Committee on Nutrition	2004	EPA plus DHA: 450 mg per day

14.3. Conclusion

It is clear that there is a wide gap between recommended intakes of n-3 fatty acids and estimated levels in the Western diet.

Nu-Mega Ingredients Pty Limited

Omega-3 DHA
and its
Importance in Human Nutrition

CHAPTER FIFTEEN - DHA AND Vitamin E

15.1 PUFA intake and vitamin E requirement

Vitamin E is a fat-soluble vitamin with antioxidant activity. The term applies to all tocopherols and tocotrienols but the biologically most active form is alpha-tocopherol (Weber, Bendich and Machlin 1997). Vitamin E protects PUFA in membrane lipids from peroxidation and its requirement is closely related to the dietary intake of PUFA (Valk and Hornstra 2000). Evidence from animal and human studies show that vitamin E requirement increases with the amount consumed and the number of double bonds in the PUFA (Meydani 1992, Jenkinson, Franklin, Wahle et al 1999). For this reason it has been advised that increased consumption of highly unsaturated fatty acids such as DHA requires increased intake of vitamin E (Weber, Bendich and Machlin 1997) and other antioxidants (Eritsland 2000).

Following a review of the literature, Valk and Hornstra (2000) recommend that an appropriate ratio would be at least 0.6 mg alpha-tocopherol per gram of PUFA. An earlier review concluded that the amount of vitamin E required was 0.4-0.8 mg per gram of PUFA but may be greater when diets contain higher than average levels of long chain PUFAs (Weber, Bendich and Machlin 1997). The authors suggested that 1.5 and 1.8 mg alpha-tocopherol would be required per gram of EPA and DHA respectively.

15.2. Conclusion

Although the evidence is not conclusive, it seems likely that advice to increase DHA intake should be accompanied by advice to increase vitamin E intake.