Essential fatty acid status in neonates after fish-oil supplementation during late pregnancy

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Healthy pregnant women (*n* 23) were supplemented with fish-oil capsules (2·7 g *n*-3 polyunsaturated fatty acids/d) from the 30th week of gestation until delivery. Subjects in a control group were either supplemented with olive-oil capsules (4 g/d, *n* 6) or received no supplementation (*n* 10). Fatty acid compositions of the phospholipids isolated from umbilical plasma and umbilical arterial and venous vessel walls were determined. Fatty acid compositions of maternal venous plasma phospholipids were determined as well. Maternal plasma phospholipids of the fish-oil-supplemented group contained more *n*-3 fatty acids and less *n*-6 fatty acids. Moreover, the amounts of the essential fatty acid deficiency markers Mead acid (20:3*n*-9) and Osbond acid (22:5*n*-6) were significantly lower. The extra amount of *n*-3 fatty acids consumed by the mothers resulted in higher contents of *n*-3 fatty acids, and of docosahexaenoic acid (22:6*n*-3) in particular, in the phospholipids of umbilical plasma and vessel walls. It is, indeed, possible to interfere with the docosahexaenoic acid status at birth: children born to mothers supplemented with fish oil in the last trimester of pregnancy start with a better docosahexaenoic acid status at birth, which may be beneficial to neonatal neurodevelopment.

Fatty acids: Phospholipids: Neonates: Fish oil

Based on fatty acid profiles of cord vessels and adult blood vessels respectively, we obtained strong indications that the biochemical essential fatty acid (EFA) status of neonates, born after normal pregnancy, is not optimal compared with that of adults (Hornstra *et al.* 1989). For its EFA, the developing fetus depends completely on the maternal supply. The fetus seems to have a high requirement of docosahexaenoic acid (DHA; 22:6*n*-3) (Hornstra *et al.* 1989; Al *et al.* 1990), which is of essential importance in fetal brain development (Crawford *et al.* 1976; Clandinin *et al.* 1980). A high intake of long-chain *n*-3 fatty acids in pregnancy has been suggested to prolong gestation (Olsen *et al.* 1986, 1992). In addition, the occurrence of obstetric complications such as pregnancy-induced hypertension (Andersen *et al.* 1989; Secher & Olsen, 1990; Baker & Broughton-Pipkin, 1991; Popeski *et al.* 1991) or intra-uterine growth retardation (Vilbergson *et al.* 1991) may decrease after higher intake of *n*-3 long chain polyenes (LCP) in pregnancy.

In the present study we investigated the feasibility of improving the fetal *n*-3 status by maternal fish-oil supplementation during the last trimester of pregnancy. To assess the fetal EFA status we analysed the fatty acid composition of phospholipids isolated from umbilical plasma and arterial and venous vessel walls of neonates at delivery. Nutrients from the mother are transported to the fetus through the umbilical vein, and blood flows

back from the fetus to the mother via the umbilical arteries. Since the umbilical vessel walls do not have a *vasa vasorum* to obtain their nutrient supply, they obtain their nutrients directly from the blood flowing through them. Therefore, the EFA composition of umbilical venous walls is considered to be a long-term reflection of the EFA supply from mother to fetus, whereas the fatty acid composition of the arterial vessel walls is likely to reflect the EFA status of the 'down-stream' tissue of the developing fetus. In addition, the effect of fish-oil supplementation on the maternal EFA status during pregnancy (30th and 37th weeks of gestation) and at delivery was studied.

SUBJECTS, MATERIALS AND METHODS

Healthy pregnant women were supplemented from the 30th week of gestation until delivery. The present biochemical study is a substudy of a larger population-based trial of fish oil in pregnancy described elsewhere (Olsen *et al.* 1992). The volunteers were allocated at random to two groups: (1) the experimental group (n 23) received four 1 g capsules of fish oil/d (Pikasol: 32 g eicosapentaenoic acid (EPA; 20:5n-3)/100 g fish oil and 23 g DHA (22:6n-3)/100 g fish oil, with 2 mg tocopherol/ml as an antioxidant). This corresponds to 2·7 g n-3 fatty acids/d; (2) the control group (n 16) either received 4 g/d encapsuled olive oil (72% oleic acid (18:1n-9) and 12% linoleic acid (18:2n-6), n 6) or received no supplementation (n 10). Patients with a history of placenta abruption in a previous pregnancy or a serious bleeding episode in the present pregnancy were excluded. Women who regularly used prostaglandin inhibitors were also ineligible. Other exclusion criteria were multiple pregnancy, allergy to fish and regular intake of fish oil.

Fatty acid compositions of phospholipids were determined in maternal venous EDTA-plasma collected in the 30th and 37th weeks of gestation and at delivery, and in venous umbilical plasma as well. In addition, a piece (50 mm) of the umbilical cord was collected immediately after birth, rinsed with saline and stored at $-80°$ until analysed. The arterial and venous vessel walls were dissected free from adjacent tissue, homogenized, and freeze dried.

Total lipid extraction was performed as described by Bligh & Dyer (1959) on 100 μl plasma and 125 μg dry tissue. L-α-Dinonadecanoyl lecithin (PC 19:0) was used as an internal standard to quantify the fatty acids.

Phospholipid fractions were isolated by solid-phase extraction according to the method of Kaluzny *et al.* (1985), after which they were hydrolysed, and their fatty acids methylated with BF$_3$ in methanol (Morissen & Smith, 1964). Fatty acid compositions were then determined by GLC using a capillary column (CP SIL 88 for the vessel walls, and CP SIL 5 for the plasma samples; Chrompack®, Middelburg, The Netherlands). The results were expressed as μg fatty acid/ml plasma or μg/g dry tissue (absolute amount) and as g/100 g total fatty acids (relative amount).

Statistics

The effects of fish-oil consumption were compared with the combined control group (olive oil and no supplement) because the amounts of linoleic acid (18:2n-6) and oleic acid (18:1n-9) given in the olive-oil supplement were very small ($< 3\%$ and $< 10\%$ respectively) compared with the average intake by Danish women (Olsen *et al.* 1992). Linear regression was used to investigate relations between maternal and neonatal values. Differences between the experimental and control groups were evaluated with Student's t tests (two-sided). P values $< 0·05$ were considered significant. The major EFA will be reported, as well as the sum of the n-6 LCP (which includes all n-6 fatty acids except linoleic acid), the sum of the n-3 fatty acids, the sum of the saturated fatty acids and finally the sum of the n-7 and

Table 1. *Clinical characteristics of the subjects*

	Fish oil (*n* 23)			Control (*n* 16)		
	Mean	SE	Range	Mean	SE	Range
Age (years)	28·0	1·02	21–39	28·0	0·584	22–31
Gestational age (d)	285	1·5	271–299	285	1·9	268–296
Gestational age (week.d)	40·5	0·2	38·5–42·5	40·5	0·2	38·2–42·2
Birth weight (g)	3716	69	2945–4350	3547	87	2880–4350
Length at birth (mm)	524	2·6	490–570	518	3·7	490–540
Head circumference (mm)	347	3·7	320–370	351	4·2	320–370

Table 2. *Fatty acid composition of venous umbilical plasma phospholipids in neonates of women consuming a fish-oil supplement and in controls**

(Mean values with their standard errors)

Fatty acid	Fish oil (*n* 18)		Control (*n* 15)		
	Mean	SE	Mean	SE	*P* value†
Total (mg/l)	698·8	33·4	727·9	23·1	NS
g/100 g total fatty acids					
20:5*n*-3	1·40	0·17	0·38	0·04	< 0·0001
22:6*n*-3	7·85	0·41	6·74	0·30	0·0428
18:2*n*-6	7·11	0·30	6·40	0·25	NS
20:4*n*-6	14·96	0·48	17·07	0·28	0·0010
22:5*n*-6	0·30	0·02	0·41	0·06	< 0·0001
20:3*n*-9	0·32	0·03	0·44	0·04	NS
Sum of *n*-3	10·33	0·55	7·78	0·34	0·0007
Sum of *n*-6 LCP	21·48	0·45	24·34	0·36	< 0·0001
Sum of *n*-7 + *n*-9	12·44	0·35	12·78	0·27	NS
Sum of saturates	45·98	0·14	45·51	0·22	NS
18:2*n*-6/20:4*n*-6	0·486	0·03	0·377	0·02	0·0036

LCP, long-chain polyenes.
* For details of procedures, see p. 724.
† Fish-oil group *v.* combined control group (Student's *t* test).

n-9 fatty acids together. Full data detailing all fatty acids separately, are available on request.

RESULTS

Random allocation generated groups that were similar at baseline with respect to available maternal characteristics as well as to the levels of variables characterizing the fetal and maternal EFA status (Table 1). Some samples were not collected at the hospital and we lost some samples during analysis. There were no significant differences in total amounts of fatty acids between the study groups; therefore, only relative values of the individual fatty acids (g/100 g) are given.

Umbilical plasma

The relative values of the fatty acid composition of venous umbilical plasma phospholipids are reported in Table 2. The levels of all *n*-3 fatty acids were significantly higher in the fish-oil group compared with the control group. This was associated with significant reductions

Table 3. *Fatty acid composition of the umbilical vessel wall phospholipids from neonates of women supplemented with fish oil, and controls**

(Mean values with their standard errors)

Fatty acid	Fish oil (n 23)		Control (n 16)		P value†
	Mean	SE	Mean	SE	
Artery					
Total (mg/kg)	15401	953	13751	743	NS
g/100 g total fatty acids					
20:5n-3	0·09	0·02	0·03	0·01	0·01
22:6n-3	6·37	0·18	5·48	0·17	0·0013
18:2n-6	1·15	0·05	1·04	0·05	NS
20:4n-6	11·83	0·32	12·35	0·50	NS
22:5n-6	2·34	0·08	2·68	0·15	0·0311
20:3n-9	2·61	0·11	2·53	0·20	NS
Sum of n-3	7·00	0·21	5·85	0·16	0·0003
Sum of n-6 LCP	17·95	0·40	19·21	0·71	NS
Sum of n-7+n-9	22·20	0·55	21·91	0·86	NS
Sum of saturates	41·71	0·39	41·92	0·60	NS
18:2n-6/20:4n-6	0·10	0·004	0·09	0·004	0·0047
Vein					
Total (mg/kg)	16918	552	16560	768	NS
g/100 g total fatty acids					
20:5n-3	0·10	0·02	0·04	0·01	0·0086
22:6n-3	6·59	0·16	5·62	0·22	0·0008
18:2n-6	1·67	0·07	1·60	0·10	NS
20:4n-6	15·16	0·29	15·84	0·41	NS
22:5n-6	1·85	0·10	2·25	0·14	0·0167
20:3n-9	0·58	0·06	0·63	0·10	NS
Sum of n-3	7·36	0·20	6·02	0·26	0·0002
Sum of n-6 LCP	23·42	0·38	24·60	0·57	NS
Sum of n-7+n-9	15·61	0·38	15·43	0·41	NS
Sum of saturates	42·60	0·43	42·96	0·70	NS
18:2n-6/20:4n-6	0·11	0·005	0·10	0·007	NS

LCP, long-chain polyenes.
* For details of procedures, see p. 724.
† Fish-oil group v. combined control group (Student's *t* test).

in arachidonic acid (20:4n-6) and Osbond acid (22:5n-6), resulting in significantly lower levels of n-6 LCP. There were no significant differences between the groups in the levels of linoleic acid (18:2n-6) and Mead acid (20:3n-9).

Umbilical vessel walls

The total amount of fatty acids in the phospholipid fraction of the arterial walls was on average 10% lower than in the venous wall of the umbilical cords (Table 3). In the arterial as well as in the venous vessel walls, significantly more of the n-3 fatty acids EPA and DHA were present in the fish-oil group than in the control group. This was associated with a significant reduction in the level of Osbond acid. Neither in the arterial nor in the venous vessel walls were significant differences between the study groups observed for the other n-6 fatty acids. In addition, no differences were observed for Mead acid and no influence of the fish-oil supplement could be observed on the difference between arteries and veins (results not shown).

Table 4. *Changes in the fatty acid composition of maternal plasma phospholipids in pregnant women consuming a fish-oil supplement, and in controls**

(Mean values with their standard errors)

Fatty acid	Value at week 30 (n 36)		Change at week 37				
			Fish oil (n 20)		Control (n 16)		
	Mean	SE	Mean	SE	Mean	SE	P value†
Total (µg/ml)	1599	37·0	+181·0	68·0	+175·5	52·5	NS
g/100 g total fatty acids							
20:5n-3	0·64	0·05	+2·19	0·22	+0·15	0·10	< 0·0001
22:6n-3	4·79	0·15	+2·66	0·29	+0·31	0·20	< 0·0001
18:2n-6	20·81	0·28	−2·89	0·41	−0·74	0·53	0·0024
20:4n-6	7·93	0·19	−0·78	0·20	+0·08	0·19	0·0189
22:5n-6	0·36	0·02	−0·20	0·02	+0·02	0·02	< 0·0001
20:3n-9	0·16	0·01	−0·05	0·01	+0·01	0·01	0·0002
Sum of n-3	6·53	0·20	+5·38	0·54	+0·48	0·31	< 0·0001
Sum of n-6 LCP	12·60	0·22	−2·09	0·29	−0·02	0·22	< 0·0001
Sum of n-7+n-9	13·01	0·17	−0·52	0·25	+0·45	0·22	0·0070
Sum of saturates	44·32	0·13	+0·24	0·15	−0·12	0·13	NS
18:2n-6/20:4n-6	0·436	0·02	−0·13	0·08	−0·06	0·12	NS

LCP, long-chain polyenes.
* For details of procedures, see p. 724.
† Fish-oil group v. combined control group (Student's t test).

Maternal plasma

The fatty acid composition of maternal plasma phospholipids immediately before the start of the supplementation at the 30th week of pregnancy and the observed changes at week 37 of gestation are given in Table 4. At week 30 there were no significant differences between the fish-oil group and the control group. Using multiple regression we were not able to find an influence of the amounts of fatty acids in maternal plasma at week 30 on the neonatal fatty acid values. In the fish-oil group the total amount of n-3 fatty acids increased by about 82%. This was accompanied by a decrease in most n-6 fatty acids. The level of Mead acid (the deficiency marker for an overall EFA deficiency, see Holman, 1960) and Osbond acid (a specific marker for the shortage of DHA see Holman, 1977) decreased significantly in the fish-oil group. In the control group no changes were observed. For all fatty acids and fatty acid combinations reported the change in the fish-oil group differed significantly from that in the control group (the sum of the saturates excluded).

For the n-3 fatty acids highly significant correlations were found between the fatty acid values in maternal plasma phospholipids at week 37 (results not shown) as well as at delivery (Table 5), and the neonatal values in plasma as well as the vessel walls (Table 5). With respect to the n-6 fatty acids, correlations were mainly observed between the values in maternal and neonatal plasma. For Osbond acid the maternal values correlated with all neonatal values.

For the arterial vessel wall significant positive correlations were found between 22:6n-3 and gestational age (r 0·35, P = 0·032), and a significant negative correlation was observed between 20:3n-9 and gestational age (r −0·46; P = 0·004).

Table 5. *Correlations between maternal and neonatal fatty acids*

Maternal plasma at delivery and:	Umbilical plasma 30		Umbilical vein 33		Umbilical artery 33	
n...						
	r	P	r	P	r	P
Total (μg/ml)	0·23	NS	0·104	NS	0·02	NS
g/100 g total fatty acids						
20:5n-3	0·81	< 0·0001	0·38	0·0288	0·51	0·0023
22:6n-3	0·36	0·0493	0·53	0·0015	0·61	< 0·0001
18:2n-6	0·63	< 0·0002	0·04	NS	0·23	NS
20:4n-6	0·71	< 0·0001	0·30	NS	0·01	NS
22:5n-6	0·75	< 0·0001	0·43	0·0115	0·39	0·0266
20:3n-9	0·12	NS	0·08	NS	0·09	NS
Sum of n-3	0·67	< 0·0001	0·62	< 0·0001	0·61	0·0002
Sum of n-6 LCP	0·83	< 0·0001	0·44	< 0·0108	0·25	NS
Sum of n-7 + n-9	0·32	NS	0·30	NS	0·06	NS
Sum of saturates	0·29	NS	0·02	NS	0·13	NS
Umbilical plasma and:	Umbilical vein 33		Umbilical artery 33		Artery v. vein 39	
n...						
	r	P	r	P	r	P
Total (μg/ml)	0·04	NS	0·01	NS	0·39	0·0153
g/100 g total fatty acids						
20:5n-3	0·56	0·0007	0·77	< 0·0001	0·82	< 0·0001
22:6n-3	0·56	0·0007	0·58	0·0004	0·58	0·0004
18:2n-6	0·43	0·0136	0·25	NS	0·33	0·0406
20:4n-6	0·51	0·0027	0·28	NS	0·38	0·0406
22:5n-6	0·60	0·0002	0·43	0·0133	0·68	0·0001
20:3n-9	0·12	NS	0·12	NS	0·06	NS
Sum of n-3	0·70	0·0001	0·66	0·0001	0·76	< 0·0001
Sum of n-6 LCP	0·58	0·0004	0·39	0·0271	0·37	0·0205
Sum of n-7 + n-9	0·34	NS	0·32	NS	0·34	0·0374
Sum of saturates	0·09	NS	0·004	NS	0·67	< 0·0001

LCP, long-chain polyenes.

DISCUSSION

Fish-oil supplementation of pregnant women during the last trimester of pregnancy affected the n-3 status of their neonates. In the umbilical vessels, veins and arteries of the fish-oil group a significantly higher amount of DHA (22:6n-3) was found compared with the control group. In the walls of both umbilical vessels this was associated with a significant reduction in the deficiency indicator of DHA, Osbond acid (22:5n-6). These results demonstrate that fish-oil administration in the last trimester of pregnancy increases the fetal DHA content at birth. Since it has been shown that the n-3 status at birth has a rather long-term influence on the postnatal n-3 status (Foreman van Drongelen *et al.* 1995), the observed increase of the n-3 status in the newborn is likely to be of benefit also on the postnatal course of the n-3 status. This might be of importance, considering the postnatal intake of these important fatty acids is much smaller if the neonate is breast-fed, or is almost nil if the neonate is fed by artificial formulas. Since the neonatal conversion of precursor fatty acids to DHA probably occurs at an insufficient rate (Anderson *et al.* 1990), promotion of breast feeding and enrichment of infant formulas with DHA are indicated to improve the postnatal DHA status of newborns. The enhancement of n-3 fatty acids in

early life is related to a better visual acuity (Uauy *et al.* 1992) and discriminative learning (Wainwright, 1992).

As a result of fish-oil supplementation, a decrease in the amount of *n*-6 fatty acid is to be expected (Houwelingen, 1988) and in maternal plasma this was indeed observed. In the venous vessel walls (reflecting the supply from the mother to the child) of the fish-oil group a tendency to a lower level of *n*-6 fatty acids was also observed, but significance was not reached in comparison with the control group. In contrast to the maternal values, the umbilical plasma phospholipid values of linoleic acid tended to be higher in the fish-oil group. Instead of this the arachidonic acid was decreased. This resulted in a significantly higher linoleic acid:arachidonic acid ratio in the fish-oil group (Table 2).

A relationship between arachidonic acid and growth has been observed (Koletzko & Braun, 1991; Carlson *et al.* 1992). However, no relationship was found between the amount of arachidonic acid and birth weight in our study. Moreover, in a large population of Dutch term infants (*n* 110) we were unable to find a correlation between the amount of arachidonic acid in umbilical plasma phospholipids and birth weight (M. D. M. Al, A. C. van Houwelingen and G. Hornstra, unpublished results). Furthermore, no influence of the supplementation could be observed on the arachidonic acid status of umbilical arteries and veins, which might give a longer-term reflection of the fetal fatty acid status of the last period of pregnancy than the umbilical plasma phospholipids.

In the neonatal material, fish-oil supplementation resulted in lower amounts of Osbond acid. Since Osbond acid is a deficiency marker for DHA (Holman, 1977), this clearly demonstrates the better DHA status of the neonates born to the fish-oil-supplemented mothers. Fish-oil supplementation did not significantly affect the average EFA status of the neonates as reflected by the insignificant effects on the neonatal amount of Mead acid.

The amounts of most fatty acids determined in the arteries correlated significantly with the amounts in the vein. However, the high levels of Mead acid in the artery did not correlate with the amount in the vein. Therefore, and because no influence of the *n*-3 supplementation on this fatty acid in the umbilical vessels was observed, it is questionable if the amount of Mead acid in this situation can be used as a deficiency marker. On the other hand, Mead acid is a deficiency marker for the overall EFA status, and because the Σn-3 + Σn-6 fatty acids changed in opposite directions and the calculated EFA status (Σn-3 + Σn-6)/(Σn-7 + Σn-9) did not change due to the *n*-3 supplementation, a change in Mead acid might not be expected. For maternal plasma phospholipids, however, the EFA status was better and the amount of Mead acid was significantly decreased in the fish-oil group.

A positive relationship was observed between gestational age and DHA in the arterial vessel walls (*r* 0·35, *P* = 0·032) and in the umbilical plasma (*r* 0·42, *P* = 0·018). This has been observed before in our Dutch populations (Hornstra, 1992). In addition, it is in agreement with the observation that the fish-oil-supplemented group in the larger study (Olsen *et al.* 1992) had, on average, a longer duration of pregnancy. Although the group selected for this 'biochemical' study did not, on average, have a longer gestation, the range of the gestational age in the fish-oil group was 3 d higher than in the control group.

In the fish-oil group the maternal plasma values showed the expected changes due to the fish-oil consumption (Table 4): an increase in *n*-3 and a decrease in *n*-6 fatty acids. These changes were maximal at week 37 of gestation. It has been reported that changes in fatty acid composition due to fish-oil supplementation result in significantly higher amounts of the EPA-derived eicosanoids thromboxane A_3 and prostaglandin I_3 (Sørensen *et al.* 1993). Since the thromboxane–prostacyclin balance may be involved in the aetiology of pregnancy-induced hypertension and pre-eclampsia (Walsh, 1985), the changes observed might be beneficial in the prevention or treatment of pre-eclampsia and intrauterine growth retardation.

The results described in this paper clearly demonstrate that fish-oil supplementation of pregnant women from the 30th week of pregnancy alters not only their own n-3 status, but that of their neonates as well. The enhancement of n-3 fatty acids in early life is likely to be of benefit (Uauy *et al.* 1992; Wainwright, 1992). Consequently, the higher neonatal $22:6n$-3 status observed after fish-oil supplementation during pregnancy is possibly beneficial. Children from the fish-oil-supplemented women started with a better DHA status. Further evidence is necessary to conclude that the extra n-3 consumption during pregnancy is of functional value for mother and child.

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