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# Changes in rat n-3 and n-6 fatty acid composition during pregnancy are associated with progesterone concentrations and hepatic FADS2 expression

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ABSTRACT

The mechanisms responsible for changes to long-chain polyunsaturated fatty acid (LC PUFA) status during pregnancy have not been fully elucidated. Tissue samples were collected from virgin and pregnant (day 12 and 20) female rats. LC PUFA status, sex hormone concentrations and hepatic mRNA expression of FADS1, FADS2 and elongase were assessed. Day 20 gestation females had higher plasma and liver docosahexaenoic acid and lower arachidonic acid content than virgin females ($P < 0.05$). There was higher FADS2 mRNA expression during pregnancy ($P=0.051$). Progesterone and oestradiol concentrations positively correlated with hepatic FADS2 mRNA expression ($P=0.043$, $P=0.004$). Progesterone concentration positively correlated with hepatic n-6 docosapentaenoic acid content ($P=0.006$), and inversely correlated with intermediates in LC PUFA synthesis including n-3 docosapentaenoic acid, γ-linolenic acid and 20:2n-6 ($P < 0.05$). Changes in progesterone and oestradiol during pregnancy may promote the synthesis of LC PUFA via increased FADS2 expression.



## 1. Introduction

Human studies have identified that women have a greater capacity than men to synthesise n-3 long-chain polyunsaturated fatty acids (LC PUFA) from their essential fatty acid (EFA) precursor α-linolenic acid (ALA; 18:3n-3) [1–3]. Women also have higher circulating concentrations of docosahexaenoic acid (DHA, 22:6n-3) than men [4–7], independent of any sex difference in dietary intake of n-3 LC PUFA. Sex differences in DHA content have also been observed within rat plasma and tissues [8–10]. It has been hypothesised that sex differences are established in order to ensure an adequate supply of LC PUFA to the developing foetus during pregnancy [11]. If this is the case, then it is possible that LC PUFA synthesis may be up-regulated during pregnancy. This may be under the control of sex hormones, and potentially mediated via an effect upon the expression of genes involved in the desaturation and elongation of EFA into LC PUFA.

Both human and rat studies of non-pregnant subjects have identified a role of sex hormones in regulation of PUFA metabolism. Human studies using stable isotope-labelled ALA identified that women using the contraceptive pill have higher rates of DHA synthesis over 21 day than women not using the pill [1]. Consistent with this, women using oral contraceptives had 60% higher total plasma DHA content, than those who did not use oral contraceptives irrespective of dietary PUFA intake [4]. Hormone replacement therapy (HRT) in post-menopausal women was associated with increased plasma eicosapentaenoic acid (EPA; 20:5n-3) and DHA concentrations [12,13]. A study of transsexual subjects demonstrated that male to female transsexuals receiving a combination of oral 17α-ethyinylestradiol and cyproterone acetate had higher DHA concentrations in their plasma cholesteryl esters (CE) within 4 months of treatment. Female to male transsexuals receiving intra-muscular testosterone had significantly lower DHA content in plasma CE within 4 months of treatment [5]. Gonadectomy in male rats resulted in higher levels of arachidonic acid (ARA; 20:4n-6) in plasma phosphatidylcholine (PC) which were comparable to those observed in females, while gonadectomy of females leads to lower ARA content similar to that of untreated males [14]. Administration of testosterone or oestrogen restored plasma PC ARA in males and females, respectively. These sex differences may be mediated through control of sex hormones by growth hormone [15], but it is not clear whether these effects are the result of changes to EFA or phospholipid metabolism.

Human studies have demonstrated that there are significant effects of pregnancy upon blood lipid fatty acid composition,

*Abbreviations:* ARA, arachidonic acid; ALA, α-linolenic acid; CE, cholesteryl ester(s); DEPC, diethyl pyrocarbonate; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EFA, essential fatty acid(s); EPA, eicosapentaenoic acid; FAME, fatty acid methyl ester(s); GLA, γ-linolenic acid; HRT, hormone-replacement therapy; LNA, linoleic acid; LC, long-chain; MUFA, monounsaturated fatty acid(s); PC, phosphatidylcholine; PE, phosphatidylethanolamine; PUFA, polyunsaturated fatty acid(s); TAG, triacylglycerol(s)

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though the effects observed have been mixed. For example, while some studies have identified a reduction in plasma phospholipid DHA status during pregnancy [16,17], others have reported increased DHA content of plasma phospholipids [18,19] or red blood cells [20]. The ARA and DHA contents of liver and plasma phospholipids are higher in rats at the end of pregnancy compared to virgin animals [21–24]. In rats, increased DHA in liver and plasma PC during pregnancy has been demonstrated to involve increased synthesis of *sn*-1 PC 16:0 molecular species *de novo*, deceased acyl remodelling of *sn*-1 16:0 to *sn*-1 18:0 molecular species, and an increased rate of total hepatic PC synthesis [24]. Such effects imply increased demand for DHA for phospholipid biosynthesis. If only ALA is provided in the diet as a source of n-3 fatty acids, such demands can only be met by increasing mobilisation of DHA from adipose tissue, and/or up-regulation of DHA biosynthesis from ALA within the diet or adipose tissue stores.

To date studies of the pregnancy-associated changes in fatty acid composition have not integrated this information with the concentrations of sex hormones in blood. The aim of this study was to determine, using a rat model, the relationship between pregnancy-associated changes in blood and tissue fatty acid composition, the concentrations of sex hormones and the expression of hepatic fatty acid desaturase and elongase enzymes.

## 2. Materials and methods

### 2.1. Animal husbandry

All animal work was carried out in accordance with the Home Office Animals (Scientific Procedures) Act (1986). Wistar rats were fed *ad libitum* on a low fat soybean oil-based diet (27.1 g/kg total fat, RM1, Special Diet Services, Witham, Essex) throughout and were aged 10 weeks at the start of experiments. The full nutrient and fatty acid composition of this diet was analysed (Online Supplementary Material). Virgin females were maintained and samples collected as described [10]. For pregnant females, mating was achieved by monogamous breeding, and when confirmed by the appearance of a vaginal plug, this was recorded as day 1 of gestation. Rats were killed by $CO_2$ asphyxiation followed by cervical dislocation at day 12 or day 20 of gestation. Blood was collected by cardiac puncture into heparinised tubes, separated into cells and plasma by centrifugation, and plasma was stored at −20 °C. Liver and adipose tissue were frozen in liquid nitrogen and stored at −80 °C.

### 2.2. Fatty acid composition analysis

The fatty acid composition of the diet, plasma and tissues was determined by gas chromatography as described previously [25]. Briefly, plasma and tissue lipids were extracted using 5 ml chloroform/methanol (2:1 by vol containing 50 mg/l BHT). PC, phosphatidylethanolamine (PE), CE and triacylglycerols (TAG) were separated by solid phase extraction using aminopropyl silica cartridges [24,25]. Fatty acid methyl esters (FAME) were synthesised by incubation with methanolic sulphuric acid (2% $H_2SO_4$ v/v) at 50 °C for 2 h followed by extraction with hexane [25]. FAME were resolved on an HP6890 Hewlett Packard gas chromatograph (Agilent, Cheshire, UK) equipped with a 30 m × 0.25 μm × 0.25 mm BPX-70 fused silica capillary column (SGE, Milton Keynes, UK) using the following temperature protocol: initial temperature 115 °C, hold 2 min, ramp 10 °C/min to 200 °C, hold 10 min, ramp 60 °C/min to 245 °C, hold 4 min. Twenty-one FAME were identified routinely by comparison with authentic standards. Area under the peak was determined using ChemStation software (Agilent, Cheshire, UK). Results for plasma and adipose tissue are presented as proportion of total fatty acids (g/100 g total fatty acids). Results for liver phospholipids are presented as absolute concentrations (μg/g liver).

### 2.3. Sex hormone concentrations

Plasma testosterone, progesterone and 17β-oestradiol concentrations were determined by radio-immunoassay using Access® kits in accordance with manufacturer's instructions (Beckman Coulter, High Wycombe, UK). Assays were performed at the Chemical Pathology Department of Southampton General Hospital.

### 2.4. Real time RTPCR

RNA was extracted from liver samples using Tri Reagent (Sigma–Aldrich, Gillingham, UK) and cDNA synthesised using M-MLV Reverse Transcriptase (Sigma–Aldrich, Gillingham, UK) according to the manufacturer's instructions. Housekeeping genes (calnexin [Canx, Gene ID: 29144], tyrosine-3-monooxygenase [Ywhaz, Gene ID: 25578] and β2-microglobulin [B2m, Gene ID: 24223]) were selected using the 12-gene geNorm rat housekeeping gene kit (PrimerDesign, Southampton, UK) [26]. Primers were obtained from Qiagen (Crawley, UK) for FADS1 (Δ5 desaturase; Rn_FADS1_1_SG), FADS2 (Δ6 desaturase; Rn_FADS2_1_SG) and Elongase 5 (Rn_Elovl5_1_SG). Real time RT PCR using SYBR green was carried out using a 7500 Fast Real-time PCR System (Applied Biosciences, Warrington, UK). mRNA content was quantified using the standard curve method [27] and is expressed in arbitrary units corresponding to the dilution factor of the standard RNA preparation, relative to geometric mean of the three housekeeping genes.

### 2.5. Statistics

SPSS Version 15.0 was used for statistical analysis. Data was assessed for normality using Kolmogorov–Smirnov test and measures of skewness and kurtosis. The effect of pregnancy upon tissue fatty acid composition, plasma sex hormones and mRNA expression was assessed by one-way ANOVA or Kruskal–Wallis test as appropriate, with post-hoc pairwise tests corrected for multiple comparisons using Bonferroni's correction. Correlation coefficients ($r$) were calculated using Pearson's or Spearman's tests as appropriate. Significance was defined as a $P$-value of $< 0.05$.

## 3. Results

### 3.1. Fatty acid content of plasma and liver lipids

Total plasma PC concentration did not significantly differ between virgin female and pregnant rats ($p = 0.985$, mean content 1.04 mg/ml). Liver PC, PE and total lipid contents were significantly higher at day 12 gestation than in virgin or day 20 gestation animals (Table 1).

### 3.2. n-3 Fatty acid composition of plasma and tissue lipids

Significantly lower levels of ALA within subcutaneous adipose tissue, but not intra-abdominal adipose tissue, were observed at day 20 of gestation (Table 2). ALA was also lowest within liver PE at day 20 of gestation, but was unchanged by pregnancy in plasma and liver PC. The EPA content of liver phospholipids was lowest at day 20 gestation, while plasma EPA content was