

Gender differences in the *n*-3 fatty acid content of tissues

Caroline E. Childs^{1*}, Meritxell Romeu-Nadal², Graham C. Burdge¹ and Philip C. Calder¹

¹*Institute of Human Nutrition and Developmental Origins of Health and Disease Division, School of Medicine, University of Southampton, Southampton SO16 6YD, UK*

²*Department of Nutrition, Faculty of Pharmacy, University of Barcelona, Barcelona, Spain*

Dietary *n*-3 PUFA have many beneficial effects on cell and tissue function and on human health. In mammals the *n*-3 essential fatty acid α -linolenic acid (ALNA) can be converted into longer-chain (LC) *n*-3 PUFA such as EPA and DHA via a series of desaturase and elongase enzymes that are mainly active in the liver. Human studies have identified that males and females appear to differ in their ability to synthesise EPA and DHA from ALNA, with associated differences in circulating concentrations. Based on studies of women using the contraceptive pill or hormone-replacement therapy and of trans-sexual subjects it is suggested that sex hormones play a role in these differences. The rat has been used to investigate gender differences in *n*-3 PUFA status since this model allows greater dietary control than is possible in human subjects. Like human subjects, female rats have higher plasma DHA concentrations than males. Rats also respond to increased dietary ALNA in a way that is comparable with available human data. The concentrations of LC *n*-3 PUFA in rat plasma and tissues are positively associated with circulating concentrations of oestradiol and progesterone and negatively associated with circulating concentrations of testosterone. These findings suggest that sex hormones act to modify plasma and tissue *n*-3 PUFA content, possibly by altering the expression of desaturase and elongase enzymes in the liver, which is currently under investigation.

PUFA: *n*-3 fatty acid: Fish oil: Gender: Phospholipid

Fatty acids: structure and naming

Fatty acids are hydrocarbon chains with a carboxyl group at one end and a methyl group at the other. The carboxyl group is reactive and readily reacts to form ester bonds. For example, fatty acids form ester bonds with glycerol or cholesterol to form TAG or cholesteryl esters (CE) respectively. Fatty acids are usually straight chains with even numbers of C (2– \geq 30), with fatty acids in the human diet typically between C₁₀ and C₃₀. Fatty acids that contain double bonds are termed unsaturated fatty acids. Unsaturated fatty acids can be further defined as MUFA (one double bond present) or PUFA (two or more double bonds).

Double bonds in a fatty acid change the shape and therefore the physical properties of the fatty acid. The effect of double bonds will depend on the number, position and configuration (e.g. *cis* or *trans* configurations) of the double bonds present. *n*-3 Fatty acids are PUFA

characterised by the presence of the first double bond on the C-3 from the methyl terminus, with subsequent double bonds separated from one another by a methylene (–CH₂–) group.

The physiological functions of fatty acids

Fatty acids are substrates for energy generation by β -oxidation and may be stored in adipose tissue in situations in which energy intake exceeds expenditure. In addition, fatty acids play a role in the modulation of membrane fluidity⁽¹⁾, interact with intracellular signalling pathways and transcription factors^(2,3) and act as substrates for the production of signalling molecules^(4–9). Unsaturated fatty acids may be subject to the process of lipid peroxidation, with susceptibility increasing with the extent of unsaturation⁽¹⁰⁾.

Abbreviations: ALNA, α -linolenic acid; CE, cholesteryl ester; DPA, docosapentaenoic acid; HRT, hormone-replacement therapy; LC, longer-chain; PC, phosphatidylcholine.

***Corresponding author:** Mrs Caroline E. Childs, fax +44 2380 795255, email cep199@soton.ac.uk

***n*-3 Fatty acids: dietary sources and typical intakes**

The essential fatty acid α -linolenic acid (ALNA; 18: 3*n*-3) is the simplest *n*-3 PUFA and is found in green leaves, some seeds, nuts and cooking oils (e.g. soyabean oil); flaxseed oil has a very high content of ALNA. The principal dietary source of the longer-chain (LC) *n*-3 PUFA EPA (20: 5*n*-3), docosapentaenoic acid (DPA; 22: 5*n*-3) and DHA (22: 6*n*-3) is fish, especially oily fish. The LC *n*-3 PUFA content of fish varies a great deal between species. For example, salmon is a rich source, with a EPA+DPA+DHA content of approximately 2.2 g/100 g portion, while cod contains approximately 300 mg/120 g portion⁽¹¹⁾.

The average dietary intake of total *n*-3 PUFA for adults in the UK is 2.27 g/d for males and 1.71 g/d for females⁽¹²⁾. For most individuals the bulk of this intake is in the form of ALNA, because dietary intake of LC *n*-3 PUFA is highly dependent on consumption of fish, which varies greatly amongst individuals⁽¹³⁾. Average adult intakes of LC *n*-3 PUFA in the UK are considered to be approximately 200 mg/d⁽¹³⁾, but the distribution of intakes is bimodal, with one peak representing non-fish eaters and another fish eaters; the former peak is larger since it is estimated that only 27% of UK adults habitually eat oily fish⁽¹³⁾.

***n*-3 PUFA and human health**

Comprehensive reviews of the evidence from epidemiological studies and human intervention trials of dietary LC *n*-3 PUFA and ALNA are available elsewhere^(14,15). LC *n*-3 PUFA have been demonstrated to convey significant benefits in CVD, some inflammatory conditions and in early brain and visual development, with benefits emerging in the areas of childhood behaviour, adult psychiatric disorders and neurological decline with ageing⁽¹⁴⁾. Benefits from ALNA are less clear and may relate to its action as a precursor of LC *n*-3 PUFA⁽¹⁵⁾.

There are substantial epidemiological and case-control study data that demonstrate that the risk of CVD is lowest among those with the highest fish or LC *n*-3 PUFA intake⁽¹⁶⁻¹⁸⁾. Supplements containing LC *n*-3 PUFA have been demonstrated to have a role in secondary prevention of CVD, reducing the risk of mortality related to cardiovascular events in subjects who had previously suffered a myocardial infarction^(18,19).

The clinical applications of LC *n*-3 PUFA in relation to chronic inflammatory disease, atopic disease and the systemic inflammatory response to surgery and injury have been reviewed^(6,7,20). There is strong evidence from randomised placebo-controlled trials that dietary fish oil supplements are beneficial for patients with the chronic inflammatory disease rheumatoid arthritis. Benefits to other inflammatory disorders have not been conclusively demonstrated, and evidence for the role of LC *n*-3 PUFA supplementation in patients receiving enteral formula feeds after surgery is complicated by the simultaneous provision of other nutrients such as certain amino acids and antioxidant vitamins.

The observations that DHA is found in high concentrations in the retina and accumulates in the brain during early life (from 3 months of gestation to 18 months after delivery in human subjects) and that feeding animals an *n*-3 PUFA-deficient diet results in visual and cognitive abnormalities suggests that an adequate supply of *n*-3 PUFA, in particular preformed DHA, is required for the development and function of the central nervous system, including the retina and brain^(21,22). Studies undertaken in preterm infants have demonstrated that formulas containing DHA improve visual function early in infancy⁽²³⁾. In term infants formulas containing DHA have been shown in some studies, although not all, to improve cognitive function⁽²⁴⁾.

Following on from the important role of DHA in promoting cognitive development in early life, research has been conducted to evaluate the potential benefits that LC *n*-3 PUFA may confer in children with neurodevelopmental disorders such as attention-deficit hyperactivity disorder⁽²⁵⁾. Studies that have evaluated the parent- and teacher-reported changes in behaviour of children with attention-deficit hyperactivity disorder have identified benefits of LC *n*-3 PUFA supplementation; these studies have used various combinations of EPA and DHA and it is not yet clear which is the more important in this setting⁽²⁵⁾. In general, this area remains controversial and larger studies are required.

Meta-analyses have identified significant benefits of supplementation with LC *n*-3 PUFA among adults with unipolar and bipolar depression⁽²⁶⁾. Other meta-analyses have identified that these benefits are only apparent in populations with an established clinical diagnosis of depressive illness⁽²⁷⁾. Epidemiological and post-mortem evidence has generated interest in the potential benefits that LC *n*-3 PUFA may have in the prevention or treatment of disorders of cognitive function in later life such as dementia and Alzheimer's disease, with further investigation from intervention studies required⁽¹⁴⁾.

Endogenous synthesis of LC *n*-3 PUFA

In addition to consumption in the diet, LC *n*-3 PUFA can be endogenously synthesised from their essential fatty acid precursor ALNA⁽²⁸⁾. This process predominantly occurs in the liver. ALNA is an essential *n*-3 PUFA in animals because of the absence of $\Delta 15$ desaturase, the enzyme required to introduce the '*n*-3' double bond into linoleic acid (18: 2*n*-6). Once consumed in the diet, ALNA can be converted via a series of elongase, desaturase and β -oxidation steps into LC *n*-3 PUFA (Fig. 1). These elongation and desaturation reactions occur in the endoplasmic reticulum, with the final β -oxidation step occurring in the peroxisome. This same series of desaturase and elongase enzymes is also involved in the metabolism of the *n*-6 PUFA linoleic acid into its LC more-unsaturated derivatives (e.g. arachidonic acid (20: 4*n*-6), adrenic acid (22: 4*n*-6)), and in situations of essential fatty acid deficiency this pathway generates mead acid (20: 3*n*-9) from oleic acid (18: 1*n*-9). The sharing of enzymes means that there is competition between linoleic acid and ALNA for

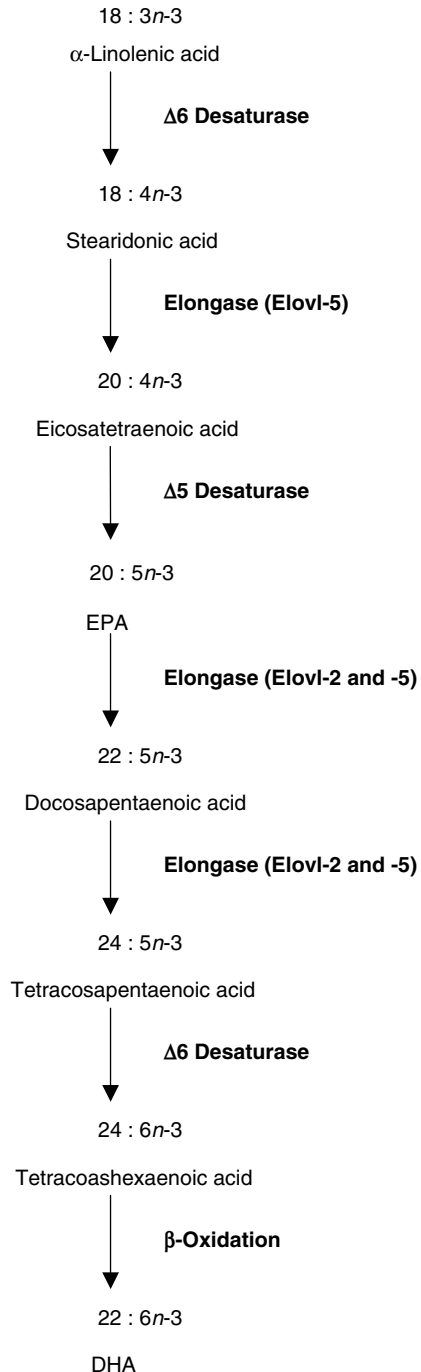


Fig. 1. Biosynthesis of longer-chain *n*-3 PUFA from α -linolenic acid.

metabolism. In most Western diets consumption of linoleic acid is about ten times that of ALNA⁽¹⁵⁾, suggesting that metabolism of the former will predominate.

The ability of human subjects to convert ALNA into LC *n*-3 PUFA has been studied using two approaches: provision of additional dietary ALNA; provision of ALNA labelled with stable isotopes. By providing additional dietary ALNA it is possible to assess whether there is a consequent increase in concentrations of LC *n*-3 PUFA in blood, cell and tissue pools, which would be indicative that synthesis of LC *n*-3 PUFA from ALNA has taken

place. In studies in which stable-isotope-labelled ALNA is provided subsequent blood samples are collected to determine the circulating concentrations of stable-isotope-labelled metabolites of ALNA such as EPA, DPA and DHA. These studies have the advantage of eliminating any doubt that these products may have come from alternative sources such as the diet or release from adipose tissue.

Comprehensive reviews of the studies that have provided increased dietary ALNA and assessed the appearance of EPA and DHA are available^(15,29). Increasing dietary ALNA intake has been found to result in dose-dependent increases in EPA in plasma phospholipids; similar effects are likely for circulating cells such as leucocytes and platelets, although there are fewer studies investigating these effects^(15,29). The relationship between increasing dietary ALNA and DHA status is less clear, with some studies reporting a reduction in DHA status with increasing dietary ALNA and others reporting no change in DHA status^(15,29).

Increasing ALNA intake might reduce DHA production as a result of the role of the $\Delta 6$ desaturase in two stages of the synthesis of LC *n*-3 PUFA (see Fig. 1). It is possible that increasing dietary ALNA increases substrate competition, inhibiting the desaturation of tetracosapentaenoic acid to DHA⁽³⁰⁾.

The studies that are currently available on the effect that increased dietary ALNA has on concentrations of LC *n*-3 PUFA vary in their design (for details, see Burdge & Calder⁽¹⁵⁾). There is wide variation between the studies in terms of duration (2–52 weeks), mode of supplementation (capsules, use of oil mixed into salads, incorporation of ALNA into dietary products such as spreads and muffins), whether linoleic acid intake was modified in parallel and the blood lipid fraction analysed. Studies to date have been conducted on men or mixed groups of men and women, apart from one study in lactating women⁽³¹⁾. The latter study has shown that increased dietary ALNA (20 g/d) increases ALNA, EPA and DPA in plasma, but does not alter plasma or breast-milk DHA. There are therefore limited data currently available to describe the effects that increased dietary ALNA may have on blood and cell LC *n*-3 PUFA status in women, and no data for non-lactating women.

Data from studies using stable-isotope-labelled ALNA has given the first indications that there are gender differences in the ability to synthesise LC *n*-3 PUFA from ALNA. Studies that have provided a dose of [¹³C]ALNA as part of a meal and subsequently collected blood and breath samples over a 3-week period have indicated that young women (average age 28 years) convert a greater proportion of ALNA into EPA and DHA compared with men (average age 36 years)^(32,33). This finding that women apparently have a higher rate of synthesis of LC *n*-3 PUFA compared with men is supported by the work of other authors who have used [²H]ALNA and mathematical modelling to determine rates of LC *n*-3 PUFA synthesis in a mixed group of healthy subjects^(34,35). When these data were assessed for gender differences it was found that women receiving a beef-based diet utilised a 3-fold greater amount of DPA to generate DHA compared with men⁽³⁶⁾.

Table 1. Studies investigating differences between males (M) and females (F) in the longer-chain *n*-3 PUFA (EPA, DHA and docosapentaenoic acid (DPA)) composition of blood lipid

| Subjects | Country | Age (years) | Dietary control | Lipid fraction | Effects of gender | | | Reference |
|----------|-----------------|-------------|-----------------------------|---|-------------------|----------------------------------|------------|--|
| | | | | | M>F | M = F | F>M | |
| 41 F | Finland | mean 40 | 7 d weighed-food record | Serum cholesteryl ester | | EPA | DHA | Nikkari <i>et al.</i> ⁽³⁸⁾ |
| 41 M | | mean 43 | | Serum TAG Serum phospholipid | | DHA EPA | DHA | |
| 103 F | The Netherlands | 18–67 | Controlled diet, 3 weeks | Plasma cholesteryl ester | | EPA, DPA | DHA | Giltay <i>et al.</i> ⁽³⁹⁾ |
| 72 M | | | | | | | | |
| 23 F | UK | 18–35 | Habitual diet (FFQ) | Plasma TAG | | EPA, DPA | DHA | Bakewell <i>et al.</i> ⁽⁴⁰⁾ |
| 13 M | | | | Plasma NEFA Plasma phosphatidylcholine Plasma cholesteryl ester | DPA | EPA, DPA EPA EPA, DPA, DHA | DHA DHA | |
| | | | | Total plasma | | DPA | EPA, DHA | |
| 1547 F | New Zealand | 15–65+ | Habitual diet (24 h recall) | Serum phospholipid | EPA, DPA | | DHA | Crowe <i>et al.</i> ⁽⁴¹⁾ |
| 1246 M | | | | Serum TAG | | EPA, DPA, DHA | | |
| | | | | Serum cholesteryl ester | EPA | | DHA | |

A similar but non-significant trend was observed in those subjects receiving an *ad libitum* diet.

The [²H]ALNA studies have also indicated that background diet may interact with gender differences in rates of LC *n*-3 PUFA synthesis. Subjects were maintained on controlled diets for 2 weeks before administration of the [²H]ALNA and remained on this diet as blood samples were collected over a 1-week period. The ability of men to generate DHA from DPA was not found to be affected by the diet received, while women were found to have lower DHA synthesis when receiving a fish diet, exhibiting a rate of synthesis comparable with that observed in men⁽³⁶⁾. The synthesis pathway in women therefore appears to be sensitive to the dietary availability of LC *n*-3 PUFA. A study of older men (mean 52 years) has demonstrated that a high-ALNA diet does not affect [¹³C]ALNA conversion to EPA, DPA or DHA, but a diet enriched in EPA+DHA decreases [¹³C]ALNA conversion to EPA and DPA, but not to DHA⁽³⁷⁾.

A limitation of the stable-isotope studies is that they do not give insight into the extent to which LC *n*-3 PUFA might have been incorporated into metabolically-relevant tissues such as the liver and adipose tissue. These studies also give little indication of whether the differences between men and women in the ability to synthesise LC *n*-3 PUFA is related to differences in the circulating concentrations of those fatty acids.

Gender differences in blood and tissue LC *n*-3 PUFA content

Studies have been undertaken in human subjects to investigate gender differences in circulating plasma concentrations of LC *n*-3 PUFA (Table 1). While these studies vary

in their sample size, extent of dietary control exerted and the range of blood lipids analysed, all have found that women have higher circulating DHA concentrations compared with men and that this difference is independent of dietary intake^(38–41). There is an indication from two of these studies that EPA and DPA are found in lower circulating concentrations in women compared with men, which may indicate either a greater rate of conversion of these fatty acids into DHA or their displacement from lipids by DHA.

Mechanisms that have been proposed to be responsible for the gender differences observed in LC *n*-3 PUFA include gender differences in rates of β -oxidation and adipose tissue composition and mobilisation, and possible influences of sex hormones on the desaturase and elongase enzymes involved in the synthesis of LC *n*-3 PUFA^(33,40).

Studies using [¹³C]ALNA that collected breath samples have enabled the rate of β -oxidation of ALNA over 24 h to be calculated via the recovery of ¹³CO₂^(32,33). These studies have estimated the extent of β -oxidation of [¹³C]ALNA to be 33 % in men and 22 % in women. These values and the gender difference observed are similar to those obtained in studies of other fatty acids (e.g. [¹³C]palmitate⁽⁴²⁾) and may therefore reflect the differences in muscle mass between men and women, rather than any specific sparing of ALNA in women. However, the lower rates of β -oxidation of fatty acids observed in women could potentially leave more ALNA available as a substrate for metabolism into LC *n*-3 PUFA.

Gender differences in subcutaneous adipose tissue composition have been observed in human studies, with women found to have more DPA and DHA in adipose tissue compared with men, although these fatty acids are relatively minor components of the total fatty acid content

(<0.3%)⁽⁴³⁾. In the fasting state plasma NEFA predominantly reflect fatty acids released from adipose tissue under the action of hormone-sensitive lipase. It has been identified that women have higher fasting plasma NEFA concentrations compared with men⁽⁴⁰⁾, reflecting the greater proportion of body fat in women compared with men. *Ex vivo* studies have identified that the release of fatty acids from human adipose tissue is selective and dependent on C chain length and the extent of unsaturation, with ALNA and EPA identified as being preferentially released in comparison with other *n*-3 PUFA^(44,45). It is therefore possible that gender differences in adipose tissue composition may directly affect circulating concentrations of LC *n*-3 PUFA or increase the availability of ALNA for synthesis of LC *n*-3 PUFA in women compared with men.

Sex hormones and *n*-3 PUFA metabolism

Human studies

A role for sex hormones in mediating the gender differences in ALNA conversion and in LC *n*-3 PUFA content of blood (and tissue) lipids has been suggested from studies of women using oral contraceptives^(33,39,40) or hormone-replacement therapy (HRT)^(46,47) and a study of sex-hormone treatment in trans-sexual subjects⁽³⁹⁾.

When [¹³C]ALNA was provided to women it was found that those women using the oral contraceptive pill have higher rates of DHA production over 21 d than women not using the pill⁽³³⁾. This finding may indicate up-regulation of the desaturase–elongase pathway by oestrogen. However, the number of female subjects in this study was low (*n* 6) and data were not available for circulating oestrogen concentrations in these women.

Data from studies of circulating LC *n*-3 PUFA concentrations support the suggestion that women using the contraceptive pill have higher rates of DHA synthesis. When thirty-two women who used oral contraceptives were compared with seventy-two women who did not use oral contraceptives, a trend was observed (*P* = 0.08) for higher concentrations of DHA in plasma CE among women using oral contraceptives⁽³⁹⁾. However, significant differences in age between these two groups of women were mentioned (but not detailed in the paper), which suggests differences in menopausal status as study participants were recruited from a wide age-range (18–67 years); furthermore data were not collected on sex hormone concentrations in this study. It is possible that the timing of blood sample collection during the menstrual cycle in this study (reported as days 5–9, follicular stage), may have been a confounding factor, as differences in the fatty acid composition of serum lipids have been identified across the menstrual cycle⁽⁴⁸⁾.

Another study that attempted to address the impact of oral contraceptive use has indicated that women using oral contraceptives have a 60% higher total plasma DHA content than those not using oral contraceptives⁽⁴⁰⁾. However, because of the small number of subjects in this study (eleven women using oral contraceptives, twelve women not using oral contraceptives) the study lacked statistical

power to identify a significant effect of contraceptive pill use. Good control over the stage of the menstrual cycle of subjects was achieved (day 10 of the menstrual cycle) but there were no data available on circulating sex hormone concentrations, which could have supported the hypothesis that higher plasma oestrogen levels are responsible for the differences in fatty acid composition between women using or not using the contraceptive pill.

The effect that use of HRT has on plasma *n*-3 PUFA composition has been assessed. A study of 104 postmenopausal Japanese women, fifty-nine of whom received HRT (0.625 mg oestrogen + 2.5 mg progesterone/d) and forty-five of whom received no treatment has found that use of HRT for 12 months is associated with increased plasma EPA and DHA⁽⁴⁶⁾, which suggests that female sex hormones may up regulate the synthesis of LC *n*-3 PUFA from ALNA. However, this study lacked a placebo group (with the subjects themselves deciding whether or not to receive HRT) and did not report any dietary assessment or control.

A placebo-controlled study to investigate the effect of HRT or an oestrogen-receptor modulator (Raloxifene) on the fatty acid composition of plasma CE has also found an increase in plasma CE-DHA content with HRT⁽⁴⁷⁾. The potential role of oestrogen rather than progesterone in these observed differences was indicated by the finding that Raloxifen also leads to an increased DHA content in CE. This study reported an inverse relationship between ALNA and DHA content of CE among women using HRT or Raloxifene, which was suggested to be indicative of an enhanced capacity to synthesise DHA from ALNA. An alternative explanation for this finding is that increased dietary ALNA can lead to reductions in DHA status⁽¹⁵⁾. As the women in this study were not controlled for dietary intake, it is possible that this inverse relationship reflects dietary variation in ALNA intake among these subjects rather than an increased conversion of ALNA into DHA.

A study of trans-sexual subjects was undertaken to assess the influence of cross-sex hormone administration on fatty acid status⁽³⁹⁾. This study has demonstrated that male-to-female trans-sexuals receiving a combination of oral ethinyl oestradiol and cyproterone acetate have higher DHA concentrations in their plasma CE within 4 months of treatment. Female-to-male trans-sexuals receiving intramuscular testosterone have a lower DHA content in plasma CE within 4 months of treatment. This finding suggests that oestradiol up regulates, while testosterone down regulates, the synthesis of DHA from ALNA. The authors have suggested that the difference seen in female-to-male trans-sexuals is not a result of the effect of testosterone treatment itself, but to the associated reductions in circulating oestrogen. However, the effect of testosterone on circulating oestrogen concentrations was only found to be significant in the subjects who received treatment for 4 months (*P* = 0.01), with the group treated for 12 months not differing significantly in their oestradiol status (*P* = 0.09). Dietary intake of subjects was assessed using the Dutch EPIC FFQ⁽⁴⁹⁾, but this was only done in the subjects receiving hormone treatment for 12 months. While the design of this FFQ allows assessment of fish intake, these data were not reported.

In vitro and animal studies

There is experimental evidence from animal studies that sex hormones might influence the activity of the desaturase enzymes involved in the conversion of *n*-3 and *n*-6 essential fatty acids into their LC more-unsaturated derivatives^(50–52). Work to date is limited to the use of *n*-6 fatty acid rather than *n*-3 fatty acid as a substrate for assessment of enzyme activities and has investigated the effect of short-term (<2 d) hormone treatment rather than the influence of normal circulating physiological concentrations of sex hormones. *In vitro* studies have identified that $\Delta 5$ desaturase activity (assessed by production of the *n*-6 PUFA arachidonic acid) in liver microsomes isolated from female rats is reduced by 17 β -oestradiol and testosterone, and unaffected by progesterone⁽⁵⁰⁾. An *ex vivo* study using rat liver microsomes to assess the effect of

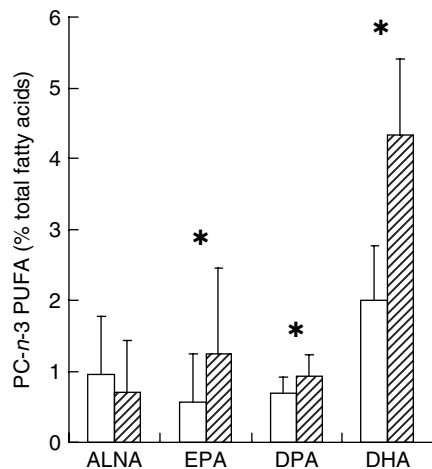


Fig. 2. *n*-3 PUFA in plasma phosphatidylcholine (PC) in 13-week-old male (□) and female (▨) rats maintained on the same diet. ALNA, α -linolenic acid; DPA, docosapentaenoic acid. Values are means and standard deviations represented by vertical bars for eighteen rats. The effect of gender was significant (Student's *t* test): **P*<0.05.

oestradiol treatment on $\Delta 6$ desaturase activity (assessed by production of the *n*-6 PUFA γ -linolenic acid (18: 3*n*-6)) has identified that treatment of female rats for 2 d with 17 β -oestradiol leads to reduced activity⁽⁵¹⁾. The effect of 17 β -oestradiol treatment on serum fatty acid composition was also assessed, with reductions in serum arachidonic acid content observed, although no significant effect on DPA or DHA content was found.

A study that assessed the effect of a single inter-peritoneal testosterone injection in both male and female rats has found that 24 h after testosterone treatment there is increased $\Delta 9$ desaturase activity, while $\Delta 6$ and $\Delta 5$ desaturase activities (assessed using *n*-6 fatty acid substrates) are inhibited⁽⁵²⁾. Fatty acid composition analysis performed on plasma and liver subcellular fractions has confirmed that these decreases in $\Delta 6$ and $\Delta 5$ desaturase activities are associated with reduced DHA content in both the male and female rats. This study has also identified that the DHA content of plasma and liver fractions in both testosterone-treated and control groups is higher in female rats compared with males, although the statistical significance of these gender differences was not presented.

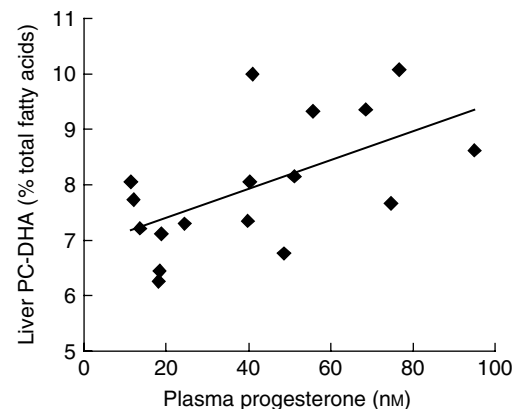


Fig. 4. Relationship between liver phosphatidylcholine (PC)-DHA content and plasma progesterone concentrations in female rats. (—), Linear-fit line; R^2 0.336.

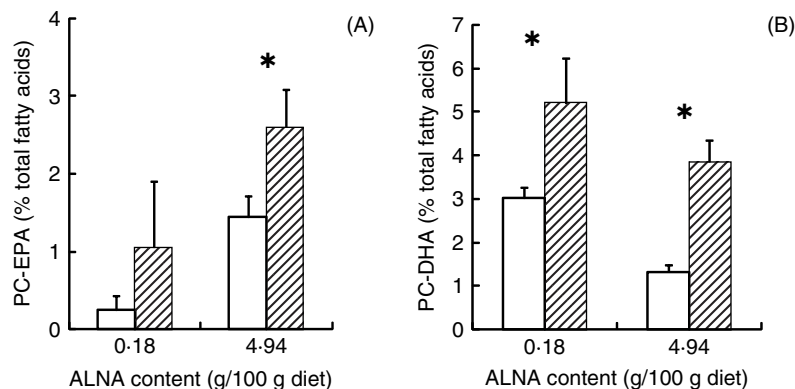


Fig. 3. Plasma phosphatidylcholine (PC)-EPA (A) and -DHA (B) in 13-week-old male (□) and female (▨) rats fed diets containing two different levels of α -linolenic acid (ALNA). Values are means and standard deviations represented by vertical bars for six rats. The effect of gender was significant (Student's *t* test): **P*<0.05.

These studies using rat models support the observations from studies of human trans-sexual subjects that testosterone treatment may reduce the activity of $\Delta 5$ and $\Delta 6$ desaturases, reducing the synthesis of LC PUFA and leading to reductions in the tissue content of LC $n-3$ PUFA such as DHA. Data also suggest that oestrogen may decrease $\Delta 5$ and $\Delta 6$ desaturase activity, although associated reductions in LC PUFA have only been identified for $n-6$ PUFA, with no significant effect of oestrogen treatment on DHA content observed. There are no data currently available on the effect that circulating sex hormones may have on the activity of the elongase enzymes involved in the synthesis of LC PUFA.

Current research aims and initial findings

The role of gender differences and altered dietary availability of ALNA on the LC $n-3$ PUFA composition of rat tissues are being investigated. Use of a rat model allows complete dietary control during the study period and enables collection of tissues that are not readily obtained from human studies. Fatty acid compositions of various lipid fractions (phosphatidylcholine (PC), TAG, CE, NEFA) in a range of tissues (plasma, liver, subcutaneous and intra-abdominal adipose tissue) have been determined. Plasma concentrations of oestradiol, progesterone and testosterone have been determined to assess any influence that these hormones may have on tissue fatty acid composition. The mRNA expression of $\Delta 5$ and $\Delta 6$ desaturase and elongase (Elovl-5) enzymes in liver is currently under investigation, in order to assess whether there are gender differences in expression, and whether expression is related to sex hormone concentrations or fatty acid composition.

It has been found that in the rat, as in human subjects, there are significant gender differences in plasma LC $n-3$ PUFA status, particularly DHA, that are independent of diet (CE Childs, M Romeu-Nadal, GC Burdge and PC Calder, unpublished results). For example, Fig. 2 shows that female rats have more EPA, DPA and DHA ($P = 0.048$, $P = 0.012$ and $P < 0.001$ respectively) in plasma PC than male rats of the same age maintained on the same diets. Gender differences were also observed in the DHA content of liver phospholipids and both subcutaneous and intra-abdominal adipose lipids.

Increasing the ALNA content of the diet results in significant ($P < 0.05$) increases in EPA in plasma PC of both the male and female rats (Fig. 3(A)). In parallel, there is a decrease in the DHA content (Fig. 3(B)), as has been observed in some human studies that have used high ALNA intakes⁽¹⁵⁾. The content of both EPA and DHA in plasma PC remains higher in female rats than in male rats irrespective of the ALNA content of the diet (Fig. 3(A,B)).

The $n-3$ PUFA content of plasma and tissue lipid fractions were examined for correlations with circulating sex hormone concentrations (CE Childs, M Romeu-Nadal, GC Burdge and PC Calder, unpublished results). LC $n-3$ PUFA (EPA, DPA and DHA) were found to be significantly inversely related to plasma testosterone concentrations across all tissues assessed. Significant positive relationships were found between plasma and liver

PC-EPA content and plasma oestradiol concentrations ($r = 0.564$, $P = 0.001$ and $r = 0.369$, $P = 0.044$ respectively). Finally significant positive relationships were found between DHA in all tissues studied and plasma progesterone concentrations. Fig. 4 shows that variation in progesterone concentration predicts 34% ($r = 0.585$, $P = 0.017$) of the variation in liver PC-DHA concentration.

Conclusions

Human studies have demonstrated that males and females differ in their ability to synthesise LC $n-3$ PUFA from ALNA and that this disparity is associated with gender differences in the circulating concentrations of LC $n-3$ PUFA, particularly DHA, which is higher in females. It has been demonstrated that the rat is a good model for investigating the effect of gender differences on fatty acid composition and the mechanisms involved, as it demonstrates similar gender differences to those observed in human subjects and comparable responses to increased dietary ALNA intake. Significant relationships between plasma and tissue fatty acid composition and circulating sex hormone concentrations have been observed, which suggest a role of sex hormones in regulating LC $n-3$ PUFA synthesis. Current investigations will provide further information on the role that the expression of desaturase and elongase enzymes in the liver may have in determining these gender differences in LC $n-3$ PUFA status.

Acknowledgements

C.E.C. is supported by a Richard Newitt Bursary. G.C.B. is supported by the British Heart Foundation.

References

1. Stubbs CD & Smith AD (1984) The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. *Biochim Biophys Acta* **779**, 89–137.
2. Schoonjans K, Staels B & Auwerx J (1996) The peroxisome proliferator activated receptors (PPARs) and their effects on lipid metabolism and adipocyte differentiation. *Biochim Biophys Acta* **1302**, 93–109.
3. Sampath H & Ntambi JM (2004) Polyunsaturated fatty acid regulation of gene expression. *Nutr Rev* **62**, 333–339.
4. Lewis RA, Austen KF & Soberman RJ (1990) Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases. *N Engl J Med* **323**, 645–655.
5. Tilley SL, Coffman TM & Koller BH (2001) Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. *J Clin Invest* **108**, 15–23.
6. Calder PC (2003) $n-3$ polyunsaturated fatty acids and inflammation: from molecular biology to the clinic. *Lipids* **38**, 343–352.
7. Calder PC (2006) $n-3$ polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am J Clin Nutr* **83**, 1505s–1519s.
8. Levy BD, Clish CB, Schmidt B, Gronert K & Serhan CN (2001) Lipid mediator class switching during acute inflammation: signals in resolution. *Nat Immunol* **2**, 612–619.

9. Serhan CN, Arita M, Hong S & Gotlinger K (2004) Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their endogenous aspirin-triggered epimers. *Lipids* **39**, 1125–1132.
10. Liu J, Yeo HC, Doniger SJ & Ames BN (1997) Assay of aldehydes from lipid peroxidation: gas chromatography-mass spectrometry compared to thiobarbituric acid. *Anal Biochem* **245**, 161–166.
11. British Nutrition Foundation (1999) *n-3 Fatty Acids and Health*. London: British Nutrition Foundation.
12. Henderson L, Gregory J, Swan G & Ruston D (2002) *The National Diet and Nutrition Survey: Adults Aged 19 to 64 Years*. London: The Stationery Office.
13. Scientific Advisory Committee on Nutrition (2004) *Advice on Fish Consumption: Benefits and Risks*. London: The Stationery Office.
14. Ruxton CHS, Calder PC, Reed SC & Simpson MJA (2005) The impact of long-chain n-3 polyunsaturated fatty acids on human health. *Nutr Res Rev* **18**, 113–129.
15. Burdge GC & Calder PC (2006) Dietary alpha-linolenic acid and health related outcomes: a metabolic perspective. *Nutr Res Rev* **19**, 26–52.
16. Kris-Etherton PM, Harris WS & Appel LJ (2002) Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* **106**, 2747–2757.
17. Calder PC (2004) n-3 Fatty acids and cardiovascular disease: evidence explained and mechanisms explored. *Clin Sci* **107**, 1–11.
18. Wang C, Harris WS, Chung M, Lichtenstein AH, Balk EM, Kupelnick B, Jordan HS & Lau J (2006) Fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. *Am J Clin Nutr* **84**, 5–17.
19. GISSI-Prevenzione (1999) Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. *Lancet* **354**, 447–455.
20. Calder PC (2006) Use of fish oil in parenteral nutrition: Rationale and reality. *Proc Nutr Soc* **65**, 264–277.
21. Farquharson J, Jamieson EC, Logan RW, Patrick WJ, Howatson AG & Cockburn F (1995) Age- and dietary-related distributions of hepatic arachidonic and docosahexaenoic acid in early infancy. *Pediatr Res* **38**, 361–365.
22. Lauritzen L, Hansen HS, Jorgensen MH & Michaelsen KF (2001) The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. *Prog Lipid Res* **40**, 1–94.
23. SanGiovanni JP, Parra-Cabrera S, Colditz GA, Berkey CS & Dwyer JT (2000) Meta-analysis of dietary essential fatty acids and long-chain polyunsaturated fatty acids as they relate to visual resolution acuity in healthy preterm infants. *Pediatrics* **105**, 1292–1298.
24. Cheatham CL, Colombo J & Carlson SE (2006) N-3 fatty acids and cognitive and visual acuity development: methodologic and conceptual considerations. *Am J Clin Nutr* **83**, 1458s–1466s.
25. Richardson AJ (2006) Omega-3 fatty acids in ADHD and related neurodevelopmental disorders. *Int Rev Psychiatry* **18**, 155–172.
26. Freeman MP, Hibbeln JR, Wisner KL *et al.* (2006) Omega-3 fatty acids: evidence basis for treatment and future research in psychiatry. *J Clin Psychiatry* **67**, 1954–1967.
27. Appleton KM, Hayward RC, Gunnell D, Peters TJ, Rogers PJ, Kessler D & Ness AR (2006) Effects of n-3 long-chain polyunsaturated fatty acids on depressed mood: systematic review of published trials. *Am J Clin Nutr* **84**, 1308–1316.
28. Leonard AE, Pereira SL, Sprecher H & Huang YS (2004) Elongation of long-chain fatty acids. *Prog Lipid Res* **43**, 36–54.
29. Arterburn LM, Hall EB & Oken H (2006) Distribution, interconversion, and dose response of n-3 fatty acids in humans. *Am J Clin Nutr* **83**, 1467S–1476S.
30. Cleland LG, Gibson RA, Pedler J & James MJ (2005) Paradoxical effect of n-3 containing vegetable oils on long-chain n-3 fatty acids in rat heart. *Lipids* **40**, 995–998.
31. Francois CA, Connor SL, Bolewicz LC & Connor WE (2003) Supplementing lactating women with flaxseed oil does not increase docosahexaenoic acid in their milk. *Am J Clin Nutr* **77**, 226–233.
32. Burdge GC, Jones AE & Wootton SA (2002) Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men. *Br J Nutr* **88**, 355–363.
33. Burdge GC & Wootton SA (2002) Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. *Br J Nutr* **88**, 411–420.
34. Pawlosky RJ, Hibbeln JR, Novotny JA & Salem N Jr (2001) Physiological compartmental analysis of alpha-linolenic acid metabolism in adult humans. *J Lipid Res* **42**, 1257–1265.
35. Pawlosky RJ, Hibbeln JR, Lin Y, Goodson S, Riggs P, Sebring N, Brown GL & Salem N Jr (2003) Effects of beef- and fish-based diets on the kinetics of n-3 fatty acid metabolism in human subjects. *Am J Clin Nutr* **77**, 565–572.
36. Pawlosky R, Hibbeln J, Lin Y & Salem N Jr (2003) n-3 Fatty acid metabolism in women. *Br J Nutr* **90**, 993–994.
37. Burdge GC, Finnegan YE, Minihane AM, Williams CM & Wootton SA (2003) Effect of altered dietary n-3 fatty acid intake upon plasma lipid fatty acid composition, conversion of [¹³C]α-linolenic acid to longer-chain fatty acids and partitioning towards β-oxidation in older men. *Br J Nutr* **90**, 311–321.
38. Nikkari T, Luukkainen P, Pietinen P & Puska P (1995) Fatty acid composition of serum lipid fractions in relation to gender and quality of dietary fat. *Ann Med* **27**, 491–498.
39. Giltay EJ, Gooren LJ, Toorians AW, Katan MB & Zock PL (2004) Docosahexaenoic acid concentrations are higher in women than in men because of estrogenic effects. *Am J Clin Nutr* **80**, 1167–1174.
40. Bakewell L, Burdge GC & Calder PC (2006) Polyunsaturated fatty acid concentrations in young men and women consuming their habitual diets. *Br J Nutr* **96**, 93–99.
41. Crowe FL, Skeaff CM & Green TJ (2008) Serum n-3 long-chain PUFA differ by sex and age in a population-based survey of New Zealand adolescents and adults. *Br J Nutr* (In the Press).
42. Jones AE, Murphy JL, Stolinski M & Wootton SA (1998) The effect of age and gender on the metabolic disposal of [1-¹³C] palmitic acid. *Eur J Clin Nutr* **52**, 22–28.
43. Tavendale R, Lee AJ, Smith WC & Tunstall-Pedoe H (1992) Adipose tissue fatty acids in Scottish men and women: results from the Scottish Heart Health Study. *Atherosclerosis* **94**, 161–169.
44. Raclot T, Langin D, Lafontan M & Groscolas R (1997) Selective release of human adipocyte fatty acids according to molecular structure. *Biochem J* **324**, 911–915.
45. Raclot T (2003) Selective mobilization of fatty acids from adipose tissue triacylglycerols. *Prog Lipid Res* **42**, 257–288.
46. Sumino H, Ichikawa S, Murakami M, Nakamura T, Kanda T, Sakamaki T, Mizunuma H & Kurabayashi M (2003) Effects of hormone replacement therapy on circulating

- docosahexaenoic acid and eicosapentaenoic acid levels in postmenopausal women. *Endocr J* **50**, 51–59.
47. Giltay EJ, Duschek EJ, Katan MB, Zock PL, Neele SJ & Netelenbos JC (2004) Raloxifene and hormone replacement therapy increase arachidonic acid and docosahexaenoic acid levels in postmenopausal women. *J Endocrinol* **182**, 399–408.
48. Mattsson LA, Silfverstolpe G & Samsioe G (1985) Fatty acid composition of serum lecithin and cholesterol ester in the normal menstrual cycle. *Horm Metab Res* **17**, 414–417.
49. Ocke MC, Bueno-de-Mesquita HB, Goddijn HE, Jansen A, Pols MA, Van Staveren WA & Kromhout D (1997) The Dutch EPIC food frequency questionnaire. I. Description of the questionnaire, and relative validity and reproducibility for food groups. *Int J Epidemiol* **26**, Suppl. 1, S37–S48.
50. Marra CA, de Alaniz MJ & Brenner RR (1988) Effect of various steroids on the biosynthesis of arachidonic acid in isolated hepatocytes and HTC cells. *Lipids* **23**, 1053–1058.
51. Gonzalez S, Nervi AM & Peluffo RO (1986) Effects of estradiol and environmental temperature changes on rat liver delta 6 microsomal desaturase activity. *Lipids* **21**, 440–443.
52. Marra CA & de Alaniz MJ (1989) Influence of testosterone administration on the biosynthesis of unsaturated fatty acids in male and female rats. *Lipids* **24**, 1014–1019.