

## Different dietary omega-3 sources during pregnancy and DHA in the developing rat brain

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**Abstract:** The essential n-3 fatty acid  $\alpha$ -linolenic acid (ALA) can be converted into eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) under the action of desaturase and elongase enzymes. Human studies have demonstrated that females convert a higher proportion of ALA into EPA and DHA than males. We have demonstrated that when fed upon an ALA rich diet, female rats have a significantly higher EPA content of plasma and liver lipids than males. When fetal tissues were collected, it was observed that pups from dams fed the ALA rich diet had a comparable brain DHA status to those from dams fed on a salmon-oil based diet, indicating that conversion of ALA to DHA during pregnancy was efficient, and that DHA accumulated in a tissue-specific manner. Similar efficacy of dietary ALA in women during pregnancy would mean that plant n-3 fatty acids would be useful alternatives to preformed EPA and DHA.

**Key words:** omega-3, sex, pregnancy

### Synthesis of long chain n-3 PUFA

Dietary sources of the essential fatty acid  $\alpha$ -linolenic acid (ALA; 18:3n-3) include green leaves, some seeds, nuts and cooking oils. The principal dietary source of the long-chain (LC) n-3 polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA; 20:5n-3), docosapentaenoic acid (DPA; 22:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) is oily fish, yet it is estimated that only 27% of UK adults habitually eat oily fish (Scientific Advisory Committee on Nutrition, 2004).

In addition to consumption in the diet, LC n-3 PUFA can be endogenously synthesised via a series of elongase, desaturase and  $\beta$ -oxidation steps from their essential fatty acid precursor ALA (Leonard *et al.*, 2004) (figure 1). This

same series of desaturase and elongase enzymes is also involved in the metabolism of the n-6 PUFA linoleic acid (LA) into its longer-chain, more unsaturated derivatives (e.g. arachidonic acid). In Western diets, consumption of LA is about 10 times that of ALA (Burdge and Calder, 2006), suggesting that synthesis of n-6 PUFA will predominate.

### Sex and plasma and tissue n-3 fatty acid composition

Studies have identified sex differences in circulating plasma concentrations of LC n-3 PUFA. While these studies vary in their sample size, degree of dietary control exerted and the range of blood lipids analysed, all have found that women have significantly higher circu-

lating DHA concentrations compared to men and that this is independent of dietary intake (Nikkari *et al.*, 1995; Giltay *et al.*, 2004; Bakewell *et al.*, 2006; Crowe *et al.*, 2008). Rat studies have also identified that the proportion of

#### Abbreviations

ALA	$\alpha$ -linolenic acid
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid
HF	high-fat
LA	linoleic acid
LC	long-chain
LF	low-fat
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PUFA	polyunsaturated fatty acid

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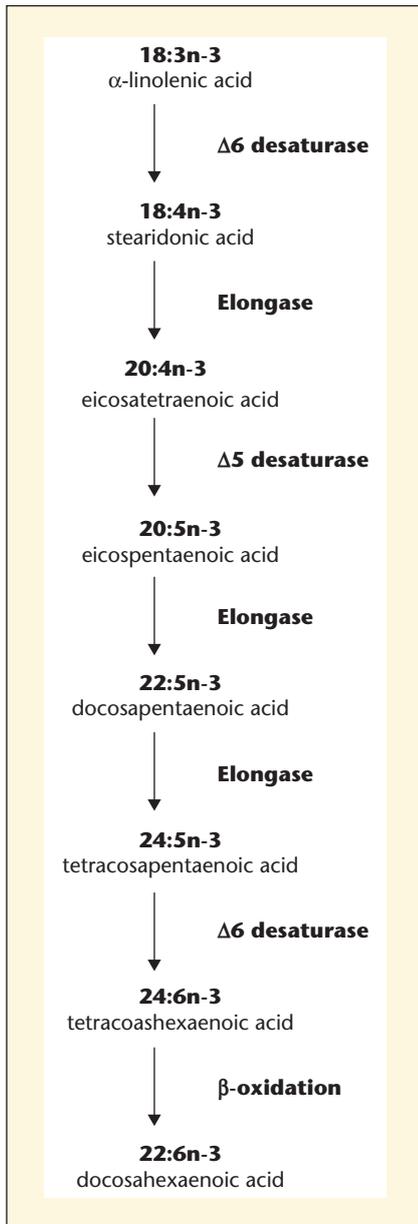


Figure 1. Biosynthesis of LC n-3 PUFA from  $\alpha$ -linolenic acid.

DHA is higher in liver and plasma phospholipids in females than males (Burdge *et al.*, 2008; Extier *et al.*, 2010; Childs *et al.*, 2010a).

Data from studies using stable isotope-labelled ALA demonstrate that there are sex differences in the ability to synthesize LC n-3 PUFA from ALA. Young women converted a greater proportion of ALA into EPA and DHA compared to men (Burdge *et al.*, 2002a; Burdge and Wootton, 2002b). It has been hypoth-

esised that sex differences are established in order to ensure an adequate supply of LC n-3 PUFA to the developing fetus during pregnancy (Bakewell, 2006). If this is the case, then it is possible that LC n-3 PUFA synthesis may be further upregulated during pregnancy.

## N-3 fatty acids and pregnancy

Specific maternal dietary fatty acids, particularly n-3 PUFA, have been demonstrated to be essential for successful fetal development and later tissue function in both humans and animals. Transfer of DHA to the developing fetus in human pregnancy predominantly occurs in the last 10 weeks of pregnancy, with the majority of this DHA accumulated within fetal adipose tissue (Haggarty, 2004). The observation that DHA is found in high concentrations in the retina and accumulates in the fetal brain during late pregnancy and in early neonatal life has led to the suggestion that an adequate dietary supply of this fatty acid is required for optimal brain and visual development (Farquharson *et al.*, 1995). Animal studies where n-3

fatty acid deficient diets have been provided demonstrate that dietary n-3 fatty acids are essential for normal cognitive and visual function, as reviewed in detail elsewhere (Lauritzen *et al.*, 2001).

Human studies have investigated the role of LC n-3 PUFA, particularly DHA, when provided in milk formula to both preterm and healthy term infants. Meta-analyses indicate that the addition of DHA to pre-term infant formula is beneficial for optimal visual development in early life (Sangiovanni *et al.*, 2000; Uauy *et al.*, 2003). Whether these effects persist beyond early life (i.e. after 4 months of age) has not yet been established. In term infants, formula containing DHA was found to improve markers of cognitive function (Cheatham *et al.*, 2006). However, the clinical relevance of the reported statistically significant differences and the validity of the neurodevelopmental tests utilised in these studies have been questioned (Koo, 2003).

Human studies have demonstrated that there are significant effects of pregnancy upon blood lipid fatty acid composition, though the effects observed have been mixed. For example, while some studies

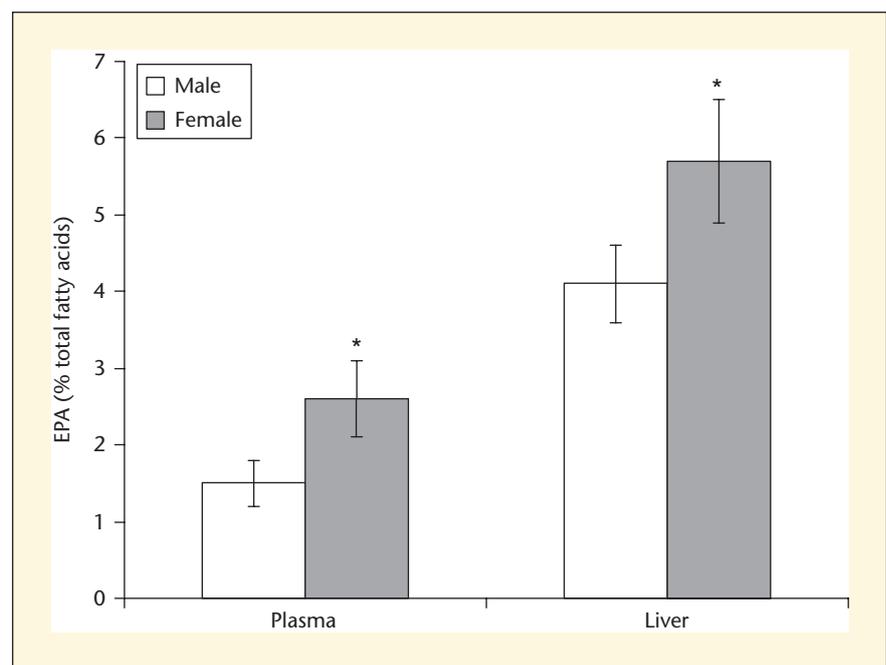


Figure 2. EPA content of plasma phosphatidylcholine in male and female rats fed an ALA-rich diet for 20 days. Values are mean  $\pm$  SD, n=6 per group. \*Significantly different from males ( $p < 0.05$ ). Data are taken from [10].

have identified a reduction in plasma phospholipid DHA status during pregnancy (Wijendran *et al.*, 1999; Hornstra, 2000), others have reported increased DHA content of plasma phospholipids (Postle *et al.*, 1995; Burdge *et al.*, 2006) or red blood cells (Stewart *et al.*, 2007). These differences between studies can most likely be attributed to variations in the type of sample analysed, whether results were expressed as a percentage or in absolute concentrations, and the possible confounding effect of maternal diet and adipose tissue composition. Studies in rats comparing virgin animals with those at the end of pregnancy have shown that the fatty acid composition of phospholipids from plasma and liver is significantly altered in response to pregnancy with higher DHA and lower arachidonic acid contents (Smith and Walsh, 1975; Cunnane, 1989; Chen *et al.*, 1992; Burdge *et al.*, 1994). In rats, higher DHA in liver and plasma phosphatidylcholine (PC) has been attributed to changes in PC synthesis (Burdge *et al.*, 1994). However, it is unclear whether the increased availability of DHA is a result of mobilisation of DHA from adipose tissue, increased dietary intake or greater synthesis by desaturation and elongation of precursors.

### Use of an ALA rich diet in rat models

We have identified that there are significant diet  $\times$  sex interactions in rat tissue n-3 fatty acid composition (Childs *et al.*, 2010a). Female rats fed an ALA rich diet had a higher proportion of EPA in plasma and liver PC compared to males (figure 2), with data suggesting that these differences may be mediated by higher expression of  $\Delta 6$  desaturase ( $\Delta 6D$ ) mRNA and greater  $\Delta 6D$  activity in females than males (Childs *et al.*, 2010a). We also identified that providing an ALA rich diet during pregnancy resulted in equivalent EPA status in fetal immune tissues (figure 3A) and equivalent DHA status in the fetal brain to that achieved in the offspring of dams fed a high-fat salmon-oil diet (figure 3B) (Childs *et al.*, 2010b). This indicates a significant role of maternal and/or fetal LC n-3 PUFA synthesis in determining fetal LC n-3 PUFA status in a tissue specific manner. The effect of maternal diet during pregnancy upon fetal brain DHA content persists until weaning (figure 4).

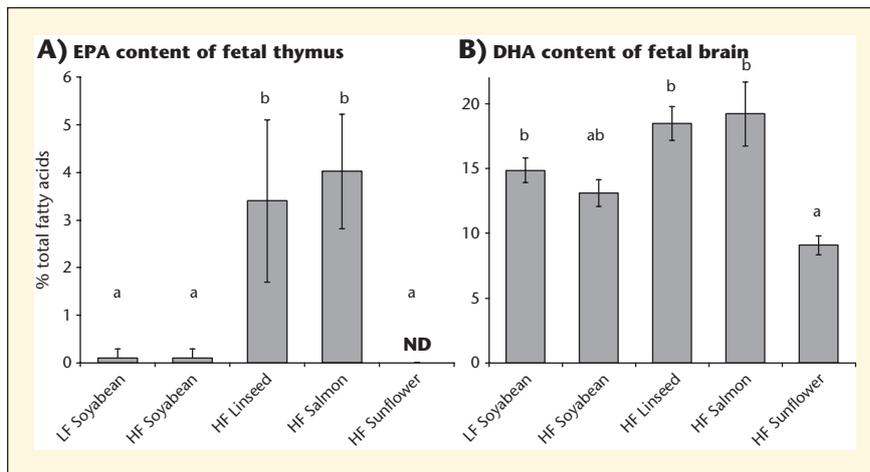


Figure 3. EPA and DHA contents of thymus and brain phosphatidylethanolamine from 20-day gestation pups from rat dams fed different experimental diets for 20 days of pregnancy. Values are mean  $\pm$  SD,  $n=6$  per group. Means without a common letter differ,  $P<0.05$ . LF, low fat diet; HF, high fat diet; ND, not detected ( $<0.1\%$ ). Data are taken from [29].

### Discussion

We have found that the percentage content of n-3 fatty acids among rats receiving standard laboratory chow *ad libitum* and the response of rats to ALA supplementation regimes compares favourably with available data from human studies. If dietary ALA during pregnancy significantly influen-

ces fetal brain and immune tissue LC n-3 PUFA content in humans this would have significant implications for strategies aimed at improving infant cognitive function or promoting infant immune development and reducing the risk of immune dysfunction (e.g. atopic sensitisation). To date, studies in pregnancy examining these infant

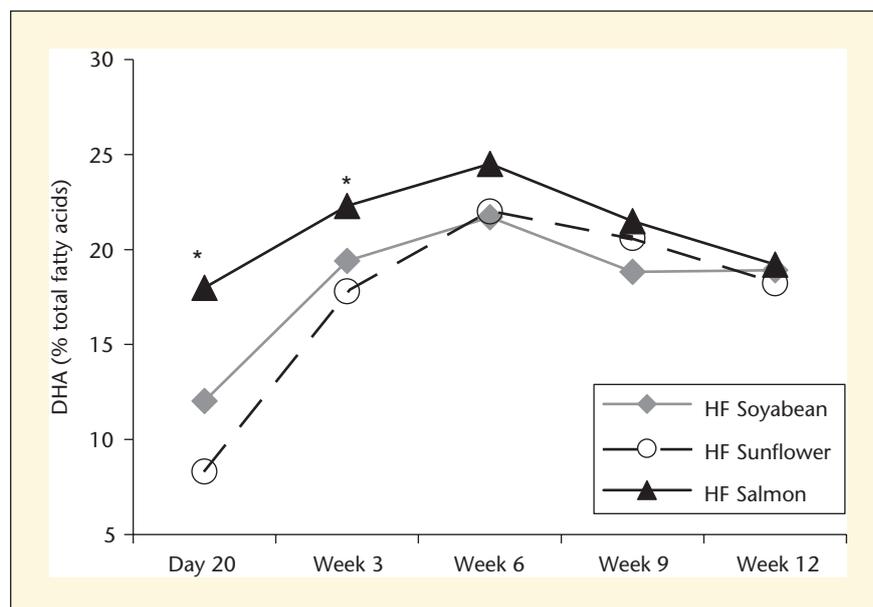


Figure 4. DHA content of brain phosphatidylethanolamine of pups from rat dams fed different high fat (HF) experimental diets for 20 days of pregnancy. Day 20 indicates day 20 of gestation; week 3, 6, 9 and 12 indicate weeks post-birth. Values are mean  $n=6$  per group. \*HF Salmon significantly different from the other two groups,  $P<0.05$ . Data are not previously published.

outcomes have largely provided marine-sources of n-3 fatty acids. The availability of plant-oil sources of n-3 fatty acids would greatly benefit vegetarian and vegan women and would have an environmental impact by reducing demand upon marine resources.

Further rat studies will be necessary to determine the threshold of ALA supplementation required to maintain equivalent brain DHA and immune tissue EPA to that achieved with a fish-oil rich diet. Whether these changes to tissue fatty acid composition result in any differences in offspring visual, cognitive or immune function is also yet to be determined. It would be of interest to conduct human studies to investigate whether there are similar sex differences in the response to dietary ALA. If the effects observed in our rat model of dietary ALA during pregnancy were replicated in human studies, this approach could be used to investigate whether there are benefits to offspring health, including women who are unwilling or unable to consume marine-based interventions (i.e. fish or fish oils).

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