Low Arachidonic Acid Rather than α-Tocopherol Is Responsible for the Delayed Postnatal Development in Offspring of Rats Fed Fish Oil Instead of Olive Oil during Pregnancy and Lactation[1]

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ABSTRACT This study was designed to compare in rats the effects of dietary fish oil and olive oil during pregnancy and lactation on offspring development, fatty acid profile and vitamin E concentration. From d 0 of pregnancy, female Sprague-Dawley rats were divided into two groups that were fed purified diets that differed only in their nonvitamin lipid components. One diet contained 10 g fish oil/100 g diet (FOD), whereas the other contained 10 g olive oil/100 g diet (OOD). At d 20 of gestation, maternal adipose tissue fatty acid profile did not differ between rats fed the two diets, whereas both maternal and fetal plasma and liver arachidonic acid (AA) contents were proportionally lower and eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid contents were higher in the FOD group than in the OOD group. α-Tocopherol concentration was lower in maternal and fetal plasma, liver and brain in the FOD group than in the OOD group. The postnatal increase in body weight and length was less and body and psychomotor maturation indices were delayed in pups from FOD-fed dams compared with those from OOD-fed dams. This difference was maintained when pups were cross-fostered at birth, with the delay in postnatal development present in the pups suckling dams fed FOD during lactation. At age 21 d, pups suckling dams fed FOD had lower AA and higher EPA and DHA concentrations in brain phospholipids. Although α-tocopherol in plasma and liver was lower in pups suckling dams fed FOD rather than OOD, brain α-tocopherol concentrations did not differ. Milk yield and milk α-tocopherol and AA concentrations were lower and EPA and DHA were higher in the milk of dams fed FOD compared with those fed OOD. Postnatal development indices and the proportion of plasma, liver and brain AA concentrations, although not plasma, liver and brain α-tocopherol concentrations, recovered to the values found in dams fed OOD when the FOD was supplemented with γ-linolenic acid. However, postnatal development indices were not recovered when the FOD was supplemented with sufficient exogenous vitamin E to increase plasma and liver α-tocopherol concentrations above those in dams fed OOD. Thus, although feeding FOD during pregnancy and lactation decreases both α-tocopherol and AA concentrations, the latter deficiency rather than the former seems to be responsible for delayed postnatal development of rat pups. J. Nutr. 130: 2855–2865, 2000.

KEY WORDS: • *fish oil diet* • *olive oil diet* • *arachidonic acid* • *α-tocopherol* • *rats*

Long-chain polyunsaturated fatty acids (PUFA)[3] are essential to normal growth and development. Docosahexaenoic [DHA, 22:6(n-3)] and arachidonic [AA, 20:4(n-6)] acids are vital components of phospholipid membranes that make up the structural matrix of cell membranes and are deposited in the central nervous system during brain growth (Arbuckle and Innis 1992, Clandinin et al. 1980, Jumpsen and Clandinin 1995). DHA is of major importance for the developing infant because is a major part of the total fatty acids in cerebral cortex and retina phospholipids (Clandinin et al. 1980, Fleisler and Anderson 1983), and AA is the precursor of prostaglandins and leukotrienes (Zurier 1993) and is essential for neonatal growth (Carlson et al. 1992). During pregnancy, these fatty acids are transported from maternal circulation across the placenta (Ruyle et al. 1990), and the fatty acid composition of developing neural tissues can be altered in animals through changes in prenatal and/or postnatal dietary fatty acid composition (Arbuckle and Innis 1992, Carlson et al. 1986, Yonekubo et al. 1993). These alterations affect neurodevelopment as shown by changes in several neurochemical and behavioral variables (Saste et al. 1998) and learning ability (Suzuki et al. 1998, Yonekubo et al. 1994). Excess intake of (n-3) fatty acids, such as that caused by high dietary concentrations of fish oil, decreased the endogenous concentrations of AA (Bourre et al. 1988 and 1990) due to an inhibitory effect on Δ6 desaturase activity (Raz et al. 1997). Dietary supplementation with fish oil is still controversial. High dietary fish oil consumption during pregnancy in rats has been shown to

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[3] Abbreviations used: AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FOD, fish oil diet; FOD-γL, fish oil diet supplemented with γ-linolenic acid; FOD-VE, fish oil diet supplemented with vitamin E; OOD, olive oil diet; PUFA, polyunsaturated fatty acids.

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TABLE 1

Composition of the diets

Ingredient	Olive oil diet	Fish oil diet (FOD)	FOD plus γ-linolenic acid	FOD plus arachidonic acid	FOD plus vitamin E
			g/kg		
Casein	170	170	170	170	170
Salt mix[1]	35	35	35	35	35
Vitamin mix[2]	10	10	10	10	10
Choline chloride	4	4	4	4	4
Cellulose	100	100	100	100	100
Cornstarch	580	580	580	580	580
Olive oil	100	—	—	—	—
Fish oil	—	100	80	80	100
Borage oil	—	—	20	—	—
ARASCO[3]	—	—	—	20	—
dl-α-Tocopherol acetate	0.1	0.1	0.1	0.1	1

[1] Salt mix (g/kg diet): copper sulfate 0.1, ammonium molybdate 0.026, sodium iodate 0.0003, potassium chromate 0.028, zinc sulfate 0.091, calcium hydrogen phosphate 0.145, ammonium ferrous sulfate 2.338, magnesium sulfate 3.37, manganese sulfate 1.125, sodium chloride 4, calcium carbonate 9.89 and potassium dihydrogen phosphate 14.75.

[2] Vitamin mix (mg/kg diet): retinyl palmitate 2.4, cholecalciferol 0.025, menadione sodium bisulfite 0.8, biotin 0.22, cyanocobalamin 0.01, riboflavin 6.6 and thiamine hydrochloride 6.6.

[3] ARASCO, high–arachidonic acid oil from the unicellular fungus *Mortierella alpina.*

improve postnatal learning ability (Yonekubo et al. 1994), and women have been advised to increase the consumption of sardines and fish oil during pregnancy to promote higher concentrations of DHA in the newborn infant (Connor et al. 1996). In addition, fetal DHA reserves have been improved by supplementing pregnant women with fish oil during the last trimester of pregnancy (Van Houwelingen et al. 1995). However, several studies have shown that postnatal supplementation with marine oil led to impaired growth, resulting in lower weight, length and head circumference (for a review on the subject, see Hamosh 1998), an effect that was related to the lower AA concentrations (Carlson and Werkman 1993).

Excess intake of PUFA enhances lipid peroxidation (Berry et al. 1991) and reduces antioxidant capacity (Cho and Choi 1994), enhancing susceptibility to oxidative damage (Mazière et al. 1998), a condition that during pregnancy may be responsible for fetal damage (Simán and Eriksson 1997, Viana et al. 1996). Therefore, the potential negative effect on offspring of high dietary fish oil intake during pregnancy could be affected not only by decreased AA concentrations but also by decreased vitamin E concentrations. On the contrary, dietary olive oil protects the (n-3) PUFA series (Navarro et al. 1994), which have been shown not to affect AA concentrations (Girón et al. 1989, Periago et al. 1990, Rao et al. 1993) and consequently have been proposed to be taken into account in nutritional recommendations (Bourre et al. 1997). In addition, monounsaturated fatty acids are much more resistant to lipid peroxidation (Berry et al. 1991, Öztezcan et al. 1996, Scaccini et al. 1992), and therefore their abundance in the diet could be protective against the loss of vitamin E, which is the main lipophilic antioxidant vitamin.

Therefore, the present study in rats was designed to compare the effects of a diet supplemented during pregnancy and lactation with fish oil versus olive oil on the fatty acid profile and vitamin E concentration of the offspring. Because a decreased postnatal growth rate, as well as a decrease in both AA and vitamin E concentrations, was found in the offspring of rats fed the fish oil–rich diet, the study was extended to determine whether dietary supplementation with either vitamin E, AA or γ-linolenic acid [18:3(n-6)], as a precursor of AA, could ameliorate these changes, as well as to determine whether the cross-fostering between the offspring and dams during lactation would affect the response.

MATERIALS AND METHODS

Animals and diets

Female Sprague-Dawley rats from our animal quarters were initially fed a standard nonpurified diet (B&K Universal, Barcelona, Spain) and housed under controlled light and temperature conditions

TABLE 2

Fatty acid composition and vitamin E concentration of the diets

	Olive oil diet	Fish oil diet	Fish oil diet-γ-linolenic acid	Fish oil diet-arachidonic acid	Fish oil diet-Vitamin E
			g/100 g fatty acids		
Fatty acid					
12:0	0.31	0.01	0.01	0.01	0.01
14:0	0.33	4.11	2.63	3.40	5.40
16:0	10.87	20.66	12.85	15.30	21.64
18:0	3.59	3.48	4.38	6.41	6.19
16:1(n-7)	0.95	6.38	5.45	6.37	8.67
18:1(n-9)	74.83	23.33	23.21	26.17	33.30
18:2(n-6)	7.39	0.01	7.50	4.04	2.50
18:3(n-6)	0.01	0.01	8.13	0.72	0.01
20:4(n-6)	0.01	0.01	0.01	9.60	0.01
20:5(n-3)	0.04	9.54	8.27	7.54	5.49
22:6(n-3)	0.01	11.44	12.38	10.28	10.06
			mmol/kg		
α-Tocopherol	0.14 ± 0.05	0.12 ± 0.03	0.18 ± 0.03	0.12 ± 0.05	1.75 ± 0.03

TABLE 3

Fatty acid composition of maternal plasma, adipose tissue and liver and fetal plasma and liver at d 20 of gestation in rats fed fish oil diet (FOD) or olive oil diet (OOD) during pregnancy[1]

	Dams						Fetuses[2]			
	Plasma		Adipose tissue		Liver		Plasma		Liver	
Fatty acid	FOD	OOD	FOD	OOD	FOD	OOD	FOD	OOD	FOD	OOD
	g/100 g fatty acids									
12:0	0.59 ± 0.09	0.28 ± 0.13	0.18 ± 0.01	0.17 ± 0.05	0.30 ± 0.08	0.25 ± 0.04	1.03 ± 0.29	0.82 ± 0.11	0.42 ± 0.07	0.37 ± 0.08
14:0	1.29 ± 0.40	0.79 ± 0.17	3.05 ± 0.19**	1.15 ± 0.06	0.26 ± 0.07	0.40 ± 0.08	2.19 ± 0.10	1.83 ± 0.23	0.97 ± 0.14	1.52 ± 0.12
16:0	17.6 ± 1.4	19.2 ± 1.9	26.0 ± 4.5	21.2 ± 0.7	23.8 ± 2.5	21.0 ± 1.8	44.3 ± 1.0*	31.7 ± 3.9	29.6 ± 2.8	31.8 ± 1.6
18:0	11.4 ± 0.4	10.4 ± 1.4	4.09 ± 0.45	2.99 ± 0.21	20.1 ± 2.0	16.1 ± 1.5	16.4 ± 1.9	12.1 ± 1.3	16.2 ± 0.6**	11.6 ± 0.4
16:1(n-7)	3.57 ± 0.07	1.91 ± 0.17	10.9 ± 0.7**	4.69 ± 0.26	1.59 ± 0.39	1.54 ± 0.21	3.78 ± 0.32	4.11 ± 0.55	3.27 ± 0.27	3.67 ± 0.39
18:1(n-9)	20.0 ± 0.8*	33.7 ± 6.1	38.7 ± 3.7**	59.6 ± 1.0	12.4 ± 1.0**	29.3 ± 2.6	19.7 ± 1.2	25.8 ± 3.4	17.1 ± 1.3*	26.1 ± 1.7
18:2(n-6)	12.7 ± 0.5	8.49 ± 1.76	8.26 ± 0.61	8.92 ± 0.48	3.40 ± 0.64	4.29 ± 0.11	3.61 ± 0.34**	4.49 ± 0.83	3.01 ± 0.22	4.42 ± 0.49
20:4(n-6)	2.30 ± 0.40*	9.41 ± 2.32	0.19 ± 0.07	0.12 ± 0.04	3.87 ± 0.52†	15.4 ± 3.3	0.84 ± 0.84*	11.1 ± 3.8	2.82 ± 0.38*	7.64 ± 0.58
20:5(n-3)	4.98 ± 0.76†	0.74 ± 0.59	1.72 ± 0.32†	0.16 ± 0.11	7.95 ± 0.97**	0.98 ± 0.60	2.23 ± 0.62	0.74 ± 0.74	8.01 ± 0.39*	4.04 ± 0.74
22:6(n-3)	7.11 ± 1.41†	1.30 ± 0.11	4.24 ± 0.52**	0.01 ± 0.01	24.7 ± 0.7**	4.55 ± 0.44	2.77 ± 0.38	1.37 ± 0.79	16.7 ± 0.8**	3.55 ± 0.53

[1] Values are expressed as means ± SEM, $n = 5$. Statistical comparison between the OOD and the FOD was made with the Student's t test. * $P < 0.05$, † $P < 0.01$, ** $P < 0.001$.

[2] Samples from all of the fetuses of the same dam were pooled and used as an experimental unit.

(12-h light/dark cycle; 22 ± 1°C). The experimental protocol was approved by the Animal Research Committee of the University San Pablo-CEU in Madrid, Spain. Rats were mated when they weighed 180–190 g, and on the day in which spermatozoids were found in vaginal smears (d 0 of pregnancy), they were divided into two groups that were fed purified diets that differed only in the nature of the nonvitamin lipid component: one contained 10 g fish oil/100 g diet acids (FOD) and the other contained 10 g olive oil/100 g diet (OOD). The composition of these diets and their proportional fatty acid contents are shown in **Tables 1** and **2**. Both diets contained a similar amount of vitamin E (Table 2); they both were isoenergetic (both providing ~16.24 kJ/g) and were fed to the rats on an ad libitum basis. Diets were prepared at the onset of the experiments and were divided into daily portions that were kept at −20°C until use. Dams were housed in collective cages (four per cage) and had free access to the assigned diet and tap water. Fresh food was provided every 24 h, and the daily food intake was estimated periodically.

Experiment 1. Some rats from each group were decapitated on d 20 of pregnancy and/or after the start of the diet, and trunk blood was collected into receptacles containing 1 g of Na_2-EDTA/L. The two uterine horns were immediately dissected and weighed with their contents to obtain the whole "conceptus" weight. Livers and lumbar adipose tissues were quickly removed and placed in liquid nitrogen before freezing at −80°C until analysis. Fetuses were weighed and decapitated, and the blood, brain and liver were collected as indicated earlier. Samples from all of the fetuses of the same dam were pooled separately and processed in parallel to the samples of the adults.

Experiment 2. Another set of pregnant rats fed either FOD or OOD as described were allowed to spontaneously deliver. Litters were adjusted to eight pups that suckled their dams. Pups and dams were fed freely the corresponding diet throughout the lactation period. The body weight and length (crown-to-rump length) were measured at different days of age. Opening of the eyelids, opening of the ear and the acquisition of both the surface righting reflex (SRR) and the air

TABLE 4

Effect of fish oil diet (FOD) and olive oil diet (OOD) during pregnancy on α-tocopherol levels in the rats[1]

α-Tocopherol level	FOD	OOD
Dam		
Plasma, *μmol/L*	15.2 ± 2.3	27.7 ± 3.0*
Fetus[2]		
Plasma, *μmol/L*	3.02 ± 0.23	7.31 ± 0.46†
Liver, *μmol/kg*	23.2 ± 2.1	61.8 ± 5.1†
Brain, *μmol/kg*	13.9 ± 0.7	19.1 ± 0.7†

[1] Values are expressed as means ± SEM. Differences between OOD ($n = 5$) and FOD ($n = 5$) groups are indicated by the P values.

[2] Samples from all of the fetuses of the same dam were pooled and used as an experimental unit.

* $P < 0.01$, † $P < 0.001$.

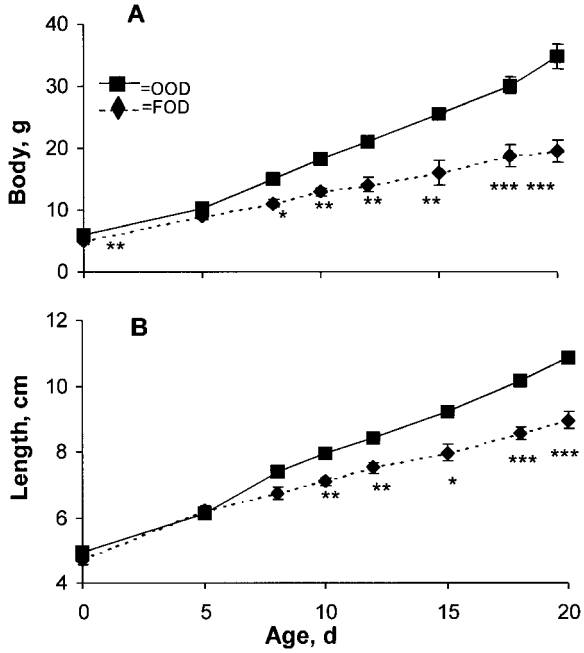

FIGURE 1 Body weight (A) and length (B) in newborn pups of rats fed fish oil diet (FOD) or olive oil diet (OOD) during gestation and lactation (expt. 2). Mean of all pups from each litter was used as experimental unit, and values are means ± SEM, $n = 6$ or 7 litters. Significant difference between the groups: *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

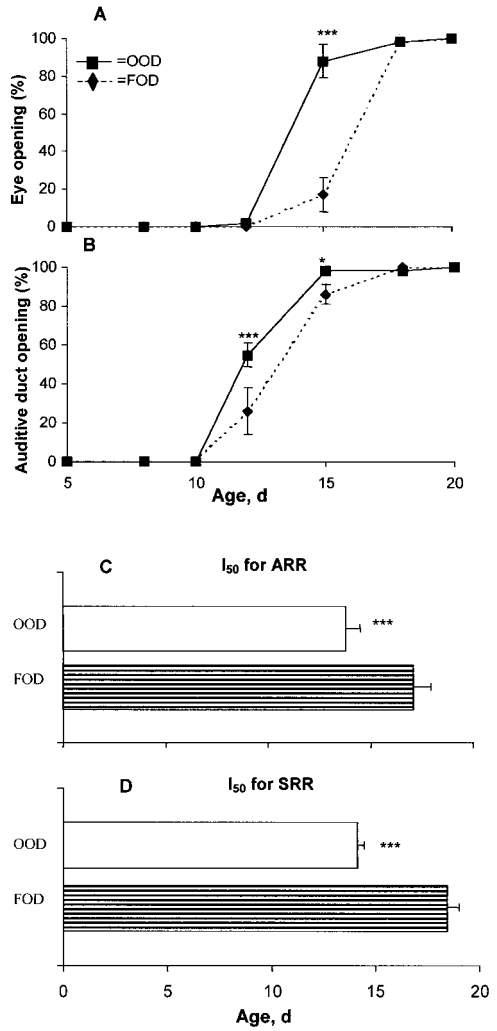

FIGURE 2 Acquisition of eyelid (A) and ear opening (B) expressed as the percentage of pups per litter attaining mature response, and air righting (ARR) (C) and surface righting (SRR) reflexes (D) expressed as the day that 50% of the litter acquired the mature response (I_{50}) in offspring of rats fed fish oil diet (FOD) or olive oil diet (OOD) (expt. 2). Mean of all pups from each litter was used as experimental unit, and values are means ± SEM, n = 6 or 7 litters. Significant difference between the groups, $^{*}P < 0.05$ and $^{***}P < 0.001$.

righting reflex (ARR) were tested as described previously (Lopez Tejero et al. 1986) on the appropriate days until the age of 20 d. Results are expressed as the cumulative percentage of pups per litter attaining mature responses. Dams and pups were decapitated as described at d 21 after delivery. The brains and livers of each pup were rapidly removed, placed into liquid nitrogen and kept at −80°C until processed.

Experiment 3. Another set of rats were fed the same diets as described earlier during pregnancy and lactation, and milk yield was estimated from pup weight and weight gain on d 7–8 and 14–15 of lactation as described previously (Sampson and Jansen 1984). On d 10, after being separated from their litters, dams were anesthetized with 0.5 mL/200 g of a cocktail containing 9 mg ketamine (Imalgene 500; Rhone Merieux, Lyon, France) and 0.25 mg chlorpromazine (Largactil; Rhone Poulenc, Madrid, Spain) administered intraperitoneally. The rats were injected intraperitoneally with 0.25 mL/200 g of a solution of oxytocin (2000 IU/L Syntocinón; Novartis Farmaceutica, Barcelona, Spain), and milk was obtained with gentle hand stripping of the teats. An aliquot of milk was immediately placed into chloroform/methanol (2:1) for lipid extraction (Folch et al. 1957), and another aliquot was kept at −80°C until processed.

Experiment 4. Another set of rats were fed FOD or OOD during pregnancy and lactation, but at the time of delivery, litters were cross-fostered. Thus, pups of dams fed FOD suckled dams fed OOD (FOD-OOD), and pups of dams fed OOD suckled dams fed FOD (OOD-FOD). Other litters were allowed to suckle from different dams that had been fed the same diet as were fed their actual dams during pregnancy (FOD-FOD and OOD-OOD), and all pups were studied in parallel. Litter size was always kept to eight per dam. Body weight, length and the different maturation tests were studied as described, and the dams and pups from all of the groups were decapitated on d 21 of lactation according to the same protocol described earlier.

Experiment 5. Other rats were fed modified FOD during pregnancy and lactation. FOD was supplemented with 1 g of *dl*-α-tocopherol acetate/kg (FOD-VE), or the fish oil content was reduced to 8% and the diet was supplemented with 2% borage oil (Larodan Fine Chemicals, Malmö, Sweden) with a γ-linolenic acid [18:3(n-6)] content of >40% (FOD-γL) or with ARASCO (Martek Biosciences, Columbia, MD), which is a triglyceride oil that contains >40% AA, no (n-3) fatty acids and small amounts of other long-chain PUFA (FOD-AA) (Tables 1, 2). The vitamin E contents in these diets were similar except for FOD-VE, in which the vitamin E was >10 times higher than that of any of the other diets. In this experiment, pups were allowed to suckle their dams, and body and psychomotor maturation were studied as described earlier, with the pups killed on d 21 after delivery.

Processing of samples

Lipid extraction and purification (Folch et al. 1957) were carried out with fresh aliquots of each diet, as well as with plasma (separated from fresh blood through centrifugation at 1500 × *g* for 15 min at 4°C), milk, frozen livers, adipose tissues and brains. Phospholipids were separated through thin layer chromatography in Silicagel 60 F_{254}, as described elsewhere (Ruiz and Ochoa 1997). Spots corresponding to phospholipids were eluted with methanol/toluene (4:1). Total lipid or phospholipid fatty acids were simultaneously saponified and methylated according to the method of Lepage and Roy (1984 and 1986). Fatty acid methyl esters were separated and quantified on a Perkin–Elmer gas chromatograph (Autosystem; Norwalk, CT) with a flame ionization detector and a 30-m × 0.25-mm Omegawax capillary column. Nitrogen was used as carrier gas, and the fatty acid methyl esters were compared with purified standards (Sigma Chemical Co., St. Louis, MO). Individual fatty acids are expressed as percent of total fatty acids in the sample.

α-Tocopherol was measured in plasma, milk, liver and brain samples through HPLC, according to methods previously described (Barbas et al. 1997, Barbas and Herrera 1998). α-Tocopherol and α-tocopheryl acetate were measured in fresh diets (Rupérez et al. 1999) and expressed as α-tocopherol.

Statistical analysis

Data are expressed as means ± SEM. Treatment effects (diet) were analyzed by one-way ANOVA with Systat Version 5.03a (Wilkinson, Evanston, IL). When treatment effects were significantly different ($P < 0.05$), means were tested by Tukey's test, and linear regressions were calculated by the least-squares method (Quaresima et al. 1996). Differences between two groups were analyzed by Student's t test. Significance was set at the $\alpha = 0.05$ error rate.

RESULTS

Neither the dam weight change during gestation nor the number of fetuses per litter or fetal weight on d 20 of gestation differed between groups (data not shown).

The fatty acid composition of plasma of rats fed FOD contained significantly less oleic acid [18:1(n-9)] and AA [20:4(n-6)] and more DHA [22:6(n-3)] and eicosapentaenoic acid [EPA, 20:5(n-3)] than the plasma of rats fed OOD (**Table 3**). A similar difference in the proportion of fatty acids was seen in both maternal liver and lumbar adipose tissue, except

TABLE 5

Fatty acid profile and vitamin E concentration in plasma, liver and brain of 21-d-old rats that suckling dams fed either fish oil diet (FOD) or olive oil diet (OOD) during pregnancy and lactation[1]

	Plasma, total fatty acids		Liver, total fatty acids		Brain, phospholipid fatty acids	
Fatty acid	FOD	OOD	FOD	OOD	FOD	OOD
			g/100 g fatty acids			
12:0	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
14:0	0.80 ± 0.17	0.69 ± 0.07	1.17 ± 0.22	1.58 ± 0.10	0.59 ± 0.04†	0.41 ± 0.04
16:0	20.1 ± 0.6	18.2 ± 1.2	20.5 ± 1.1	18.5 ± 0.2	26.8 ± 1.3	25.3 ± 1.1
18:0	11.4 ± 1.3	11.1 ± 0.9	12.6 ± 0.7*	10.8 ± 0.4	23.3 ± 0.3	22.1 ± 0.9
16:1(n-7)	2.33 ± 0.18†	1.66 ± 0.11	1.90 ± 0.27	1.92 ± 0.29	1.03 ± 0.06	0.88 ± 0.12
18:1(n-9)	19.7 ± 1.1**	39.4 ± 1.9	16.1 ± 1.3**	44.0 ± 1.0	14.0 ± 1.4	13.5 ± 0.6
18:2(n-6)	7.05 ± 0.56**	10.8 ± 0.4	4.17 ± 0.26†	5.50 ± 0.20	0.71 ± 0.02	0.60 ± 0.05
20:4(n-6)	4.51 ± 0.34**	13.0 ± 1.3	4.03 ± 0.35**	9.72 ± 0.32	6.46 ± 0.25**	11.7 ± 0.5
20:5(n-3)	13.0 ± 1.8**	0.66 ± 0.32	7.24 ± 0.17**	1.05 ± 0.58	3.98 ± 0.74*	1.77 ± 0.49
22:6(n-3)	11.0 ± 1.3**	1.24 ± 0.11	26.4 ± 1.1**	2.33 ± 0.15	17.4 ± 0.5*	12.9 ± 1.3
	μmol/L		*μmol/kg*			
α-Tocopherol	16.2 ± 7.5*	38.3 ± 1.2	65.5 ± 9.1*	103 ± 13	36.2 ± 1.4	35.3 ± 1.9

[1] Values are expressed as means ± SEM, $n = 6$ or 7. Statistical comparison between the OOD and the FOD group was made with the Student's t test. * $P < 0.05$, † $P < 0.01$, ** $P < 0.001$.

that in adipose tissue, the amount of AA was practically undetectable in both groups and the proportion of both myristic [14:0] and palmitoleic [16:1(n-7)] acids was higher in rats fed FOD than in rats fed OOD (Table 3). In fetal plasma, the proportions of the different fatty acids did not differ between the groups, except for palmitic acid [16:0], AA and linoleic acid. Palmitic acid was higher and both AA and linoleic acid [18:2(n-6)] were lower in fetuses of dams fed FOD rather than OOD; the difference was especially striking for AA, being barely detectable in plasma of fetuses of dams fed FOD (Table 3). In fetal liver, the proportions of stearic acid [18:0], EPA and DHA were higher, whereas the proportions of oleic acid and AA were lower in the former (Table 3). Similarities in the fatty acid profile between maternal plasma and fetal liver prompted us to calculate linear correlations with individual values, and we found that although the correlation was not significant when all saturated fatty acids were considered ($r = 0.04$, $n = 10$), it was significant when either monounsaturated fatty acids ($r = 0.73$, $n = 10$, $P < 0.05$) or (n-6)- ($r = 0.89$, $n = 9$, $P < 0.01$) or (n-3)- ($r = 0.86$, $n = 10$, $P < 0.01$) PUFA were considered.

The concentrations of α-tocopherol in both maternal and fetal plasma as well as in fetal liver and brain were significantly lower in the FOD group than in the OOD group (**Table 4**).

Pregnant rats fed both diets were allowed to deliver (expt. 2). Although no difference was found in litter size between groups (13.8 ± 1.2 in FOD and 12.5 ± 0.8 in OOD), this variable was adjusted to eight pups per litter, and pups were allowed to suckle from their own dams. From the 1st d after birth, pups of rats fed FOD had lower body weights, with this difference especially striking from d 8 on (**Fig. 1***A*). The length of the pups did not differ between groups up to age 10 d, but from then on, pups of dams fed FOD were shorter than those of dams fed OOD (Fig. 1*B*). Several tests related to body and psychomotor maturation were carried out in these pups, and results showed that either eye or auditive duct opening, as well as ARR or SRR acquisition, occurred earlier in the pups of dams fed OOD than in the pups of dams fed FOD (**Fig. 2**). These pups were killed at d 21 of suckling, and their plasma had higher palmitoleic acid concentrations, whereas both EPA and DHA were much higher in those suckling dams fed FOD rather than OOD. Oleic and linoleic acids and AA were lower in those suckling dams fed FOD rather than OOD (**Table 5**). Livers of pups suckling dams fed FOD had higher stearic acid, EPA and DHA and lower oleic and linoleic acid and AA than those suckling dams fed OOD (Table 5). In brain phospholipids, the proportion of AA was lower and the proportions of myristic acid, EPA and DHA were higher in pups suckling dams fed FOD compared with those suckling dams fed OOD (Table 5). The concentration of α-tocopherol was significantly lower in plasma and liver of pups suckling dams fed FOD than of pups of dams fed OOD, whereas α-tocopherol in brain did not differ between the two groups (Table 5).

To determine whether the differences between pups of dams fed FOD and those of dams fed OOD were a consequence of the intrauterine milieu or were affected by suckling, newborns from rats fed either FOD or OOD during pregnancy were cross-fostered (expt. 4). Both body weight and length were lower in suckling rat pups whose dams were fed OOD during pregnancy but that suckled dams fed FOD (OOD-FOD), to the concentration found in the FOD-FOD group, whereas both variables recovered in FOD-OOD pups to the concentration found in OOD-OOD pups (**Fig. 3**). Similar intergroup relationships were found in the body and psychomotor maturation indices studied, because both the eye and auditive duct opening and both the ARR and the SRR acquisition occurred later in the FOD-FOD and OOD-FOD pups than in either FOD-OOD or OOD-OOD pups, although for both ARR and SRR, the difference between OOD-FOD and FOD-OOD was not significant ($P = 0.506$ and 0.257) (**Fig. 4**).

At d 21 of suckling, plasma, liver and brain phospholipid fatty acid profiles did not differ in pups suckling dams fed the same diet, independent of the diet fed during pregnancy, whereas they were substantially different between those suckling dams fed FOD compared with those fed OOD during lactation (**Table 6**). In plasma, EPA and DHA were lower and oleic acid and AA were higher in both FOD-OOD and OOD-OOD than in FOD-FOD or OOD-FOD pups. In liver, myristic

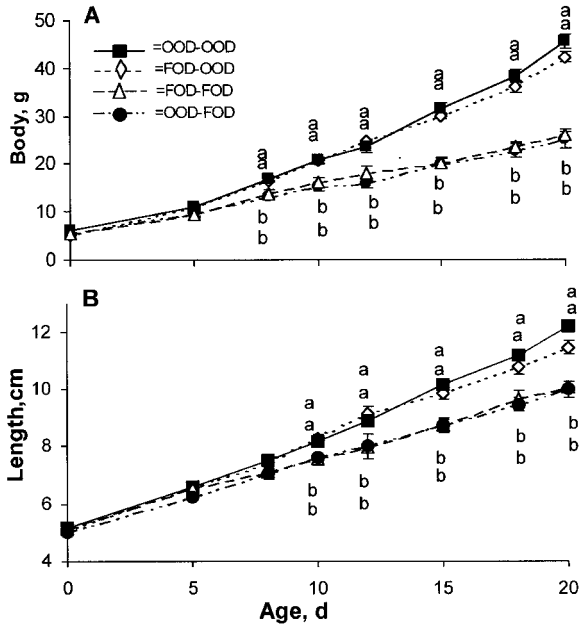

FIGURE 3 Body weight (A) and length (B) in newborn pups from rats fed fish oil diet (FOD) during pregnancy and lactation (FOD-FOD), FOD during pregnancy and olive oil diet (OOD) during lactation (FOD-OOD), OOD during pregnancy and FOD during lactation (OOD-FOD) or OOD during pregnancy and lactation (OOD-OOD) (expt. 4). Mean of all pups from each litter was used as experimental unit, and values are means ± SEM, $n = 6$ or 7 litters. Pairwise differences were analyzed by Tukey's test after ANOVA. Different letters indicate significant differences between the groups ($P < 0.05$) for pups of the same age.

and oleic acids and AA were higher, whereas both EPA and DHA were lower in FOD-OOD and OOD-OOD than in FOD-FOD or OOD-FOD. In brain phospholipids, EPA and DHA were lower and linoleic acid and AA were higher in FOD-OOD and OOD-OOD than in either FOD-FOD or OOD-FOD (Table 6). α-Tocopherol concentrations in both plasma and liver were much higher in pups from the FOD-OOD and OOD-OOD groups than in pups from the FOD-FOD and OOD-FOD groups, whereas in brain, no differences were found among the four groups (Table 6).

The differences detected in growth and fatty acid profile between the pups suckled by dams fed FOD and OOD prompted us to determine milk production and composition (expt. 3). The feeding of FOD during pregnancy and lactation decreased milk yield measured at d 7–8 and 15–16 after delivery compared with dams fed OOD (**Table 7**), although food intake did not differ between the two groups (data not shown). Although the concentration of total fatty acids in milk (mainly in the form of triglycerides) did not differ between the two groups (data not shown), the proportions of myristic acid, palmitic acid, stearic acid, palmitoleic acid, DHA and EPA were higher and the proportions of oleic acid and AA were lower in milk from rats fed FOD than in milk from those fed OOD (Table 7). The concentration of α-tocopherol in milk from FOD-fed rats was much lower than that from OOD-fed rats (Table 7).

A negative effect of the deficient concentration of either AA or α-tocopherol in pups of lactating dams fed FOD may have contributed to their decreased growth and body and psychomotor maturation. To determine which of these two components was responsible for the effects, during lactation the FOD was supplemented with either γ-linolenic acid [18:3(n-6)] as substrate for endogenous AA synthesis (FOD-γL), AA (FOD-AA) or vitamin E (FOD-VE) (expt. 5). Substantial amounts of γ-linolenic acid were present in FOD-γL and of AA in FOD-AA, both of which were practically absent in the other diets (Table 2). A similar concentration of vitamin E was present in all diets, except for the FOD-VE, in which the vitamin E concentration was >10 times higher than that in any other diet (Table 2). Compared with pups suckling dams fed OOD, both body weight and length were significantly lower in FOD or FOD-VE pups, whereas no difference was found between those of OOD and FOD-γL pups, and the pups of dams fed FOD-AA had a lower body weight than those of dams fed OOD, although length did not differ (**Table 8**). The date of acquisition of either ARR or SRR was also delayed in pups of dams fed FOD, FOD-AA or FOD-VE compared with those from dams fed OOD, whereas no differences were found between pups of dams fed OOD or FOD-γL diets (Table 8).

Except for lower oleic acid in plasma and liver of pups of dams fed FOD with any supplement compared with those of

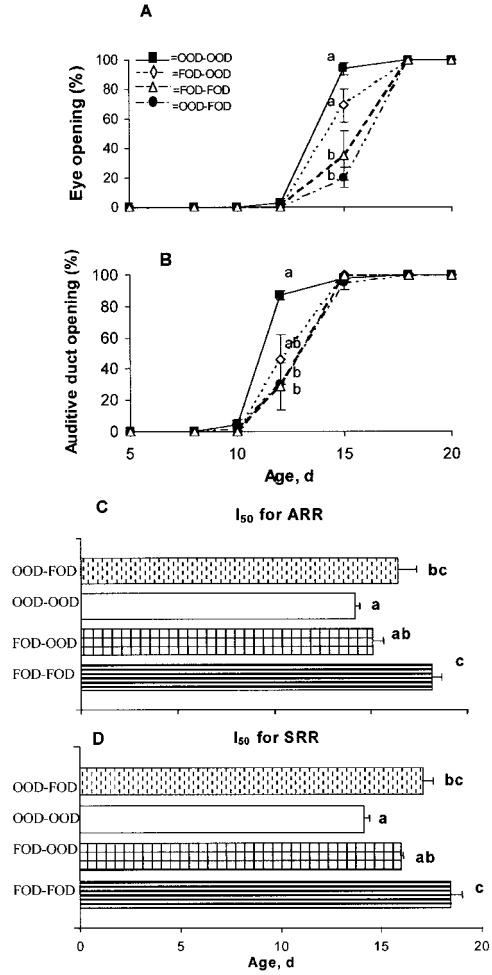

FIGURE 4 Acquisition of eyelid (A) and ear opening (B) expressed as the percent of pups per litter attaining mature response, and air righting (ARR) (C) and surface righting (SRR) reflexes (D) expressed as the day that 50% of the litter acquired the mature response (I_{50}) in newborn pups from rats fed fish oil diet (FOD) during pregnancy and lactation (FOD-FOD), FOD during pregnancy and olive oil diet (OOD) during lactation (FOD-OOD), OOD during pregnancy and FOD during lactation (OOD-FOD) or OOD during pregnancy and lactation (OOD-OOD) (expt. 4). Mean of all pups from each litter was used as experimental unit, and values are means ± SEM, $n = 6$ or 7 litters. Pairwise differences were analyzed by Tukey's test after ANOVA. Different letters indicate significant differences between the groups ($P < 0.05$).

TABLE 6

Fatty acid profile and α-tocopherol concentration in plasma, liver and brain of 21-d-old rats suckling dams fed either the fish oil diet (FOD) or the olive oil diet (OOD) during pregnancy and cross-fostered during lactation[1,2]

	FOD-FOD	FOD-OOD	OOD-OOD	OOD-FOD
Plasma fatty acids				
		g/100 g fatty acids		
12:0	0.98 ± 0.18	0.92 ± 0.31	1.47 ± 0.21	0.65 ± 0.24
14:0	2.78 ± 0.17	1.91 ± 0.38	2.76 ± 0.30	2.48 ± 0.40
16:0	23.3 ± 1.1^{a}	19.5 ± 1.1^{ab}	18.1 ± 0.3^{b}	22.1 ± 0.7^{a}
18:0	9.87 ± 0.38	10.2 ± 0.4	10.2 ± 0.4	9.77 ± 0.36
16:1(n-7)	2.75 ± 0.21^{a}	1.88 ± 0.42^{ab}	1.43 ± 0.18^{b}	2.95 ± 0.27^{a}
18:1(n-9)	15.3 ± 0.7^{b}	39.8 ± 3.4^{a}	41.3 ± 1.2^{a}	14.7 ± 1.1^{b}
18:2(n-6)	6.22 ± 0.23^{b}	9.55 ± 0.30^{a}	9.85 ± 0.18^{a}	7.49 ± 0.37^{b}
20:4(n-6)	5.27 ± 0.15^{b}	11.1 ± 1.0^{a}	12.8 ± 0.6^{a}	5.95 ± 0.38^{b}
20:5(n-3)	18.5 ± 0.7^{a}	1.48 ± 1.48^{b}	0.01 ± 0.01^{b}	19.1 ± 0.3^{a}
22:6(n-3)	13.4 ± 0.7^{a}	3.01 ± 1.59^{b}	1.47 ± 0.31^{b}	13.9 ± 0.4^{a}
Plasma α-tocopherol, *μmol/L*	29.1 ± 7.6^{b}	59.2 ± 2.0^{a}	60.3 ± 1.8^{a}	23.4 ± 1.9^{b}
		g/100 g fatty acids		
Liver fatty acids				
12:0	0.23 ± 0.05^{ab}	0.40 ± 0.04^{a}	0.28 ± 0.07^{ab}	0.23 ± 0.03^{b}
14:0	1.05 ± 0.19^{b}	2.34 ± 0.10^{a}	1.93 ± 0.20^{a}	1.16 ± 0.09^{b}
16:0	22.1 ± 0.9^{a}	19.7 ± 0.4^{ab}	17.5 ± 1.2^{b}	20.2 ± 1.1^{ab}
18:0	13.2 ± 0.5^{a}	10.3 ± 0.3^{bc}	9.50 ± 0.70^{c}	11.9 ± 0.6^{ab}
16:1(n-7)	1.48 ± 0.17	2.01 ± 0.16	1.51 ± 0.43	1.50 ± 0.11
18:1(n-9)	12.5 ± 0.8^{c}	44.0 ± 0.7^{a}	37.7 ± 2.2^{b}	12.8 ± 0.3^{c}
18:2(n-6)	3.31 ± 0.68	4.77 ± 0.14	4.68 ± 0.27	3.90 ± 0.37
20:4(n-6)	4.24 ± 0.32^{b}	8.66 ± 0.28^{a}	8.06 ± 0.79^{a}	3.76 ± 0.37^{b}
20:5(n-3)	6.03 ± 0.59^{a}	0.07 ± 0.03^{b}	0.08 ± 0.03^{b}	5.45 ± 0.54^{a}
22:6(n-3)	26.2 ± 1.1^{a}	2.95 ± 0.11^{b}	2.83 ± 0.30^{b}	23.5 ± 1.9^{a}
Liver α-tocopherol, *μmol/kg*	142 ± 13^{b}	560 ± 48^{a}	558 ± 41^{a}	121 ± 14^{b}
		g/100 g fatty acids		
Brain phospholipid fatty acids				
12:0	0.01 ± 0.01	0.01 ± 0.14	0.01 ± 0.01	0.01 ± 0.01
14:0	0.90 ± 0.14	0.22 ± 0.14	0.42 ± 0.28	0.30 ± 0.18
16:0	30.6 ± 2.5^{a}	25.9 ± 1.1^{ab}	22.9 ± 1.3^{b}	30.8 ± 2.3^{a}
18:0	18.7 ± 2.3^{c}	30.8 ± 1.4^{a}	27.6 ± 1.2^{b}	23.4 ± 1.7^{bc}
16:1(n-7)	1.10 ± 0.58^{a}	0.01 ± 0.01^{b}	0.01 ± 0.01^{b}	0.13 ± 0.12^{ab}
18:1(n-9)	15.6 ± 4.0^{ab}	15.4 ± 1.4^{ab}	17.8 ± 2.2^{a}	9.65 ± 1.27^{b}
18:2(n-6)	5.38 ± 0.67^{b}	8.36 ± 0.52^{a}	8.69 ± 0.72^{a}	4.81 ± 0.86^{b}
20:4(n-6)	2.73 ± 0.47^{b}	5.32 ± 0.64^{a}	6.97 ± 0.59^{a}	1.46 ± 0.45^{b}
20:5(n-3)	6.41 ± 1.43^{ab}	4.62 ± 0.76^{b}	1.68 ± 0.61^{b}	9.89 ± 1.95^{a}
22:6(n-3)	9.53 ± 3.16^{a}	1.83 ± 0.42^{c}	2.33 ± 0.26^{bc}	8.82 ± 2.37^{ab}
Brain α-tocopherol, *μmol/kg*	41.1 ± 0.9	38.1 ± 0.9	36.5 ± 0.2	35.8 ± 2.3

[1] Values are expressed as means ± SEM, $n = 6$ or 7.
[2] Tukey's test was used to determine differences between groups after one-way ANOVA. Different superscripts in a row indicate significant differences ($P < 0.05$). No superscript letters in a row indicate no significant differences.

dams fed OOD and a similar content of monounsaturated fatty acids in brain phospholipids in pups from all the groups, major differences in fatty acid profile included significantly lower AA in plasma and liver fatty acids and in brain phospholipids in pups of dams fed either FOD or FOD-VE and more AA in plasma and liver fatty acids in pups of dams fed FOD-AA compared with pups of dams fed OOD (**Table 9**). Compared with pups of dams fed OOD, α-tocopherol concentration in plasma and liver was greater in pups of dams fed FOD-VE than in any of the other groups, whereas values in pups of dams fed OOD were higher than those of pups of dams fed FOD, FOD-γL or FOD-AA (Table 9). However, brain α-tocopherol did not differ among the groups (Table 9).

DISCUSSION

The present study shows that a deficiency of AA and α-tocopherol occurs in both in dams and fetuses when rats are fed a diet with a moderate amount of fish oil (10%) as the only nonvitamin fat component in comparison with those fed the same diet but containing olive oil instead of fish oil during pregnancy, and a similar effect is found 21 d after delivery in pups when the dietary treatment is maintained during lactation. In fact, when pups from rats fed FOD were studied during suckling, a decreased growth rate and a delay in the acquisition of body and psychomotor maturation indices were found with the AA and α-tocopherol deficiencies. The effect was also

TABLE 7

Milk yield at d 7/8 and 15/16 and composition at d 10 after delivery in rats fed either fish oil diet (FOD) or olive oil diet (OOD) during lactation[1]

	FOD	OOD
	mL · pup^{-1} · d^{-1}	
Milk yield		
d 7/8 of lactation	2.1 ± 0.1	2.9 ± 0.1*
d 15/16 of lactation	2.8 ± 0.3	4.1 ± 0.1*
	g/100 g fatty acids	
Fatty acids		
12:0	5.55 ± 1.15	4.39 ± 0.54
14:0	8.18 ± 1.30	5.19 ± 0.55†
16:0	25.2 ± 0.5	19.0 ± 0.3*
18:0	4.44 ± 0.19	3.76 ± 0.05*
16:1(n-7)	6.65 ± 0.30	2.21 ± 0.11*
18:1(n-9)	31.3 ± 1.7	59.2 ± 1.0*
18:2(n-6)	3.79 ± 0.53	3.99 ± 0.09
20:4(n-6)	0.38 ± 0.07	0.93 ± 0.06*
20:5(n-3)	2.95 ± 0.49	0.01 ± 0.01*
22:6(n-3)	7.94 ± 0.37	0.15 ± 0.02*
α-Tocopherol, *mmol/L*	13.7 ± 1.9	49.9 ± 7.4*

[1] Values are expressed as means ± SEM, $n = 13$ or 14. Statistical comparison was made with the Student's t test. * $P < 0.001$, † $P < 0.05$.

found when newborns of rats fed OOD during pregnancy were cross-fostered to rats fed FOD.

The fatty acid profile in adipose tissue in rats fed either diet during pregnancy was very similar to the composition of the diet, including a lack of AA in rats from either group and an enhanced proportion of DHA and EPA at the expense of oleic acid in adipose tissue of rats fed FOD. Most of the fat accumulated in adipose tissue comes from the diet due to its low capacity to synthesize fatty acids (Shargo et al. 1969), and although lipogenesis is enhanced in this tissue during pregnancy (Palacín et al. 1991), maternal hyperphagia and unchanged or even enhanced adipose tissue lipoprotein lipase activity during early gestation (Knopp et al. 1975) allow dietary fatty acids circulating in plasma in the form of triglyceride-rich lipoproteins (chylomicrons and VLDL) to be taken up by the tissue. Different from adipose tissue, a substantial amount of AA appeared in both liver and plasma of rats fed OOD, probably as result of its active synthesis from linoleic acid [18:2(n-6)] in liver. This was, however, not the case in rats fed FOD, in which the enhanced content of both DHA and EPA could have caused the competitive inhibition of Δ6-desaturase (Christiansen et al. 1991, Raz et al. 1997 and 1998), the rate-limiting reaction for the conversion of linoleic acid to γ-linolenic acid in the synthesis of AA. Our finding that supplementation with γ-linolenic acid in lactating rats fed FOD overcame the deficiency of AA in their suckling newborns further supports this hypothesis.

Except for saturated fatty acids, which show a higher proportion in fetal compared with maternal structures, probably as result of the well-recognized lipogenic capability of fetal liver (Lorenzo et al. 1981), similar changes in the fatty acid profile were detected in both fetal plasma and liver, as well as in maternal sites. Preferential uptake of both monounsaturated and PUFA by the placenta and their consequent transfer to the fetus seem to be mediated via the placental plasma membrane fatty acid–binding protein (p-FABP$_{pm}$), as recently reviewed (Dutta-Roy 2000), and although the process has different preferences depending on the type of fatty acid, the present findings show that the correlation is significant for monounsaturated and for either (n-6) or (n-3) PUFA. Although precursor PUFA may be elongated and desaturated in the rat fetus and no exogenous supply of 20:4(n-6) or 22:6(n-3) should be required if the precursor lipids, 18:2(n-6) and 18:3(n-3), respectively, are adequate in the diet (Hachey 1994), the present findings show that an excess of (n-3) fatty acids in maternal diet causes a specific deficiency of AA in the fetus, with the effect being a consequence of either the inhibitory action of (n-3) fatty acids on Δ6 desaturation within the fetus or an altered proportional fatty acid placental transfer secondary to the changes taking place in the maternal side or both.

The present findings also show that a proportional excess of dietary PUFA enhances the depletion of α-tocopherol, causing a deficient condition of this antioxidant in both the dam and the fetus. The feeding of fish oil enhances vitamin E requirements (Cho and Choi 1994), probably as a consequence of the effect of PUFA enrichment on enhancement of lipid peroxidation (Mazière et al. 1998). This condition contrasts with the decreased susceptibility to lipid peroxidation that occurs when rats are fed diets supplemented with olive oil (Öztezcan et al. 1996), allowing appropriate endogenous concentrations of α-tocopherol, as seen in the present study in both pregnant rats and fetuses. Despite the decreased AA and α-tocopherol concentrations in fetuses of pregnant rats fed FOD, litter size and fetal weight were unaffected, and a similar finding was reported by others who subjected rats during pregnancy to different dietary fat compositions (Buison et al. 1997) or even under conditions of decreased α-tocopherol concentration (Schinella et al. 1999). These findings support the notion that during pregnancy in rats, neither substantial

TABLE 8

Effect on pups of supplementation with γ-linolenic acid (γL), arachidonic acid (AA) or vitamin E (VE) to the diet of lactating rats fed fish oil diet (FOD) compared with those fed olive oil diet (OOD)[1]

	OOD	FOD	FOD-γL	FOD-AA	FOD-VE
d 21 body weight, *g*	42.2 ± 2.7^{a}	26.3 ± 4.0^{b}	32.0 ± 2.3^{ab}	24.8 ± 1.7^{b}	22.0 ± 1.9^{b}
d 21 body length, *cm*	11.3 ± 0.3^{a}	9.7 ± 0.5^{b}	10.6 ± 0.3^{ab}	9.92 ± 0.21^{ab}	9.39 ± 0.30^{b}
I_{50} for ARR[2]	11.9 ± 0.6^{c}	16.5 ± 0.5^{ab}	13.4 ± 0.6^{c}	14.3 ± 0.5^{bc}	17.6 ± 0.8^{a}
I_{50} for SRR	15.2 ± 0.6^{b}	18.8 ± 0.7^{a}	15.4 ± 0.4^{b}	16.9 ± 0.5^{ab}	19.1 ± 0.5^{a}

[1] Values are expressed as means ± SEM, $n = 4–6$. Tukey's test was used to determine differences between groups after one-way ANOVA. Different superscript letters in a row indicate significant differences ($P < 0.05$).

[2] I_{50}, day that 50% of the litter acquired the mature response; ARR, air righting reflex; SRR, surface righting reflex.

TABLE 9

Fatty acid profile and vitamin E concentration in plasma, liver and brain of 21-d-old rats suckling dams fed fish oil diet (FOD) or FOD supplemented with γ-linolenic acid (FOD-γL), arachidonic acid (FOD-AA) or vitamin E (FOD-VE) compared with those fed olive oil diet (OOD)[1]

	OOD	FOD	FOD-γL	FOD-AA	FOD-VE
			g/100 g fatty acids		
Plasma fatty acids					
12:0	1.66 ± 0.33^{ab}	2.22 ± 0.27^{a}	1.19 ± 0.35^{ab}	1.23 ± 0.15^{ab}	1.10 ± 0.13^{b}
14:0	2.51 ± 0.33^{ab}	3.75 ± 0.60^{a}	2.36 ± 0.27^{ab}	2.13 ± 0.21^{b}	2.37 ± 0.19^{ab}
16:0	20.1 ± 1.8^{b}	25.7 ± 1.0^{a}	20.2 ± 1.0^{b}	19.6 ± 0.4^{b}	20.6 ± 1.0^{b}
18:0	10.1 ± 0.6	10.6 ± 0.8	9.74 ± 0.73	11.6 ± 0.6	9.11 ± 0.33
16:1(n-7)	1.73 ± 0.25	2.18 ± 0.24	1.78 ± 0.15	1.68 ± 0.08	2.28 ± 0.14
18:1(n-9)	34.4 ± 3.0^{a}	16.0 ± 1.0^{b}	14.6 ± 1.2^{b}	13.7 ± 0.8^{b}	19.0 ± 1.7^{b}
18:2(n-6)	10.4 ± 0.5^{a}	6.09 ± 0.59^{bc}	8.06 ± 0.73^{ab}	3.86 ± 0.26^{c}	7.11 ± 0.79^{b}
18:3(n-6)	0.01 ± 0.01^{b}	0.01 ± 0.01^{b}	1.01 ± 0.48^{a}	0.01 ± 0.01^{b}	0.27 ± 0.20^{b}
20:4(n-6)	13.8 ± 0.4^{b}	3.89 ± 0.43^{c}	16.4 ± 1.9^{b}	30.4 ± 0.7^{a}	5.01 ± 0.55^{c}
20:5(n-3)	1.37 ± 0.97^{b}	14.3 ± 1.0^{a}	8.17 ± 0.66^{a}	4.38 ± 0.16^{b}	14.0 ± 2.2^{a}
22:6(n-3)	1.80 ± 0.20^{b}	11.8 ± 0.9^{a}	11.4 ± 0.7^{a}	9.69 ± 0.49^{a}	13.4 ± 0.8^{a}
Plasma α-tocopherol, *μmol/L*	41.1 ± 4.0^{b}	24.3 ± 2.5^{c}	18.0 ± 2.7^{c}	20.8 ± 2.0^{c}	57.4 ± 7.1^{a}
			g/100 g fatty acids		
Liver fatty acids					
12:0	0.36 ± 0.06	0.21 ± 0.09	0.19 ± 0.03	0.31 ± 0.05	0.25 ± 0.06
14:0	1.84 ± 0.27^{a}	0.89 ± 0.12^{b}	0.85 ± 0.06^{b}	0.98 ± 0.17^{b}	1.17 ± 0.22^{ab}
16:0	20.6 ± 0.7	23.5 ± 0.4	22.1 ± 1.0	21.7 ± 1.0	23.5 ± 2.0
18:0	12.4 ± 0.5^{b}	15.6 ± 0.4^{a}	14.5 ± 0.9^{ab}	16.4 ± 0.6^{a}	13.7 ± 0.5^{ab}
16:1(n-7)	1.54 ± 0.24	1.17 ± 0.17	1.45 ± 0.47	1.39 ± 0.22	2.09 ± 0.35
18:1(n-9)	38.8 ± 1.7^{a}	11.2 ± 0.6^{b}	13.8 ± 3.6^{b}	11.8 ± 0.7^{b}	16.2 ± 1.5^{b}
18:2(n-6)	6.17 ± 0.24	3.54 ± 0.36	5.22 ± 0.44	3.56 ± 0.23	4.70 ± 0.33
18:3(n-6)	0.21 ± 0.08	0.01 ± 0.01	0.93 ± 0.53	0.58 ± 0.43	0.03 ± 0.02
20:4(n-6)	12.3 ± 0.8^{b}	4.69 ± 0.44^{c}	9.19 ± 1.67^{b}	18.1 ± 0.4^{a}	4.71 ± 0.61^{c}
20:5(n-3)	0.32 ± 0.31^{c}	6.36 ± 0.49^{ab}	4.53 ± 0.69^{bc}	2.43 ± 0.25^{c}	7.90 ± 1.23^{a}
22:6(n-3)	3.59 ± 0.32^{c}	32.1 ± 1.3^{a}	22.5 ± 2.7^{b}	20.3 ± 1.8^{b}	23.2 ± 2.3^{ab}
Liver α-tocopherol, *μmol/kg*	300 ± 36^{b}	139 ± 19^{c}	128 ± 12^{c}	139 ± 12^{c}	477 ± 37^{a}
			g/100 g fatty acids		
Brain phospholipid fatty acids					
12:0	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
14:0	0.41 ± 0.04	0.51 ± 0.05	0.45 ± 0.05	0.35 ± 0.02	0.40 ± 0.04
16:0	24.8 ± 2.2	24.4 ± 1.6	24.9 ± 1.0	24.4 ± 1.3	24.9 ± 1.4
18:0	22.9 ± 0.7	22.8 ± 0.4	23.7 ± 0.6	23.7 ± 0.5	22.4 ± 0.3
16:1(n-7)	0.91 ± 0.07	1.13 ± 0.11	1.00 ± 0.05	0.92 ± 0.07	1.19 ± 0.07
18:1(n-9)	17.7 ± 2.5	18.0 ± 2.1	15.8 ± 1.2	14.9 ± 1.6	16.2 ± 0.3
18:2(n-6)	0.77 ± 0.04^{a}	0.88 ± 0.18^{a}	0.53 ± 0.05^{a}	0.16 ± 0.07^{b}	0.73 ± 0.08^{a}
18:3(n-6)	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
20:4(n-6)	12.0 ± 0.8^{a}	6.03 ± 0.70^{b}	10.5 ± 0.6^{a}	11.6 ± 0.8^{a}	7.09 ± 0.54^{b}
20:5(n-3)	1.74 ± 0.52	3.48 ± 0.54	2.14 ± 0.46	2.88 ± 0.30	2.71 ± 0.53
22:6(n-3)	11.4 ± 0.4^{c}	18.2 ± 1.2^{ab}	16.3 ± 0.7^{b}	14.2 ± 0.3^{c}	20.9 ± 1.7^{a}
Brain α-tocopherol, *μmol/kg*	37.4 ± 1.9	36.2 ± 1.9	30.9 ± 2.0	28.6 ± 1.3	36.0 ± 2.6

[1] Values are expressed as means ± SEM, $n = 4–6$. Tukey's test was used to determine differences between groups after ANOVA. Different superscript letters in a row indicate significant differences ($P < 0.05$). No superscript letters in a row indicate that differences are not significant, $P \geq 0.05$.

changes in dietary fatty acids nor an antioxidant-deficient condition affects pregnancy outcome, probably because in this species a substantial part of the development of neural tissue occurs postnatally (Dobbing and Sands 1979), as is also the case in humans (Pomeroy and Segal 1998). This view is supported in the present study by the findings in the cross-fostering experiments, in which pups born to dams fed FOD during pregnancy showed a postnatal growth rate and psychomotor maturation indices similar to those of pups of dams fed OOD when allowed to suckle from dams fed OOD.

An important delay in growth rate and psychomotor development was seen in pups suckling dams fed FOD during lactation. The effect could be the result of either the decreased milk yield or the altered fatty acid composition in milk detected in these rats, with the latter reflecting their plasma fatty acids profile, or both. A decreased availability of milk during suckling, such as that caused by maternal underfeeding in pair-fed nutritional controls of lactating rats fed alcohol, decreases pup growth rate (Tavares do Carmo et al. 1999), but sensory maturation indices seem to be less affected (Lopez Tejero et al. 1986). The feeding of FOD during lactation also caused a depletion of α-tocopherol in plasma and liver and a major alteration in neural fatty acid composition, with a specific decline in AA in brain phospholipids. Both of these

changes, the α-tocopherol deficiency and the decline in AA in brain phospholipids, could have affected neurodevelopment in the pups, because similar results were reported for pups of dams fed a fish oil–supplemented diet throughout pregnancy and lactation (Saste et al. 1998). In adult rats fed a fish oil–supplemented diet, monoaminergic neurotransmission and behavior were affected (Chalon et al. 1998), and in adults subjected to a vitamin E deficiency regimen, a disturbance of monoamine metabolism in brain was observed (Adachi et al. 1999). In an attempt to determine which of these two effects (decreased AA in brain phospholipids or α-tocopherol deficiency) was responsible for the delayed growth rate and neurodevelopment in pups of dams fed FOD, a dietary supplement experiment was carried out. In this experiment, conditions that restored brain AA content rather than plasma and liver concentrations of α-tocopherol avoided the negative effects of the feeding of FOD during lactation. The effect was more evident when γ-linolenic acid rather than AA was supplemented to FOD, although both treatments restored brain phospholipid AA content to the same concentration as in pups whose dams were fed OOD. The only difference was the absence of linoleic acid [18:2(n-6)] in brain phospholipids when rats were supplemented with AA, whereas it was present in those supplemented with γ-linolenic acid at a concentration that did not differ from that of those whose dams were fed OOD. Regarding the different response to supplementation with γ-linolenic acid versus with AA, although it was previously found in humans that diets rich in AA decrease the proportion of linoleic acid in plasma phospholipids (Sinclair and Mann 1996), the effect is likely a consequence of the replacement by AA of linoleic acid in tissues (Whelan 1996). It is, however, worth emphasizing the exquisite capability of the brain to buffer exaggerated increments in plasma concentrations of AA, as shown in pups of dams fed FOD supplemented with AA, which had much higher plasma concentrations than any of the other groups, whereas the proportional content in brain phospholipids did not differ from those of dams fed either OOD or FOD supplemented with γ-linolenic acid.

The supplementation of vitamin E to lactating rats fed FOD enhanced plasma and liver α-tocopherol concentrations in pups but did not modify the concentration in brain compared with pups of dams fed OOD. It has previously been shown in humans that even high oral α-tocopherol supplementation did not increase ventricular cerebrospinal fluid α-tocopherol concentrations (Pappert et al. 1996), and in rats, α-tocopherol intake modestly increases brain α-tocopherol (Martin et al. 1999, Vatassery et al. 1988), with the change being much smaller than that in plasma and other tissues, including the liver. In fact, the turnover or exchange half-life rate of α-tocopherol in the nervous system is much slower than that in plasma (Vatassery 1992), and the brain uptake index of α-tocopherol in mice is very low (Adams and Wang 1994). Furthermore, the high content of PUFA and, more specifically, DHA in brain phospholipids in pups suckling dams fed FOD supplemented with vitamin E may have enhanced the consumption of antioxidants and thus may impeded the increase in brain α-tocopherol concentration over the values for pups of dams fed OOD. Because vitamin E deficiency plays a role in the disturbance of monoamine metabolism in rat brain (Adachi et al. 1999), there is no way to determine whether this condition aggravates the nervous system function caused by the altered fatty acid profile of these animals. However, the fact that supplementation of γ-linolenic acid to the lactating rats fed FOD increased the AA content of brain phospholipids in their pups and normalized body weight and psychomotor maturation variables despite their low α-tocopherol concentrations supports a more important role of the appropriate availability of AA rather than vitamin E on postnatal development.

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