Effects of maternal n-3 fatty acid supplementation on placental cytokines, pro-resolving lipid mediators and their precursors

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Abstract

The aim of this study was to determine whether supplementation with fish oil-derived n-3 polyunsaturated fatty acids (n-3 PUFA) during pregnancy modifies placental PUFA composition, the accumulation of specialised pro-resolving lipid mediators (SPMs, specifically resolvins (Rv), protectins (PD) and upstream precursors) and inflammatory gene expression. Placentas were collected from women (n=51) enrolled in a randomised, placebo controlled trial of n-3 PUFA supplementation from 20-week gestation. Lipids were extracted for fatty acid analysis and SPMs were quantitated by mass spectrometry. Gene expression was determined by qRT-PCR. Using multiple regression analysis, data were correlated for placental n-3 PUFA and SPM levels with PUFA levels in maternal and cord blood erythrocytes. Supplementation with n-3 PUFA increased placental docosahexaenoic acid (DHA) levels, but not eicosapentaenoic acid (EPA) levels (P<0.05), and increased the levels of the SPM precursors 18-hydroxyeicosapentaenoic acid and 17-hydroxydocosahexaenoic acid (17-HDHA) by two- to threefold (P<0.0005). RvD1, 17R-RvD1, RvD2 and PD1 were detectable in all placentas, but concentrations were not significantly increased by n-3 PUFA supplementation. Placental DHA levels were positively associated with maternal and cord DHA levels (P<0.005), and with placental 17-HDHA concentrations (P<0.0001). Placental mRNA expression of PTGS2, IL18, IL6 and IL10 was unaffected by n-3 PUFA supplementation, but TNFα expression was increased by 14-fold (P<0.05).

We conclude that n-3 PUFA supplementation in pregnancy i) enhances placental accumulation of DHA and SPM precursors, ii) does not alter placental EPA levels, and iii) has no stimulatory effects on inflammatory gene expression. Further studies are required to ascertain the biological significance of SPMs in the placenta and the potential immunomodulatory effects of elevating placental SPM levels.


Introduction

Epidemiological and interventional studies have suggested that consumption of a diet high in fish oil-derived n-3 polyunsaturated fatty acids (n-3 PUFAs) may be beneficial in reducing the incidence of common pregnancy complications such as preterm birth, pre-eclampsia and intrauterine growth restriction (Larque et al. 2012, Mozurkewich & Klemens 2012). Maternal n-3 PUFA supplementation has also been shown in some studies to benefit the fetus/neonate by improving neurodevelopment and behavioural outcomes (Campoy et al. 2012, Larque et al. 2012, Rogers et al. 2013) and reducing the risk of developing allergic diseases in childhood (Dunstan & Prescott 2005, Klemens et al. 2011, Palmer et al. 2012). Many of the potential benefits ascribed to n-3 PUFA can be attributed to their well-documented immunomodulatory/anti-inflammatory actions (Calder 2013a).

The cellular mechanisms through which n-3 PUFAs exert their effects include their ability to modify the production and actions of a range of distinct classes of lipid-derived mediators, thereby mitigating the harmful effects of inflammation and oxidative stress (Calder 2012). n-3 PUFA-enriched diets i) increase the ratio of 3-series:2-series prostaglandins via a reduction in the levels of pro-inflammatory prostaglandin E2, ii) decrease production of prothrombotic thromboxane A2, iii) reduce production of pro-inflammatory cysteinyl pro-resolution lipids (SPMs) (specifically resolvins (Rv), protectins (PD) and related upstream precursors (RvD1, 17R-RvD1, RvD2, PD1)) (Calder 2013b, 2013c). The SPMs are referred to as ‘pro-resolution lipids’ as they promote resolution of inflammation via the upregulation of anti-inflammatory transcription factors such as Nrf2, and the reprogramming of gene expression toward a resolution phenotype (Calder & Howlett 2013). The cellular mechanisms by which SPMs exert their effects include the upregulation of anti-inflammatory transcription factors, the promotion of resolution of pro-inflammatory cell subsets, the inhibition of apoptosis and the promotion of cell survival (Calder et al. 2013a, 2013b, 2013c).
leukotrienes and iv) increase production of anti-inflammatory lipoxins (Calder 2013b). n-3 PUFAs also directly downregulate the nucleotide-binding oligomerisation domain, leucine-rich repeat and pyrin domain-containing 3 (NLRP3) inflammasome via G protein-coupled receptor 40 (GPR40) and GPR120 (Yan et al. 2013).

Recent studies have identified a class of n-3 PUFA-derived mediators known as ‘specialised pro-resolving lipid mediators’ (SPMs), which include the resolvins (Rv), protectins (PD) and maresins (Serhan & Chiang 2013). The SPMs are enzymatically produced autacoids that exert potent receptor-mediated immuno-resolving effects on the activated immune system to prevent the destructive effects of uncontrolled chronic inflammation by promoting the return to homeostasis (Arita et al. 2007, Bannenberg & Serhan 2010). The E-series Rv (e.g. RvE1 and RvE2) are derived from metabolism of eicosapentaenoic acid (EPA; 20:5n-3) via the intermediate 18-hydroxyeicosapentaenoic acid (18-HPEE), whereas the D-series Rv (RvD1, RvD2 and RvD3) are derived from docosahexaenoic acid (DHA; 22:6n-3) via 17-hydroxydocosahexaenoic acid (17-HDHA) (Bannenberg & Serhan 2010). PD (e.g. PD1, 10R and 17S-PD1) are also derived from DHA.

Most of the characterisation of SPMs has used in vitro studies or rodent models of inflammation, and limited data are available on SPMs in human tissues and their role and significance in inflammatory pathophysiology. Circulating SPMs have been measured in healthy subjects (Mas et al. 2012), with concentrations shown to be associated with n-3 PUFA intake (Colas et al. 2014) and produced in greater amounts after exercise in patients taking ibuprofen (Markworth et al. 2013). SPMs have been measured in breast milk (Weiss et al. 2013), but to date have not been investigated in the context of human pregnancy.

Several pregnancy conditions associated with excessive systemic and placental inflammation, such as preterm birth and preeclampsia (Bowen et al. 2002), could presumably benefit from the anti-inflammatory/pro-resolving effects of SPMs. We have recently shown that maternal dietary supplementation with n-3 PUFAs in the pregnant rat increased the placental levels of n-3 PUFAs, SPMs and their precursors (Jones et al. 2013a, b). However, there are no data on the production of SPMs in human placenta, or the effects of dietary n-3 PUFA supplementation on placental cytokine, SPM or n-3 PUFA concentrations. Therefore, this study reports the levels of SPMs and their precursors in placental tissues from women taking n-3 PUFA supplements during pregnancy. The expression of a range of inflammation-associated genes (Bowen et al. 2002) was also measured to determine whether there was any relationship with placental n-3 PUFA or SPM concentrations and placental inflammatory activation.

Materials and methods

Trial participants
The trial design and participant demographics have been described in detail elsewhere (Dunstan et al. 2003). The trial protocol was approved by the Human Research Ethics Committees at St John of God Hospital and Princess Margaret Hospital, Perth, and all women gave informed written consent. In brief, 98 pregnant, allergic, non-smoking women with uncomplicated pregnancies who ate not more than two fish meals per week were recruited into the study between 1999 and 2001 and block-randomised according to parity, age, pre-pregnancy BMI and maternal allergy status (allergic rhinitis or asthma). Women in the n-3 PUFA group received 4×1 g capsules/day, comprising 3.7 g of n-3 PUFAs (56.0% DHA and 27.7% EPA). The control group received 4×1 g capsules of olive oil/day, containing 66.6% oleic acid (C18:1 n-9) and <1% n-3 PUFAs. The participants completed a validated, semi-quantitative food frequency questionnaire at 20- and 30-week gestation to monitor fish consumption. Compliance was monitored by maternal diaries and measurement of DHA and EPA content of erythrocyte membranes from maternal blood samples collected at 36-week gestation (Barden et al. 2006) and cord blood collected at birth. Eighty-three participants remained in the study at delivery. From these, placentas were sampled from 53 participants, of which 51 (n=28 control and n=23 n-3 PUFA) were successfully processed for n-3 PUFA analysis. The samples were taken from a single transection of the placenta within 5 cm of the cord insertion and targeted the foetal villous portion of the placenta, below the amniotic membrane, after the surface was swabbed with alcohol. The samples were collected within 5–10 min of delivery and placed into liquid nitrogen within 30 min. They were stored at –80°C without thawing before retrieval for this study. The demographic, obstetric and neonatal characteristics of the participants are listed in Table 1. The two groups were similar in terms of maternal, neonatal and pharmacological characteristics, although on average the women in the n-3 PUFA group were 2.7 years younger (P=0.017) and their infants 1.2 cm longer at birth (P=0.02).

Fatty acid analysis
Fatty acids were extracted from placental tissue (500 mg) using methanol and chloroform and analysed by gas chromatography (Mori et al. 2000, Jones et al. 2013b). Individual fatty acids were represented as a percentage of the total fatty acids measured.

SPM analysis in placenta
SPMs and their precursors were extracted using a modification of the method of Masoodi et al. (Masoodi et al. 2008, Nicolaou et al. 2010). Placental tissue (~200 mg) were thawed at room temperature and 500 pg LTB4-d4 added as the internal standard. The samples were homogenised using a Dounce homogeniser in 2 ml of 100 mmol/l sodium acetate (pH 3)/15% methanol and adjusted to pH 3 using 0.125 M hydrochloric acid. The homogenates were allowed to stand for 20 min and centrifuged for 7 min at 2400 g at 4°C. The supernatants were applied to methanol-primed solid-phase C18 extraction cartridges (Alltech/
**Table 1** Fish oil supplementation trial participants, placental sub-group: demographic, obstetric and neonatal characteristics (mean ± s.d. or n, %).

<table>
<thead>
<tr>
<th>Maternal characteristics</th>
<th>Olive oil (n=28)</th>
<th>Fish oil (n=22)</th>
<th>Significance&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age</td>
<td>32.5 ± 3.7</td>
<td>29.8 ± 4.0</td>
<td>0.017</td>
</tr>
<tr>
<td>BMI pre-pregnancy</td>
<td>23.7 ± 3.7</td>
<td>22.5 ± 2.8</td>
<td>0.22</td>
</tr>
<tr>
<td>BMI at 20 weeks</td>
<td>25.5 ± 4.2</td>
<td>24.8 ± 3.92</td>
<td>0.55</td>
</tr>
<tr>
<td>Pregnancy weight gain&lt;sup&gt;a&lt;/sup&gt; (kg)</td>
<td>8.3 ± 2.7</td>
<td>7.8 ± 2.3</td>
<td>0.49</td>
</tr>
<tr>
<td>Parity ≥ 1 (%)</td>
<td>16 (55.2)</td>
<td>9 (40.9)</td>
<td>0.39</td>
</tr>
<tr>
<td>Gravidity</td>
<td>2.3 ± 1.2</td>
<td>2.3 ± 1.8</td>
<td>0.99</td>
</tr>
<tr>
<td>Smoking in pregnancy (%)</td>
<td>15 (51.7)</td>
<td>6 (30)</td>
<td>0.08</td>
</tr>
<tr>
<td>GA at delivery (days)</td>
<td>275 ± 73</td>
<td>276 ± 8.6</td>
<td>0.66</td>
</tr>
<tr>
<td>Vaginal delivery (%)</td>
<td>14 (48.3)</td>
<td>16 (80)</td>
<td>0.15</td>
</tr>
</tbody>
</table>

**Neonatal characteristics**

| Neonatal BMI               | 1.39 ± 0.23     | 1.31 ± 0.16     | 0.17                   |
| Birth weight (g)           | 3429 ± 385      | 3339 ± 348      | 0.40                   |
| Birth length (cm)          | 49.8 ± 1.86     | 51.0 ± 1.64     | 0.02                   |
| Appgar score               | 8.4 ± 0.8       | 8.5 ± 0.6       | 0.63                   |
| Head circumference (cm)    | 34.8 ± 1.12     | 35.2 ± 1.27     | 0.24                   |
| Sex, male (%)              | 14 (48.3)       | 11 (55.0)       | 0.99                   |
| Sepsis (%)                 | 4 (13.9)        | 2 (9.1)         | 0.68                   |

| Relevant drug information (%) | 0.05. At a = 0.05, based on the observed variance in placental SPM concentrations, the study had an 80% power to determine a twofold difference between groups (G*Power, version 3.1.9, Heinrich-Heine-Universitét Düsseldorf, Düsseldorf, Germany). |

**Results**

Total placental PUFA levels were similar between the n-3 PUFA and control groups (Table 3). Supplementation with n-3 PUFAs significantly increased placental DHA levels by ~80%, whereas EPA levels were unaltered (Table 3 and Fig. 1A). Total n-3 and n-6 PUFA levels were not significantly different between the groups, although the n3:n6 ratio was significantly increased in the n-3 PUFA group vs controls (mean ± s.d.: 0.356 ± 0.078 vs 0.288 ± 0.089, respectively; P < 0.01).

17-HDHA was the most abundant SPM precursor measured in placental extracts, and levels were significantly increased by approximately twofold in the n-3

**Statistical analyses**

Statistical analyses were carried out using Statistical Analysis Software SAS 9.2 (SAS Institute, Inc., Cary, NC, USA) and Instat 3 (GraphPad Software, Inc., La Jolla, CA, USA). ANOVA with post hoc tests was used to determine the level of significance in normally distributed data, while Kruskal–Wallis test was employed for non-parametric data. Linear correlation analyses were carried out on the combined data from both groups using multiple regression with the model adjusted for group. The values are given as mean ± s.d. or median and interquartile range. Significance was set at P < 0.05. At a = 0.05, based on the observed variance in placental SPM concentrations, the study had an 80% power to determine a twofold difference between groups (G*Power, version 3.1.9, Heinrich-Heine-Universitét Düsseldorf, Düsseldorf, Germany).

**Table 2** Cytokine PCR primers and qPCR conditions.

<table>
<thead>
<tr>
<th>Gene (accession no.)</th>
<th>Forward sequence</th>
<th>Reverse sequence</th>
<th>Amplicon size (bp)</th>
<th>MgCl2 (mM)</th>
<th>Primer concentration (pmol)</th>
<th>Annealing temperature (°C)</th>
<th>Maximum cycle number</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH (NM_002030)</td>
<td>TCCACGACCCACTCTGAGG</td>
<td>GCGATCGACCTGTCACGAGG</td>
<td>80</td>
<td>3</td>
<td>5</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>IL1β (NM_000575)</td>
<td>GCCACAAGTGCTGCTTCTC</td>
<td>CAGATCTTTCTCAGGCC</td>
<td>91</td>
<td>3</td>
<td>5</td>
<td>60</td>
<td>45</td>
</tr>
<tr>
<td>IL6 (NM_000560)</td>
<td>CAGACGCCCACTCCCTCTCTCA</td>
<td>TCTCAGCGGAAAGTCTCTCTCA</td>
<td>211</td>
<td>3</td>
<td>5</td>
<td>60</td>
<td>45</td>
</tr>
<tr>
<td>IL10 (NM_000575)</td>
<td>TCCAGCGACTTAAAGGTTAC</td>
<td>TCTTTGTTTCTGACGG</td>
<td>94</td>
<td>3</td>
<td>5</td>
<td>60</td>
<td>45</td>
</tr>
<tr>
<td>TNFa (NM_000594)</td>
<td>CTGACGCTTCTCTCTCTCTC</td>
<td>AGAAAGATGATGATGACTGCC</td>
<td>147</td>
<td>3</td>
<td>5</td>
<td>58</td>
<td>45</td>
</tr>
<tr>
<td>PTGS2 (NM_000963)</td>
<td>AAGAGGCCTAATACTGTAGG</td>
<td>TGTTGAAATGATGTTCTGG</td>
<td>113</td>
<td>3</td>
<td>5</td>
<td>60</td>
<td>45</td>
</tr>
</tbody>
</table>

**RNA extraction and quantitative real-time RT-PCR**

Total RNA was extracted from ~500 mg placent al tissue using RNAqueous 4PCR Purification kits (Ambion/Thermo Fisher Scientific), followed by treatment with DNase-I to remove genomic DNA contamination. RNA yield and quality were determined by measuring 260:280 nm ratios using a Nanodrop 1000 (Thermo Fisher Scientific). cDNA was synthesised from 1 µg RNA using Moloney murine leukemia virus reverse transcriptase with random hexamers (Bioline Pty Ltd, Alexandria, NSW, Australia) according to the manufacturer’s instructions. The quantification of gene transcripts was determined by quantitative real-time PCR on a Rotor-Gene Q cycler (Qiagen Pty Ltd, Chadstone Centre, VIC, Australia). KiQStart SYBR Green PCR primers were designed and supplied by Sigma–Aldrich Pty Ltd (Castle Hill, NSW, Australia). PCRs were carried out in triplicate with 2 µl of cDNA using the SensiFast SYBR No-ROX Mix (Bioline Pty Ltd). PCR primer sequences, amplification conditions and amplicon size are given in Table 2. The amplicons were analysed by melt-curve analysis to confirm the absence of non-specific amplification products. Relative expression was computed using the 2−ΔΔCT method, with reference to the housekeeping gene GAPDH (Murthi et al. 2008), and normalised to a control sample.
PUFA group relative to the controls (81.6 ± 54.2 vs 39.2 ± 19.9 pg/mg tissue, respectively; P < 0.005; Fig. 1B). 18-HEPE concentrations were significantly increased with n-3 PUFA supplementation relative to the controls (8.87 ± 6.66 vs 2.80 ± 1.73; P < 0.0001). PD1, RvD1, 17R-RvD1 and RvD2 were present in placental tissues, but SPM concentrations were not significantly affected by n-3 PUFA supplementation. Placental 17-HDHA and DHA levels were significantly correlated with each other (r² = 0.328; P = 0.0001), but surprisingly there was no correlation between placental 18-HEPE and EPA levels. Placental concentrations of 17-HDHA (but not DHA) were significantly correlated with those of RvD1 (r² = 0.244; P < 0.005), 17R-RvD1 (r² = 0.364; P < 0.0001), 10S,17S-diHDHA (r² = 0.199; P < 0.005) and PD1 (r² = 0.379; P < 0.0001) (Fig. 2).

There was no significant change in mRNA expression of IL1β, IL6, IL10 or PTGS2 with n-3 PUFA supplementation (Fig. 3). However, the expression of TNF-α was increased 14-fold with supplementation (P = 0.039; Mann–Whitney U test). Inflammation-associated gene expression was not correlated with placental levels of DHA, EPA, SPMs or their precursors.

Umbilical cord blood erythrocyte DHA levels were significantly correlated with DHA (r² = 0.31; P < 0.0005), 17-HDHA (r² = 0.299; P < 0.0005), PD1 (r² = 0.156; P = 0.02) and 10S,17S-diHDHA (r² = 0.148; P = 0.025) levels in the placenta. There was also a significant correlation between cord blood erythrocyte levels of EPA and placental 18-HEPE (r² = 0.339; P < 0.0001).

Maternal circulating DHA levels were correlated with placental DHA and 17-HDHA (P < 0.0005), but not with placental RvD1, RvD2, 17R-RvD1 and PD1 concentrations. Maternal EPA levels were significantly correlated with placental 18-HEPE levels (P < 0.0005; Fig. 4).

**Discussion**

This is the first study describing the presence and concentrations of SPMs and their precursors in human pregnancy. We report that the concentrations of the two major SPM precursors, namely 18-HEPE and 17-HDHA, were significantly increased in the placenta after maternal n-3 PUFA supplementation during pregnancy, as were placental DHA levels. The concentrations of the SPMs themselves were not significantly increased with supplementation, although there was a trend towards an increase in SPM levels in the n-3 PUFA group. These findings could be explained by limited hydrolase/peroxidase activity in the placenta, leading to inefficient conversion of the precursors to the more active SPM metabolites. We have previously examined SPM levels in the rat placenta after maternal n-3 PUFA supplementation and shown that levels of 17-HDHA, 18-HEPE, 10S,17S-diHDHA, PD1, RvD1, RvD2 and 17R-RvD1 were all significantly increased after n-3 PUFA supplementation in the Labyrinth zone of the placenta at day 22.
of gestation (Jones et al. 2013a). The concentrations of the major D-series Rv precursor 17-HDHA in the rat placenta were approximately fourfold higher than those measured here, while levels of the E-series precursor 18-HEPE were ~20-fold higher in the rat; PD1, RvD1, RvD2 and 17R-RvD1 concentrations, however, were similar between rat and human (Jones et al. 2013a). The difference in dietary n-3 PUFA content and duration of supplementation in the rat versus human studies may be responsible for these differences. The amounts of SPMs detected in this study would equate to low-mid nanomolar tissue concentrations, which are biologically significant. SPMs and their precursors have also previously been detected in normal human plasma and lymphoid organs (Mas et al. 2012, Markworth et al. 2013, Colas et al. 2014) and in human milk (Weiss et al. 2012). Our findings expand the range of LOX-Rv precursor metabolites identified in the placenta to include the n-3 PUFA-derived SPMs. Interestingly, 15-LOX levels might be particularly enhanced with maternal DHA supplementation. Studies on circulating and placental SPM levels in preeclampsia might provide some interest.

This study is also the first to describe the changes in n-3 and n-6 PUFA composition in the placentas of women on n-3 PUFA supplements in pregnancy. We report, for the first time, a positive relationship between placental DHA levels and corresponding levels in maternal erythrocytes. Our study showed that n-3 PUFA supplementation resulted in a significant increase in placental DHA levels, but interestingly not EPA levels. This contrasts with our finding that maternal EPA levels increased by ~300%, whereas maternal DHA levels were only increased by ~65% in the n-3 PUFA group (Barden et al. 2006). This suggests that placental EPA levels may be regulated independently of maternal levels, reflecting differences in placental uptake, accumulation or transfer of the two n-3 PUFAs (Jones et al. 2014). Consistent with this view, we have recently shown that in rats, placental levels of EPA and DHA at term were increased as a result of n-3 PUFA supplementation by approximately two- and fourfold, respectively, compared with maternal levels which increased by 16- and threefold respectively (Jones et al. 2013a).

Previous studies of fatty acid levels in pregnancy have shown that for some fatty acids (e.g. EPA, linoleic acid and linolenic acid), cord blood levels (as % total fatty acids) are maintained at similar or lower levels than maternal plasma, consistent with limited passive diffusion across the placenta (Larque et al. 2011). On the other hand, relative levels of other fatty acids in cord blood (e.g. DHA, dihomo-γ-linolenic acid and arachidonic acid) are higher than maternal levels and show a greater degree of independence (Berghaus et al. 1998, Haggarty et al. 1999, Herrera et al. 2004, Sakamoto & Kubota 2004, Larque et al. 2011). These differences can be explained by selective placental transfer of maternal fatty acids and faetal de-novo fatty acid synthesis and metabolism.

Figure 2 Correlations between placental 17-HDHA and SPM concentrations. Correlations were determined by multiple regression, adjusted for group (circles, controls; diamonds, n-3 PUFA treated).

Figure 3 Cytokine mRNA expression in placental tissues from n-3 PUFA-supplemented pregnancies or controls. Placental mRNA expression of PTGS2, IL1β, IL6, IL10 and TNF-α was determined by qRT-PCR in the placentas of women taking n-3 PUFAs vs controls from the middle of pregnancy until delivery. Data are shown as median and interquartile range (IQR). There were no significant differences between the levels of expression in two groups, with the exception of TNF-α which was markedly increased. *P<0.039 by Mann–Whitney U test. Note the log scale for the vertical axis.
with our study in pregnant rats (Jones et al. 1999, Duttaroy 2009, Larque et al. 2011). Neonatal and maternal plasma DHA levels are strongly correlated (Sakamoto & Kubota 2004), and our present study has also found significant positive correlations between placental DHA and maternal and cord blood DHA levels, as well as correlations between maternal DHA and SPM precursor levels. Our data suggest that maternal DHA intake is a significant modulator of placental DHA and SPM precursor levels. We also observed a relationship between maternal EPA levels and placental 18-HEPE concentrations, in agreement with our study in pregnant rats (Jones et al. 2013b).

It has not yet been determined whether/how placental tissues respond to Rv or PD. E-series Rv act via the G-protein-coupled receptors ChemR23 (chemerin receptor) and BLT1 (leukotriene B4 receptor) (Arita et al. 2007), while the D-series Rv act through ALX/FPR2 (lipoxin A4 receptor), and GPR32 (Im 2012, Norling et al. 2012). ChemR23 and BLT1 are both expressed in the human placenta (Mognetti et al. 2000), while ALX/FPR2 has also been shown to be present and active within the placenta (Xu et al. 2014). Low levels of GPR32 (lysophosphatidic acid receptor 4) mRNA expression have been reported in the placenta (Noguchi et al. 2003), although its functionality remains unknown. Thus, the signalling pathways necessary to respond to local Rv production in the placenta appear to be present, and the concentrations of SPMs detected are sufficient to activate these receptors.

Despite the increases in placental SPM precursor levels, there was no significant effect on PTGS2, IL1β, IL6 or IL10 expression with n-3 PUFA supplementation, and no correlation between placental inflammatory gene expression and SPM concentrations. These genes are expressed constitutively in the human placenta and are considered a robust and reliable marker of inflammatory activation (Bowen et al. 2002). In contrast, the expression of the proinflammatory cytokine TNF-α was markedly elevated in the n-3 PUFA-supplemented placentas, an interesting finding which should be confirmed independently in a different cohort. To date, studies assessing the effects of n-3 PUFAs on human placental cytokine production are lacking; however, in our rodent model, n-3 PUFA supplementation had no effects on placental PTGS2 and TNF-α gene expression, but increased the levels of IL6 and IL1β mRNA expression at term (Jones et al. 2013a). The prevailing evidence, therefore, does not support cytokine suppressive role for SPMs and their precursors in the placenta in normal pregnancy, and if anything the data indicate a pro-inflammatory effect. These findings do not rule out other immunomodulatory effects in placental tissues. It should be noted that this study was conducted in women with normal pregnancies, unlikely to be complicated by excessive placental inflammation; whether or not n-3 PUFAs, SPMs and their precursors might be able to suppress placental cytokine expression in the presence of an inflammatory response remains to be determined.

In conclusion, we report that the levels of DHA and the major SPM precursors were increased in the placentas of women at term following dietary supplementation with n-3 PUFAs from 20-week gestation. Further studies are needed to explore the biological effects of SPMs in the human placenta in order to ascertain their significance in mediating the beneficial effects of n-3 PUFA supplementation in pregnancy and their pharmacological applications in treating and preventing placental complications.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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