

Maternal adipose tissue becomes a source of fatty acids for the fetus in fasted pregnant rats given diets with different fatty acid compositions

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Abstract

Purpose The utilization of long-chain polyunsaturated fatty acids (LCPUFA) by the fetus may exceed its capacity to synthesize them from essential fatty acids, so they have to come from the mother. Since adipose tissue lipolytic activity is greatly accelerated under fasting conditions during late pregnancy, the aim was to determine how 24 h fasting in late pregnant rats given diets with different fatty acid compositions affects maternal and fetal tissue fatty acid profiles. **Methods** Pregnant Sprague–Dawley rats were given iso-energetic diets containing 10% palm-, sunflower-, olive- or fish-oil. Half the rats were fasted from day 19 of pregnancy and all were studied on day 20. Triacylglycerols (TAG), glycerol and non-esterified fatty acids (NEFA) were analyzed by enzymatic methods and fatty acid profiles were analyzed by gas chromatography.

Results Fasting caused increments in maternal plasma NEFA, glycerol and TAG, indicating increased adipose tissue lipolytic activity. Maternal adipose fatty acid profiles paralleled the respective diets and, with the exception of animals on the olive oil diet, maternal fasting increased the plasma concentration of most fatty acids. This maintains the availability of LCPUFA to the fetus during brain development.

Conclusions The results show the major role played by maternal adipose tissue in the storage of dietary fatty acids during pregnancy, thus ensuring adequate availability of

LCPUFA to the fetus during late pregnancy, even when food supply is restricted.

Keywords Dietary fatty acids · Adipose tissue lipolysis · Polyunsaturated fatty acids · Fatty acids in fetal brain · Maternal–fetal fatty acid transfer

Introduction

Fatty acid composition of adipose tissue results from fatty acids synthesized in situ and from the uptake of blood fatty acids, which originated either in the diet or from synthesis in the liver [1–4]. The amount of fatty acids synthesized in situ by adipose tissue declines as animals grow older, while the deposition of lipid from exogenous sources increases [5–7]. Consequently, dietary fat intake modulates the fatty acid composition of adipose tissue in adult humans [8, 9] and rats [10, 11] and a linear positive correlation between fatty acids in the diet and maternal adipose tissue has also been found in pregnant rats [12].

Fat deposition during pregnancy occurs in both women and experimental animals and takes place during the first two-thirds of gestation, whereas during the last third of gestation, maternal fat deposition stops or even declines (for reviews see refs [13–15]). Changes in adipose tissue lipoprotein lipase (LPL) activity could contribute to the changes of fat accumulation throughout early pregnancy. During early and mid-gestation, LPL activity in adipose tissue is either slightly increased [16, 17] or unchanged [18, 19], which together with maternal hyperphagia [20–22] may facilitate the uptake of dietary lipids. However, during late pregnancy LPL activity in rat adipose tissue has consistently been found to be decreased [23–25] and in pregnant women decreased plasma LPL activity has been also found

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after heparin administration [18]. Thus, fat uptake by adipose tissue decreases during late pregnancy, and this change, together with an increased lipolytic activity [26–28], results in an accelerated net breakdown of fat depots during the last trimester of pregnancy. Such increased adipose tissue lipolytic activity is especially accelerated under fasting conditions [27, 29]. The products of adipose tissue lipolysis, non-esterified fatty acids (NEFA) and glycerol are released, in large part, into the circulation. Since the placental transfer of these products is quantitatively low [29], their main fate is maternal liver [30], where they are partially re-esterified to synthesize triacylglycerols (TAG), which are released into the circulation as part of very low density lipoproteins (VLDL). The insulin-resistant condition of late pregnancy contributes to both the increased lipolysis of fat stores [31] and the increased production of liver VLDL although, for the later, the increased concentration of estrogens in late pregnancy seems to be its major activator [32].

The increased liver VLDL production and their decreased removal from the circulation, as a consequence of reduced adipose tissue LPL activity, actively contribute to maternal hyperlipidemia, which also corresponds to an increase in plasma TAG even in lipoprotein fractions that normally do not transport them, like low density lipoproteins (LDL) and high density lipoproteins (HDL) [29]. Maternal lipoproteins do not cross the placental barrier [29], but polyunsaturated fatty acids (PUFA) from maternal circulation, which are mainly transported in their esterified form in maternal plasma lipoproteins, must become available to the fetus to sustain its growth and development [33]. Although the process is not completely understood, the placental transfer of maternal PUFA is quite efficient [15, 29]. Moreover, by feeding female rats with diets containing different fatty acid compositions during just the first 12 days of pregnancy, we found that maternal adipose tissue stores dietary-derived fatty acids, which are released into the blood during late pregnancy [34]. Following this reasoning, we hypothesize that PUFA from maternal diet are accumulated in adipose tissue during pregnancy and, once released by lipolysis, they become an important source for PUFA to be transferred to the fetus, thus enabling their availability during critical periods of prenatal development. Long-chain polyunsaturated fatty acids (LCPUFA) and especially docosahexaenoic (DHA, 22:6n-3) and arachidonic-acid (AA, 20:4n-6) are major structural components of the neuronal plasma membrane. Fetal tissues, including fetal brain, may convert the essential fatty acids (EFA), α -linolenic (ALA, 18:3n-3) and linoleic (LA, 18:2n-6) acids, into LCPUFA of the n-3 and n-6 series, respectively [35, 36]. In the late prenatal stage in the rat, there is an active neurogenesis [37, 38], with a preferential accumulation of DHA compared to other fatty acids [39, 40], which may exceed the fetus's capacity to convert EFA into LCPUFA. Changes in dietary fatty acids in

pregnant rats cause major changes in the fatty acid profile of maternal and fetal plasma and liver, in maternal adipose tissue [12, 34] as well as in fetal brain [40]. Adipose tissue lipolytic activity is greatly accelerated under fasting conditions during late pregnancy [27]; to determine the potential role of adipose tissue on the availability of PUFA in the fetus, the aim of this study was to determine how 24 h fasting in late pregnant rats, given diets with different fatty acid compositions, affects maternal and fetal tissue fatty acid profiles.

Materials and methods

Animals and diets

Female Sprague–Dawley rats from our animal quarters were initially fed a standard non-purified diet (B&K Universal, Barcelona, Spain) and housed under controlled light and temperature conditions (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 spermatozooids were found in vaginal smear (day 0 of pregnancy), they were randomly divided into four groups. All groups were given free access to semi-purified diets that differed only in the nature of the non-vitamin lipid component. The diets contained per kg: 170 g casein, 100 g cellulose, 580 g maize starch, 35 g salt mix,¹ 10 g vitamin mix² and 100 g of the non-vitamin lipid component, which corresponded to either palm- (POD), olive- (OOD), sunflower- (SOD), or fish-oil (FOD). The fatty acid composition of each diet is given in Table 1, where it can be seen that the highest proportion of saturated fatty acids was in the POD, the highest of LA (18:2n-6) in the SOD, the highest OA (18:1n-9) in the OOD and the highest proportion of ALA (18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3) docosapentaenoic acid (DPA, 22:5n-3) and DHA (22:6n-3) in the FOD. Diets were prepared at the beginning of the experiment and were kept at -20 °C in daily portions until use. Rats were housed in collective cages (four per

¹ Salt mix (g/kg diet): copper sulfate 0.1; ammonium molybdate 0.026; sodium iodate 0.000310; potassium chromate 0.028; zinc sulfate 0.091; calcium hydrogen phosphate 0.145; ammonium ferrous sulfate 2.228; 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 1 Fatty acid composition of the experimental diets

Fatty acid (g/100 g)	POD	SOD	OOD	FOD
Total saturated FA	59.2	13.1	15.4	18.8
OA, 18:1n-9	30.5	28.0	77.0	20.0
LA, 18:2n-6	8.2	58.6	5.6	2.9
GLN, 18:3n-6	ND	ND	0.7	1.2
AA, 20:4n-6	ND	0.08	0.06	0.19
DPA, 22:5n-6	ND	ND	ND	ND
ALA, 18:3n-3	1.6	ND	0.4	2.3
EPA, 20:5n-3	ND	ND	ND	9.5
DPA, 22:5n-3	ND	ND	ND	3.1
DHA, 22:6n-3	ND	ND	ND	11.7

Values correspond to the mean of two separate samples processed independently

POD palm oil diet, SOD soy oil diet, OOD olive oil diet, FOD fish oil diet, ND not detected

cage) and had free access to the assigned diet and tap water. Every 24 h fresh food was provided.

On day 19 of pregnancy, food was completely removed from the cages of half of the rats allowing free access only to tap water, whereas the other half remained with access ad libitum to their diet. On day 20 of pregnancy both fed and fasted rats were decapitated and trunk blood was collected in ice-cold tubes that contained 1 mg Na₂-EDTA. Plasma was separated from fresh blood in a centrifuge at 3000×g for 20 min at 4 °C and kept frozen until the day of analysis. Lumbar adipose tissue was quickly removed and placed in liquid nitrogen before storing at –80 °C until analysis. The two uterine horns were immediately dissected and each placenta was separated from its corresponding fetus and was weighed. Fetuses were decapitated, and the blood collected as above. Fetal plasma, liver and brain from all the fetuses of the same dam were pooled and processed in parallel to the samples of the adults.

Processing of samples

Plasma glucose (Spinreact Reactives, Spain), TAG (Spinreact Reactives, Spain), glycerol (Sigma Chemical Co., St. Louis, MO), β-hydroxybutyrate (Sigma Chemical Co., St. Louis, MO) and NEFA (Wako Chemicals, Germany) were determined enzymatically using commercial kits.

Pentadecanoic acid (15:0) (Sigma Chemical Co.) was added as the internal standard to fresh aliquots of frozen plasma which, together with fresh aliquots of each diet, frozen maternal lumbar adipose tissue and fetal liver and brain aliquots, were used for lipid extraction and purification [41]. The final lipid extract was evaporated to dryness under vacuum and resuspended in methanol/toluene and subjected to methanolysis in the presence of acetyl chloride at 80 °C

for 2.5 h [42]. 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). Maternal and fetal plasma quantification of the fatty acids was performed as a function of the corresponding peak areas compared to that of the internal standard. Individual fatty acids in the remaining lipid extracts were expressed as a percentage of total fatty acids in the sample. Aliquots of fetal lipid extracts were dried down and suspended in propan-2-ol in the presence of activated alumina to remove phospholipids for the analysis of TAG (Sigma, St. Louis, MO).

Statistical analysis

Data are expressed as means ± SEM. Treatment effects (diet) were analyzed by one-way ANOVA with SPSS 14:0 (Chicago, IL). When treatment effects were significantly different ($P < 0.05$), means were tested by SNK test. Differences between the fed and fasted groups were analyzed by Student's *t* test. Lineal correlations were tested by Pearson's method.

Results

The different dietary fatty acid compositions given during 20 days of pregnancy did not affect maternal body weight or placental weight (data not shown). Under maternal fed conditions fetal weight was highest in the POD group and lowest in the SOD whereas no differences between the groups was seen for fetal liver or brain weights (Table 2). Maternal fasting for 24 h significantly reduced fetal body and fetal liver weights in all the groups, without modifying fetal brain weight although it was increased when corrected for body weight. As shown in Fig. 1, the concentration of glucose in maternal plasma did not differ between the groups when fed, whereas it was greatly decreased by fasting in every group, values being higher in rats on POD than in the other groups. Plasma concentrations of both TAG and NEFA in fed-maternal plasma did not differ between the groups, whereas in fasted rats both variables increased, although the effect was less pronounced in rats on OOD than in any of the other groups (Fig. 1). Plasma glycerol concentrations, which were not affected by the diets in fed pregnant rats, did increase after fasting although the effect was only significant in rats on FOD and SOD (Fig. 1). Plasma β-hydroxybutyrate concentrations were slightly higher in pregnant rats fed the SOD and greatly increased with fasting in the four groups without significant differences between the groups (Fig. 1).

Table 2 Fetal body weight and fetal liver and brain weights at day 20 of gestation in fed and 24 h fasted rats that were given palm oil (POD), sunflower oil (SOD), olive oil (OOD) or fish oil (FOD) diets during pregnancy

Group		Fetal body wt, g	Fetal liver wt, g	Fetal brain wt, g	Fetal liver wt/body wt%	Fetal brain wt/body wt%
POD	Fed	4.42 ± 0.07 ^A	0.23 ± 0.01	0.16 ± 0.01	5.3 ± 0.3 ^A	3.7 ± 0.1
POD	Fasted	4.03 ± 0.07 ^{a***}	0.19 ± 0.01 [*]	0.17 ± 0.01	5.1 ± 0.1 ^a	4.2 ± 0.1 [*]
SOD	Fed	3.99 ± 0.04 ^B	0.20 ± 0.01	0.16 ± 0.003	5.1 ± 0.2 ^A	3.9 ± 0.1
SOD	Fasted	3.79 ± 0.04 ^{b***}	0.17 ± 0.01 ^{**}	0.16 ± 0.01	5.0 ± 0.1 ^a	4.3 ± 0.1 [*]
OOD	Fed	4.17 ± 0.06 ^C	0.23 ± 0.01	0.16 ± 0.01	5.5 ± 0.2 ^{AB}	3.8 ± 0.2
OOD	Fasted	3.85 ± 0.04 ^{b***}	0.19 ± 0.01 ^{**}	0.16 ± 0.01	5.0 ± 0.1 ^a	4.2 ± 0.1
FOD	Fed	4.18 ± 0.04 ^C	0.23 ± 0.01	0.13 ± 0.02	5.9 ± 0.2 ^B	3.1 ± 0.4
FOD	Fasted	3.75 ± 0.03 ^{b***}	0.18 ± 0.01 ^{**}	0.16 ± 0.01	5.1 ± 0.1 ^{a***}	4.2 ± 0.1 [*]

Values are expressed as means ± SEM of 75–110 fetuses per group for the fetal body weight and 6–8 for the fetal liver and fetal brain weight. An SNK test was used to determine differences between groups after ANOVA. Statistical comparisons for each variable between fed groups are shown with capital superscript letters and between fasted groups with lower-case superscript letters. Different capital or lower-case letters indicate that the difference is statistically significant ($P < 0.05$). Differences between fed and fasted groups were analyzed by the Student's *t* test and are shown by asterisks (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Fig. 1 Plasma metabolites in fed and 24 h fasted, 20 day pregnant, rats that were given palm oil (POD), sunflower oil (SOD), olive oil (OOD) or fish oil (FOD) diets during pregnancy. **a** Plasma glucose in fed (white bars) and fasted (black bars) rats, **b** plasma Triacylglycerols in fed (white bars) and fasted (black bars) rats, **c** plasma NEFAs in fed (white bars) and fasted (black bars) rats, **d** plasma glycerol in fed (white bars) and fasted (black bars) rats and (E) plasma β -hydroxybutyrate in fed (white bars) and fasted (black bars) rats. Values are expressed as means ± SEM of 6–8 rats per group. An SNK test was used to determine differences between groups after ANOVA. Statistical comparisons for each variable between fed groups are shown with capital superscript letters and between fasted groups with lower-case superscript letters. Different capital or lower-case letters indicate that the difference is statistically significant ($P < 0.05$). Differences between fed and fasted groups were analyzed by Student's *t* test and are shown by asterisks (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

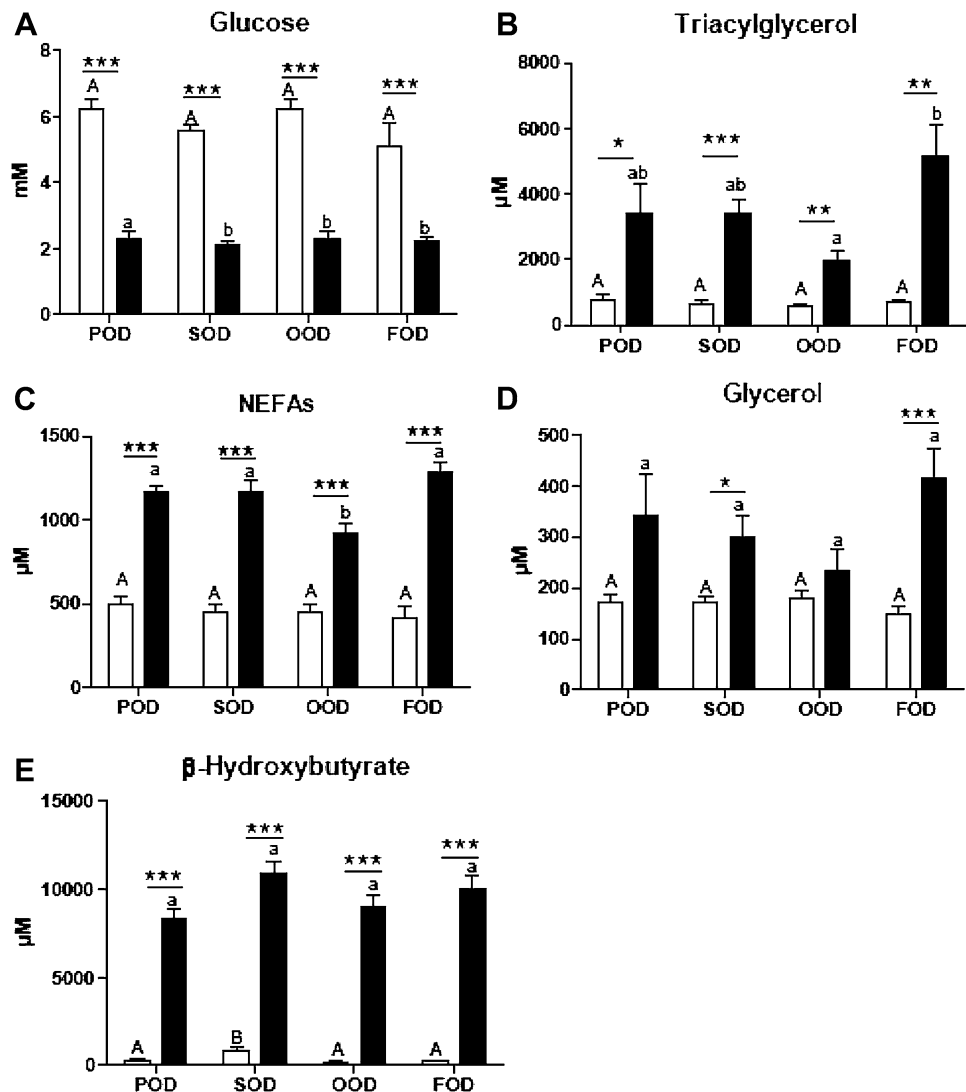
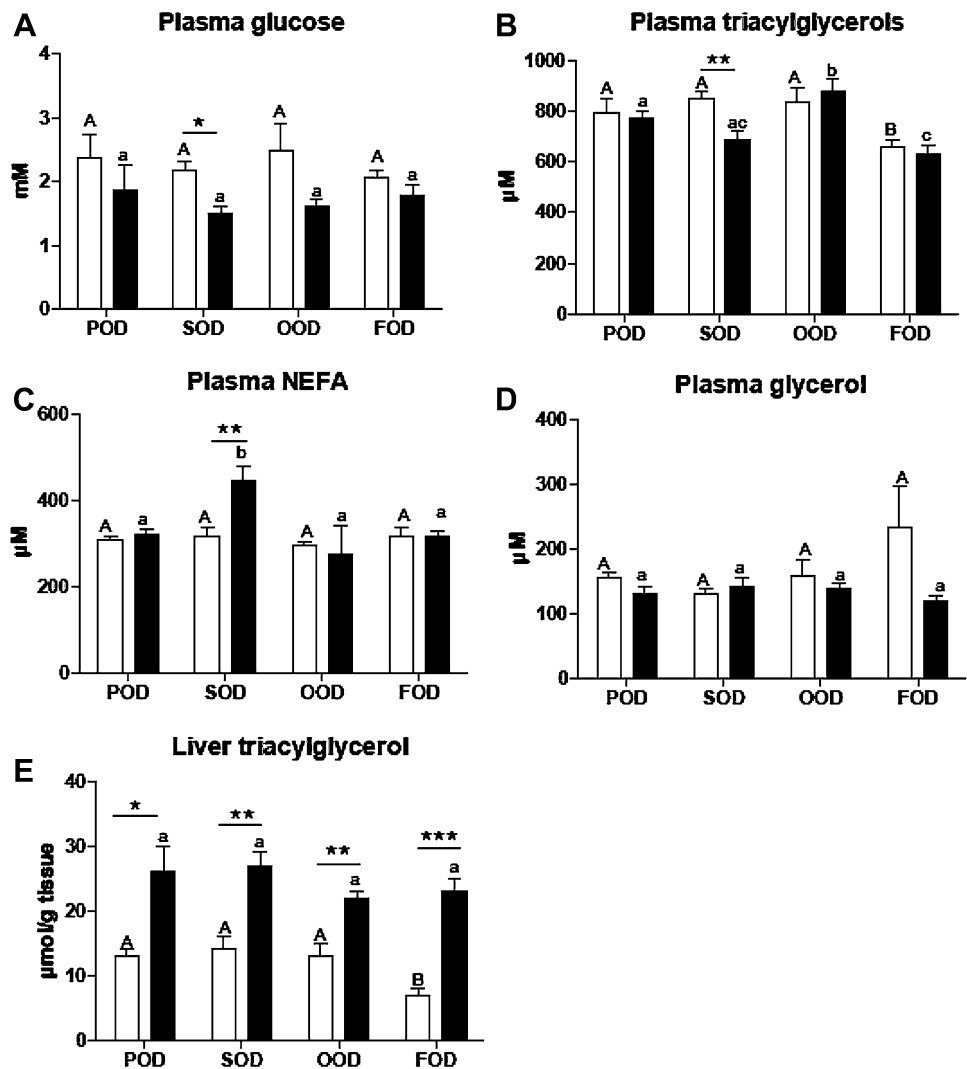


Fig. 2 Plasma metabolites and liver triacylglycerols (TAG) concentrations in fetuses of fed and 24 h fasted, 20 day pregnant, rats that were given palm oil (POD), sunflower oil (SOD), olive oil (OOD) or fish oil (FOD) diets during pregnancy. **a** Plasma glucose in fed (white bars) and fasted (black bars) rats, **b** plasma triacylglycerols in fed (white bars) and fasted (black bars) rats, **c** plasma NEFA in fed (white bars) and fasted (black bars) rats, **d** plasma glycerol in fed (white bars) and fasted (black bars) rats and **e** liver Triacylglycerols in fed (white bars) and fasted (black bars) rats. Values are expressed as means \pm SEM of 6–8 rats per group. An SNK test was used to determine differences between groups after ANOVA. Statistical comparisons for each variable between fed groups are shown with capital superscript letters and between fasted groups with lower-case superscript letters. Different capital or lower-case letters indicate that the difference is statistically significant ($P < 0.05$). Differences between fed and fasted groups were analyzed by Student's *t* test and are shown by asterisks * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$



In fetuses of fed dams, there were no differences between the four dietary groups in plasma glucose, NEFA or glycerol but both plasma and liver TAG were lower in FOD than in the other groups (Fig. 2). The effect of maternal fasting on the fetus showed only a significant decrease in plasma glucose and TAG and an increase in NEFA in fetuses of the SOD group, but an increase in liver TAG in all four groups (Fig. 2).

Analysis of maternal lumbar adipose tissue fatty acids showed that the proportion of individual fatty acids in fed rats depended on the type of diet eaten by the animals. Saturated fatty acids, i.e., palmitic (PA, 16:0) and stearic (SA, 18:0) acids, were higher in rats on the POD than in any of the other groups, OA (18:1n-9) was highest in rats on the OOD, LA (18:2 n-6), γ -linolenic acid (GLN, 18:3n-6) and AA (20:4n-6) were highest in rats on the SOD, and the proportions of ALA (18:3n-3) and DHA (22:6n-3) were highest in rats on the FOD (Table 3). With the exception of a slight significant increase in LA in the SOD group and in DHA in

the POD group, the 24 h fast did not affect the proportion of any of the individual fatty acids in maternal adipose tissue. With the exception of only ALA (18:3n-3), AA (20:4n-6) and EPA (20:5n-3) the percentage value of all individual fatty acids in maternal lumbar adipose tissue correlated significantly ($P < 0.001$) with those in the diet.

The plasma concentrations of individual FA in maternal plasma are shown in Fig. 3. Under fed conditions, the concentration of palmitic acid (PA, 16:0) and stearic acid (SA, 18:0) were highest in rats given the POD although in those on OOD, SA did not differ statistically. OA concentrations were highest in rats fed the OOD; for the n-6 fatty acids, plasma concentrations of LA were highest in rats given the SOD and lowest in those given the FOD; and the lowest plasma concentrations of AA were in the FOD rats followed by those fed SOD—in both cases concentrations were lower than in the OOD and POD groups. Gamma-linolenic acid (GLN, 18:3n-6) concentrations were undetectable in any of the groups. For the n-3 fatty acids in fed dams, plasma

Table 3 Fatty acid profile (g/100 g fatty acids) in lumbar adipose tissue of fed and 24 h fasted, 20 day pregnant rats that were given palm oil (POD), sunflower oil (SOD), olive oil (OOD) or fish oil (FOD) diets during pregnancy

Fatty acid		POD	SOD	OOD	FOD
PA, 16:0	Fed	27.2 ± 1.3 ^A	18.2 ± 0.7 ^B	18.4 ± 0.5 ^B	25.2 ± 0.9 ^A
PA, 16:0	Fasted	25.7 ± 0.8 ^a	17.5 ± 0.8 ^b	20.1 ± 0.7 ^b	25.0 ± 1.3 ^a
SA, 18:0	Fed	8.5 ± 0.4 ^A	7.0 ± 0.4 ^B	6.1 ± 0.3 ^B	6.7 ± 0.5 ^B
SA, 18:0	Fasted	8.5 ± 0.3 ^a	6.5 ± 0.3 ^b	5.9 ± 0.4 ^b	6.1 ± 0.3 ^b
OA, 18:1n-9	Fed	40.1 ± 1.8 ^A	32.1 ± 0.7 ^B	56.1 ± 1.4 ^C	31.1 ± 1.0 ^B
OA, 18:1n-9	Fasted	40.1 ± 1.1 ^a	31.7 ± 0.8 ^b	55.2 ± 0.9 ^c	30.7 ± 1.0 ^b
LA, 18:2n-6	Fed	12.2 ± 0.5 ^A	32.8 ± 1.0 ^B	9.9 ± 0.6 ^A	11.8 ± 0.6 ^A
LA, 18:2n-6	Fasted	14.6 ± 1.4 ^a	35.7 ± 0.7 ^{b,*}	9.7 ± 0.3 ^c	11.3 ± 0.9 ^c
AA, 20:4n-6	Fed	0.34 ± 0.03 ^A	0.87 ± 0.07 ^B	0.36 ± 0.03 ^A	0.59 ± 0.04 ^C
AA, 20:4n-6	Fasted	0.46 ± 0.06 ^a	0.89 ± 0.06 ^b	0.29 ± 0.04 ^c	0.52 ± 0.04 ^a
ALA, 18:3n-3	Fed	0.69 ± 0.03 ^{AB}	0.51 ± 0.04 ^A	0.64 ± 0.04 ^{AB}	0.89 ± 0.13 ^B
ALA, 18:3n-3	Fasted	0.79 ± 0.08 ^a	0.44 ± 0.04 ^b	0.63 ± 0.05 ^a	0.99 ± 0.05 ^c
EPA, 20:5n-3	Fed	0.92 ± 0.60 ^A	1.34 ± 0.71 ^A	2.50 ± 0.94 ^A	2.58 ± 0.38 ^A
EPA, 20:5n-3	Fasted	2.24 ± 0.81 ^a	0.25 ± 0.06 ^a	1.69 ± 1.0 ^a	2.24 ± 0.32 ^a
DHA, 22:6n-3	Fed	0.00 ± 0.00 ^A	0.17 ± 0.07 ^A	0.10 ± 0.02 ^A	4.27 ± 0.34 ^B
DHA, 22:6n-3	Fasted	0.17 ± 0.04 ^{a,*}	0.00 ± 0.00 ^a	0.16 ± 0.03 ^a	3.97 ± 0.22 ^b

Values are expressed as mean ± SEM. An SNK test was used to determine differences between groups after ANOVA. Statistical comparisons for each variable between fed groups are shown with capital superscript letters and between fasted groups with lower-case superscript letters. Different capital or lower-case letters indicate that the difference is statistically significant ($P < 0.05$). Differences between fed and fasted groups were analyzed by Student's *t* test and are shown by asterisks (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

concentrations of ALA were undetectable in the POD and SOD groups, but both EPA and DHA concentrations were highest in rats given the FOD with no difference between the other groups except for a lower concentration of DHA in rats given the SOD.

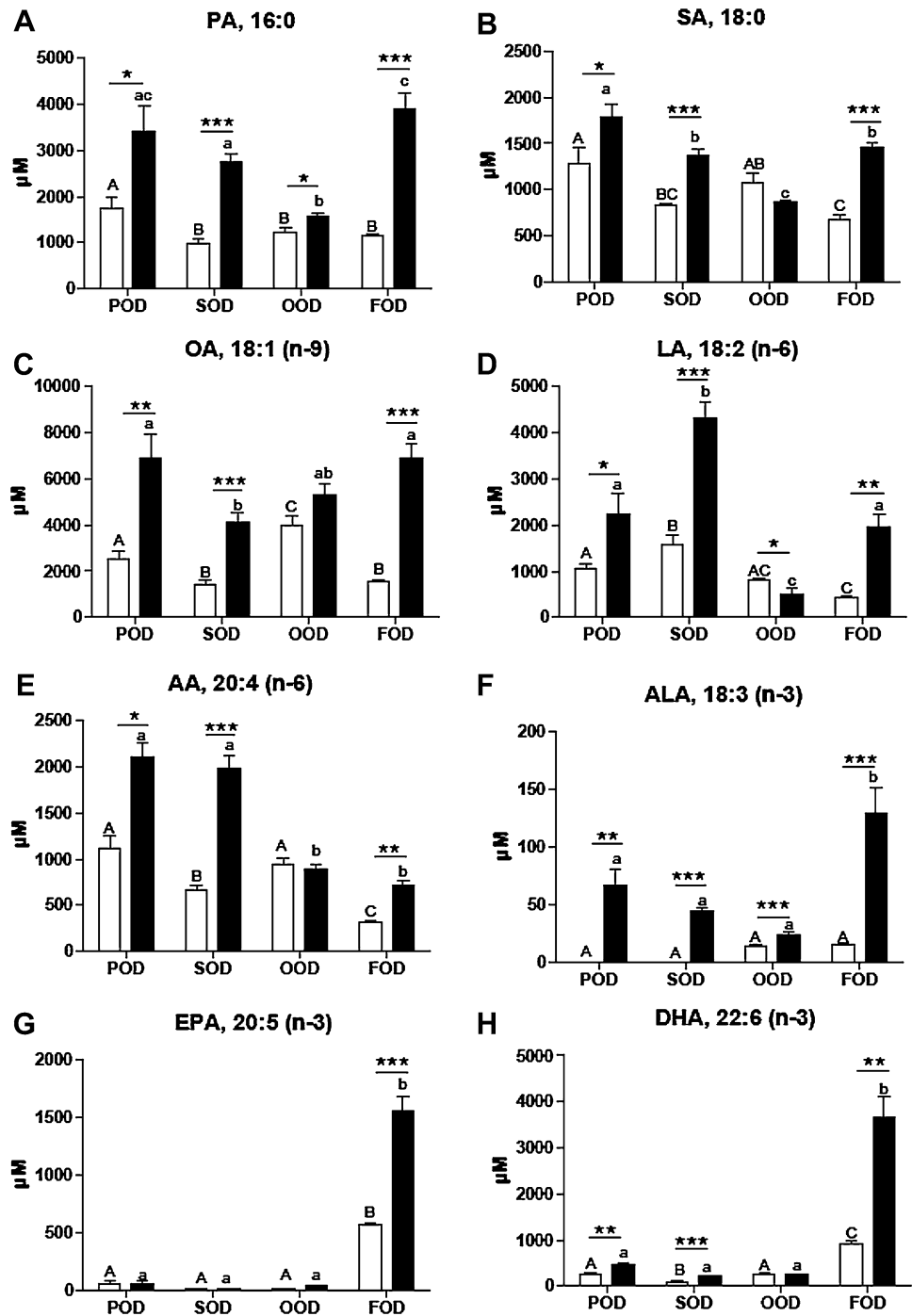
The response to 24 h fasting in rats given the OOD was quite different to the responses of the other three groups: with the exception of a slight increase in plasma PA neither SA nor the concentration of any of the unsaturated fatty acids differed between fed and fasted rats in that group. The only exception to that rule was a much less pronounced increase in the concentrations of ALA than seen in the other groups. In all the other groups (i.e., POD, SOD and FOD), maternal fasting produced a significant increase in all the individual fatty acids detected (Fig. 3).

Fetal plasma FA concentrations showed different responses to those described for their mothers. PA, SA and OA concentrations did not differ between the four groups of fed animals (Fig. 4). However, fetal plasma concentrations of most PUFA paralleled those of their fed mothers, e.g., highest n-6 fatty acids (LA and AA) in fetus of dams given the SOD and of n-3 fatty acids (EPA and DHA) in those of dams given the FOD. Whereas GLN was undetectable in maternal plasma, it was present in fetal plasma, its concentration being higher in the SOD group than in the other groups (Fig. 4). Maternal fasting decreased the concentration of PA, SA and OA in the fetal plasma of FOD compared to the fed group, but did not modify the concentration of any PUFA in fetal plasma in any group (Fig. 4).

In fetal liver the proportion of neither saturated fatty acids or nor OA followed the trends found in maternal plasma; in fed animals they did not differ between the four groups except for a lower OA value in the FOD group and, whereas fasting only produced an increase in fetal liver PA in the POD group and OA in the OOD group, the proportion of SA declined in both groups (Table 4). The proportion of LA and GLN in fetal liver appears highest in those from dams given SOD and of all of the n-3 fatty acids in those from dams given FOD. The proportion of both AA and GLN was lower in fetal liver of the FOD group than in any of the other groups. Maternal fasting caused a decline of AA in the fetal liver of the FOD group but caused a significant increase in both LA and ALA in all four groups, an increase in GLN in the SOD group and an increase in n-3 DPA in the FOD group, with no change in any of the remaining PUFA.

The FA profile in fetal brain also showed some specific changes (Table 5). There were no significant changes in the proportions of any of the saturated fatty acids between the groups either fed or fasted, except that fasting increased the proportion of SA in the SOD and FOD groups. No differences were found between the groups in the proportion of OA when fed, and although fasting did not have a significant effect on the proportion of OA in fetal brain, values in both OOD and FOD were higher than in the POD or SOD groups. Results for the proportions of PUFA in fetal brain showed that values of LA were higher in fetuses from SOD dams and the proportion of DHA was higher in

Fig. 3 Plasma fatty acid concentration (μM) in fed and 24 h fasted, 20 day pregnant, rats that were given palm oil (POD), sunflower oil (SOD), olive oil (OOD) or fish oil (FOD) diets during pregnancy. **a** Palmitic acid (PA) in fed (white bars) and fasted (black bars) rats, **b** stearic acid (SA) in fed (white bars) and fasted (black bars) rats, **c** oleic acid (OA) in fed (white bars) and fasted (black bars) rats, **d** linoleic acid (LA) in fed (white bars) and fasted (black bars) rats, **e** arachidonic acid (AA) in fed (white bars) and fasted (black bars) rats, **f** α -linolenic acid (ALA) in fed (white bars) and fasted (black bars) rats and **g** eicosapentanoic acid (EPA) in fed (white bars) and fasted (black bars) rats and **h** docosahexanoic acid (DHA) in fed (white bars) and fasted (black bars) rats. Values are expressed as means \pm SEM of 6–8 rats per group. An SNK test was used to determine differences between groups after ANOVA. Statistical comparisons for each variable between fed groups are shown with capital superscript letters and between fasted groups with lower-case superscript letters. Different capital or lower-case letters indicate that the difference is statistically significant ($P < 0.05$). Differences between fed and fasted groups were analyzed by Student's *t* test and are shown by asterisks * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$



fetuses from FOD dams than in any of the other groups. The proportion of DHA was lowest in the SOD group and that of AA and adrenic acid (ADA, 22:4n-6) were lowest in the FOD group (Table 5). Maternal fasting increased the proportion of LA in brain of fetuses from the OOD, and that of EPA in brain of fetuses from the SOD rats, with no effect in any of the other groups.

Discussion

The results presented here show that when pregnant rats were given diets containing different fatty acid compositions, the fatty acid profile of maternal adipose tissue during late pregnancy mirrored that of the diets. With the exception of animals fed the OOD, maternal fasting greatly increased the plasma concentration of most fatty acids. This allows

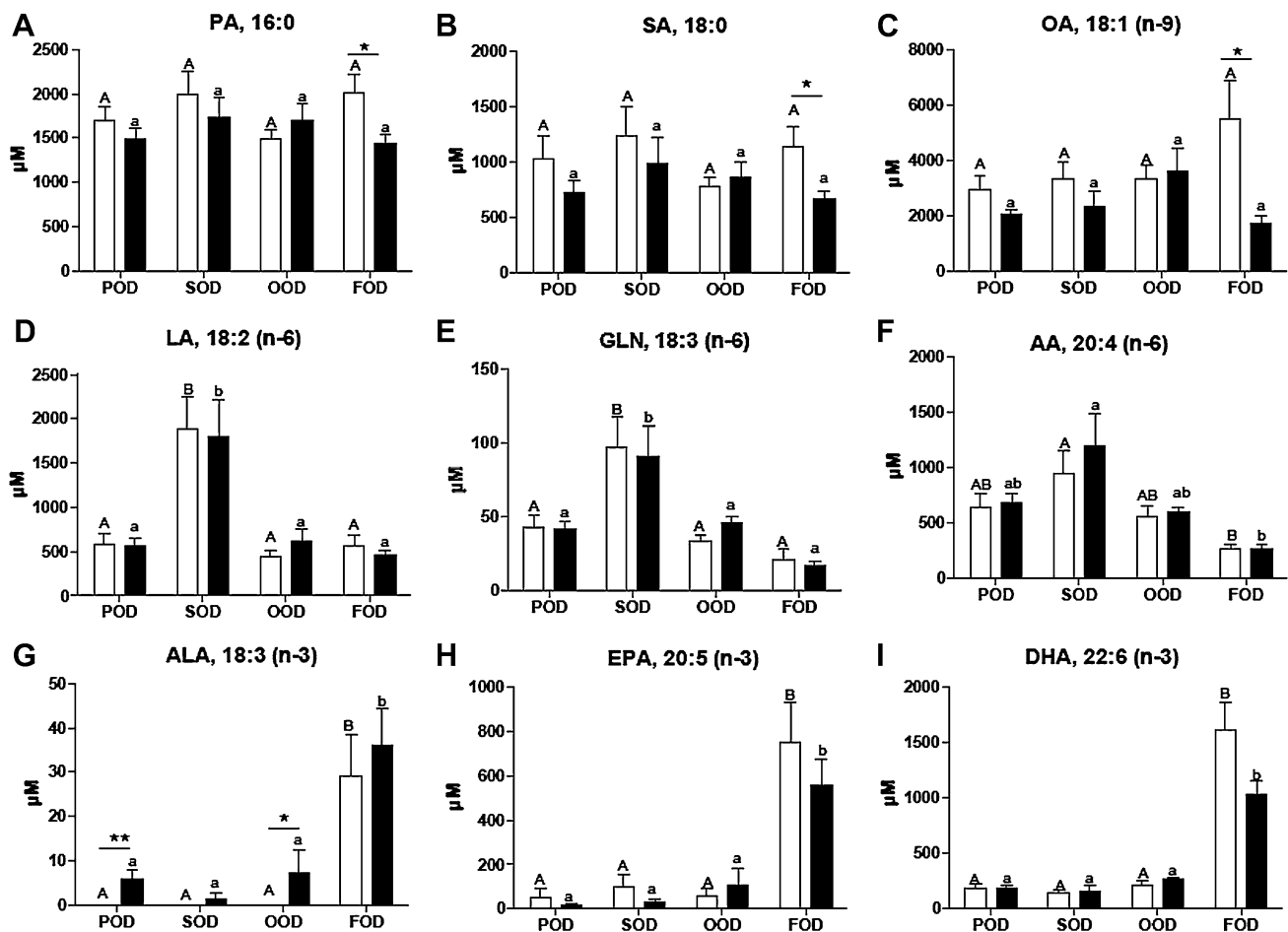


Fig. 4 Plasma fatty acid concentration (μM) in fetuses of fed and 24 h fasted, 20 day pregnant, rats that were given palm oil (POD), sunflower oil (SOD), olive oil (OOD) or fish oil (FOD) diets during pregnancy. **a** Palmitic acid (PA) in fed (white bars) and fasted (black bars) rats, **b** stearic acid (SA) in fed (white bars) and fasted (black bars) rats, **c** oleic acid (OA) in fed (white bars) and fasted (black bars) rats, **d** linoleic acid (LA) in fed (white bars) and fasted (black bars) rats, **e** γ -linolenic acid (GLN) in fed (white bars) and fasted (black bars) rats, **f** arachidonic acid (AA) in fed (white bars) and fasted (black bars) rats, **g** α -linolenic acid (ALA) in fed (white bars) and fasted (black bars) rats **h** eicosapentanoic acid (EPA) in fed (white

bars) and fasted (black bars) rats and **i** docosahexanoic acid (DHA) in fed (white bars) and fasted (black bars) rats. Values are expressed as means \pm SEM of 6–8 rats per group. An SNK test was used to determine differences between groups after ANOVA. Statistical comparisons for each variable between fed groups are shown with capital superscript letters and between fasted groups with lower-case superscript letters. Different capital or lower-case letters indicate that the difference is statistically significant ($P < 0.05$). Differences between fed and fasted groups were analyzed by Student's *t* test and are shown by asterisks * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

for the continued availability of PUFA to the fetus and preserves the normal (i.e., fed-state) amount of the PUFA in fetal brain, which may contribute to the maintenance of brain weight while both fetal body and liver weights decline. These findings indicate that PUFA from the maternal diet, accumulated in maternal adipose tissue during pregnancy, represent the main source of placentally transferred PUFA under the conditions of maternal fasting. Increments during fasting of maternal plasma NEFA, glycerol and TAG all indicate an active adipose tissue lipolytic activity, which is known to be highly accelerated during late pregnancy in both women [26, 43] and rats [27] and results from hypoglycemia, as found in all the groups, and subsequent increased

catecholamine release [44] and activation of the lipolytic cascade. The smaller increments in those indices of lipolytic activity associated with smaller or no rise in individual plasma fatty acids in the OOD group indicate a less dramatic increase in adipose tissue lipolytic activity with maternal fasting. This particular response may occur as a consequence of the accumulation of OA in adipose tissue of rats given the OOD, which can reach values over 50% of the total fatty acids in the tissue. It is known that the key enzyme controlling lipolysis, hormone sensitive lipase, shows a specificity for esterified fatty acids that results in a selective mobilization of stored fatty acids. Monounsaturated acids are more slowly mobilized than more unsaturated fatty acids [45, 46].

Table 4 Liver fatty acid profile (g/100 g of fatty acids) in fetuses of fed and 24 h fasted, 20 day pregnant rats that were given palm oil (POD), sunflower oil (SOD), olive oil (OOD) or fish oil (FOD) diets during pregnancy

Fatty acid		POD	SOD	OOD	FOD
PA, 16:0	Fed	21.5 ± 1.2 ^A	20.7 ± 0.6 ^A	23.6 ± 1.6 ^A	24.8 ± 1.3 ^A
PA, 16:0	Fasted	25.3 ± 0.7 ^{a*}	19.7 ± 0.8 ^b	22.3 ± 1.3 ^{ab}	24.6 ± 1.3 ^a
SA, 18:0	Fed	18.4 ± 1.0 ^A	16.2 ± 1.0 ^A	17.9 ± 1.1 ^A	18.9 ± 0.3 ^A
SA, 18:0	Fasted	14.4 ± 0.3 ^{a**}	13.5 ± 0.9 ^a	13.5 ± 0.4 ^{a**}	16.3 ± 1.3 ^a
OA, 18:1n-9	Fed	25.5 ± 0.3 ^A	22.5 ± 0.8 ^A	25.8 ± 0.9 ^A	15.0 ± 1.7 ^B
OA, 18:1n-9	Fasted	23.6 ± 1.1 ^a	20.7 ± 0.8 ^a	33.6 ± 0.8 ^{b***}	12.9 ± 1.7 ^c
LA, 18:2n-6	Fed	5.5 ± 0.5 ^A	14.0 ± 1.0 ^B	4.0 ± 0.5 ^{AC}	3.2 ± 0.1 ^C
LA, 18:2n-6	Fasted	7.4 ± 0.3 ^{a**}	18.7 ± 0.8 ^{b**}	5.4 ± 0.2 ^{c*}	5.5 ± 0.3 ^{c***}
GLN, 18:3n-6	Fed	0.38 ± 0.02 ^A	0.68 ± 0.12 ^B	0.34 ± 0.03 ^A	0.10 ± 0.01 ^C
GLN, 18:3n-6	Fasted	0.46 ± 0.03 ^a	0.94 ± 0.04 ^{b*}	0.38 ± 0.05 ^a	0.15 ± 0.02 ^c
AA, 20:4n-6	Fed	10.4 ± 0.5 ^A	10.4 ± 0.4 ^A	8.8 ± 1.1 ^A	4.3 ± 0.1 ^B
AA, 20:4n-6	Fasted	8.6 ± 0.6 ^a	11.4 ± 0.6 ^b	7.9 ± 0.3 ^a	3.6 ± 0.1 ^{c**}
ALA, 18:3n-3	Fed	0.00 ± 0.00 ^A	0.04 ± 0.02 ^A	0.02 ± 0.02 ^A	0.11 ± 0.02 ^B
ALA, 18:3n-3	Fasted	0.20 ± 0.01 ^{a***}	0.21 ± 0.03 ^{a***}	0.14 ± 0.01 ^{a***}	0.49 ± 0.02 ^{b***}
EPA, 20:5n-3	Fed	0.7 ± 0.4 ^A	0.6 ± 0.3 ^A	3.8 ± 1.1 ^B	8.2 ± 0.6 ^C
EPA, 20:5n-3	Fasted	1.2 ± 0.6 ^a	1.0 ± 0.4 ^a	1.3 ± 0.5 ^a	9.6 ± 0.7 ^b
DPA, 22:5n-3	Fed	0.14 ± 0.07 ^A	0.11 ± 0.04 ^A	0.34 ± 0.1 ^A	1.65 ± 0.14 ^B
DPA, 22:5n-3	Fasted	0.12 ± 0.04 ^a	0.13 ± 0.03 ^a	0.17 ± 0.04 ^a	2.44 ± 0.24 ^{b*}
DHA, 22:6n-3	Fed	3.4 ± 0.3 ^A	1.6 ± 0.3 ^B	3.3 ± 0.5 ^A	16.1 ± 0.7 ^C
DHA, 22:6n-3	Fasted	3.3 ± 0.4 ^a	1.9 ± 0.2 ^a	3.3 ± 0.2 ^a	16.5 ± 1.1 ^b

Values are expressed as means ± SEM of 6–8 rats per group. An SNK test was used to determine differences between groups after ANOVA. Statistical comparisons for each variable between fed groups are shown with capital superscript letters and between fasted groups with lower-case superscript letters. Different capital or lower-case letters indicate that the difference is statistically significant ($P < 0.05$). Differences between fed and fasted groups were analyzed by Student's *t* test and are shown by asterisks (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

AA concentrations were present in the plasma of all the studied rats, although its value was lowest in rats fed the FOD. Since this fatty acid is practically absent in the diet and very low in adipose tissue, it is mainly synthesized endogenously in liver from LA catalyzed by highly active $\Delta 5$ - and $\Delta 6$ -desaturases present in the organ [47, 48]. The known inhibition of the $\Delta 6$ -desaturase by excessive concentrations of EPA and DHA [49, 50] explains the low amounts of AA present in maternal plasma of the FOD group. Alongside the increases in lipolysis, maternal fasting increased the proportion of practically all the individual fatty acids, the effect being more pronounced in those animals given the FOD, SOD or POD than in those given the OOD. These findings lead us to propose the following scheme:

1. Dietary PUFA are taken up by adipose tissue throughout pregnancy, and especially during early pregnancy, when adipose tissue LPL is either increased [17] or unchanged [18, 19].
2. Increased lipolytic activity during fasting at late pregnancy releases these fatty acids as NEFA into the circulation from where they taken up by the liver, returning to the circulation in the form of TAG associated with VLDL. Here they are transferred to lipoproteins

of higher density. In fact, as found in women [13] and in rats [51], most PUFA in maternal plasma are carried in their esterified form associated to circulating lipoproteins rather than as NEFA.

3. Although these lipoproteins do not directly cross the placental barrier [15], they are taken up by the placenta, from where fatty acids diffuse to the fetal side.

With the exception of saturated and monounsaturated fatty acids, the concentrations of most individual fatty acids in plasma of fetuses from fed mothers very closely paralleled the changes of plasma fatty acids seen in their respective mothers. It is not surprising that the concentration of neither saturated nor monounsaturated fatty acids in fetal plasma follow the changes in their maternal side, since it is known that at this late stage of intrauterine development, both fatty acid synthesis and $\Delta 9$ desaturase activity are well developed in fetal liver [52, 53]. Taken together with the limited ability of placenta to transfer these fatty acids [54], this explains the lack of dependence on the mother of the fetus for the supply of these acids.

As well as the trend to mirror maternal plasma fatty acid concentrations, certain PUFAs exhibit more extreme intergroup differences in their fetal plasma concentrations, which

Table 5 Brain fatty acid profile (g/100 g of fatty acids) in fetuses of fed and 24 h fasted, 20 day pregnant, rats that were given palm oil (POD), sunflower oil (SOD), olive oil (OOD) or fish oil (FOD) diets during pregnancy

Fatty acid		POD	SOD	OOD	FOD
PA, 16:0	Fed	28.7 ± 0.8 ^A	29.8 ± 0.6 ^A	26.3 ± 3.2 ^A	29.7 ± 1.4 ^A
PA, 16:0	Fasted	28.4 ± 1.5 ^a	18.2 ± 3.9 ^{a*}	28.0 ± 1.4 ^a	27.3 ± 2.2 ^a
SA, 18:0	Fed	16.2 ± 0.3 ^A	16.7 ± 0.5 ^A	17.9 ± 1.5 ^A	16.6 ± 0.9 ^A
SA, 18:0	Fasted	16.9 ± 0.6 ^a	21.3 ± 1.5 ^{a*}	17.5 ± 1.0 ^a	20.0 ± 1.5 ^{a*}
OA, 18:1n-9	Fed	16.1 ± 0.2 ^A	15.0 ± 0.2 ^A	16.0 ± 0.9 ^A	20.5 ± 3.4 ^A
OA, 18:1n-9	Fasted	15.7 ± 0.4 ^a	15.6 ± 0.4 ^a	17.3 ± 0.6 ^b	17.9 ± 0.6 ^b
LA, 18:2n-6	Fed	0.5 ± 0.01 ^A	1.1 ± 0.09 ^B	0.4 ± 0.06 ^A	0.5 ± 0.02 ^A
LA, 18:2n-6	Fasted	0.5 ± 0.02 ^a	1.1 ± 0.05 ^b	0.6 ± 0.05 ^{a*}	0.6 ± 0.06 ^a
AA, 20:4n-6	Fed	12.3 ± 0.3 ^A	12.8 ± 0.4 ^A	11.9 ± 1.2 ^A	6.6 ± 0.3 ^B
AA, 20:4n-6	Fasted	11.9 ± 0.5 ^a	14.2 ± 1.0 ^a	12.6 ± 0.3 ^a	6.9 ± 0.7 ^b
ADA, 22:4n-6	Fed	0.6 ± 0.1 ^A	0.9 ± 0.1 ^A	0.8 ± 0.2 ^A	0.0 ± 0.0 ^B
ADA, 22:4n-6	Fasted	0.7 ± 0.1 ^a	1.2 ± 0.1 ^{b*}	0.5 ± 0.1 ^{ab}	0.0 ± 0.0 ^c
ALA, 18:3n-3	Fed	0.0 ± 0.0 ^A	0.0 ± 0.0 ^A	0.0 ± 0.0 ^A	0.5 ± 0.2 ^A
ALA, 18:3n-3	Fasted	0.3 ± 0.1 ^{a**}	0.1 ± 0.1 ^a	0.5 ± 0.2 ^a	1.8 ± 0.5 ^{b*}
EPA, 20:5n-3	Fed	1.9 ± 0.3 ^A	1.6 ± 0.3 ^A	3.3 ± 1.4 ^A	2.6 ± 0.3 ^A
EPA, 20:5n-3	Fasted	2.4 ± 0.1 ^a	5.3 ± 1.5 ^{a*}	1.9 ± 0.6 ^a	5.2 ± 1.5 ^a
DHA, 22:6n-3	Fed	6.2 ± 0.4 ^A	3.3 ± 0.1 ^B	6.2 ± 1.1 ^A	13.2 ± 0.8 ^C
DHA, 22:6n-3	Fasted	6.3 ± 0.6 ^a	3.5 ± 0.4 ^b	6.2 ± 0.3 ^a	12.4 ± 1.5 ^c

Values are expressed as means ± SEM of 6–8 rats per group. An SNK test was used to determine differences between groups after ANOVA. Statistical comparisons for each variable between fed groups are shown with capital superscript letters and between fasted groups with lower-case superscript letters. Different capital or lower-case letters indicate that the difference is statistically significant ($P < 0.05$). Differences between fed and fasted groups were analyzed by Student's *t* test and are shown by asterisk (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

may be higher than on the maternal side of the placenta. This is the case for LA and especially the immediate product of its $\Delta 6$ desaturation, GLN the concentration of which is much higher in fetal plasma of SOD dams than in the maternal plasma. In fact, GLN is absent both in maternal diet, and in maternal plasma. Therefore, its presence in fetal plasma and fetal liver must be the result of its synthesis from LA, en route to the synthesis of AA, which is also present at higher concentrations in fetal plasma of the SOD group than in their mothers (and indeed, more than in any of the other groups). As well as indicating the efficient placental transfer of LA, these findings indicate an efficient $\Delta 6$ desaturation process in fetal tissues, which agrees with the known presence of $\Delta 5$ and $\Delta 6$ desaturases in both rat and human fetal liver [55, 56]. Other PUFA in fetal plasma, such as the n-3 fatty acids follow the same trend as maternal plasma, as can be most clearly seen in the case of the high concentrations of both DHA and EPA in the FOD group. In this specific group, fetal plasma concentrations of AA are lower than in any other group, and the same is true in fetal liver. The same low AA concentration was also present in plasma of FOD mothers. The present results do not allow us to determine whether the low AA concentration in the FOD group results from inhibition of the $\Delta 6$ desaturase by DHA and EPA in the fetal liver, or as a direct consequence of the events taking place on maternal side.

Maternal fasting has very little effect on fetal plasma fatty acid concentrations, with the only exception being a decline in some saturated fatty acids, which may be the result of a reduced lipogenic activity. The effect of maternal fasting is more obvious in the fatty acid composition of fetal liver, where the proportion of the two essential fatty acids, LA and ALA, increase in all the groups. These overall findings indicate that in maternal fasting, maternal plasma is the main source of these fatty acids for the fetus. Furthermore, under fasting conditions, these fatty acids must, in turn, be derived from lipolysis in maternal adipose tissue, which, therefore, plays a major role in their availability to the fetus.

The situation in fetal brain requires special comment. The fatty acid profile of fetal brains from fed dams parallel those found in fetal liver as influenced by the maternal diet. In contrast, and unlike the fetal liver, fasting the mothers had virtually no effect on the composition of fetal brain. These findings indicate again that fatty acids stored in maternal fat depots seems to be an important source of PUFA for fetal brain development, especially when dietary restrictions occur during late pregnancy. The rapid accumulation of some of the PUFA occurs to sustain the active neurogenesis taking place during this critical stage of brain development in rats, as has been shown previously for both DHA and AA [39, 40]. Some specific contributions of brain fatty acid biochemistry may also occur at this stage: it is known that

fetal brain is able to convert the essential precursors ALA and LA into DHA and AA, respectively [36]. Except for the FOD, the other diets used in present study were clearly n-3 PUFA deficient and resulted in the low proportions of DHA in fetal liver and brain, which was counterbalanced by a higher content of AA and its elongation product, ADA, a finding that agrees with previous results showing an inverse interrelationship between these n-6 LCPUFA and DHA PUFA in fetal liver and brain [40, 57].

As has been consistently found in all the fetal and maternal sites studied here, rats fed the FOD show a lower proportion of AA than any of the other groups. A decline in brain phospholipid AA content was also previously reported in pregnant rats receiving dietary supplements of DHA [40]. We know that in rats fed the FOD during pregnancy and lactation, the deficiency in offspring brain AA is maintained post-natally, and seems to be responsible for their delayed development, including the acquisition of psycho-motor reflexes [58]. Thus, although intervention with maternal dietary supplements of DHA or fish oil during pregnancy may replace potential deficiencies of DHA in fetal brain, which may be indicated in certain pathophysiological conditions, such action should only be done taking care to avoid a concomitant depletion of AA.

Although extrapolation of these findings to the human condition is not possible for obvious reasons, they show the major role of maternal adipose tissue fatty acid deposition during pregnancy as a guarantee of the availability of PUFA to the fetus under conditions of food restriction. They also emphasize the need for a diet of balanced PUFA composition to ensure that the appropriate composition is available for the developing fetus.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

- Awad AB (1981) Effect of dietary lipids on composition and glucose utilization by rat adipose tissue. *J Nutr* 111:34–39
- Becker W, Bruce A (1986) Retention of linoleic acid in carcass lipids of rats fed different levels of essential fatty acids. *Lipids* 21:121–126
- Nelson GJ, Kelley DS, Schmidt PC, Serrato SM (1987) The influence of dietary fat on the lipogenic activity and fatty acid composition of rat adipose tissue. *Lipids* 22:338–344
- Sanchez-Blanco C, Amusquivar E, Bispo K, Herrera E (2016) Influence of cafeteria diet and fish oil in pregnancy and lactation on pups' body weight and fatty acid profiles in rats. *Eur J Nutr* 55:1741–1753
- Anderson DB, Kauffman RG (1973) Cellular and enzymatic changes in porcine adipose tissue during growth. *J Lipid Res* 14:160–168
- Etherton TD, Allen CE (1980) Effects of age and adipocyte size on glucose and palmitate metabolism and oxidation in pigs. *J Anim Sci* 50:1073–1084
- Gandemer G, Pascal G, Durand G (1985) Comparative changes in the lipogenic enzyme activities and in the in vivo fatty acid synthesis in liver and adipose tissues during the post-weaning growth of male rats. *Comp Biochem Physiol B* 82:581–586
- Baylin A, Kabagambe EK, Siles X, Campos H (2002) Adipose tissue biomarkers of fatty acid intake. *Am J Clin Nutr* 76:750–757
- Leaf DA, Connor WE, Barstad L, Sexton G (1995) Incorporation of dietary n-3 fatty acids into the fatty acids of human adipose tissue and plasma lipid classes. *Am J Clin Nutr* 62:68–73
- Lhuillery C, Mebarki S, Lecourtier MJ, Demarne Y (1988) Influence of different dietary fats on the incorporation of exogenous fatty acids into rat adipose glycerides. *J Nutr* 118:1447–1454
- Weber N, Klein E, Mukherjee KD (2002) The composition of the major molecular species of adipose tissue triacylglycerols of rats reflects those of dietary rapeseed, olive and sunflower oils. *J Nutr* 132:726–732
- Amusquivar E, Herrera E (2003) Influence of changes in dietary fatty acids during pregnancy on placental and fetal fatty acid profile in the rat. *Biol Neonate* 83:136–145
- Herrera E (2002) Lipid metabolism in pregnancy and its consequences in the fetus and newborn. *Endocr* 19:43–55
- Herrera E, Ortega-Senovilla H (2014) Lipid metabolism during pregnancy and its implications for fetal growth. *Curr Pharm Biotechnol* 15:24–31
- Herrera E, Desoye G (2016) Maternal and fetal lipid metabolism under normal and gestational diabetic conditions. *Horm Mol Biol Clin Investig* 26:109–127
- Herrera E, Lasunción MA, Martín A, Zorzano A (1992) Carbohydrate-lipid interactions in pregnancy. In: Herrera E, Knopp RH (eds) *Perinatal biochemistry*. CRC Press, Boca Raton, pp 1–18
- Knopp RH, Boroush MA, O'Sullivan JB (1975) Lipid metabolism in pregnancy. II. Postheparin lipolytic activity and hypertriglyceridemia in the pregnant rat. *Metabolism* 24:481–493
- Alvarez JJ, Montelongo A, Iglesias A, Lasuncion MA, Herrera E (1996) Longitudinal study on lipoprotein profile, high density lipoprotein subclass, and postheparin lipases during gestation in women. *J Lipid Res* 37:299–308
- Martin-Hidalgo A, Holm C, Belfrage P, Schotz MC, Herrera E (1994) Lipoprotein lipase and hormone-sensitive lipase activity and mRNA in rat adipose tissue during pregnancy. *Am J Physiol* 266:E930–E935
- Moore BJ, Brasel JA (1984) One cycle of reproduction consisting of pregnancy, lactation or no lactation, and recovery: effects on fat pad cellularity in ad libitum-fed and food-restricted rats. *J Nutr* 114:1560–1565
- Murphy SP, Abrams BF (1993) Changes in energy intakes during pregnancy and lactation in a national sample of US women. *Am J Public Health* 83:1161–1163
- Piers LS, Diggavi SN, Thangam S, van Raaij JM, Shetty PS, Hautvast JG (1995) Changes in energy expenditure, anthropometry, and energy intake during the course of pregnancy and lactation in well-nourished Indian women. *Am J Clin Nutr* 61:501–513

23. Hamosh M, Clary TR, Chernick SS, Scow RO (1970) Lipoprotein lipase activity of adipose and mammary tissue and plasma triglyceride in pregnant and lactating rats. *Biochim Biophys Acta* 210:473–482
24. Herrera E, Lasuncion MA, Gomez-Coronado D, Aranda P, Lopez-Luna P, Maier I (1988) Role of lipoprotein lipase activity on lipoprotein metabolism and the fate of circulating triglycerides in pregnancy. *Am J Obstet Gynecol* 158:1575–1583
25. Ramirez I, Llobera M, Herrera E (1983) Circulating triacylglycerols, lipoproteins, and tissue lipoprotein lipase activities in rat mothers and offspring during the perinatal period: effect of post-maturity. *Metabolism* 32:333–341
26. Elliott JA (1975) The effect of pregnancy on the control of lipolysis in fat cells isolated from human adipose tissue. *Eur J Clin Invest* 5:159–163
27. Knopp RH, Herrera E, Freinkel N (1970) Carbohydrate metabolism in pregnancy. 8. Metabolism of adipose tissue isolated from fed and fasted pregnant rats during late gestation. *J Clin Invest* 49:1438–1446
28. Williams C, Coltart TM (1978) Adipose tissue metabolism in pregnancy: the lipolytic effect of human placental lactogen. *Br J Obstet Gynaecol* 85:43–46
29. Herrera E, Lasunción MA (2017) Maternal-fetal transfer of lipid metabolites. In: Polin RA, Abman SH, Rowitch DH, Benitz WE, Fox WW (eds) *Fetal and neonatal physiology*. Elsevier, Philadelphia, pp 342–353
30. Mampel T, Villarroya F, Herrera E (1985) Hepatectomy-nephrectomy effects in the pregnant rat and fetus. *Biochem Biophys Res Commun* 131:1219–1225
31. Ramos P, Herrera E (1995) Reversion of insulin resistance in the rat during late pregnancy by 72-h glucose infusion. *Am J Physiol* 269:E858–E863
32. Knopp RH, Bonet B, Lasunción MA, Montelongo A, Herrera E (1992) Lipoprotein metabolism in pregnancy. In: Herrera E, Knopp RH (eds) *Perinatal biochemistry*. CRC Press, Boca Raton, pp 19–51
33. Herrera E, Ortega-Senovilla H (2016) The roles of fatty acids in fetal development. In: Duttaroy AK, Basak S (eds) *Human placental trophoblasts impact of maternal nutrition*. CRC Press, Boca Raton, pp 93–111
34. Fernandes FS, Tavares do Carmo M, Herrera E (2012) Influence of maternal diet during early pregnancy on the fatty acid profile in the fetus at late pregnancy in rats. *Lipids* 47:505–517
35. Crawford MA, Hassam AG, Stevens PA (1981) Essential fatty acid requirements in pregnancy and lactation with special reference to brain development. *Prog Lipid Res* 20:31–40
36. Green P, Yavin E (1993) Elongation, desaturation, and esterification of essential fatty acids by fetal rat brain in vivo. *J Lipid Res* 34:2099–2107
37. Bayer SA, Altman J, Russo RJ, Zhang X (1993) Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. *Neurotoxicology* 14:83–144
38. Frederiksen K, McKay RD (1988) Proliferation and differentiation of rat neuroepithelial precursor cells in vivo. *J Neurosci* 8:1144–1151
39. Green P, Glozman S, Kamensky B, Yavin E (1999) Developmental changes in rat brain membrane lipids and fatty acids. The preferential prenatal accumulation of docosahexaenoic acid. *J Lipid Res* 40:960–966
40. Schiefermeier M, Yavin E (2002) n-3 Deficient and docosahexaenoic acid-enriched diets during critical periods of the developing prenatal rat brain. *J Lipid Res* 43:124–131
41. Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 226:497–509
42. Amusquivar E, Schiffner S, Herrera E (2011) Evaluation of two methods for plasma fatty acid analysis by GC. *Eur J Lipid Sci Technol* 113:711–716
43. Sivan E, Homko CJ, Chen X, Reece EA, Boden G (1999) Effect of insulin on fat metabolism during and after normal pregnancy. *Diabetes* 48:834–838
44. Herrera EM, Knopp RH, Freinkel N (1969) Urinary excretion of epinephrine and norepinephrine during fasting in late pregnancy in the rat. *Endocrinology* 84:447–450
45. Raclot T, Groscolas R (1995) Selective mobilization of adipose tissue fatty acids during energy depletion in the rat. *J Lipid Res* 36:2164–2173
46. Raclot T, Mioskowski E, Bach AC, Groscolas R (1995) Selectivity of fatty acid mobilization: a general metabolic feature of adipose tissue. *Am J Physiol* 269:R1060–1067
47. Brenner RR (1974) The oxidative desaturation of unsaturated fatty acids in animals. *Mol Cell Biochem* 3:41–52
48. Cook HW (1991) Fatty acid desaturation and chain elongation in eucaryotes. In: Vance DE, Vance J (eds) *Biochemistry of lipids, lipoproteins and membranes*. Elsevier, Amsterdam, pp 141–169
49. Garg ML, Thomson AB, Clandinin MT (1990) Interactions of saturated, n-6 and n-3 polyunsaturated fatty acids to modulate arachidonic acid metabolism. *J Lipid Res* 31:271–277
50. Raz A, Kamin-Belsky N, Przeddecki F, Obukowicz M (1998) Dietary fish oil inhibits delta6-desaturase activity. *J Am Oil Chem Soc* 75:241–245
51. Herrera E, Amusquivar E, Lopez-Soldado I, Ortega H (2006) Maternal lipid metabolism and placental lipid transfer. *Horm Res* 65(Suppl 3):59–64
52. Lorenzo M, Caldes T, Benito M, Medina JM (1981) Lipogenesis in vivo in maternal and foetal tissues during late gestation in the rat. *Biochem J* 198:425–428
53. Mercuri O, de Tomas ME, Itarte H (1979) Prenatal protein depletion and Delta9, Delta6 and Delta5 desaturases in the rat. *Lipids* 14:822–825
54. Campbell FM, Gordon MJ, Dutta-Roy AK (1996) Preferential uptake of long chain polyunsaturated fatty acids by isolated human placental membranes. *Mol Cell Biochem* 155:77–83
55. Ravel D, Chambaz J, Pepin D, Manier MC, Bereziat G (1985) Essential fatty acid interconversion during gestation in the rat. *Biochim Biophys Acta* 833:161–164
56. Chambaz J, Ravel D, Manier MC, Pepin D, Mulliez N, Bereziat G (1985) Essential fatty acids interconversion in the human fetal liver. *Biol Neonate* 47:136–140
57. Green P, Kamensky B, Yavin E (1997) Replenishment of docosahexaenoic acid in n-3 fatty acid-deficient fetal rats by intraamniotic ethyl-docosahexaenoate administration. *J Neurosci* 17:264–272
58. Amusquivar E, Ruperez FJ, Barbas C, Herrera E (2000) Low arachidonic acid rather than alpha-tocopherol is responsible for the delayed postnatal development in offspring of rats fed fish oil instead of olive oil during pregnancy and lactation. *J Nutr* 130:2855–2865