n−3 Fatty acid supplementation during pregnancy in women with allergic disease: effects on blood pressure, and maternal and fetal lipids

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ABSTRACT

n−3 Fatty acids derived from fish oil reduce plasma triacylglycerols (triglycerides) and increase HDL-C (high-density lipoprotein cholesterol); however, the effect of n−3 fatty acid supplementation during pregnancy, a hyperlipidaemic state, remains unknown. We took the opportunity to investigate maternal lipid levels and blood pressure during and after pregnancy, and fetal lipid levels at birth, in a study that aimed primarily to examine the effect of fish oil supplementation during pregnancy on immune function in infants born to women with allergic disease. Eighty-three pregnant women who had allergic disease, but were otherwise healthy, completed the study. They were randomly allocated to receive fish oil or olive oil capsules, taken as 4 g/day, from 20 weeks of pregnancy until delivery. Compared with olive oil, fish oil supplementation did not alter triacylglycerols, total cholesterol, LDL-C (low-density lipoprotein cholesterol) or HDL-C during or after pregnancy. There was also no effect of fish oil on cord blood triacylglycerols, total cholesterol, LDL-C or HDL-C. Fish oil supplementation during pregnancy did not alter maternal blood pressure during or after pregnancy. The effects of fish oil on lipids and blood pressure in non-pregnant individuals appear to be lost when it is administered during pregnancy.

INTRODUCTION

Pregnancy is associated with hyperlipidaemia. The raised levels of triacylglycerols (triglycerides) in the third trimester of pregnancy maintain the supply of fatty acids and cholesterol to the fetus and are critical for normal fetal development. Pregancies complicated by pre-eclampsia are associated with a further elevation in triacylglycerols and NEFAs [non-esterified fatty acids (‘free-fatty acids’)] [1–4]. Raised cholesterol and triacylglycerol levels have been shown to persist in women with previous pre-eclampsia at 6 months [5] and 2 years [6] after the index pregnancy, but were not observed 20 years after the index pregnancy [7]. An altered lipid profile in pregnancies complicated by pre-eclampsia or gestational diabetes may be important beyond pregnancy, as these women are at increased risk of developing cardiovascular disease later in life [8,9].
Supplementation with $\omega$–3 fatty acids [EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid)] given as either fish or fish oil has been shown to significantly reduce serum triacylglycerols in non-pregnant individuals who have both normal and altered lipid profiles [16–18]. Fish oils have also been shown to lower BP (blood pressure) and improve vascular reactivity in the non-pregnant state [19,20]. There is some evidence that supplementation with $\omega$–3 fatty acids during pregnancy may also benefit maternal and fetal outcomes [21,22]. However, to our knowledge the effect of fish oil supplementation from 20 weeks of gestation on BP and maternal and fetal lipids has not been studied. We aimed to investigate the effects of fish oil supplementation from 20 weeks of gestation on BP and maternal and fetal lipids in a study that primarily aimed to examine the effect of fish oil supplementation during pregnancy on immune function in infants born to women with allergic disease. We have previously reported data on immune function in infants from the same study [22].

**METHODS**

**Subject recruitment and study design**

Ninety-eight pregnant women with allergic disease were recruited before 20 weeks of pregnancy from St John of God Hospital, Subiaco, Western Australia, Australia. The research was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and was approved by the Human Ethics Committees of St John of God Hospital and Princess Margaret Hospital. All women gave their written consent to participate, and 83 women completed the study. Allergic disease was defined as a history of doctor-diagnosed allergic rhinitis and/or asthma and one or more positive skin prick tests to common allergens (house dust mite, grasses, moulds, cat, dog, feathers and cockroach; Hollister-Stier Laboratories). Women were excluded from the study if they were smokers or had other pre-existing medical conditions, complicated pregnancies, seafood allergies or were consuming more than two fish meals per week or taking fish oil supplements. They were stratified on the basis of allergy (allergic rhinitis or asthma), parity (nulliparous compared with primiparous and multiparous), age and pre-pregnancy BMI (body mass index). They were then randomly assigned to receive either a daily supplement of fish oil or olive oil capsules in a double blind placebo-controlled parallel study from 20 weeks of pregnancy until delivery. The fish oil group received four (1 g) capsules per day containing 56 % DHA and 27.7 % EPA (Ocean Nutrition); the olive oil group received four (1 g) capsules per day containing 67 % oleic acid (Pan Laboratories).

**Samples and measurements**

BP was measured at 20, 25, 30 and 36 weeks of gestation and at 5 days and 6 weeks after delivery using a Dinamap 1846 SX oscillometric monitor (Critikon) after 5 min of seated rest. BP at each time point was calculated as the average of six readings, taken 1 min apart. Fasting maternal blood samples were collected for lipid measurements from the antecubital vein after 10 min of seated rest at 20, 30 and 36 weeks of gestation and at 6 weeks after delivery. Cord blood samples were collected from the placental vessels by venipuncture immediately after delivery. Total cholesterol, triacylglycerols, HDL-C (high-density lipoprotein cholesterol), HDL$_2$-C and HDL$_3$-C were measured in the Department of Clinical Biochemistry at Royal Perth Hospital using standardized methods [18]. LDL-C (low-density lipoprotein-cholesterol) was derived using the Friedewald formula [18]. Compliance with the intervention was assessed by measurement of RBC (red blood cell) phospholipid fatty acids in maternal blood at 36 weeks and cord blood at delivery, as described previously [23].

**Statistical analysis**

Between-group differences in lipids and BP during pregnancy were analysed using a mixed model in SAS (SAS Institute). This repeated-measures model takes into account the correlated nature of the data and adjusts for any baseline differences between the groups. Between-group differences in cord blood lipids were assessed using a general linear model and univariate analysis. Baseline (20 weeks of gestation) between-group differences in BP and lipids were assessed using an unpaired Student’s $t$ test.

**RESULTS**

Four mothers whose babies were delivered before 36 weeks were excluded from the analysis. Two infants with significant disease were excluded from the analysis. Although both were in the fish oil group, neither condition was linked in any apparent way to the supplementation. Seven women in the fish oil group and one in the control group withdrew from the study due to nausea which they attributed to consumption of the capsules. Cord blood was not collected at one delivery. A total of 83 women and their babies completed the study;
Table 1 Characteristics of the women receiving fish oil or olive oil supplementation during pregnancy

<table>
<thead>
<tr>
<th></th>
<th>Fish oil (n = 40)</th>
<th>Olive oil (n = 43)</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>31.0 ± 0.6</td>
<td>32.4 ± 0.5</td>
</tr>
<tr>
<td>Pre-pregnancy BMI (kg/m²)</td>
<td>23.7 ± 0.6</td>
<td>24.1 ± 0.6</td>
</tr>
<tr>
<td>Nulliparous women (%)</td>
<td>45</td>
<td>47</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>3503 ± 53</td>
<td>3430 ± 57</td>
</tr>
</tbody>
</table>

40 in the fish oil group and 43 in the olive oil group. There were no differences in age, BMI or parity between the groups (Table 1). Birth weight of the babies was also similar between the groups. Fish oil supplementation significantly increased RBC EPA and DHA compared with controls (P < 0.001 for each fatty acid). In the fish oil group, fatty acid changes from 20 weeks to 36 weeks were: EPA, 0.63 ± 0.03 to 1.99 ± 0.13 µg/10⁶ RBCs (P < 0.001); DHA, 5.25 ± 0.27 to 8.46 ± 0.42 µg/10⁶ RBCs (P < 0.001); and AA (arachidonic acid), 10.79 ± 0.51 to 8.09 ± 0.32 µg/10⁶ RBCs (P < 0.001). Respective changes in the olive oil group were: EPA, 0.57 ± 0.03 to 0.51 ± 0.03 µg/10⁶ RBCs (P = not significant); DHA, 4.62 ± 0.20 to 4.71 ± 0.24 µg/10⁶ RBCs (P = not significant); and AA, 10.33 ± 0.36 to 9.92 ± 0.41 µg/10⁶ RBCs (P = not significant).

Effect of fish oil supplementation on maternal and fetal lipids

Triacylglycerols were not statistically different in the two groups prior to supplementation at 20 weeks of gestation and there was no significant effect of fish oil given during the last 20 weeks of pregnancy compared with olive oil during or after pregnancy (P = 0.49; Figure 1, lower panel). Total cholesterol levels were similar between the two groups prior to supplementation at 20 weeks of gestation and did not differ between the groups during and after pregnancy (P = 0.745; (Figure 1, upper panel). Although HDL-C was significantly higher at 20 weeks of gestation in the fish oil group (P < 0.01), there was no significant effect of fish oil supplementation relative to the olive oil group on HDL-C (P = 0.908). HDL2-C was significantly elevated at 20 weeks of gestation in the fish oil group; however, HDL2-C and HDL3-C subfractions were also not altered by fish oil supplementation relative to the olive oil group during pregnancy (Figure 2). LDL-C was similar between the two groups prior to supplementation and was not affected by fish oil during or after pregnancy (P = 0.51; Figure 3).

In cord blood, the levels of all lipid fractions were lower than in the maternal circulation. There was no significant effect of fish oil on total cholesterol, HDL-C, LDL-C or triacylglycerols relative to olive oil (Figure 4). There was a significant correlation between cord blood HDL-C and maternal HDL-C (r = 0.296, P = 0.03; n = 52), but there were no significant correlations between maternal and cord blood total cholesterol, LDL-C or triacylglycerols respectively.

Effect of fish oil on BP and heart rate during pregnancy

SBP (systolic BP) was lower in the fish oil group at baseline (P = 0.03), whereas there were no significant differences in DBP (diastolic BP). SBP and DBP rose in both groups as gestation progressed, were highest at 5 days after delivery, and subsequently fell in the post-partum period. There was no significant effect of fish oil relative to olive oil on either SBP or DBP during or after pregnancy (Figure 5). There was a rise in heart rate from 20 weeks of gestation in both groups that plateaued at 30 weeks of gestation and fell post-partum. However, there were no significant differences between the fish oil and olive oil groups (Figure 5).
Figure 2  Maternal serum HDL-C levels (upper panel), and HDL₂-C and HDL₃-C levels (lower panels) during and after pregnancy in women supplemented with 4 g/day of either fish oil (▲) or olive oil (■) from 20 weeks of pregnancy until delivery. Values are means ± S.E.M. P = 0.91, P = 0.53 and P = 0.29 for between-group differences in HDL-C, HDL₂-C and HDL₃-C respectively, as determined using a mixed model in SAS. †P < 0.01 for the group differences at baseline in HDL-C and HDL₂-C.

Figure 3  Maternal LDL-C levels during and after pregnancy in women supplemented 4 g/day of either fish oil (▲) or olive oil (■) from 20 weeks of pregnancy until delivery. Values are means ± S.E.M. P = 0.51 for the between-group differences, as determined by using a mixed model in SAS.

DISCUSSION

Supplementation with 4 g/day of fish oil from 20 weeks of gestation until delivery did not affect the concentration of maternal serum triacylglycerols, total cholesterol, HDL₃-C, HDL₂-C, HDL₃-C or LDL-C. Fetal (cord blood) total cholesterol, HDL-C, LDL-C and triacylglycerol levels were lower than those of the mothers studied either during or after pregnancy, confirming previous studies [24–26], but were not different between infants whose mothers received fish oil compared with those who received olive oil.

BP and heart rate recorded during and after pregnancy were not significantly affected by fish oil supplementation. Most studies in non-pregnant subjects using...
similar levels of supplementation of $n-3$ fatty acids have shown reduced BP in hypertensive but not normotensive subjects [20]. However, it may not be surprising that BP was not lowered by fish oil in the present study, given that these women had an uncomplicated pregnancy which is associated with a state of vasodilatation and low BP, particularly during the second trimester, compared with the non-pregnant state [27].

The present study contrasts with previous trials in the non-pregnant state, where similar doses of fish oil have consistently resulted in a significant fall of 25–30% in triacylglycerols and an increase in HDL-C, in particular the HDL$_2$ subfraction, in normolipidaemic and hyperlipidaemic subjects [17]. Our present study was powered to show a fall in triacylglycerols of $\geq 15$% with 40 subjects per group and, although it was confined to pregnant women with allergic disease, there is no evidence to suggest that their response to fish oil with respect to lipids would differ from other pregnant women.

Fish oil lowers serum triacylglycerol levels in humans by reducing hepatic triacylglycerol synthesis and secretion of triacylglycerol-rich lipoproteins from the liver into the circulation, rather than increasing clearance of VLDL (very-low-density lipoprotein) [16]. This effect is mediated by an effect of EPA and DHA to increase peroxisomal $\beta$-oxidation of fatty acids [28] and decrease hepatic fatty acid synthesis [29], reducing substrate availability for triacylglycerol synthesis. During pregnancy, the regulation of triacylglycerol synthesis is under the control of a number of factors. In the first half of pregnancy, maternal body fat accumulates. In the third trimester, an accelerated breakdown of fat depots occurs to help sustain fetal metabolism [30,31]. Maternal insulin resistance is thought to be responsible for enhanced adipose tissue lipolytic activity, leading to increased NEFA and glycerol substrate for hepatic triacylglycerol synthesis [32]. The insulin-resistant state also reduces adipose tissue lipoprotein lipase preventing breakdown of triacylglycerols [33]. At the same time, an increase in oestrogen levels in the third trimester stimulates release of VLDL triacylglycerols [34] and suppresses hepatic lipase activity, causing accumulation of triacylglycerols by preventing the breakdown of non-VLDL–triacylglycerol-containing lipoproteins [35]. Therefore, although $n-3$ fatty acids have profound effects on triacylglycerol levels in the non-pregnant state, in pregnancy the modest nutritional intervention of $n-3$ fatty acids is offset by the physiological drivers that maintain high levels of circulating triacylglycerols.

Maternal triacylglycerols are not transported intact across the placenta [36]. Fetal triacylglycerol concentrations are determined by a number of factors, including fatty supply from the maternal circulation, efficiency of fatty acid uptake by the fetal liver, hepatic synthesis of triacylglycerols and secretion and turnover of triacylglycerols in the plasma compartment. Our present results suggest that fish oil supplementation has no adverse effect on fetal triacylglycerol levels.

In conclusion, supplementation with 4 g of fish oil per day during the last 20 weeks of pregnancy does not affect maternal or fetal lipid profiles compared with supplementation with olive oil. Therefore efforts to alter lipid levels during pregnancy to help manage conditions associated with altered lipid profiles may need to concentrate on altering the total dietary fatty intake, rather than replacing one type of fat with another.

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REFERENCES
