British Journal of Nutrition (2003), **90**, 135–145
DOI: 1079/BJN2003888

Maternal docosahexaenoic acid supplementation and fetal accretion

Colette Montgomery[1], Brian K. Speake[4], Alan Cameron[2], Naveed Sattar[2] and Lawrence T. Weaver[1]*

[1]*Department of Child Health,* [2]*Department of Fetal Medicine and* [3]*Department of Clinical Biochemistry, University of Glasgow, Scotland, UK*
[4]*Scottish Agricultural College, Auchincruive, Ayr, Scotland, UK*

(Received 21 August 2002 – Revised 12 February 2003 – Accepted 11 March 2003)

Docosahexaenoic acid (DHA) (22 : 6*n*-3) is a polyunsaturated fatty acid that is an essential constituent of membranes, particularly of the nervous system. Infants acquire DHA from their mothers, either prenatally via the placenta or postnatally in milk. The present study aimed to test the hypothesis that maternal supplementation during the second and third trimesters of pregnancy enriches maternal and/or fetal DHA status. In a randomised, prospective, double-blind study 100 mothers received either fish-oil capsules containing 400 mg DHA/g (200 mg/d) (*n* 50), or placebo containing 810 mg oleic acid/g (400 mg/d) (*n* 50) from 15 weeks gestation until term. Venous blood samples were obtained from mothers at 15, 28 and 40 weeks, and from the umbilical cord at birth. Total fatty acids in plasma and erythrocytes were analysed by GC–MS. There were no significant differences between maternal groups in baseline DHA, as a proportion of total fatty acids (g/100 g total fatty acids) or concentration (nmol/ml), in plasma and erythrocytes. DHA concentrations in plasma at 28 weeks (P=0·02) and erythrocytes at both 28 weeks (P=0·03) and term (P=0·02) were 20 % higher in supplemented mothers than the placebo group. DHA accounted for a higher proportion of total fatty acids in erythrocytes of supplemented mothers at 28 weeks (P=0·003) and term (P=0·01). There were no significant differences between groups in DHA (g/100 g total fatty acids or nmol/l) in cord blood. Maternal DHA status was maximal in mid-trimester and declined to term, at a lower rate in supplemented compared with unsupplemented mothers. Maternal DHA supplementation significantly increases maternal DHA status and limits the last trimester decline in maternal status, aiding preferential transfer of DHA from mother to fetus.

Polyunsaturated fatty acid: Docosahexaenoic acid: Mother: Infant

Docosahexaenoic acid (DHA) (22 : 6*n*-3) is a polyunsaturated fatty acid (PUFA) that is an essential constituent of cell membranes, particularly of the nervous system, where it is found in relatively high concentrations in the brain and retina. In adults, DHA is obtained by endogenous conversion from its parent essential fatty acid (EFA), α-linolenic acid (18 : 3*n*-3), dependent on an adequate dietary intake of α-linolenic acid and the α-linolenic acid:*n*-6 EFA ratio, linoleic acid (18 : 2*n*-6), or preformed in the diet, largely from fish, particularly oily fish. Circulating DHA concentrations correlate with fish intake, even in the west of Scotland where consumption is low and intakes of DHA and oily fish by women of reproductive age are below current dietary recommendations (Berry *et al.* 2001).

The developing brain and nervous system have an essential requirement for DHA. Neither the fetal retina nor brain initially synthesise DHA and their capacity to do so is a function of gestational age, making placental transfer crucial (Clandinin, 1999). The postnatal period is as critical as gestation for the accumulation of long-chain PUFA (Connor, 2000). In both term and preterm neonates, conversion of α-linolenic acid to DHA occurs, but in limited amounts that may not adequately meet requirements (Carnielli *et al.* 1996; Salem *et al.* 1996; Sauerwald *et al.* 1997). This is of particular importance as only breast-fed infants receive preformed dietary DHA in the postnatal period.

Maternal PUFA status varies with fish and/or *n*-3 consumption during pregnancy. Regular consumption of oily fish (Olsen *et al.* 1991; Sanjurjo *et al.* 1995) or supplementation with *n*-3 PUFA (van Houwelingen *et al.* 1995; Connor *et al.* 1996) results in an increased circulating maternal DHA status during pregnancy and at term. Following pregnancies in which mothers consume oily fish or *n*-3 fatty acid supplements, umbilical cord erythrocytes (ERY) and plasma have elevated levels of *n*-3 PUFA, including DHA, and lower *n*-6 PUFA status (Sanjurjo *et al.* 1995; van Houwelingen *et al.* 1995; Connor *et al.* 1996). In addition, the proportion of DHA

Abbreviations: DHA, decosahexaenoic acid; EFA, essential fatty acid; ERY, erythrocyte; PUFA, polyunsaturated fatty acid.
* **Corresponding author:** Professor Lawrence T. Weaver, fax +44 141 201 0837, email lweaver@clinmed.gla.ac.uk

in cord ERY and plasma correlates not only with maternal blood DHA, but also with maternal dietary *n*-3 fatty acids (Connor *et al.* 1996).

During gestation, accumulation of long-chain PUFA by the fetus elevates the fetal levels of these fatty acids above those in the mother. Generally, relative levels of linoleic acid and α-linolenic acid are higher, while arachidonic acid and DHA are lower in maternal compared with cord plasma triacylglycerols (Berghaus *et al.* 2000), plasma phospholipids (Al *et al.* 1990, 1995*a*; Hoving *et al.* 1994) and ERY total lipids (Reece *et al.* 1997). Such preferential accumulation or biomagnification (Crawford *et al.* 1976) of long-chain PUFA by the fetus may be the result of maternal long-chain PUFA mobilisation and preferential placental transfer to the fetus (Campbell *et al.* 1996; Dutta-Roy *et al.* 1996).

The contribution of maternal diet to maternal fatty acid status and the subsequent impact of maternal status on that of the fetus is well known. However, previous studies of antenatal maternal supplementation have investigated short periods of supplementation, which often cease prior to delivery, and in determining functional benefits of elevated *n*-3 fatty acid intake to the neonate have considered the relationship between postnatal diet and development. We are aware of only one study on development in relation to fetal nutrition (Helland *et al.* 2001). The effect of maternal supplementation during early pregnancy has therefore not been adequately addressed when considering infant development, although there is evidence that maternal antenatal nutrition influences later visual development (Williams *et al.* 2001). The inter-dependence of maternal and fetal nutrition is thus reason to examine the hypothesis that manipulation of maternal diet will enhance both maternal and infant PUFA status, and provide a means by which to optimise infant development.

The aim of the present study, therefore, was to test the hypothesis that antenatal maternal DHA supplementation enriches maternal DHA status and thereby increases the amount available to the fetus and neonate, and influences neonatal visual development. The effect of maternal supplementation on maternal and fetal nutrition, as reflected in maternal ERY and plasma, and (fetal) umbilical cord ERY and plasma, is presented here; the effects on placental tissue, umbilical cord tissue and breast milk, and on infant visual development will be reported elsewhere.

Subjects and methods

A double-blind, placebo-controlled trial was undertaken in which 100 expectant mothers were randomised to receive either fish-oil (*n* 50) or placebo (*n* 50) capsules from 15 weeks gestation until term (Table 1). Fish-oil capsules contained a blended fish oil, Marinol D40 (400 mg DHA/g, 100 mg per capsule); the amount of eicosapentaenoic acid per capsule (approximately 20 mg) was not sufficient to have any pharmacological effect on bleeding time or platelet aggregation (Department of Health, Committee on Medical Aspects of Food Policy, 1994). The placebo consisted of high-oleic-acid sunflower oil containing 810 mg oleic acid/g (18 : 1*n*-9, 200 mg per capsule) and was devoid of any *n*-3 fatty acid. Participants took two capsules per d, providing the fish oil-supplemented group with 200 mg DHA/d. The capsules were identical in appearance, taste and odour. The antioxidant used for capsule stability was vitamin E. Both capsule types were donated by RP Scherer Ltd (Swindon, Wilts., UK).

Table 1. Composition of active supplement and placebo capsules (g/100 g total fatty acids)*

Fatty acid	Marinol D40 Active supplement†	HOSF placebo‡
14 : 0	3·7	
16 : 0	6·7	4·0
16 : 1*n*-7	4·3	
18 : 0	2·4	5·0
18 : 1*n*-9	15·6	81·0
18 : 2*n*-6	1·2	7·2
18 : 3*n*-3	0·8	
18 : 4	1·4	
20 : 0	0·1	
20 : 1	2·0	
20 : 5*n*-3	7·2	
22 : 1	2·7	
22 : 5*n*-6	4·1	
22 : 6*n*-3	40·4	
∑ Fatty acids	92·6	97·2
Other fatty acids	9·4	3·0
Total	102·0	100·2

HOSF, high-oleic-acid sunflower oil.
* Values were certified by the manufacturer (RP Scherer Ltd, Swindon, Wilts., UK).
† 400 mg docosahexaenoic acid/g, 100 mg per capsule (two capsules per d).
‡ 810 mg oleic acid/g, 200 mg per capsule (two capsules per d).

Mothers who were expected to deliver their babies at term and planned to feed their babies on breast and/or formula milk were eligible to participate in the study. Mothers with diabetes, twin pregnancies, pre-eclampsic toxaemia, a past history of abruption or postpartum haemorrhage, allergy to fish products, a thrombophilic tendency or who were receiving drugs that affect thrombocyte function (non-steroidal anti-inflammatory drugs) were excluded. Samples collected at delivery from pregnancies concluded prematurely before 36 weeks, or in which the neonate had an Apgar score >7 at 10 min, weight below the 3rd centile for gestational age or had visual, medical or developmental problems were not included in the final analysis of results.

Sample size was based on the DHA status of non-pregnant and unsupplemented women within the study population, which is generally low (mean ERY DHA 4·57 (SD 0·82) g/100 g total fatty acids and with little variation (Berry *et al.* 2001). Assuming the same range of values at 15 weeks gestation, we hypothesised that supplementation of mothers would increase maternal DHA status by 20 % of baseline (approximately 1 SD). The physiological importance of such an increase could not be determined prospectively, but was measured by postnatal assessment of infant visual development, as reported elsewhere (Malcolm *et al.* 2003). The number required per study group to detect a 1 SD difference with a significance level (α) of 0·05 and power (β) of 90 % was 21. Allowing for a 20 % 'drop-out' rate between recruitment, each measurement time and the final measurement at 6 months postpartum, a total of 100 participants (fifty per group) entered the study.

A questionnaire was used to collect information regarding the maternal dietary intake of fish, smoking and exercise patterns, and alcohol consumption. Frequency of dietary fish intake was recorded at 15 weeks gestation, 28 weeks gestation and birth. Fish-intake frequency was classified as never or less than once per week, or once or more per week. Most recent fish consumption prior to obtaining a maternal blood sample at both 15 weeks and 28 weeks gestation was determined to find out if it occurred within the 24 h period prior to sampling. Thus, consumption was classified as either more than 24 h before sampling, or 24 h or less prior to sampling.

At 15 and 28 weeks gestation, participants were given a bottle containing 200 capsules. Compliance in consumption of study capsules was measured by asking participants to return these bottles at the next study time-point (28 weeks gestation or delivery). The number of capsules and time period over which they were consumed were determined, indicating the dosage obtained (Clelend *et al.* 1992).

Of the 100 original participants, twenty-nine (*n* 15 fish-oil group, *n* 14 placebo group) withdrew from the study between 15 and 28 weeks and seven (*n* 4 fish oil group, *n* 3 placebo group) withdrew between 28 weeks and birth. Reasons for withdrawal included *nausea gravidarum*, poor compliance and loss of contact. It was not possible to obtain blood samples from three mothers at 15 weeks, nine mothers at 28 weeks, three mothers and six neonates at delivery. Two mother–infant pairs in the placebo group were excluded at delivery; one was delivered prematurely, and one was below the 3rd centile for weight for gestational age. Only those with three maternal blood samples (15 weeks, 28 weeks and birth) and an umbilical blood sample were included in the longitudinal analyses.

All pregnancies were singleton and delivered at term. Mothers were representative of the Glasgow population in terms of socio-economic status, as measured using the Carstairs deprivation categories based on residential postcode (Carstairs & Morris, 1990). The two maternal groups were similar with regard to maternal age, anthropometry, socio-economic status, fish consumption, smoking and alcohol habits. Their infants were of comparable gestational age and anthropometry, and were similar for gender, mode of delivery and feeding practice. Nulliparous women were those for whom the current pregnancy was their first and only; parous women were defined as those with any previously confirmed pregnancy, regardless of outcome or duration.

Antenatal maternal inter-prandial blood samples were collected at enrolment (*n* 97) and 28 weeks gestation (*n* 62). Maternal term blood samples (*n* 59) were obtained within 20 h (mean value) of delivery. Umbilical cord blood samples (*n* 56) were obtained within 2 h of delivery.

Venous maternal or umbilical cord blood (5 ml) was collected in potassium EDTA (Teklab, Sacriston, Durham, UK). Samples were centrifuged at 550 ***g*** (2500 rpm) for 5 min. Plasma was removed and ERY were washed with sterile saline (9 g NaCl/l, 2·5 ml) three times. Both plasma and ERY were stored at −70°C until analysis.

The fatty acid composition of total lipids in ERY and plasma was analysed. Fatty acids were extracted via a modified Folch extraction (chloroform–methanol (1:1, v/v)) (Folch *et al.* 1957) and derivatised with methanolic hydrochloric acid. An external standard of sixteen available non-esterified fatty acids of known concentration was derivatised concurrently with the samples. The external standard and the samples included an internal standard (15:0 triacylglycerol; Sigma-Aldrich, Poole, Dorset, UK) for calculation of response factors and concentrations. Fatty acid methyl esters were analysed by GC–MS. The GC (Hewlett Packard 5890 Series II; Agilent Technologies, Stockport, Cheshire, UK) was used in split mode (20:1). The carrier gas was He (BOC, Guildford, Surrey, UK), at a flow rate of 1 ml/min. The analytical column was a fused silica capillary column (length 30 m, internal diameter 0·25 mm and film thickness 0·25 μm; BPX70, SGE Europe Ltd, Milton Keynes, UK). GC injector temperature was 250°C and the injection volume was 2 μl. The GC temperature programme was as follows: initial temperature 120°C for 2 min, increasing by 4°C per min to 180°C, then by 2°C per min to 194°C, followed by 30°C per min to 240°C, which was maintained for 1 min. The temperature of the transfer line between the GC and MS was 280°C. The MS (Hewlett Packard 5972) with electron impact ionisation was operated in scanning mode. Fatty acid methyl esters were quantified in both relative and absolute terms: results were expressed as g/100 g total fatty acids based on peak area, and as concentrations (nmol/ml). Relative values were obtained for nineteen individual fatty acids; the concentrations of three fatty acids (22 : 5*n*-6, 24 : 0 and 24 : 1) could not be determined due to the lack of their non-esterified fatty acid standards and their exclusion from the external standard. In addition, the *n*-6 and *n*-3 PUFA classes were each summed (total *n*-6 and total *n*-3 fatty acids respectively) and expressed relative to each other (*n*-6:*n*-3 fatty acids). The low values for 22 : 5*n*-6 (docosapentaenoic acid) precluded the use of the DHA:docosapentaenoic acid ratio.

Because the data were not normally distributed, statistical analyses were performed using non-parametric tests. Differences between groups were tested using Mann–Whitney two-sample rank tests, with a chosen significance level of $P<0·05$. The longitudinal changes in maternal status and the differences between mother and fetus were not symmetrically distributed and were therefore assessed by one-sample Sign tests at a significance level of $P<0·01$. All statistical analyses were performed using Minitab for Windows version 10.51 software (1995; Minitab Inc., State College, PA, USA).

Approval for the study was obtained from both Glasgow University Ethics Committee and Yorkhill Research Ethics Committee, and it was performed with the informed consent of the mothers.

Results

There were no significant differences between supplemented and placebo groups in terms of the number of parous women (χ^2 1·478, $P > 0·2$), or in the frequency of maternal fish consumption at 15 weeks, 28 weeks or delivery (all $P > 0·2$).

The maternal DHA status of parous women did not differ from that of nulliparous women during gestation or at birth. Cord plasma DHA did, however, differ between the groups: DHA accounted for a higher proportion of total fatty acids (median value 3·6 *v.* 2·8 g/100 g total fatty acids, P=0·02), and was of a higher concentration (median value 701 *v.* 350 nmol/ml, P=0·01), in cord plasma obtained from nulliparous women. There were no differences in DHA status of ERY or plasma samples between those whose last previous pregnancy was < 1 year before the current pregnancy, and those whose last previous pregnancy was > 1 year before.

Consumption of fish once or more per week was associated with a higher proportion of DHA in both maternal ERY (median value 3·1 *v.* 2·4 g/100 g total fatty acids, P=0·003) and plasma (median value 1·8 *v.* 1·6 g/100 g total fatty acids, P=0·02) at 15 weeks. DHA concentration was not significantly higher at 15 weeks in those who consumed fish once or more per week. Moreover, weekly fish consumption was not associated with higher DHA status after supplementation began, at either 28 weeks or birth.

Fish intake in the 24 h prior to sampling was associated with a higher DHA status, expressed as both a proportion of total fatty acids (median value 1·9 *v.* 1·6 g/100 g total fatty acids, P=0·004) and as a concentration (median value 154 *v.* 122 nmol/ml, P=0·005) in maternal plasma at 15 weeks gestation. However, this effect was not observed at 28 weeks, following the commencement of supplementation.

There were no significant differences between fish oil-supplemented and placebo groups in baseline PUFA status at 15 weeks (Table 2). In both groups, there was an increase in the concentration and proportion of DHA in maternal ERY and plasma between 15 and 28 weeks, followed by a decrease between 28 weeks and term (Figs 1 and 2). However, at 28 weeks, the concentration of DHA was 22 % higher in plasma (P=0·02) and 13 % higher in ERY (P=0·02) in the fish-oil compared with the placebo group (Table 3). Arachidonic acid (20 : 4*n*-6) accounted for a significantly (P=0·02) lower proportion of fatty acids in ERY of fish oil-group mothers at 28 weeks (Table 3). In maternal plasma at 28 weeks, the concentration of eicosapentaenoic acid (20 : 5*n*-3) was significantly (P=0·04) higher in the fish-oil group (Table 3). These were the only incidences in which the amount of either arachidonic acid or eicosapentaenoic acid was significantly different between treatment groups. At term, ERY DHA concentration remained 42 % higher (P=0·02) in the fish oil-supplemented group. DHA also accounted for a higher proportion of total fatty acids in ERY of fish oil-supplemented mothers at 28 weeks (P=0·003) (Table 3) and at term (P=0·01) (Table 4). Total *n*-3 fatty acids were elevated, with a concomitant lower *n*-6:*n*-3 fatty acid ratio, in the fish-oil group at 28 weeks (Table 3) and at birth (Table 4) in both maternal ERY and plasma, for both relative and absolute measurements (all $P < 0·05$). Thus, fish-oil supplementation enhanced the overall maternal DHA and *n*-3 fatty acid status (Tables 3 and 4).

There were no significant differences between supplemented and placebo groups in DHA as a proportion of total fatty acids or concentration in cord blood (Table 5). In both groups, DHA (proportion of total fatty acids and concentration) was higher in cord than maternal ERY and plasma at birth (Figs 1 and 2). The relative and absolute amounts of DHA in cord plasma and ERY were most similar to the maximal maternal DHA observed at 28 weeks (Figs 1 and 2).

Discussion

Fetal and neonatal DHA status are dependent on maternal DHA status. Maternal supplies of DHA are transferred to the fetus via the placenta, and to the neonate via breast milk. Babies who are inadequately supplied with DHA either *in utero* or postnatally accumulate lower amounts in blood and tissue (Jamieson *et al.* 1999), and may be at a disadvantage with regard to neurodevelopment.

In the present study, expectant mothers were supplied with preformed DHA at a dose (200 mg/d) comparable with current dietary recommendations (Department of Health, Committee on Medical Aspects of Food Policy, 1994) and previously shown to be effective in lactating women (Makrides *et al.* 1996; Helland *et al.* 1998; Fidler *et al.* 2000), to determine its effects on the nutritional status of both mother and fetus.

A differential elevation of circulating DHA and total *n*-3 fatty acids, with concomitant lowering of the *n*-6:*n*-3 fatty acid ratio, was observed in mothers receiving fish-oil compared with placebo supplements. The enrichment of maternal *n*-3 PUFA status has been noted previously in mothers with a habitually higher amount of fish in their diet throughout pregnancy (Olsen *et al.* 1991; Sanjurjo *et al.* 1995) and in mothers supplemented during the last trimester of pregnancy (van Houwelingen *et al.* 1995; Connor *et al.* 1996).

In all measures of circulating DHA status (relative and absolute levels in both ERY and plasma), the same pattern for DHA was noted: an increase in maternal status between 15 and 28 weeks, followed by a decline between 28 weeks and birth, and a subsequently higher cord than maternal DHA status (Figs 1 and 2). Differences were noted in the magnitude of these changes and in the materno–fetal difference between the groups. The fish-oil group exhibited either an enhanced maternal DHA status, a less pronounced decline in maternal status during the last trimester, and/or a less compromised maternal status relative to fetal status.

Fluctuations in maternal DHA and other PUFA during pregnancy are well reported (Otto *et al.* 1997). In particular, the elevation of DHA and long-chain PUFA in the early and mid trimesters (Al *et al.* 1995*a*; Ashby *et al.* 1997; Otto *et al.* 2001) and subsequent decrease in the last trimester (Al *et al.* 1995*b*) accord with the longitudinal maternal changes observed in both supplemented and placebo groups. The early increase in maternal DHA probably indicates mobilisation of maternal stores to facilitate preferential transfer to and accumulation by the fetus (Al *et al.* 1995*a*), inevitably causing an elevation of fetal relative to maternal DHA pool. The larger maternal increase noted in the fish-oil group of the current study

Table 2. Fatty acid status of baseline maternal erythrocyte and plasma total lipids at 15 weeks gestation in fish oil-supplemented and placebo groups*
(Median values and 95 % Wilcoxon confidence intervals)

	Erythrocyte fatty acids										Plasma fatty acids									
	g/100 g total fatty acids					nmol/ml					g/100 g total fatty acids					nmol/ml				
	Fish oil		Placebo			Fish oil		Placebo			Fish oil		Placebo			Fish oil		Placebo		
Group…†	Median	95 % CI	Median	95 % CI	P‡	Median	95 % CI	Median	95 % CI	P‡	Median	95 % CI	Median	95 % CI	P‡	Median	95 % CI	Median	95 % CI	P‡
10:0	0·0	0·0, 0·0	0·0	0·0, 0·0		0	0, 0	0	0, 0		0·0	0·0, 0·0	0·0	0·0, 0·0		0	0, 0	0	0, 0	
12:0	0·0	0·0, 0·0	0·0	0·0, 0·0		0	0, 0	0	0, 0		0·1	0·1, 0·2	0·1	0·1, 0·1		8	8, 13	5	4, 8	
14:0	0·2	0·2, 0·3	0·0	0·1, 0·3	0·41	7	5, 12	2	4, 8	0·38	1·4	1·3, 1·6	1·2	1·1, 1·4	0·13	92	85, 108	77	66, 90	0·02
16:0	32·4	32·0, 33·8	32·9	32·5, 34·4	0·52	855	850, 998	873	840, 981	0·87	29·7	29·2, 30·6	30·2	29·4, 30·4	0·91	1953	1785, 2017	1618	1563, 1803	0·02
16:1*n*-7	0·1	0·1, 0·4	0·1	0·1, 0·3	0·56	7	6, 11	5	3, 9	0·32	2·3	2·1, 2·6	1·9	1·8, 2·2	0·17	125	113, 149	101	91, 116	0·03
18:0	16·8	16·5, 17·5	16·9	16·7, 17·4	0·88	383	362, 419	372	346, 406	0·51	7·4	7·2, 7·8	7·5	7·1, 7·6	0·44	356	345, 395	322	298, 348	0·02
18:1*n*-9	13·4	13·4, 14·5	13·2	13·0, 13·8	0·12	366	348, 418	353	327, 397	0·38	20·4	20·0, 21·5	19·8	19·1, 20·8	0·14	1221	1136, 1328	1054	950, 1129	0·004
18:2*n*-6	9·1	8·8, 9·6	9·0	8·7, 9·3	0·49	272	238, 284	230	220, 267	0·41	27·3	25·4, 28·1	27·3	26·7, 28·7	0·25	1579	1477, 1726	1437	1350, 1565	0·09
18:3*n*-3	0·0	0·0, 0·0	0·0	0·0, 0·0		0	0, 0	0	0, 0		0·5	0·5, 0·6	0·5	0·4, 0·5	0·38	61	52, 66	50	44, 57	0·06
20:0	0·5	0·3, 0·5	0·6	0·3, 0·6	0·19	10	7, 12	11	8, 12	0·64	0·3	0·3, 0·4	0·3	0·3, 0·4	0·78	13	12, 14	12	11, 13	0·15
20:3*n*-6	1·2	1·0, 1·2	1·0	1·0, 1·1	0·20	111	120, 255	142	136, 217	0·68	1·6	1·4, 1·7	1·5	1·5, 1·7	0·81	272	257, 311	238	234, 296	0·27
20:4*n*-6	10·9	10·6, 11·4	10·9	10·4, 11·4	0·89	326	302, 381	319	283, 374	0·59	5·4	4·9, 5·7	5·4	5·2, 5·8	0·38	237	218, 260	220	206, 249	0·35
20:5*n*-3	0·1	0·1, 0·2	0·0	0·1, 0·2	0·25	5	6, 15	0	3, 13	0·39	0·3	0·3, 0·4	0·3	0·3, 0·4	0·59	23	21, 33	24	21, 29	0·93
22:4*n*-6	1·9	1·6, 2·1	1·7	1·6, 2·1	0·74	151	116, 183	112	110, 186	0·96	0·1	0·1, 0·2	0·2	0·1, 0·2	0·79	12	10, 17	13	10, 15	0·70
22:5*n*-6	0·0	0·0, 0·1	0·0	0·0, 0·0		ND		ND			0·0	0·0, 0·0	0·0	0·0, 0·0		ND		ND		
24:0	3·7	3·1, 3·9	3·9	3·2, 4·1	0·56	ND		ND			0·2	0·2, 0·3	0·3	0·2, 0·3	0·29	ND		ND		
24:1*n*-9	4·3	4·0, 4·7	4·6	4·1, 4·9	0·78	ND		ND			0·9	0·7, 0·9	0·9	0·8, 1·0	0·19	ND		ND		
22:5*n*-3	1·0	0·9, 1·2	0·9	0·9, 1·1	0·60	55	47, 120	38	36, 123	1·00	0·2	0·2, 0·2	0·2	0·2, 0·2	0·49	14	13, 17	13	11, 15	0·24
22:6*n*-3	2·8	2·4, 3·0	2·9	2·5, 3·1	0·80	144	122, 179	134	122, 185	0·98	1·5	1·4, 1·8	1·7	1·6, 1·9	0·15	132	123, 152	134	124, 148	0·98
Total *n*-3 fatty acids	3·9	3·4, 4·4	4·0	3·4, 4·3	0·86	205	177, 294	169	159, 273	0·66	2·5	2·4, 3·0	2·7	2·5, 3·0	0·57	233	217, 262	229	203, 247	0·36
Total *n*-6 fatty acids	23·5	22·7, 23·9	23·0	22·2, 23·5	0·29	893	805,1099	827	758, 1048	0·51	35·0	32·5, 35·4	35·1	34·0, 36·0	0·23	2141	2007, 2286	1867	1829, 2138	0·09
n-6:*n*-3 fatty acids	5·8	5·6, 7·5	5·8	5·6, 7·2	0·92	5	4, 6	5	4, 6	0·96	13·4	12·0, 14·9	12·7	12·1, 14·6	0·98	9	8, 10	9	8, 10	0·93

ND, not determined.
* For details of subjects, supplements and procedures, see Table 1 and p. 136.
† Fish-oil group: erythrocytes *n* 47, plasma *n* 48; placebo group *n* 49.
‡ Statistical significance of effect (Mann–Whitney test).

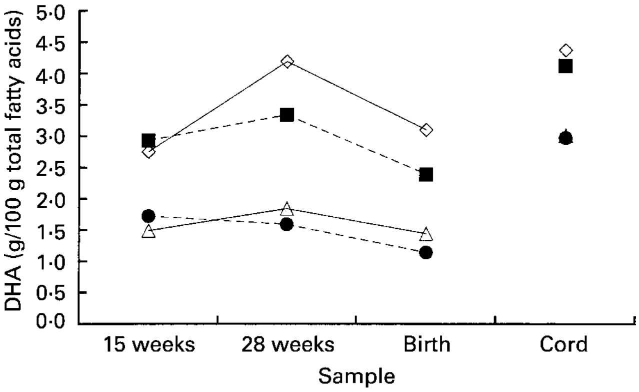

Fig. 1. Docosahexaenoic acid (DHA) measured as g/100 g total fatty acids in erythrocytes and plasma in the fish oil-supplemented and placebo groups. Values are medians in maternal blood at 15 and 28 weeks gestation and at birth, and in cord blood. For details of subjects, supplements and procedures, see Table 1 and p. 136. –◇–, Fish-oil group erythrocytes (15 weeks *n* 47, 28 weeks *n* 30, birth *n* 29, cord *n* 27); --■--, placebo group erythrocytes (15 weeks *n* 49, 28 weeks *n* 33, birth *n* 28, cord *n* 29); –△–, Fish-oil group plasma (15 weeks *n* 48, 28 weeks *n* 30, birth *n* 30, cord *n* 27); --●--, placebo group plasma (15 weeks *n* 49, 28 weeks *n* 33, birth *n* 28, cord *n* 29).

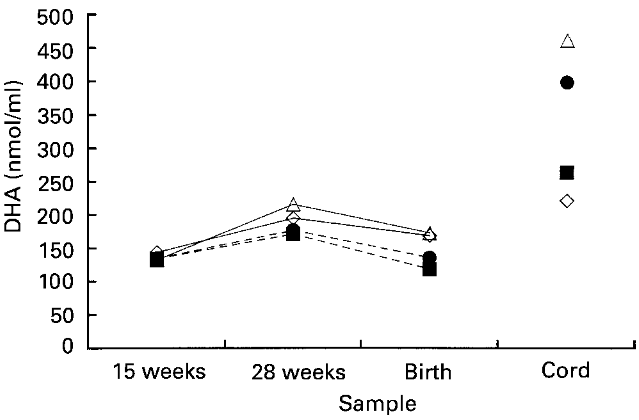

Fig. 2. Docosahexaenoic acid (DHA) measured as a concentration in erythrocytes and plasma in the fish oil-supplemented and placebo groups. Values are medians in maternal blood at 15 and 28 weeks gestation and at birth, and in cord blood. For details of subjects, supplements and procedures, see Table 1 and p. 136. –◇–, Fish-oil group erythrocytes (15 weeks *n* 47, 28 weeks *n* 30, birth *n* 29, cord *n* 27); --■--, placebo group erythrocytes (15 weeks *n* 49, 28 weeks *n* 33, birth *n* 28, cord *n* 29); –△–, fish-oil group plasma (15 weeks *n* 48, 28 weeks *n* 30, birth *n* 30, cord *n* 27); ---●---, placebo group plasma (15 weeks *n* 49, 28 weeks *n* 33, birth *n* 28, cord *n* 29).

may be attributable to both a greater circulating pool and a larger store in adipose tissue from which to mobilise DHA. The subsequent decline observed between 28 weeks and birth indicates that elevated maternal levels cannot be sustained and that accretion by the fetus probably occurs at the expense of maternal status. Maternal fish-oil supplementation, however, reduces the inevitable last trimester decline in maternal DHA status, and attenuates the difference between maternal and fetal status. It is therefore suggested that while maternal DHA supplementation may not increase fetal ERY status *per se*, the increase in the overall maternal ERY status during pregnancy may make available to the fetus a greater reservoir from which DHA can be supplied.

The relative and absolute amounts of DHA in cord ERY and plasma were consistently higher than in the corresponding maternal sample at birth. Moreover, the materno–fetal difference was generally characterised by lower EFA and higher long-chain PUFA, including arachidonic acid, in cord than maternal blood samples. These findings are in agreement with other comparisons of maternal and fetal fatty acids (Crawford *et al.* 1989; Al *et al.* 1990, 1995*a*; van der Schouw *et al.* 1991; Hoving *et al.* 1994; Otto *et al.* 1997; Reece *et al.* 1997; Berghaus *et al.* 2000).

It is perhaps surprising that no differences were observed between groups in the circulating fatty acids of umbilical cord blood, as other studies have reported variations in cord plasma and ERY in relation to maternal diet (Reddy *et al.* 1994; Al *et al.* 1995*c*; Sanjurjo *et al.* 1995; van Houwelingen *et al.* 1995; Connor *et al.* 1996). However, habitually high intakes of marine foods in the maternal diet are not consistently associated with elevated *n*-3 PUFA in cord blood (Hornstra *et al.* 1992), suggesting that other factors may be involved when the maternal-to-fetal supply is adequate over long periods.

We reported the fatty acid composition of total lipids and therefore do not distinguish between the various lipid fractions of triacylglycerols, phospholipids and cholesteryl esters. Previous studies also differ in the lipid fraction(s) analysed, which may account for some of the differences reported in the literature. Analysis of total lipids determines overall fatty acid status, but does not identify class specific changes or differences. It is therefore possible that the analysis of total lipids in the present study obscured changes during pregnancy, differences between supplemented and placebo groups and/or differences between mother and fetus, which may have been more evident on analysis of distinct lipid fractions. The relative and absolute measurements of fatty acid status are reported here, as together they provide more useful information when considering maternal–fetal transfer of fatty acids (Al *et al.* 1990; Matorras *et al.* 1994).

Conservation of the differences between mother and fetus (in both the current and previous studies) suggests a physiological requirement by the fetus to accrue greater amounts of and to attain a higher status of DHA and long-chain PUFA, relative to the mother. Moreover, the requirement appears specific for preformed long-chain PUFA as the fetus accumulates relatively less EFA compared with the mother. Indeed, the lack of difference in cord blood samples between the supplemented and placebo groups suggests that the differential EFA and long-chain PUFA status of mother and infant is a natural physiological process during pregnancy. That manipulation of the maternal diet and DHA status did not influence fetal DHA status is further evidence that DHA accretion by the fetus is a necessity and may be largely pre-determined. Moreover, there may be a threshold level (relative or absolute) that the fetus must attain, which could explain the current and previous (Otto *et al.* 1997) observations that the materno–fetal difference is greater when maternal

Table 3. Fatty acid status of maternal erythrocyte and plasma total lipids at 28 weeks gestation in fish oil-supplemented and placebo groups*
(Median values and 95 % Wilcoxon confidence intervals)

	Erythrocyte fatty acids										Plasma fatty acids									
	g/100 g total fatty acids					nmol/ml					g/100 g total fatty acids					nmol/ml				
	Fish oil		Placebo			Fish oil		Placebo			Fish oil		Placebo			Fish oil		Placebo		
Group…†	Median	95 % CI	Median	95 % CI	P‡	Median	95 % CI	Median	95 % CI	P‡	Median	95 % CI	Median	95 % CI	P‡	Median	95 % CI	Median	95 % CI	P‡
10 : 0	0·0	0·0, 0·0	0·0	0·0, 0·0		0	0, 0	0	0, 0		0·0	0·0, 0·0	0·0	0·0, 0·0		0	0, 0	0	0, 0	
12 : 0	0·0	0·0, 0·0	0·0	0·0, 0·0		0	0, 0	0	0, 0		0·1	0·1, 0·2	0·1	0·1, 0·1		18	12, 24	11	8, 15	
14 : 0	0·4	0·4, 0·5	0·4	0·3, 0·4	0·29	12	11, 14	11	9, 13	0·38	1·6	1·4, 1·8	1·6	1·4, 1·7	0·43	157	140, 179	148	124, 159	0·20
16 : 0	31·4	30·8, 32·0	31·4	30·6, 31·6	0·53	885	860, 917	859	826,911	0·39	31·6	30·5, 31·9	30·7	30·0, 31·6	0·48	2807	2655,3084	2546	2425,2939	0·10
16 : 1n-7	0·7	0·6, 0·8	0·6	0·5, 0·7	0·20	16	14, 18	15	12, 17	0·28	2·5	2·2, 3·0	2·5	2·3, 2·9	0·90	226	179, 238	183	165, 221	0·35
18 : 0	16·0	15·8, 16·4	16·1	15·8, 16·4	0·99	369	353, 390	369	351,384	0·93	6·6	6·3, 6·9	6·5	6·3, 6·6	0·61	425	375, 456	374	339, 403	0·07
18 : 1n-9	12·9	12·6, 13·4	13·4	12·9, 13·6	0·22	366	345, 392	365	346,392	0·95	21·5	20·4, 22·9	21·1	20·7, 22·1	0·59	1621	1464,1790	1501	1317,1580	0·14
18 : 2n-6	8·7	8·3, 9·2	8·7	8·3, 8·9	0·68	253	239, 278	250	236,262	0·50	25·4	24·4, 27·3	27·1	25·6, 27·7	0·33	1886	1679,2103	1645	1498,1881	0·16
18 : 3n-3	0·0	0·0, 0·0	0·0	0·0, 0·0		0	0, 1	0	0, 1		0·6	0·7, 0·7	0·6	0·5, 0·7	0·26	82	68, 110	66	54, 82	0·10
20 : 0	0·6	0·5, 0·6	0·6	0·5, 0·6	0·63	11	10, 12	11	9, 12	0·99	0·3	0·3, 0·4	0·3	0·3, 0·4	0·43	19	18, 23	19	17, 22	0·50
20 : 3n-6	1·5	1·3, 1·5	1·4	1·4, 1·7	0·30	126	113, 140	134	116,158	0·56	1·4	1·3, 1·5	1·5	1·5, 1·7	0·01	343	322, 382	365	335, 414	0·44
20 : 4n-6	10·6	10·1, 10·9	11·2	10·8, 11·5	0·02	264	250, 279	277	262,298	0·17	4·2	4·0, 4·8	4·6	4·4, 5·0	0·24	254	244, 310	303	253, 308	0·86
20 : 5n-3	0·3	0·2, 0·3	0·2	0·2, 0·3	0·37	11	9, 14	11	7, 12	0·42	0·3	0·3, 0·5	0·3	0·3, 0·4	0·14	33	30, 45	27	22, 37	0·04
22 : 4n-6	1·9	1·7, 2·0	2·2	1·9, 2·3	0·07	105	91, 112	121	101,127	0·12	0·1	0·1, 0·1	0·1	0·1, 0·1	0·90	9	8, 15	9	6, 13	0·66
22 : 5n-6	0·0	0·0, 0·0	0·0	0·0, 0·0		ND		ND			0·0	0·0, 0·0	0·0	0·0, 0·0		ND		ND		
24 : 0	4·1	3·8, 4·3	4·2	3·9, 4·3	0·78	ND		ND			0·1	0·1, 0·1	0·1	0·1, 0·1	0·41	ND		ND		
24 : 1n-9	5·2	4·8, 5·3	5·1	4·9, 5·4	0·79	ND		ND			0·8	0·7, 0·9	0·7	0·5, 0·8	0·29	ND		ND		
22 : 5n-3	1·3	1·2, 1·4	1·3	1·2, 1·5	0·34	58	51, 61	60	52, 66	0·34	0·2	0·2, 0·2	0·2	0·1, 0·2	0·37	18	15, 21	15	12, 19	0·18
22 : 6n-3	4·2	3·9, 4·4	3·3	3·2, 3·8	0·003	194	181, 211	171	153,185	0·02	1·8	1·7, 2·1	1·6	1·5, 1·9	0·09	215	205, 265	176	155, 217	0·02
Total n-3 fatty acids	5·7	5·3, 6·1	5·0	4·7, 5·5	0·02	257	244, 287	246	213,262	0·12	3·1	2·9, 3·4	2·6	2·4, 3·2	0·05	359	334, 426	275	256, 354	0·01
Total n-6 fatty acids	23·1	22·2, 23·4	23·5	22·8, 24·0	0·10	753	708, 788	780	723,839	0·45	31·7	30·1, 33·4	33·3	32·1, 34·2	0·13	2499	2286,2772	2387	2124,2589	0·36
n-6:n-3 fatty acids	4·0	3·8, 4·4	4·6	4·4, 5·3	0·01	3	3, 3	3	3, 4	0·01	10·1	9·6, 11·2	12·2	11·1, 14·2	0·02	7	6, 7	8	7, 9	0·03

ND, not determined.
* For details of subjects, supplements and procedures, see Table 1 and p. 136.
† Fish-oil group n 30, placebo n 33.
‡ Statistical significance of effect (Mann–Whitney test).

Table 4. Fatty acid status of maternal erythrocyte and plasma total lipids at delivery in fish oil-supplemented and placebo groups*
(Median values and 95 % Wilcoxon confidence intervals)

	Erythrocyte fatty acids										Plasma fatty acids									
	g/100 g total fatty acids					nmol/ml					g/100 g total fatty acids					nmol/ml				
	Fish oil		Placebo			Fish oil		Placebo			Fish oil		Placebo			Fish oil		Placebo		
Group…†	Median	95 % CI	Median	95 % CI	*P*‡	Median	95 % CI	Median	95 % CI	*P*‡	Median	95 % CI	Median	95 % CI	*P*‡	Median	95 % CI	Median	95 % CI	*P*‡
10:0	0·0	0·0, 0·0	0·0	0·0, 0·0		0	0, 0	0	0, 0		0·0	0·0, 0·0	0·0	0·0, 0·0		0	0, 0	0	0, 0	
12:0	0·0	0·0, 0·0	0·0	0·0, 0·0		0	0, 0	0	0, 0		0·0	0·0, 0·0	0·0	0·0, 0·0		0	0, 4	0	0, 4	
14:0	0·3	0·2, 0·3	0·3	0·2, 0·4	0·19	10	5, 11	10	7, 12	0·46	1·0	0·9, 1·1	1·0	0·9, 1·1	0·22	78	70, 96	96	77, 107	0·37
16:0	35·1	34·1, 36·1	35·0	34·4, 36·6	0·67	1000	906,1063	971	827,1013	0·63	32·5	31·9, 33·1	33·7	32·8, 34·5	0·04	2749	2404,3032	2572	2400,2963	0·90
16:1*n*-7	0·6	0·4, 0·7	0·6	0·5, 0·8	0·43	16	10, 18	14	9, 17	0·68	2·6	2·4, 3·1	3·0	2·8, 3·5	0·10	178	155, 216	217	186, 244	0·12
18:0	16·1	15·6, 16·7	16·1	14·9, 16·4	0·45	380	337, 413	377	287, 397	0·45	6·4	6·0, 6·5	6·1	5·8, 6·3	0·12	445	380, 467	413	359, 448	0·49
18:1*n*-9	14·7	14·4, 15·6	15·1	14·9, 16·4	0·10	421	379, 466	432	368, 476	0·97	23·1	22·4, 24·9	23·8	22·8, 24·9	0·67	1853	1610,2107	1750	1594,2003	0·77
18:2*n*-6	8·7	8·3, 8·9	8·5	8·2, 8·9	0·72	251	227, 290	242	203, 270	0·36	26·2	24·3, 27·1	24·2	23·0, 25·4	0·08	2006	1830,2305	1830	1640,2157	0·25
18:3*n*-3	0·0	0·0, 0·0	0·0	0·0, 0·0		0	0, 0	0	0, 0		0·5	0·4, 0·6	0·5	0·4, 0·6	0·96	60	53, 79	63	57, 82	0·64
20:0	0·5	0·5, 0·5	0·5	0·3, 0·5	0·17	11	8, 11	9	6, 10	0·17	0·3	0·1, 0·3	0·2	0·1, 0·3	0·34	13	8, 15	13	8, 15	0·85
20:3*n*-6	1·0	0·9, 1·2	1·0	0·9, 1·2	0·92	81	74, 116	91	69, 106	0·74	1·2	1·1, 1·3	1·3	1·1, 1·3	0·64	296	255, 340	308	275, 356	0·43
20:4*n*-6	8·5	7·5, 9·1	9·2	7·9, 9·6	0·27	225	190, 262	236	175, 260	0·96	3·7	3·5, 4·3	3·9	3·7, 4·2	0·63	251	219, 289	258	227, 288	0·74
20:5*n*-3	0·0	0·0, 0·1	0·0	0·0, 0·1	0·23	0	0, 7	0	0, 2	0·24	0·2	0·2, 0·3	0·2	0·1, 0·2	0·23	23	17, 31	19	12, 26	0·32
22:4*n*-6	1·2	0·9, 1·2	1·1	1·0, 1·3	0·52	57	51, 78	69	50, 82	0·85	0·0	0·0, 0·1	0·0	0·0, 0·1	0·80	0	0, 8	0	0, 7	0·91
22:5*n*-6	0·0	0·0, 0·0	0·0	0·0, 0·0		ND		ND			0·0	0·0, 0·0	0·0	0·0, 0·0		ND		ND		
24:0	3·6	3·5, 4·3	3·7	3·5, 4·2	0·88	ND		ND			0·0	0·0, 0·1	0·0	0·0, 0·1	0·70	ND		ND		
24:1*n*-9	5·4	5·1, 5·9	5·0	4·8, 5·9	0·34	ND		ND			0·6	0·5, 0·7	0·6	0·5, 0·7	0·64	ND		ND		
22:5*n*-3	0·9	0·7, 1·0	0·8	0·6, 0·9	0·49	40	31, 49	32	23, 41	0·19	0·1	0·1, 0·1	0·1	0·1, 0·1	0·42	12	8, 16	10	6, 13	0·35
22:6*n*-3	3·1	2·5, 3·5	2·4	2·0, 2·7	0·01	168	128, 194	118	92, 143	0·02	1·4	1·2, 1·6	1·1	1·0, 1·4	0·06	172	152, 207	135	119, 179	0·11
Total *n*-3 fatty acids	3·9	3·3, 4·5	3·3	2·7, 3·6	0·02	207	164, 244	149	118, 185	0·03	2·4	1·9, 2·5	1·9	1·7, 2·2	0·07	281	237, 336	232	206, 295	0·25
Total *n*-6 fatty acids	19·8	18·1, 20·3	19·9	18·5, 20·7	0·60	639	555, 742	612	499, 709	0·63	30·7	29·5, 32·1	29·5	28·1, 30·7	0·17	2569	2360,2919	2384	2171,2805	0·40
n-6:*n*-3 fatty acids	4·9	4·5, 6·6	6·2	5·8, 7·6	0·01	3	3, 4	4	4, 5	0·005	13·9	12·7, 17·2	15·1	14·1, 20·5	0·16	9	8, 11	10	9, 14	0·16

ND, not determined.
* For details of subjects, supplements and procedures, see Table 1 and p. 136.
† Fish-oil group: erythrocytes *n* 29, plasma *n* 30; placebo group: erythrocytes *n* 28, plasma *n* 29.
‡ Statistical significance of effect (Mann–Whitney test).

Table 5. Fatty acid status of umbilical cord erythrocyte and plasma total lipids at delivery in fish oil-supplemented and placebo groups*
(Median values and 95 % Wilcoxon confidence intervals)

	Erythrocyte fatty acids										Plasma fatty acids									
	g/100 g total fatty acids					nmol/ml					g/100 g total fatty acids					nmol/ml				
	Fish oil		Placebo			Fish oil		Placebo			Fish oil		Placebo			Fish oil		Placebo		
Group…†	Median	95 % CI	Median	95 % CI	P‡	Median	95 % CI	Median	95 % CI	P‡	Median	95 % CI	Median	95 % CI	P‡	Median	95 % CI	Median	95 % CI	P‡
10 : 0	0·0	0·0, 0·0	0·0	0·0, 0·0		0	0, 0	0	0, 0		0·0	0·0, 0·0	0·0	0·0, 0·0		0	0, 0	0	0, 0	
12 : 0	0·0	0·0, 0·0	0·0	0·0, 0·0		0	0, 0	0	0, 0		0·0	0·0, 0·0	0·0	0·0, 0·0		0	0, 1	0	0, 1	
14 : 0	0·4	0·2, 0·4	0·4	0·2, 0·4	0·55	11	6, 12	12	7, 13	0·42	1·0	0·9, 1·0	1·0	1·0, 1·1	0·10	22	19, 26	26	23, 34	0·10
16 : 0	34·8	34·4, 35·9	35·1	34·6, 36·2	0·63	784	728, 906	874	832, 989	0·06	33·9	33·3, 34·5	33·9	33·4, 35·0	0·72	833	710,932	827	770,1016	0·45
16 : 1*n*-7	0·4	0·3, 0·4	0·3	0·3, 0·4	0·80	13	11, 17	14	12, 17	0·76	4·4	4·2, 5·0	4·7	4·4, 5·2	0·53	73	69, 95	87	83, 109	0·07
18 : 0	18·1	17·4, 18·5	18·1	17·4, 18·3	0·63	328	304, 387	368	341, 389	0·29	11·2	10·6, 11·7	10·8	10·0, 11·2	0·11	198	172,230	208	183, 232	0·83
18 : 1*n*-9	11·0	10·8, 11·5	11·1	10·5, 11·5	0·76	278	253, 330	306	283, 356	0·14	19·1	18·1, 19·7	17·7	17·4, 19·7	0·47	401	350,463	398	368, 506	0·72
18 : 2*n*-6	3·4	3·2, 4·0	3·5	3·3, 3·9	0·85	85	80, 106	98	87, 118	0·43	10·9	10·5, 13·3	11·2	10·4, 12·0	0·77	265	233,313	256	233, 346	0·96
18 : 3*n*-3	0·0	0·0, 0·0	0·0	0·0, 0·0		0	0, 0	0	0, 0		0·1	0·1, 0·2	0·1	0·1, 0·2	0·81	3	2, 5	3	2, 4	0·82
20 : 0	0·7	0·7, 0·8	0·8	0·7, 0·8	0·51	12	11, 16	16	14, 18	0·12	0·6	0·5, 0·7	0·6	0·5, 0·6	0·47	11	8, 12	10	8, 11	0·99
20 : 3*n*-6	2·0	1·9, 2·3	2·1	2·0, 2·4	0·50	482	416, 584	573	500, 662	0·18	2·6	2·4, 2·9	2·5	2·3, 2·9	0·63	76	61, 101	89	82, 136	0·12
20 : 4*n*-6	12·4	11·6, 12·7	12·4	11·8, 12·8	0·66	349	321, 426	420	384, 465	0·06	10·6	9·1, 11·2	11·1	9·8, 11·7	0·38	147	109,157	165	138, 183	0·17
20 : 5*n*-3	0·0	0·0, 0·1	0·0	0·0, 0·1	0·88	2	1, 4	2	1, 6	0·96	0·3	0·2, 0·3	0·2	0·1, 0·2	0·07	8	5, 10	5	4, 7	0·17
22 : 4*n*-6	2·5	2·3, 2·7	2·7	2·5, 2·9	0·08	195	175, 251	251	217, 295	0·14	0·4	0·2, 0·4	0·4	0·3, 0·5	0·24	10	6, 12	12	9, 15	0·12
22 : 5*n*-6	0·3	0·2, 0·4	0·3	0·3, 0·5		ND		ND			0·0	0·0, 0·0	0·0	0·0, 0·1		ND		ND		
24 : 0	5·9	3·5, 6·1	5·6	3·2, 5·7	0·29	ND		ND			0·2	0·1, 0·4	0·2	0·1, 0·4	0·78	ND		ND		
24 : 1*n*-9	4·1	3·9, 4·4	4·5	4·1, 4·7	0·13	ND		ND			1·1	0·8, 1·3	0·9	0·8, 1·2	0·64	ND		ND		
22 : 5*n*-3	0·3	0·2, 0·4	0·3	0·2, 0·4	0·78	26	16, 33	19	11, 33	0·78	0·1	0·1, 0·2	0·1	0·1, 0·2	0·91	4	3, 14	8	5, 13	0·87
22 : 6*n*-3	4·4	4·1, 4·8	4·1	3·9, 4·5	0·28	221	207, 283	263	225, 288	0·63	3·0	2·5, 3·4	3·0	2·3, 3·2	0·49	460	354,660	397	268, 555	0·50
Total *n*-3 fatty acids	4·6	4·3, 5·2	4·7	4·1, 5·0	0·42	255	228, 310	296	243, 311	0·81	3·3	3·1, 3·9	3·3	2·7, 3·6	0·41	467	358,685	416	281, 583	0·47
Total *n*-6 fatty acids	20·8	20·1, 21·3	21·4	20·7, 21·8	0·22	1150	1008,1371	1373	1199,1525	0·10	25·8	24·5, 26·2	26·0	24·7, 26·9	0·46	519	435,577	559	493, 692	0·27
n-6:*n*-3 fatty acids	4·7	4·1, 4·8	4·6	4·4, 5·3	0·18	5	4, 5	5	5, 6	0·07	7·5	6·6, 8·7	8·2	7·4, 10·7	0·19	1	1, 3	2	1, 5	0·14

ND, not determined.
* For details of subjects, supplements and procedures, see Table 1 and p. 136.
† Fish-oil group *n* 27, placebo group *n* 29.
‡ Statistical significance of effect (Mann–Whitney test).

status is low. Fetal requirements appear to be met at the expense of maternal status, which becomes relatively depleted by term. Maternal DHA supplementation apparently limits the extent to which maternal status is compromised by fetal accretion. This may have implications for maternal and fetal status in subsequent pregnancies (Al *et al.* 1997).

In conclusion, DHA supplementation of pregnant women enhances maternal plasma and ERY status (proportion of total fatty acids and concentration) and limits the last trimester decline in maternal DHA status. Maternal supplementation may not directly increase fetal DHA status. The biomagnification (Crawford *et al.* 1976) of DHA from mother to fetus appears to be physiologically pre-determined. The gradient between mother and fetus may, however, be enhanced on the maternal side by fatty acid supplementation, thereby aiding transfer of DHA from mother to fetus.

Acknowledgements

We thank the mothers and babies for participating in the study, and RP Scherer Ltd (Swindon, Wilts., UK) for donating the study capsules. Funding was provided by the University of Glasgow and the Chief Scientist Office, Scotland, UK. We thank Ms Christine Slater, Dr Tom Preston and Mr Martin McMillan for help and advice regarding sample analysis. Professor Forrester Cockburn, Dr Jim Farquharson and Dr Cherry Jamieson are thanked for their interest and advice.

References

Al MD, van Houwelingen AC, Badart-Smook A, Hasaart TH, Roumen FJ & Hornstra G (1995*a*) The essential fatty acid status of mother and child in pregnancy-induced hypertension: a prospective longitudinal study. *Am J Obstet Gynecol* **172**, 1605–1614.

Al MD, van Houwelingen AC, Badart-Smook A & Hornstra G (1995*b*) Some aspects of neonatal essential fatty acid status are altered by linoleic acid supplementation of women during pregnancy. *J Nutr* **125**, 2822–2830.

Al MDM, Hornstra G, van der Schouw YT, Bulstra-Ramakers MTEW & Huisjes HJ (1990) Biochemical EFA status of mothers and their neonates after normal pregnancy. *Early Hum Dev* **24**, 239–248.

Al MDM, van Houwelingen AC & Hornstra G (1997) Relation between birth order and the maternal and neonatal docosahexaenoic acid status. *Eur J Clin Nutr* **51**, 548–553.

Al MDM, van Houwelingen AC, Kester ADM, Hasaart TH, De Jong AEP & Hornstra G (1995*c*) Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status. *Br J Nutr* **74**, 55–68.

Ashby AM, Robinette B & Kay HH (1997) Plasma and erythrocyte profiles of nonesterified polyunsaturated fatty acids during normal pregnancy and labor. *Am J Perinatol* **14**, 623–629.

Berghaus TM, Demmelmair H & Koletzko B (2000) Essential fatty acids and their long-chain polyunsaturated metabolites in maternal and cord plasma triglycerides during late gestation. *Biol Neonat* **77**, 96–100.

Berry C, Montgomery C, Sattar N, Norrie J & Weaver LT (2001) Fatty acid status of women of reproductive age. *Eur J Clin Nutr* **55**, 518–524.

Campbell FM, Gordon MJ & Dutta-Roy AK (1996) Preferential uptake of long chain polyunsaturated fatty acids by isolated human placental membranes. *Mol Cell Biochem* **155**, 77–83.

Carnielli VP, Wattimena DJL, Luijendijk IHT, Boerlage A, Degenhart HJ & Sauer PJJ (1996) The very low birth weight premature infant is capable of synthesizing arachidonic and docosahexaenoic acids from linoleic and linolenic acids. *Pediatr Res* **40**, 169–174.

Carstairs V & Morris R (1990) Deprivation and health in Scotland. *Health Bull (Edinb)* **48**, 162–175.

Clandinin MT (1999) Brain development and assessing the supply of polyunsaturated fatty acid. *Lipids* **34**, 131–137.

Cleland LG, James MJ, Neumann MA, D'Angelo M & Gibson RA (1992) Linoleate inhibits EPA incorporation from dietary fish oil supplements in human subjects. *Am J Clin Nutr* **55**, 395–399.

Connor WE (2000) Importance of *n*-3 fatty acids in health and disease. *Am J Clin Nutr* **71**, 171S–175S.

Connor WE, Lowensohn R & Hatcher L (1996) Increased docosahexaenoic acid levels in human newborn infants by administration of sardines and fish oil during pregnancy. *Lipids* **31**, Suppl., S183–S187.

Crawford MA, Doyle W, Drury P, Lennon A, Costeloe K & Leighfield M (1989) *n*-6 and *n*-3 fatty acids during early human development. *J Intern Med* **225**, Suppl. 1, 159–169.

Crawford MA, Hassam AG & Williams G (1976) Essential fatty acids and fetal brain growth. *Lancet* **i**, 452–453.

Department of Health, Committee on Medical Aspects of Food Policy (1994) *Nutritional Aspects of Cardiovascular Disease: Report on Health and Social Subjects* no. 46. London: H. M. Stationery Office.

Dutta-Roy AK, Campbell FM, Taffess S & Gordon MJ (1996) Transport of long chain polyunsaturated fatty acids across the human placenta: role of fatty acid-binding proteins. In *γ-Linolenic acid: Metabolism and Role in Nutrition and Medicine*, pp. 45–52 [YS Huang and DE Mills, editors]. New York: AOCS Press.

Fidler N, Sauerwald T, Pohl A, Demmelmair H & Koletzko B (2000) Docosahexaenoic acid transfer into human milk after dietary supplementation: a randomized clinical trial. *J Lipid Res* **41**, 1376–1383.

Folch J, Lees M & Stanley GHS (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* **226**, 497–509.

Helland IB, Saarem K, Saugstad OD & Drevon CA (1998) Fatty acid composition in maternal milk and plasma during supplementation with cod liver oil. *Eur J Clin Nutr* **52**, 839–845.

Helland IB, Saugstad OD, Smith L, *et al.* (2001) Similar effects on infants of *n*-3 and *n*-6 fatty acids supplementation to pregnant and lactating women. *Pediatrics*. www.pediatrics.org/cgi/content/full/108/5/e82

Hornstra G, Al MDM, Gerrard JM & Simonis MMG (1992) Essential fatty acid status of neonates born to Inuit mothers: comparison with Caucasian neonates and effect of diet. *Prostaglandins Leukot Essent Fatty Acids* **45**, 125–130.

Hoving EB, van Beusekom CM, Nijeboer HJ & Muskiet FAJ (1994) Gestational age dependency of essential fatty acids in cord plasma cholesterol esters and triglycerides. *Pediatr Res* **35**, 461–469.

Jamieson EC, Farquharson J, Logan RW, *et al.* (1999) Infant cerebellar gray and white matter fatty acids in relation to age and diet. *Lipids* **34**, 1065–1071.

Makrides M, Neumann MA & Gibson RA (1996) Effect of maternal docosahexaenoic acid (DHA) supplementation on breast milk composition. *Eur J Clin Nutr* **50**, 352–357.

Malcolm C, McCulloch DL, Shepher AJ, Montgomery C & Weaver LT (2003) Effect of maternal docosahexaenoic acid (DHA) supplementation during pregnancy on development of the visual evoked potential in term infants: a double blind randomised trial. *Arch Dis Child* (In the Press).

Matorras R, Perteagudo L, Nieto A & Sanjurjo P (1994) Intrauterine growth retardation and plasma fatty acids in the mother and the fetus. *Eur J Obstet Gynecol Reprod Biol* **57**, 189–193.

Olsen SF, Hansen HS, Sommer S, *et al.* (1991) Gestational age in relation to marine *n*-3 fatty acids in maternal erythrocytes: a study of women in the Faroe Islands and Denmark. *Am J Obstet Gynecol* **164**, 1203–1209.

Otto SJ, Houwelingen ACv, Antal M, *et al.* (1997) Maternal and neonatal essential fatty acid status in phospholipids: an international comparative study. *Eur J Clin Nutr* **51**, 232–242.

Otto SJ, van Houwelingen AC, Badart-Smook A & Hornstra G (2001) Changes in the maternal essential fatty acid profile during early pregnancy and the relation of the profile to diet. *Am J Clin Nutr* **73**, 302–307.

Reddy S, Sanders TAB & Obeid O (1994) The influence of maternal vegetarian diet on essential fatty acid status of the newborn. *Eur J Clin Nutr* **48**, 358–368.

Reece MS, McGregor JA, Allen KDG & Harris MA (1997) Maternal and perinatal long-chain fatty acids: possible roles in preterm birth. *Am J Obstet Gynecol* **176**, 907–914.

Salem N Jr, Wegher B, Mena P & Uauy R (1996) Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants. *Proc Nat Acad Sci USA* **93**, 49–54.

Sanjurjo P, Matorras R & Perteagudo L (1995) Influence of fatty fish intake during pregnancy in the polyunsaturated fatty acids of erythrocyte phospholipids in the mother at labor and newborn infant. *Acta Obstet Gynecol Scand* **74**, 594–598.

Sauerwald TU, Hachey DL, Jensen CL & Heird WC (1997) New insights into the metabolism of long chain polyunsaturated fatty acids during infancy. *Eur J Med Res* **2**, 88–92.

van der Schouw YT, Al MDM, Hornstra G, Bulstra-Ramakers MTEW & Huisjes HJ (1991) Fatty acid composition of serum lipids of mothers and their babies after normal and hypertensive pregnancies. *Prostaglandins Leukot Essent Fatty Acids* **44**, 247–252.

van Houwelingen AC, Sørensen JD, Hornstra G, *et al.* (1995) Essential fatty acid status in neonates after fish-oil supplementation during late pregnancy. *Br J Nutr* **74**, 723–731.

Williams C, Birch EE, Emmett PM, Northstone K & The Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC) Study Team (2001) Stereoacuity at age 3.5y in children born full-term is associated with prenatal and postnatal dietary factors: a report from a population-based cohort study. *Am J Clin Nutr* **73**, 316–322.