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Influence of Changes in Dietary Fatty Acids during Pregnancy on Placental and Fetal Fatty Acid Profile in the Rat

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Key Words

Pregnancy, rat · Dietary fatty acids · Palm oil · Sunflower oil · Olive oil · Fish oil · Arachidonic acid · Fatty acid profile · Placenta

Abstract

To determine whether the composition of long-chain polyunsaturated fatty acids (PUFA) could be modified in the fetus by maternal dietary fatty acids, pregnant Sprague-Dawley rats were fed semipurified diets that differed only in the non-vitamin lipid component. The diets contained either 10 g palm, sunflower, olive or fish oil (FOD)/100 g diet. A total of 5–6 rats were studied in each group. At day 20 of gestation, corresponding to 1.5 days prior parturition, the fatty acids in maternal adipose tissue were closely related to the fatty acid composition in the corresponding diet. An important proportion of arachidonic acid (AA) appeared in maternal liver and plasma, although it was lower in the FOD than in the other groups. Except for saturated fatty acids, the proportion of individual fatty acids in the placenta correlated linearly with that in maternal plasma. Also, PUFA in fetal plasma and liver showed significant correlations with PUFA in maternal plasma. Again, AA showed the lowest proportion in the plasma and liver of the FOD group. Therefore, the maternal dietary fatty acid composition influences maternal and fetal plasma and tissue composition, and an increase in dietary ω–3 fatty acids decreases the amount of AA in maternal and fetal tissues.

Introduction

Long-chain polyunsaturated fatty acids (LCPUFA) are essential for normal growth and development of placenta and fetuses. Considerable amounts of arachidonic acid (AA) and docosahexaenoic acid (DHA) are deposited in the human brain and other tissues during intrauterine and postnatal growth [1]. These LCPUFA are part of the structural components of cell membranes and so affect many aspects of membrane function such as membrane permeability, receptor functions and membrane-associated enzyme activities [2]. Moreover, DHA is known to play a key role in membrane lipid-dependent functions in the brain and retina [1], while AA is the main precursor for eicosanoids [3, 4] and is known to play a key role in neonatal growth [5]. LCPUFA synthesis from essential fatty acids (EFA), linoleic and α-linolenic acids, involves Δ6- and Δ5-desaturases and peroxisomal β-oxidation retroconversion [6, 7], constituting the ω–6 and ω–3 PUFA metabolic pathways, respectively.

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Although studies in rats have demonstrated that the fetus is capable of metabolizing EFA to LCPUFA to some extent [8, 9], the needs are not fulfilled and therefore LCPUFA and the nutritional EFA linoleic and α-linolenic acids should be obtained from the maternal circulation by passage across the placenta. LCPUFA in maternal plasma must derive either from the maternal diet during gestation, from maternal stores in adipose tissue given the enhanced lipolysis found in the adipose tissue in the last trimester of pregnancy [10], and/or from the endogenous synthesis in maternal liver from parental EFA. Thus, EFA derived from the maternal diet and their LCPUFA derivatives, transported mainly as triglycerides, phospholipids or esterified cholesterol in lipoproteins in maternal plasma, become available to the fetus thanks to the presence of the very low-density lipoprotein/apoE receptor, low-density lipoprotein receptor and low-density lipoprotein receptor-related protein [11–13] as well as lipoprotein lipase [14] and other lipase activities in the placenta [12], all of which have been found in human placental cells. Placental uptake of free fatty acids occurs via a process of facilitated membrane translocation involving plasma membrane fatty acid-binding protein [15], which is known to selectively transfer LCPUFA and EFA to the fetal circulation in preference to the non-essential fatty acids [16]. Although Δ6- or Δ5-desaturase activities have not been detected in the placenta [17, 18], phospholipase A_2 [19] as well as intracellular lipase activities [20, 21] are expressed in human and rat placenta. Once in the placenta, fatty acids can either be metabolized, as is the case for the conversion of AA to eicosanoids, or can be reesterified and hydrolyzed again to release fatty acids to the fetus. PUFA in fetal plasma can reach the brain, where they can be incorporated into phospholipids of cell membranes, or can be desaturated and elongated to their long-chain derivatives, as has been shown in rat fetal brain in vivo [9].

Although supplementation of pregnant women with LCPUFA has been shown to improve neonatal LCPUFA status [22–24], an excess may have detrimental effects. Thus, excessive dietary intake of linoleic acid may inhibit Δ6-desaturase resulting in a decrease in the formation of DHA from α-linolenic acid [24]. An excess of DHA, as that caused by fish oil treatment, has also been demonstrated to inhibit Δ6-desaturase activity which is responsible for major declines in AA levels in rats [25, 26] and in turn is responsible for the impaired neonatal growth and delayed psychomotor maturation of pups from mothers given a fish oil diet as compared to those on an olive oil diet [25]. Since the benefits and risks of modifying maternal fat intake in pregnancy have not yet been completely established, the present study is addressed to determine how changes in dietary fatty acids affect maternal and fetal blood and tissue fatty acid composition. To determine the relationship between maternal and fetal fatty acid profile, rats were fed diets supplemented with either palm, sunflower, olive or fish oil during pregnancy.

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 (12-hour 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 spermatozoids appeared in vaginal smears (d0 of pregnancy), they were randomly divided into 4 groups (5–6 rats/group). Rats were housed in collective cages (4/cage) and had free access to the assigned diet and tap water. Diets containing 10 g of the nonvitamin lipid component/100 g diet, which corresponded to either palm (POD), sunflower (SOD), olive (OOD) or fish oil (FOD) diets, were isoenergetic and their composition and the proportional fatty acid profiles of each diet are shown in table 1 and 2, respectively. Diets were prepared at the beginning of the experiment and were kept at −20 °C in daily portions until use. Every 24 h fresh food was provided and the daily food intake was estimated periodically.

On d20 of pregnancy corresponding also to the 20th day under the corresponding diet, rats were decapitated and trunk blood was collected at 4 °C into tubes that contained 1 g Na_2-EDTA/L. Plasma was separated from fresh blood by centrifugation at 1,500 *g* for 15 min at 4 °C and kept frozen until the day of analysis. Livers and lumbar adipose tissue were quickly removed and placed in liquid nitrogen before freezing at −80 °C until analysis. The two uterine horns were immediately dissected and each placenta was separated from its corresponding fetus and immediately frozen in liquid nitrogen. Fetuses were decapitated, and the blood and liver were collected as above. Samples from all the fetuses of the same dam were pooled separately and processed in parallel to the samples of the adults.

Processing of Samples

Fresh aliquots of each diet, frozen plasma, livers, adipose tissue and placentas were used for lipid extraction and purification [27].

In the placental lipid extracts, phospholipids and neutral lipids were separated by eluting neutral lipids in chloroform as phospholipids were retained in Silicagel 60 (Sigma Chemical Co., St. Louis, Mo.). Phospholipids were recovered in methanol.

Total lipid, neutral lipid or phospholipid fatty acids were simultaneously saponified and methylated following the method of Lepage and Roy [28, 29]. Fatty acid methyl esters were separated and quantified on a Perkin-Elmer gas chromatograph (Autosystem; Norwalk, Conn.) 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.

Table 1. Composition of the diets (g/kg)

Ingredient	Palm oil diet	Sunflower oil diet	Olive oil diet	Fish oil diet
Casein	170	170	170	170
Salt mix[1]	35	35	35	35
Vitamin mix[2]	10	10	10	10
Choline chloride	4	4	4	4
Cellulose	100	100	100	100
Cornstarch	580	580	580	580
Palm oil	100	–	–	–
Sunflower oil	–	100	–	–
Olive oil	–	–	100	–
Fish oil	–	–	–	100

[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; 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; thiamin hydrochloride 6.6; α-tocopherol acetate 100.

Table 2. Fatty acid composition of diets

Fatty acid g/100 g fatty acids	Palm oil diet	Sunflower oil diet	Olive oil diet	Fish oil diet
12:0	40.68	0.28	0.31	0.01
14:0	15.13	0.29	0.33	4.11
16:0	16.79	7.08	10.87	20.66
18:0	22.43	4.87	3.59	3.48
16:1 ω–7	0.01	0.32	0.95	6.38
18:1 ω–9	4.97	23.63	74.83	23.33
18:2 ω–6	0.01	62.13	7.39	0.01
20:4 ω–6	0.01	0.01	0.01	0.01
20:5 ω–3	0.01	0.01	0.04	9.54
22:6 ω–3	0.01	0.01	0.01	11.44

Statistical Analysis

Data are expressed as means ± SEM. Treatment effects (diet) were analyzed by one-way ANOVA with Systat Version 5.03 (Wilkinson, Evanston, Ill.,). When treatment effects were significantly different ($p < 0.05$), means were tested by Tukey's test. Linear regressions were calculated by the least-squares method [30].

Results

The fatty acid composition of the lumbar adipose tissue of rats fed semisynthetic diets containing palm, sunflower, olive or fish oil during pregnancy (table 3) appeared to be close to the fatty acid composition of the diets eaten by each group (table 2). Of the saturated fatty acids, palmitic acid (16:0) showed the highest proportion in all groups. Lauric (12:0), myristic (14:0) and stearic (18:0) fatty acids were higher in the lumbar adipose tissue of rats fed POD than in any of the other groups. Oleic acid (18:1 ω–9) was the predominant monounsaturated fatty acid in adipose tissue in all the groups except for those fed SOD, being highest in the OOD group. Among ω–6 PUFA, linoleic acid (18:2 ω–6) was higher in adipose tissue from the SOD group than in any of the other groups, while AA (20:4 ω–6) was almost absent in all of them. The proportions of ω–3 fatty acids were practically undetectable in all the groups except for eicosapentaenoic acid (EPA; 20:5 ω–3) and DHA, present in significant proportions only in the FOD group. The similarities found between the fatty acid composition of the diet and the lumbar adipose tissue of the rats led us to compare the proportion of each fatty acid in the diet and in the adipose tissue of individual rats. A significant linear correlation was always found except for AA (20:4 ω–6), which was not present in either the diet or the adipose tissue of 20d pregnant rats (table 4).

As shown in table 5 and 6, the liver and plasma fatty acid composition were similar and followed a similar trend to that of each diet. However, a few differences deserve to be pointed out. Whereas in POD, lauric acid (12:0) represented almost 40% and stearic acid (18:0) 23% of the total fatty acids and these fatty acids appeared to be much lower in the other diets (table 2), there were no differences in the proportions of lauric and stearic acids in the livers of rats fed the different diets. As to the composition of the diets, myristic acid (14:0) was higher in the liver and plasma of the POD group than in any of the other groups. Although the proportion of oleic acid was much higher in the OOD than in any other diet, this fatty acid reached a similar proportion in the liver and plasma of the rats fed OOD and POD, the values being higher than in the other groups. Similar to the diets, linoleic acid was in a higher proportion in the liver of the SOD group than in any of the other groups. In spite of being practically undetectable in the diets, AA reached an important proportion in the liver and plasma of the rats, reaching the highest values under the SOD and the lowest under the FOD (table 5, 6). DHA and EPA values were highest in

Table 3. Fatty acid composition of maternal adipose tissue at day 20 of gestation in rats fed palm oil (POD), sunflower oil (SOD), olive oil (OOD) or fish oil diets (FOD) during pregnancy

Fatty acid g/100 g fatty acids	POD	SOD	OOD	FOD
12:0	8.82±0.34[a]	0.16±0.02[b]	0.17±0.05[b]	0.18±0.01[b]
14:0	7.91±0.34[a]	1.24±0.03[b]	1.15±0.06[b]	3.05±0.19[a,b]
16:0	28.77±0.67[a]	21.99±1.11[a]	21.2±0.66[a]	25.97±4.46[a]
18:0	5.55±0.21[a]	3.44±0.12[b]	2.99±0.21[b]	4.09±0.45[a,b]
18:1 ω–9	34.59±1.19[a]	31.61±0.89[a]	59.57±0.97[b]	38.69±3.67[a]
18:2 ω–6	5.75±0.69[a]	34.74±2.19[b]	8.92±0.48[a]	8.26±0.61[a]
20:4 ω–6	0.01±0.01[a]	0.96±0.19[b]	0.12±0.04[a]	0.19±0.07[a]
20:5 ω–3	0.01±0.01[a]	0.09±0.03[a]	0.16±0.11[a]	1.72±0.32[b]
22:6 ω–3	0.01±0.01[a]	0.01±0.01[a]	0.01±0.01[a]	4.24±0.52[b]

Values are expressed as means ± SEM. Tukey's test was used to determine differences between groups after ANOVA. Different superscripts in a row indicate significant differences ($p < 0.05$).

Table 4. Linear correlations between fatty acids in the diets and maternal adipose tissue at 20 days of pregnancy

Fatty acids	n	r	p
12:0	37	0.91	<0.001
14:0	37	0.90	<0.001
16:0	35	0.61	<0.001
18:0	36	0.75	<0.001
18:1 ω–9	35	0.84	<0.001
18:2 ω–6	37	0.97	<0.001
20:4 ω–6	37	–	–
20:5 ω–3	35	0.58	<0.001
22:6 ω–3	36	0.97	<0.001

Table 5. Fatty acid profile in liver at day 20 of gestation in rats fed palm oil (POD), sunflower oil (SOD), olive oil (OOD) or fish oil diets (FOD) during pregnancy

Fatty acid g/100 g fatty acids	POD	SOD	OOD	FOD
12:0	1.44±0.69[a]	0.39±0.05[a]	0.25±0.04[a]	0.30±0.08[a]
14:0	1.80±0.41[a]	0.39±0.09[b]	0.40±0.08[b]	0.26±0.07[b]
16:0	25.79±2.23[a]	18.70±0.70[a]	21.00±1.83[a]	23.76±2.54[a]
18:0	21.64±0.59[a]	20.08±0.94[a]	16.15±1.53[a]	20.12±1.96[a]
18:1 ω–9	20.57±1.28[a,c]	11.44±0.35[b]	29.30±2.63[a]	12.36±0.96[b,c]
18:2 ω–6	4.85±0.43[a]	14.91±0.71[b]	4.29±0.11[a]	3.40±0.64[a]
20:4 ω–6	10.93±1.26[a]	17.33±1.29[a]	15.42±3.29[a]	3.87±0.52[b]
20:5 ω–3	1.22±0.53[a]	0.31±0.24[a]	0.98±0.60[a]	7.95±0.97[b]
22:6 ω–3	5.24±0.47[a]	2.37±0.14[a]	4.55±0.44[a]	24.71±0.74[b]

Values are expressed as means ± SEM. Tukey's test was used to determine differences between groups after ANOVA. Different superscripts in a row indicate significant differences ($p < 0.05$).

Table 6. Fatty acid profile in plasma at day 20 of gestation in rats fed palm oil (POD), sunflower oil (SOD), olive oil (OOD) or fish oil diets (FOD) during pregnancy

Fatty acid g/100 g fatty acids	POD	SOD	OOD	FOD
12:0	1.16 ± 0.13[a]	0.24 ± 0.14[a]	0.28 ± 0.13[a]	0.29 ± 0.08[a]
14:0	2.70 ± 0.29[a]	0.88 ± 0.06[a,b]	0.79 ± 0.17[b]	1.29 ± 0.40[a,b]
16:0	21.11 ± 0.94[a]	17.26 ± 2.75[a]	19.15 ± 1.89[a]	17.56 ± 1.36[a]
18:0	14.58 ± 1.07[a]	12.77 ± 1.62[a]	10.43 ± 1.38[a]	11.37 ± 0.36[a]
18:1 ω–9	28.69 ± 0.39[a]	18.21 ± 1.88[b]	33.66 ± 6.05[a]	19.96 ± 0.79[a,b]
18:2 ω–6	10.81 ± 1.24[a]	20.92 ± 2.58[b]	8.49 ± 1.76[a]	12.69 ± 0.48[a]
20:4 ω–6	4.94 ± 1.01[a,b]	16.37 ± 5.75[a]	9.41 ± 2.32[a,b]	2.30 ± 0.40[b]
20:5 ω–3	1.56 ± 1.09[a]	0.43 ± 0.27[a]	0.74 ± 0.59[a]	4.98 ± 0.76[b]
22:6 ω–3	1.29 ± 0.28[a]	0.21 ± 0.14[a]	1.30 ± 0.11[a]	7.11 ± 1.41[b]

Values are expressed as means ± SEM. Tukey's test was used to determine differences between groups after ANOVA. Different superscripts in a row indicate significant differences ($p < 0.05$).

Table 7. Fatty acid profile in placenta at day 20 of gestation in rats fed palm oil (POD), sunflower oil (SOD), olive oil (OOD) or fish oil diets (FOD) during pregnancy

Fatty acid g/100 fatty acids	POD	SOD	OOD	FOD
Placental phospholipids				
12:0	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.11 ± 0.06[a]	0.10 ± 0.09[a]
14:0	0.76 ± 0.07[a]	0.31 ± 0.09[b]	0.17 ± 0.10[b]	0.46 ± 0.09[a,b]
16:0	19.39 ± 0.80[a]	19.35 ± 1.29[a]	20.18 ± 0.84[a]	23.50 ± 1.60[a]
18:0	23.97 ± 0.52[a]	22.54 ± 2.01[a]	21.98 ± 0.61[a]	21.64 ± 0.66[a]
18:1 ω–9	17.47 ± 0.35[a]	16.77 ± 3.69[a]	22.34 ± 1.32[a]	15.17 ± 2.05[a]
18:2 ω–6	8.98 ± 0.43[a]	16.82 ± 2.20[b]	6.30 ± 1.34[a]	6.21 ± 1.72[a]
20:4 ω–6	18.28 ± 0.41[a]	15.12 ± 1.76[a]	20.63 ± 1.68[a]	8.45 ± 0.63[b]
20:5 ω–3	0.07 ± 0.06[a]	0.01 ± 0.01[a]	0.20 ± 0.19[a]	4.98 ± 0.76[b]
22:6 ω–3	3.52 ± 0.84[a]	0.86 ± 0.38[b]	0.25 ± 0.24[b]	15.23 ± 0.59[c]
Placental neutral lipids				
12:0	0.19 ± 0.12[a]	0.58 ± 0.39[a]	0.14 ± 0.09[a]	0.42 ± 0.11[a]
14:0	1.08 ± 0.41[a]	1.88 ± 0.78[a]	0.97 ± 0.59[a]	1.24 ± 0.18[a]
16:0	21.32 ± 1.90[a]	13.69 ± 0.83[b]	14.79 ± 0.89[b]	26.37 ± 1.30[a]
18:0	21.35 ± 3.89[a]	17.35 ± 2.03[a]	16.64 ± 1.37[a]	18.17 ± 2.08[a]
18:1 ω–9	26.03 ± 4.59[a,b]	14.39 ± 1.64[a]	32.51 ± 4.34[b]	22.87 ± 1.19[a,b]
18:2 ω–6	8.80 ± 1.03[a,b]	17.06 ± 3.76[b]	6.80 ± 1.37[a]	6.56 ± 0.96[a]
20:4 ω–6	6.02 ± 1.94[a,b]	9.56 ± 2.55[a]	9.52 ± 1.91[a]	1.80 ± 0.75[b]
20:5 ω–3	0.58 ± 0.45[a]	0.75 ± 0.53[a]	1.33 ± 0.98[a]	4.44 ± 0.40[b]
22:6 ω–3	3.54 ± 2.00[a]	5.49 ± 3.83[a]	4.11 ± 2.89[a]	6.23 ± 2.72[a]

Values are expressed as means ± SEM. Tukey's test was used to determine differences between groups after ANOVA. Different superscripts in a row indicate significant differences ($p < 0.05$).

the liver and plasma of the rats fed FOD due to their high proportions in this diet.

The fatty acid composition of phospholipids in the placenta differed from that of neutral lipids in the same tissue (table 7). Although the proportions of palmitic and stearic acids were the highest in saturated fatty acids in both lipidic fractions, palmitic acid was higher in neutral lipids of placenta of rats fed POD and FOD than in the other groups, whereas in phospholipids there were no differences in the proportion of palmitic acid between the

Table 8. Fatty acid profile in fetal plasma at day 20 of gestation in rats fed palm oil (POD), sunflower oil (SOD), olive oil (OOD) or fish oil diets (FOD) during pregnancy

Fatty acid g/100 g fatty acids	POD	SOD	OOD	FOD
12:0	0.72±0.16[a]	0.90±0.10[a]	0.82±0.11[a]	1.03±0.29[a]
14:0	3.59±0.63[a]	1.43±0.12[b]	1.83±0.23[b]	2.19±0.10[b]
16:0	29.50±3.34[a]	27.13±1.36[a]	31.65±3.93[a]	44.27±1.04[b]
18:0	11.80±0.58[a]	12.64±0.94[a]	12.14±1.30[a]	16.35±1.90[a]
18:1 ω–9	28.45±2.99[a]	19.58±2.31[b]	25.79±3.39[a]	19.74±1.19[b]
18:2 ω–6	4.10±0.72[a]	11.59±1.86[b]	4.49±0.83[a]	3.61±0.34[a]
20:4 ω–6	7.51±2.87[a]	16.55±4.62[b]	11.08±3.79[a,b]	0.84±0.84[c]
20:5 ω–3	0.69±0.69[a]	0.39±0.33[a]	0.74±0.74[a]	2.23±0.62[b]
22:6 ω–3	1.66±0.67[a]	0.53±0.27[a]	1.37±0.79[a]	2.77±0.38[b]

Values are expressed as means ± SEM. Tukey's test was used to determine differences between groups after ANOVA. Different superscripts in a row indicate significant differences ($p < 0.05$).

Table 9. Fatty acid profile in fetal liver at day 20 of gestation in rats fed palm oil (POD), sunflower oil (SOD), olive oil (OOD) or fish oil diets (FOD) during pregnancy

Fatty acid g/100 g fatty acids	POD	SOD	OOD	FOD
12:0	0.28±0.12[a]	0.20±0.09[a]	0.37±0.08[a]	0.42±0.07[a]
14:0	2.04±0.23[a]	1.21±0.09[b]	1.52±0.12[a,b]	0.97±0.14[b]
16:0	21.72±1.05[a]	23.09±3.04[a]	21.76±1.56[a]	29.57±2.75[a]
18:0	13.23±0.24[a]	12.53±0.60[a]	11.55±0.37[a]	16.17±0.63[b]
18:1 ω–9	32.53±3.69[a]	18.54±1.44[b,c]	26.12±1.74[a,c]	17.11±1.29[b]
18:2 ω–6	4.34±0.37[a]	11.42±1.07[b]	4.42±0.49[a]	3.01±0.22[a]
20:4 ω–6	10.62±2.64[a]	8.88±0.84[a]	7.64±0.58[a,b]	2.82±0.38[b]
20:5 ω–3	2.73±1.22[a]	2.27±0.73[a]	4.04±0.74[a]	8.01±0.39[b]
22:6 ω–3	2.54±0.09[a]	3.78±1.16[a]	3.55±0.53[a]	16.73±0.84[b]

Values are expressed as means ± SEM. Tukey's test was used to determine differences between groups after ANOVA. Different superscripts in a row indicate significant differences ($p < 0.05$).

different groups of animals. There was a lower proportion of oleic acid in the phospholipids of the placenta than in neutral lipids, except for the SOD group, and, whereas in phospholipids there were no differences in the proportion of this fatty acid between groups, in neutral lipids, the OOD group showed the highest proportion of this fatty acid (table 7). The PUFA profile was also different in the two lipidic fractions of the placenta. Whereas AA was the most abundant PUFA in phospholipids, the most abundant in neutral lipids was linoleic acid (18:2 ω–6). AA in placental phospholipids reached a similar proportion in rats fed POD, SOD and OOD, but a lower proportion in the FOD group. In neutral lipids, the proportion of AA was also lower in the FOD group than in any of the other groups. On the other hand, ω–3 fatty acids were in a higher proportion in both phospholipids and neutral lipids of the placenta of rats fed FOD than in the other groups, with the exception of DHA, (22:6 ω–3) in neutral lipids of the placenta which was found in similar proportion in the different groups (table 7).

The fatty acid composition of fetal plasma (table 8) was similar to that of fetal liver (table 9) in all the groups with the exception of palmitic acid, which was higher in the fetal plasma in the FOD group than in other groups (table 8).

In fetal liver, there were no differences in the proportion of saturated fatty acids in the different groups (table 9), except for myristic acid (14:0) which was higher in

Table 10. Linear correlations of the proportion of each fatty acid in the mothers and their fetuses

Fatty acid	Maternal plasma vs. placenta		Placenta vs. fetal plasma		Maternal plasma vs. fetal plasma		Maternal plasma vs. fetal liver	
	r	p	r	p	r	p	r	p
Saturated	0.16	NS	0.05	NS	0.26	NS	0.04	NS
18:1 ω–9	0.46	<0.01	0.42	<0.05	0.15	NS	0.62	<0.001
18:2 ω–6	0.66	<0.001	0.54	<0.001	0.55	<0.001	0.63	<0.001
20:4 ω–6	0.65	<0.001	0.48	<0.01	0.46	<0.05	0.46	<0.01
20:5 ω–3	0.56	<0.001	0.51	<0.01	0.58	<0.001	0.56	<0.001
22:6 ω–3	0.83	<0.001	0.67	<0.001	0.67	<0.001	0.79	<0.001

the group fed POD than in the SOD and FOD groups. The POD group also showed a greater proportion of oleic acid than SOD or FOD. SOD showed the highest proportion of linoleic acid, whereas the FOD group showed the lowest proportion of AA and the highest proportion of EPA and DHA (table 9).

To test the potential interrelationship in fatty acid composition between maternal plasma, placenta and fetal liver and plasma, correlations of individual values were performed. As shown in table 10, the linear correlations of fatty acids in maternal plasma and placenta were not significant for saturated fatty acids, whereas the correlations were significant for oleic, linoleic, AA, EPA or DHA acids. No significant correlation was found for saturated fatty acids between placenta and fetal plasma, whereas a significant correlation was found for oleic acid and for any of the other fatty acids studied. Finally, we also correlated the proportion of each fatty acid in maternal plasma and in fetal plasma and liver. It was found that all PUFA were significantly correlated, whereas oleic acid correlated significantly in maternal and fetal liver but not between maternal and fetal plasma, and again saturated fatty acids did not correlate in either maternal and fetal liver or in maternal and fetal plasma (table 10).

Discussion

The present study shows that the fatty acid composition of the lumbar adipose tissue of rats fed semisynthetic diets containing palm, sunflower, olive or fish oil during pregnancy is influenced by the dietary fatty acid composition. This fits with either the high lipoprotein lipase activity in this tissue [31], which facilitates the uptake of fatty acids associated with plasma triglycerides, or with the low capacity of the adipose tissue to synthesize fatty acids [32]. Moreover, AA was not present in either the diet or the adipose tissue which could be related to the low Δ6-desaturase activity described in the adipose tissue [33].

The fatty acid composition of the liver is influenced not only by the diet but also by the endogenous synthesis and transformation of fatty acids that occur principally in this organ. Moreover, during late gestation there is an increased arrival of free fatty acids coming from adipose tissue to the liver due to an enhanced lipolytic activity in adipose tissue during the last third of pregnancy [34, 35]. A similar proportion of saturated fatty acids in liver was found here in all groups independent of the diet, probably as a result of the active lipogenesis normally taking place in this organ [36]. A similar proportion of oleic acid in POD and OOD groups was also found in spite of the much lower proportion of oleic acid in the diet of the former group. This finding could be a consequence of the Δ9 desaturation of stearic acid (which was in high proportion in the POD) in the liver [8]. Also, an important proportion of AA appeared in the livers of all groups except in those fed the FOD, where it was much lower than in any of the other groups, despite its undetectable amount in any of the diets. The synthesis of AA from linoleic acid in the liver is known to be related to the high Δ5- and Δ6-desaturase activities present in this organ [8, 37]. It was found here that the proportion of linoleic acid in the liver and adipose tissue was similar in the POD, OOD and FOD groups and higher in the SOD group, indicating enough substrate availability for AA synthesis in all groups. However, the group fed FOD also showed the greatest proportion of EPA and DHA, and thus it may be proposed that the presence of such high proportions of EPA and DHA in the FOD group inhibited the synthesis of AA from linoleic acid due to a competitive inhibition of Δ6-desaturase (the rate-limiting enzyme in the synthesis of LCPUFA) by ω–3 LCPUFA. Inhibition of AA synthe-

sis by enhanced proportions of EPA and DHA in the FOD has been described previously [26, 38, 39], and it is therefore proposed that this effect is responsible for the low proportions of AA detected in the liver of the FOD group.

PUFA in maternal plasma were mainly transported as triglycerides in very low-density lipoprotein (data not shown) which are synthesized in the liver [40]. The enhanced production of these lipoproteins during pregnancy is known [41], and this may be the reason why the fatty acid composition of the maternal plasma showed a similar pattern to the fatty acid composition of the maternal liver in all groups studied.

Our results show significant correlations in the PUFA proportions of maternal plasma and placenta, indicating that the fatty acid composition of the placenta could be influenced by dietary fatty acids. The binding and transfer of both EFA and LCPUFA to human placenta has been shown to be selective and preferential to the nonessential fatty acids [15, 16, 42]. This is in accordance with our findings showing a lower significant correlation ($p < 0.01$) in the proportion of oleic acid in maternal plasma and placenta than in any of the PUFA ($p < 0.001$). On the other hand, the fact that saturated fatty acids did not correlate linearly between maternal plasma and placenta could be due to the active lipogenetic activity in this organ [43]. In relation to fatty acids crossing the placenta to reach the fetus, significant positive correlations in the proportion of each of the PUFA between placenta and fetal plasma were found, and were higher than that of oleic acid in these compartments (table 10). However, AA showed a lower correlation ($p < 0.01$) between the placenta and fetal plasma than linoleic acid or DHA ($p < 0.001$). The correlation for AA in these compartments was also lower than the one for the same fatty acid between maternal plasma and placenta ($p < 0.001$). The difference in the correlations of AA in maternal plasma versus placenta and in placenta versus fetal plasma is in accordance with studies reporting that, although there is a higher affinity for plasma membrane fatty acid-binding protein to bind AA than other PUFA [44], there is also a lower transfer of AA to the fetal side than of other fatty acids [42]. The limited capability of the placenta to transfer AA seems to be due to its accumulation of AA as an intermediate state before its conversion into thromboxane A_2, eicosatrienoic and hydroxyeicosatetraenoic acids, which have been found in placenta [45–47]. In relation with AA in the placenta, it was also found here that the presence of AA is greater in phospholipids than in neutral lipids, different from DHA that is accumulated in a higher proportion in neutral lipids. This difference in their respective incorporations into placental lipids could play an important role in the specific transfer of these fatty acids to the fetus [48].

The present results also show that the proportion of PUFA in the fetus is directly influenced by the proportional PUFA content in maternal plasma because the proportions of each ω–3 and ω–6 PUFA correlated significantly between maternal and fetal plasma and between maternal plasma and fetal liver. Among the different PUFA, AA showed the lowest correlation between maternal plasma and fetal plasma ($p < 0.05$). This could be due either to the active metabolism of AA in the placenta, or to the capacity of the fetus to synthesize AA from linoleic acid coming from the maternal side, due to the presence of Δ5 and Δ6 activities in fetal liver and brain [49, 50], or to both factors together.

With regard to oleic acid, a positive linear correlation was found between maternal plasma and fetal liver, but not between maternal and fetal plasma. Therefore, we think that oleic acid supplied by the mother is rapidly transported to the fetal liver bound to α-fetoprotein [51, 52], whereas the lack of correlation between maternal and fetal plasma could indicate that oleic acid is synthesized by the fetus from stearic acid, using Δ9-desaturase [8]. On the other hand, the significant correlation found in the proportions of this fatty acid between maternal plasma and fetal liver could indicate that in some way, the fetus depends on its mother to obtain all the necessary oleic acid. This is in accordance with a study of Bourre et al. [53] showing that oleic acid could not be synthesized in an optimal amount if it is not supplied in an adequate amount by the diet.

For saturated fatty acids, a linear correlation in the proportion of these fatty acids between maternal plasma and either fetal liver or plasma was not found. This shows that fetal lipogenesis is sufficient to satisfy the needs for saturated fatty acids, which agrees with the active lipogenesis that has been described in fetal liver [54].

Thus, our study has shown that changes in the proportion of ω–3 and ω–6 PUFA in the maternal diet influence the fatty acid composition in the mother and in the fetus. Present findings show that although LCPUFA in the fetus should be supplied by the mother, they can also be synthesized from their precursor EFA. It is also found that when there is an excess of ω–3 LCPUFA in the maternal diet there is a decrease in AA content in the mother and the fetal liver and plasma, which may have a negative influence on postnatal development [25].

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