Fate of orally administered radioactive fatty acids in the late pregnant rat

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Abstract

In order to investigate the biodisponibility of placental transfer of fatty acids, rats pregnant for 20 days were given tracer amounts of $^{14}$C-palmitic- (PA), oleic- (OA), linoleic- (LA), $\alpha$-linolenic- (LNA) or docosahexaenoic-acid (DHA) orally and sacrificed at 0.5, 1.0, 2.0 or 8.0 h thereafter. Maternal plasma radioactivity in lipids initially increased to decline at later times. Most of the label appeared first as triacylglycerols (TAG); later, the proportion in phospholipids (PhL) increased. The percentage of label in placental lipids was also always highest shortly after administration and declined later; again PhL increased with time. Fetal plasma radioactivity increased with time, its highest value at 8.0 h after DHA or LNA administration. DHA initially appeared primarily in the non-esterified fatty acids (NEFA), and PA, OA, LA and LNA as TAG followed by NEFA; in all cases there was an increase in PhL at later times. Measurement of fatty acid concentrations allowed calculation of specific (radio)activities and the ratio (fetal/maternal) of these in the plasmas gave an index of placental transfer activity, which was LNA>LA>DHA=OA>PA. It is proposed that a considerable proportion of most fatty acids transferred through the placenta are released into the fetal circulation in the form of TAG.

Keywords: fatty acids – placental transfer – pregnancy – rats – fetal triacylglycerols

Abbreviations: AA, arachidonic acid, 20:4n-6; DAG, diacylglycerols; DHA, docosahexaenoic acid, 22:6n-3; EC, esterified cholesterol; EFA, essential fatty acids; LA, linoleic acid, 18:2n-6; LCPUFA, long-chain polyunsaturated fatty acids; LNA, alpha-linolenic acid, 18:3n-3; MAG, monoacylglycerols; NEFA, non-esterified fatty acids; OA, oleic acid, 18:1n-9; PA, palmitic acid, 16:0; PhL, phospholipids; TAG, triacylglycerols
The developing fetus requires fatty acids to maintain the conformation and function of membranes, as precursors of bioactive compounds like prostaglandins, prostacyclins, thromboxanes and leukotrienes, and as a source of energy. All fatty acids can act as an energy source, the structural functions rely upon the presence of unsaturated and polyunsaturated acids with 18 carbons or more, and the bioactive prostanoids are derived from the long chain polyunsaturated fatty acids (LCPUFA). Of these unsaturated acids, the essential fatty acids (EFA), linoleic (18:2n-6, LA) and α-linolenic (18:3n-3, LNA) acids, and their respective long-chain derivatives, mainly arachidonic (20:4n-6, AA) and docosahexaenoic (22:6n-3, DHA) acids are required to sustain fetal tissue growth and development (7, 17, 18, 33). Dietary-derived fatty acids of the mother have been related to fetal growth and, in general, reduced dietary intake of EFA and/or their LCPUFA derivates has been related with reduced birth size, as recently reviewed (22). Deposition of lipid in the fetus increases very rapidly with gestational age, especially during the last weeks of intrauterine life (45). Although some of these lipids are derived from fetal lipogenesis, which has been shown to be active in both human (9) and rat fetuses (34), a major proportion comes from maternal circulation by placental transfer. This is especially true for the n-3 and n-6 fatty acids both for the 18-carbon EFA and for their longer chain derivatives such as AA and DHA, which are needed for normal brain development and function (6, 11, 13, 37, 46).

Several studies on the fatty acid composition of neonatal and maternal plasma at term delivery have been published (for reviews on the subject see (11, 17, 32)). Although it has been emphasized that the concentration of LCPUFA is higher in the fetal than in maternal blood, this is only true when they are expressed in terms of the percentage of total fatty acids but not in terms of absolute values compared to all plasma lipids (33) or as actual plasma concentrations, which have consistently been found to be higher in maternal plasma than in cord plasma (38, 42).

Placental transfer of fatty acids has been studied in humans using vesicles prepared from syncytiotrophoblast of term placentas (26, 27), by perfusion of term placentas in vitro (19, 20), by determining the expression and specific fatty acid affinity of fatty acid binding proteins (5, 10, 29, 36) or by comparing paired maternal and umbilical cord plasma fatty acid profiles either under basal conditions or after fatty acid supplementation (38). In rats, transplacental fatty acid transport has been studied by examining the effects of changes in maternal dietary fatty acids on the fatty acid profiles of fetal tissue.
(16,31), the expression of fatty acid regulatory proteins in placenta and trophoblast cell culture models (24) and transfer of fatty acids from palmitic acid or triacylglycerol in a short-term perfusion system in situ (21).

Although these approaches have given important information on the transplacental fatty acid transport system, they all have limitations when it comes to extrapolating the results to late pregnancy in vivo, where there are other uncontrolled variables like uterine blood flow, placental aging and differences in maternal/fetal circulating concentrations, any of which could affect the rate of placental transfer. In order to overcome these problems, the use of stable isotopes at the time of caesarean section has been applied in humans (25, 28), but the impossibility (for obvious reasons) of obtaining samples either at different time points or from fetal tissue limits the ability to draw conclusions. In the study reported here, 20 d pregnant rats were given orally 14C-labeled palmitic-, oleic-, linoleic-, α-linolenic- or docosahexaenoic-acid and were studied at 0.5, 1, 2 or 8 h thereafter. Results show that during the first hours after their administration the major part of each of the fatty acids appeared in maternal plasma esterified as triacylglycerols (TAG), this also being the main form in the placenta. When fetal/maternal plasma specific activities ratios were used as an index of maternal to fetal transfer, the data showed that the rate transfer was in the order α-linolenic- > linoleic- > docosahexaenoic- = oleic- > palmitic-acid.

MATERIALS AND METHODS

Animals

Female Sprague-Dawley rats from the animal quarter of University San Pablo-CEU given a standard non-purified diet (B&K Universal, Barcelona, Spain) were housed under conditions of controlled light and temperature (12-hour light/dark cycle; 22 ± 1º C). The experimental protocol was approved by the Animal Research Committee of the University San Pablo-CEU and by the National Nuclear Commission, Madrid, Spain (CSN/CCS/IRA-2187/02 and CSN-C-DPR-245/02). Rats were mated when they weighed 180-190 g and the day spermatozoa appeared in vaginal smears was considered day 0 of pregnancy. On day 20 of pregnancy fed rats were given, via a gastric tube, in a final volume of 0.5 mL, 0.5-1 µCi of the corresponding 14C-labeled fatty acid: palmitic acid [1-14C] (55 mCi/mmol), oleic acid [1-14C] (51 mCi/mmol), α-linolenic acid [1-14C] (53.7 mCi/mmol), linoleic acid [1-14C] (51-mCi/mmol) or docosahexaenoic acid [1-14C] (55 mCi/mmol) from Perkin Elmer (Boston, MAS) suspended in a 2% Tween 20/water vehicle. An aliquot of the
administered suspension was saved for use as an experimental standard as described below. From the time
that the animals received the tracer they were maintained without access to food and were decapitated at 0.5,
1, 2 or 8 h thereafter, with the exception of those receiving 14C-docosahexaenoic acid, which were
decapitated only at 1 or 8 h. Trunk blood was collected into tubes containing Na2-EDTA and kept on ice until
centrifugation. The two uterine horns were dissected and placentas were immediately placed in ice-chilled
saline. All the fetuses except one from each dam were weighed and decapitated, and blood was collected as
described above for maternal blood. The remaining fetus was directly placed into liquid nitrogen. Plasma
was prepared from fresh blood by centrifugation at 1,500 g for 15 min at 4ºC and kept frozen until analysis.
Collected samples from all the fetuses of individual dams were pooled and processed in parallel to the
samples from the corresponding dam. Placentas were rinsed thoroughly in ice-chilled saline, blotted with
Whatman filter paper and placed in liquid nitrogen. All frozen samples were kept at -80ºC until processed.

**Processing of samples**

Samples of frozen plasma, placentas and of the remaining fetuses were used for lipid extraction and
purification (15). Some aliquots of the lipid extracts were dried in a stream of nitrogen and used to determine
their content of radioactivity; other aliquots were used for thin layer chromatographic separation on silicagel
F60 (Merck, Germany), developed using heptane/diisopropylether/acetic acid (60/40/3 by volume) and
identified with reference to authentic purified lipid standards run in parallel on the same plates. Distribution
of radioactivity was analyzed using the Imaging Screen K and Personal Molecular Imager system (Bioimag,
Bio-Rad, California) and the spots were visualized under ultraviolet light after spraying with a solution of
95% methanol:2',7'-dichlorofluorescein (0.1%, w/v). Radioactivity values were corrected by the label
administered to each rat and were normalized to an initial administered amount of 1.0 x 10⁸ dpm/rat and all
values adjusted accordingly (named “corrected dpm” (cdpm)).

To determine fatty acid concentrations in diet and plasma, total lipids were extracted in chloroform-
methanol (15) and an internal standard of nonadecanoic acid (19:0) was added to each sample. Simultaneous
saponification and methylation was carried out as previously described (1, 14). 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 x 0.25 mm Omegawax capillary column (Supelco,Bellefonte, PA). Nitrogen
was used as carrier gas and the fatty acid methyl esters were identified by comparison with purified standards
(Sigma Chemical Co., St. Louis, Mo.).
Statistical analysis

Data are expressed as means ± SEM. The statistical differences between groups were determined by ANOVA with a computer software package (Statgraphics Centurion XV, version 15.2.06, Statistical Graphics Corp.). When differences were statistically significantly ($P<0.05$), comparisons were tested by Tukey’s post hoc test.

RESULTS

Because fed 20 d pregnant rats received orally labeled palmitic acid (PA, 16:0), oleic acid (OA, 18:1n-9), linoleic acid (LA, 18:2n-6), α-linolenic acid (LNA, 18:3n-3) or docosahexaenoic acid (DHA, 22:6n3) and differences in the dilution with dietary fatty acids could affect the appearance of radioactivity in blood, the fatty acids profile of the diet was measured and is shown in Table 1. LA was the fatty acid present in the greatest amount in the diet, followed by OA, PA and LNA; the amount of DHA was undetectable. As shown in Table 2, radioactivity in the total lipid fraction of plasma after oral administration of labeled (PA, OA or LA to 20 d pregnant rats increased from 0.5 to 1.0 h, although the differences between each time point did not reach statistical significance due to the high standard error values. Later, values progressively declined at 2 and 8 h. In the case of LNA plasma radioactivity in total lipids almost reached the highest value at the 0.5 h time point, with a small increase at 1 h and a decline thereafter. When DHA was the administered tracer, plasma radioactivity values at 1 h were not significantly lower than those of any of the other fatty acids, and values further declined at 8 h, although at this time the values were comparable to those found in the other groups.

In order to determine the distribution of radioactivity in maternal plasma lipids, the proportion of label present in the different lipid fractions was determined. As shown in Figure 1, up to the 2 h time, more than 80% of the radioactivity in plasma appeared in the form of TAG when any of the labeled fatty acids was administered, whereas at 8 h there was always a decline in the proportion of labeled TAG in favor of phospholipids, an increase which was especially striking between 1 h and 8 h when DHA was the administered tracer. The proportion of label in the esterified cholesterol fraction also increased in maternal plasma over the course of the experiment, although the values reached were lower than those of phospholipids and the incorporation of PA and DHA into cholesterol ester after 8 h was lower than in the other groups.
The radioactivity present in the placenta total lipids always increased from 0.5 to 1 h after the administered tracer, showing its highest values at these two times when LNA was the administered tracer (Table 3). At 2 h and 8 h after administration, the radioactivity in placental lipids remained stable with PA, OA, LA and LNA, but it increased non-significantly at 8 h when DHA was administered, its value being the highest of the five groups. When determining the distribution of placental radioactivity in the different lipid fractions (Figure 2), it was found that most of the radioactivity at 0.5 and 1 h appeared in the TAG fraction (80-90%) to decline progressively with time in favor of phospholipids, this change being greatest for DHA, which in comparison to the other administered tracers at 8 h showed the lowest proportion of radioactivity in the TAG fraction and the highest in phospholipids. Irrespective of the time point or of the tracer administered, the proportion of label in the other lipid fractions in the placenta (i.e. mono- and diacylglycerols, NEFA or esterified cholesterol) was very low.

In contrast to the maternal circulation, the radioactivity present in fetal plasma progressively increased with time; for all acids the highest value always occurred at 8 h, with both LNA and DHA showing higher values than the other acids at this time (Table 4). The distribution of radioactivity in the different lipid fractions in fetal plasma is shown in Figure 3. At 0.5 or 1 h the highest proportion of radioactivity appeared in the form of TAG followed by NEFA, but major differences between the different administered tracers were evident. In case of palmitic and oleic acids the proportion of label in TAG was stable up to 2 h to decline at 8 h and the proportion of NEFA remained stable at 0.5 h and 1 h after administration to progressively decline at 2 h and 8 h; the opposite change was evident for phospholipids, where the label progressively increased with time after the administration attaining the highest value at 8 h. When the administered tracer was either LA or LNA the proportion of label in TAG increased from 0.5 to 1 h and declined later, whereas the label present in NEFA was highest at 0.5 h to decline greatly at 1 h and remained very low at 2 and 8 h. However, when either LA or LNA were administered, radioactivity present in the phospholipid fraction in fetal plasma progressively increased at 2 and 8 h after the tracer. In the case of DHA, which behaved differently to the other acids, most of the label in fetal plasma lipids after 1 h was in the form of NEFA rather than TAG or phospholipids; by 8 h the proportion of label in NEFA had declined greatly and had appeared in the phospholipid fraction reaching a higher proportion of radioactivity in phospholipid than any of the other groups. An interesting complement to these data occurred when the proportion of label in the different lipid classes present in fetal liver was examined (Figure 4). The pattern
differed among the fatty acids administered, but the most striking change was that when DHA was administered, there was practically no radioactive incorporation into any of the different lipid classes except for phospholipids which exceeded 90% at both time points studied.

In order to obtain a more precise estimation of the total amount of radioactivity that reached the fetus, its presence in total fetal lipids was measured. Values are shown in Figure 5, where it can be seen that values parallel those in plasma (Table 4), increasing with the time after the administration, with values at 8 h being the lowest for palmitic and oleic acids and progressively higher for LA, LNA and DHA.

Since some of the observed results could be affected by differences in the endogenous dilution of the tracer by the corresponding fatty acid, the concentrations of the fatty acids in the study were determined in maternal and fetal plasma. Since all the labeled fatty acids were administered in tracer concentrations, the identity of the fatty acid administered had no bearing on the fatty acid profiles at any given time, so only compiled values at each time point are presented. The concentrations of the different fatty acids in maternal plasma (Table 5) were as would be expected, with the highest values being for PA, OA and LA, followed by DHA and LNA being the lowest. The concentrations of PA, OA and LA remained stable from the 0 to the 8 h sampling times, but a significant decline occurred for LNA and a non-significant increase was found for DHA. When fetal fatty acid concentrations were considered (Table 6), the highest values were for PA and OA followed by LA and DHA whereas LNA was practically undetectable. In every case, the concentrations of the fatty acids in fetal plasma were of the order of 5-10 times lower than in maternal plasma and it was found that in fetal plasma the concentration values for OA, LA and DHA progressively increased during the 8-hour course of the experiment (Table 6).

The interpretation of the radioactivity values in plasma requires knowledge the specific radioactivity of each of the fatty acids, which corresponds to the correction of the radioactivity values by the concentration of the same fatty acid (i.e. dpm/mg). As shown in Table 7, specific activity in maternal plasma of PA at 0.5 and 1 h is non-significantly lower than all the other fatty acids, with either OA or LA being the next higher, the highest values being attained at 1 h, declining later. The specific activity of LNA in maternal plasma reached much higher values than any of the other fatty acids at any of the time point studied, progressively declining with the time after its administration. In the case of DHA maternal plasma specific activity was higher than that of PA, OA and LA and also declined from 1 to 8 h.
In case of the fetal plasma (Table 8), there was a progressive increase in the specific activities of all the fatty acids over time, with the highest values always measured at 8 h. The specific activity for polyunsaturated fatty acids in fetal plasma was higher than for either saturated (i.e. PA) or monounsaturated fatty acid (i.e. OA) the highest value being attained for LNA followed by DHA and then LA.

While it is not possible to determine from these data the actual rate of placental transfer of each of these fatty acids, the ratio of fetal/maternal plasma specific activity for each of the studied fatty acids gives an indirect index of the rate of the transfer. As shown in Table 9, fetal/maternal plasma specific activity ratio for PA and OA is the lowest of the five fatty acids, and values remain low during the first 2 h after the tracer administration to increase at 8 h. Values of fetal/maternal specific activity ratio for LA during the first 2 h appeared stable but higher than that seen for any of the other fatty acids except LNA, and values further increased at 8 h. Fetal/maternal specific activity ratio for LNA was the highest of all the fatty acids studied at all the time points studied, values progressively increasing with time from the 0.5 h up to the 8 h time point. In the case of DHA, the fetal/maternal specific activity ratio at either 1 or 8 h appeared lower than that seen for either LA or LNA, values reached at the 8 h time point being of the same order of magnitude than those attained by OA.

DISCUSSION

This study compares the kinetics of five different labeled fatty acids administered orally to late pregnant rats. The fatty acids were given in tracer amounts and were rapidly incorporated into their endogenous pool, attaining their highest level of radioactivity in plasma at 1.0 h after their administration, with small differences between them except that LNA had already attained its highest value at 0.5 h. These differences should be interpreted with caution since they depend not only on their actual intestinal absorption, but also on their respective endogenous isotopic dilution with unlabeled fatty acid present in the intestinal lumen. Because rats received the corresponding tracer under fed conditions, the quantities of unlabeled fatty acids in the lumen depends on the fatty acid composition of the diet. As found here (Table 1), the concentration of LNA in the rat standard diet used is lower than that of PA, OA and LA, resulting in a smaller isotope dilution. Therefore, it is not surprising that the LNA radioactivity in plasma rose more quickly and to a higher value than that of the other acids. No possibility exists to apply this same argument to DHA, whose content in the diet was undetectable, but in this particular case only two time points after its administration were studied.
The appearance of label in plasma lipids and, more importantly, its change with time after the oral administration would depend on the isotopic dilution of the individual fatty acid. Of the five fatty acids studied, the one with the highest (mass) concentration in maternal plasma is PA, followed by OA and LA, with DHA and LNA being much lower. Accordingly, the specific activities of the first three fatty acids at all time points studied were low compared to the specific activities of LNA and especially DHA. Moreover, the profile of the different fatty acids over time differed according to the fatty acid studied; the plasma concentration of labeled fatty acid, and its specific activity, continued to increase between 0.5 h and 1.0 h in cases of PA, OA and LA, whereas LNA values reached a maximum earlier and remained stable between 0.5 h and 1 h. These variables progressively declined for all the fatty acids thereafter. These findings suggest that the actual intestinal absorption process for PA, OA and LA was slower than for LNA. Insufficient measurements were made with DHA to allow direct comparison, although its higher specific activity compared to PA, OA and LA would also point to a more rapid intestinal absorption.

As would be predicted by the accepted pathway for fatty acid absorption by the intestine (23, 39), in every case practically all the label in maternal plasma during the first 2 h appeared as TAG, probably as chylomicrons or as very low density lipoproteins (VLDL), these being the forms that deliver TAG to the placenta. Supporting this interpretation is the finding that the pattern of radioactivity in placental lipids changed in parallel to that in plasma and that the highest radioactivity present in the placental lipids at the early time points (i.e. 0.5 and 1.0 h) was always in the TAG form. The suspicion that some of the labeled TAG in the placenta could have been the result of its contamination with blood was found not to be the case because exhaustive rinsing of the fresh placental tissue with saline before its lipid extraction did not affect the proportion of labeled TAG found (results not shown). The presence of a high proportion of labeled TAG in the placenta shortly after the oral administration of labeled fatty acids indicates that TAG play an important role in the placental transfer of the studied fatty acids to the fetal circulation in the rat. This has already been proposed for certain fatty acids in human placenta (10). From 2 h onwards, the proportion in placental TAG progressively declined in favor of phospholipids, which may have been derived from either a process of lipolysis of the TAG followed by incorporation of the released fatty acids into phospholipids within the placenta, or as result of the uptake of labeled phospholipids circulating in the maternal system by the placenta. This later possibility is supported by the observation that at 8 h but not earlier after their oral administration a considerable proportion of circulating labeled fatty acids appeared in their phospholipid
form rather than TAG, the highest proportion found for DHA, which was also the fatty acid where the highest proportion of placental lipids were phospholipids at this same time. The preferential incorporation of DHA into placental phospholipids may be related to the reported correlation between the expression of placental fatty acid transport proteins (FATPs) and the percentage of DHA in human placental phospholipids (29). In animal models of maternal obesity, where placental fatty acid transfer is expected to be increased, placental mRNA expression of these FATPs are increased (43, 47). It is therefore proposed that the incorporation of DHA to placental phospholipids could be also involved in its efficient placental transfer.

The appearance of labeled fatty acids in fetal plasma followed the expected pattern, with a progressive time-dependent enrichment in the corresponding fatty acid. As discussed above, the appearance of label in fetal plasma fatty acids should be affected not only by the rate of their placental transfer but also by the degree of their isotopic dilution. The plasma concentration of all the studied fatty acids was lower in fetal plasma than in maternal plasma. Values for both PA and OA were higher than for any other fatty acid probably as result of their endogenous synthesis, which is active in the fetus (34). By the same logic, in fetal plasma, the specific activities of PA and OA were lower than for any of the polyunsaturated fatty acids studied, also suggesting a lower rate of placental transfer of PA and OA. Of the polyunsaturated fatty acids studied, the specific activity reached in fetal plasma for LNA was the highest, followed by that of DHA and then LA.

Since the values of fetal plasma specific activity were calculated in the same manner as in maternal plasma, we reasoned that the fetal/maternal specific activity ratio could be used as an index of the rate of maternal-fetal transfer for the corresponding fatty acid, although differences in the kinetics of the administered fatty acids and between maternal and fetal sites in vivo could disturb the order. The result of calculating the ratio of fetal and maternal specific activities at the longest time studied (i.e. 8 h after their oral administration), indicated that the rates of fatty acid maternal-to-fetal transfer were in the following order: LNA > LA > DHA = OA > PA.

The results reported above permit some speculation on the biodisponibility of placental transfer of fatty acids and their release to the fetal circulation, which has not been clearly established by earlier reports. With the exception of DHA (see below), it was found that at the shortest time point studied after their oral administration to the mother (i.e. 0.5 h), the major proportion of all of the labeled fatty acids (PA, OA, LA and LNA) appeared in the TAG fraction, with smaller amounts being non-esterified (i.e. NEFA). This trend
continued at 1 h, when the proportion as TAG further increased to about 80% of the total plasma radioactivity to decline later; meanwhile the proportion of NEFA progressively decreased with time. However the presence of label in the phospholipid fraction increased from the 2 h on. These findings would support the possibility that a considerable proportion of fatty acids crossing the rat placenta are released to fetal circulation in their esterified form in the TAG fraction, probably associated with specific lipoproteins which pattern in fetal circulation differs from that in adults in rats (2), as has also been found in humans (3, 4, 40). This suggestion is consistent with the report that rat yolk sac can synthesize apo B-containing lipoproteins (41) and the placenta can express apo-B (8); similar findings have also been reported in mouse yolk sac (12,44) and in the human placenta (35).

The fact that a proportion (12-20%) of the label found in fetal plasma shortly after administration (0.5 h) appeared in the form of NEFA before disappearing, does not preclude the possibility of the classical picture obtaining (21), i.e. that a portion of the transferred fatty acids are also released from the placenta to the fetal circulation in their non-esterified form, probably bound to specific carrier proteins (i.e. alpha-feto protein and serum albumin) and rapidly transported to fetal liver. There the fatty acids would be esterified for the synthesis of TAG, phospholipids or both, as suggested by the distribution of label found here in fetal liver in the different lipid fractions, and then released back into circulation as lipoproteins.

Special attention must be paid to DHA, a higher proportion of the radioactivity of which was found in fetal plasma at a short time after its administration (1 h) as its non-esterified fatty acid form, compared to all the other acids studied. This proportion had greatly declined by 8 h, when its main proportion was phospholipids. It was also the fatty acid with the highest proportion incorporated into phospholipids in fetal liver. Thus it may be proposed that unlike the other fatty acids studied, DHA was released from the placenta to the fetal circulation mainly in the form of NEFA which, upon its arrival at the liver, was incorporated into phospholipids to be released back into the circulation.

In conclusion, the high proportion of label present as TAG in both maternal plasma and placenta and the low proportion in any other lipid classes in both sites shortly after the oral administration of labeled fatty acids supports the concept that, at least in the late pregnant rat, the main fate of dietary fatty acids after their gut absorption is their release into the circulation in their esterified form as TAG, which are directly taken up by the placenta. From there, fatty acids are released into the fetal circulation, where they mainly appear in their TAG form rather than as NEFA, with DHA being an exception in comparison to the remaining studied
fatty acids. This suggests that, although the fatty acids may have been released and re-esterified during their passage through the placenta, a substantial proportion are released into fetal circulation in the form of TAG, probably associated with specific lipoproteins. We also suggest that fetal/maternal specific activity ratios may be a useful index of comparative rates of placental transfer, but they should be used with caution for reasons give above.

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Author contributions

PLL, conducted the research; HO-S, conducted the research and analyzed data; IL-S, conducted the research; EH, designed research, had primary responsibility for final content and wrote the paper. The authors report no conflicts of interest.
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Figure CAUTIONS

Figure 1.- Percentage distribution of radioactivity in maternal plasma lipids at different times after oral administration of $^{14}$C-palmitic acid (PA, 16:0), $^{14}$C-oleic acid (OA, 18:1n-9), $^{14}$C-linoleic acid (18:2n-6), $^{14}$C-$\alpha$-linolenic acid (18:3n-3) or $^{14}$C-docosahexaenoic acid (22:6n-3) to 20 d pregnant rats. Means ± S.E.M. from 4 rats per fatty acid and time point. TAG, triacylglycerols; MAG, monoacylglycerols; DAG, diacylglycerols; NEFA, non-esterified fatty acids; PhL, phospholipids and EC, esterified cholesterol. Lower letters over the columns indicate significant differences between hours for a specific fatty acid.

Figure 2.- Percentage distribution of radioactivity in placental lipids at different times after oral administration of $^{14}$C-palmitic acid (PA, 16:0), $^{14}$C-oleic acid (OA, 18:1n-9), $^{14}$C-linoleic acid (18:2n-6), $^{14}$C-$\alpha$-linolenic acid (18:3n-3) or $^{14}$C-docosahexaenoic acid (22:6n-3) to 20 d pregnant rats. Means ± S.E.M. from 4 rats per fatty acid and time point. TAG, triacylglycerols; MAG, monoacylglycerols; DAG, diacylglycerols; NEFA, non-esterified fatty acids; PhL, phospholipids and EC, esterified cholesterol. Lower letters over the columns indicate significant differences between hours for a specific fatty acid.

Figure 3.- Percentage distribution of radioactivity in fetal plasma lipids at different times after oral administration of $^{14}$C-palmitic acid (PA, 16:0), $^{14}$C-oleic acid (OA, 18:1n-9), $^{14}$C-linoleic acid (18:2n-6), $^{14}$C-$\alpha$-linolenic acid (18:3n-3) or $^{14}$C-docosahexaenoic acid (22:6n-3) to 20 d pregnant rats. Means ± S.E.M. from 4 rats per fatty acid and time point. TAG, triacylglycerols; NEFA, non-esterified fatty acids and PhL, phospholipids. Lower letters over the columns indicate significant differences between hours for a specific fatty acid.

Figure 4.- Percentage distribution of radioactivity in fetal liver lipids at different times after oral administration of $^{14}$C-palmitic acid (PA, 16:0), $^{14}$C-oleic acid (OA, 18:1n-9), $^{14}$C-linoleic acid (18:2n-6), $^{14}$C-$\alpha$-linolenic acid (18:3n-3) or $^{14}$C-docosahexaenoic acid (22:6n-3) to 20 d pregnant rats. Means ± S.E.M. from 4 rats per fatty acid and time point. TAG, triacylglycerols; NEFA, non-esterified fatty acids and PhL, phospholipids. Lower letters over the columns indicate significant differences between hours for a specific fatty acid.

Figure 5.- Radioactivity in total lipids of whole rat fetuses at different times after oral administration of $^{14}$C-palmitic acid (PA, 16:0), $^{14}$C-oleic acid (OA, 18:1n-9), $^{14}$C-linoleic acid (18:2n-6), $^{14}$C-$\alpha$-linolenic acid (18:3n-3) or $^{14}$C-docosahexaenoic acid (22:6n-3) to 20 d pregnant rats. Means ± S.E.M. from 4 fetuses from 4 different rats per fatty acid and time point. Lower letters over the columns indicate significant differences between hours for a specific fatty acid.
### Table 1. Fatty Acid Composition of the Experimental Diet

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Content in standard diet (pellets) (mg/g diet)</th>
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<tbody>
<tr>
<td>PA (16:0)</td>
<td>5.0</td>
</tr>
<tr>
<td>18:0</td>
<td>0.72</td>
</tr>
<tr>
<td>OA (18:1, n-9)</td>
<td>6.4</td>
</tr>
<tr>
<td>LA (18:2, n-6)</td>
<td>16.7</td>
</tr>
<tr>
<td>AA (20:4, n-6)</td>
<td>ND</td>
</tr>
<tr>
<td>LNA (18:3, n-3)</td>
<td>1.1</td>
</tr>
<tr>
<td>EPA (20:5, n-3)</td>
<td>ND</td>
</tr>
<tr>
<td>DHA (22:6, n-3)</td>
<td>ND</td>
</tr>
</tbody>
</table>

Mean of the analysis of three samples. ND, not detectable
TABLE 2. Radioactivity in maternal plasma lipids at different times after the oral administration of $^{14}$C-palmitic acid (PA, 16:0), $^{14}$C-oleic acid (OA, 18:1n-9), $^{14}$C-linoleic acid (LA, 18:2n-6), $^{14}$C-α-linolenic acid (LNA, 18:3n-3) or $^{14}$C-docosahexaenoic acid (DHA, 22:6n-3) to 20 d pregnant rats.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>dpm$^1$/mL</th>
<th>½ h</th>
<th>1 h</th>
<th>2 h</th>
<th>8 h</th>
<th>$p^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA (16:0)</td>
<td>304,108 ±  39,901 $^{\text{a},\text{A}}$</td>
<td>542,710 ± 185,277 $^{\text{a},\text{A}}$</td>
<td>351,597 ± 44,412 $^{\text{a},\text{A}}$</td>
<td>184,047 ± 40,793 $^{\text{a},\text{A}}$</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>OA (18:1, n-9)</td>
<td>523,859 ±  46,782 $^{\text{ab},\text{AB}}$</td>
<td>720,682 ± 158,934 $^{\text{a},\text{A}}$</td>
<td>354,479 ± 33,577 $^{\text{bc},\text{A}}$</td>
<td>88,150 ± 25,845 $^{\text{c},\text{A}}$</td>
<td>0.0015</td>
<td></td>
</tr>
<tr>
<td>LA (18:2, n-6)</td>
<td>446,634 ± 110,420 $^{\text{ab},\text{A}}$</td>
<td>679,468 ± 105,199 $^{\text{a},\text{A}}$</td>
<td>478,050 ± 80,022 $^{\text{ab},\text{A}}$</td>
<td>147,893 ± 30,169 $^{\text{b},\text{A}}$</td>
<td>0.0083</td>
<td></td>
</tr>
<tr>
<td>LNA (18:3, n-3)</td>
<td>773,488 ±  83,101 $^{\text{a},\text{B}}$</td>
<td>837,771 ±  91,189 $^{\text{a},\text{A}}$</td>
<td>534,478 ±  99,448 $^{\text{a},\text{A}}$</td>
<td>108,032 ±  1,777 $^{\text{b},\text{A}}$</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>DHA (22:6, n-3)</td>
<td>366,403 ±  57,368 $^{\text{a},\text{A}}$</td>
<td>366,403 ±  57,368 $^{\text{a},\text{A}}$</td>
<td>366,403 ±  57,368 $^{\text{a},\text{A}}$</td>
<td>170,227 ±  49,614 $^{\text{b},\text{A}}$</td>
<td>0.0414</td>
<td></td>
</tr>
</tbody>
</table>

$^1$Corrected as explained in the text. Different lowercase letters indicate significant differences between hours for a specific fatty acid of which its ANOVA $p$ value is shown by $p^2$; different uppercase letters indicate significant differences between radioactive fatty acids at the same time of which its ANOVA $p$ value is shown by $p^3$.

Mean ± S.E.M. from 4 rats per fatty acid and time point.
TABLE 3. Radioactivity in placental lipids at different times after the oral administration of $^{14}$C-palmitic acid (PA, 16:0), $^{14}$C-oleic acid (OA, 18:1n-9), $^{14}$C-linoleic acid (LA, 18:2n-6), $^{14}$C-$\alpha$-linolenic acid (LNA, 18:3n-3) or $^{14}$C-docosahexaenoic acid (DHA, 22:6n-3) to 20 d pregnant rats.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>dpm $^{\text{dpm/g}}$</th>
<th>½ h</th>
<th>1 h</th>
<th>2 h</th>
<th>8 h</th>
<th>$p^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA (16:0)</td>
<td>37,090 ± 6,800 $^{a,A}$</td>
<td>82,017 ± 16,608 $^{a,A}$</td>
<td>65,931 ± 8,193 $^{a,A}$</td>
<td>63,103 ± 4,632 $^{a,A}$</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>OA (18:1, n-9)</td>
<td>73,565 ± 9,454 $^{a,AB}$</td>
<td>115,795 ± 16,207 $^{a,AB}$</td>
<td>83,184 ± 7,694 $^{a,AB}$</td>
<td>70,143 ± 6,092 $^{a,AB}$</td>
<td>0.0379</td>
<td></td>
</tr>
<tr>
<td>LA (18:2, n-6)</td>
<td>53,825 ± 12,909 $^{a,A}$</td>
<td>95,968 ± 7,659 $^{b,AB}$</td>
<td>110,359 ± 3,485 $^{b,AB}$</td>
<td>94,769 ± 2,863 $^{b,AB}$</td>
<td>0.0016</td>
<td></td>
</tr>
<tr>
<td>LNA (18:3, n-3)</td>
<td>113,239 ± 12,019 $^{a,B}$</td>
<td>164,981 ± 27,402 $^{a,B}$</td>
<td>106,226 ± 18,633 $^{a,B}$</td>
<td>82,638 ± 4,209 $^{a,B}$</td>
<td>0.0392</td>
<td></td>
</tr>
<tr>
<td>DHA (22:6, n-3)</td>
<td>74,843 ± 9,925 $^{a,A}$</td>
<td>170,125 ± 55,569 $^{a,B}$</td>
<td></td>
<td></td>
<td>N.S.</td>
<td></td>
</tr>
</tbody>
</table>

$p^3$ 0.0016 0.0137 N.S. 0.0180

Mean ± S.E.M. from 4 rats per fatty acid and time point. $^{1}$Corrected as explained in the text. Different lowercase letters indicate significant differences between hours for a specific fatty acid of which its ANOVA $p$ value is shown by $p^2$; different uppercase letters indicate significant differences between radioactive fatty acids at the same time of which its ANOVA $p$ value is shown by $p^3$. 
TABLE 4. Radioactivity in fetal plasma lipids at different times after the oral administration of $^{14}$C-palmitic acid (PA, 16:0), $^{14}$C-oleic acid (OA, 18:1n-9), $^{14}$C-linoleic acid (LA, 18:2n-6), $^{14}$C-$\alpha$-linolenic acid (LNA, 18:3n-3) or $^{14}$C-docosahexaenoic acid (DHA, 22:6n-3) to 20 d pregnant rats.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>0.5 h</th>
<th>1 h</th>
<th>2 h</th>
<th>8 h</th>
<th>$p^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA (16:0)</td>
<td>2,586 ± 116 $^{a,A}$</td>
<td>3,102 ± 254 $^{a,A}$</td>
<td>5,649 ± 909 $^{a,A}$</td>
<td>12,432 ± 2,071 $^{b,A}$</td>
<td>0.0002</td>
</tr>
<tr>
<td>OA (18:1, n-9)</td>
<td>4,150 ± 422 $^{a,A}$</td>
<td>6,525 ± 2,574 $^{a,A}$</td>
<td>11,305 ± 105 $^{a,AB}$</td>
<td>14,998 ± 4,813 $^{a,A}$</td>
<td>N.S.</td>
</tr>
<tr>
<td>LA (18:2, n-6)</td>
<td>6,483 ± 556 $^{a,B}$</td>
<td>6,913 ± 1,247 $^{a,A}$</td>
<td>8,499 ± 717 $^{a,AB}$</td>
<td>16,667 ± 1,797 $^{b,AB}$</td>
<td>0.0002</td>
</tr>
<tr>
<td>LNA (18:3, n-3)</td>
<td>6,848 ± 252 $^{a,B}$</td>
<td>8,370 ± 2,111 $^{a,A}$</td>
<td>13,523 ± 2,985 $^{a,B}$</td>
<td>29,153 ± 1,450 $^{b,B}$</td>
<td>0.0000</td>
</tr>
<tr>
<td>DHA (22:6, n-3)</td>
<td>6,476 ± 705 $^{a,A}$</td>
<td>23,185 ± 4,312 $^{b,AB}$</td>
<td>23,185 ± 4,312 $^{b,AB}$</td>
<td>0.0087</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± S.E.M. from 4 rats per fatty acid and time point. $^3$Corrected as explained in the text. Different lowercase letters indicate significant differences between hours for a specific fatty acid of which its ANOVA $p$ value is shown by $p^2$; different uppercase letters indicate significant differences between radioactive fatty acids at the same time of which its ANOVA $p$ value is shown by $p^3$. 


TABLE 5. Fatty acid concentrations in maternal plasma at different times after the oral administration of tracer amounts of $^{14}$C-palmitic acid (PA, 16:0), $^{14}$C-oleic acid (OA, 18:1n-9), $^{14}$C-linoleic acid (LA, 18:2n-6), $^{14}$C-α-linolenic acid (LNA, 18:3n-3) or $^{14}$C-docosahexaenoic acid (DHA, 22:6n-3) to 20 d pregnant rats.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>mg/L</th>
<th></th>
<th></th>
<th></th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>½ h</td>
<td>1 h</td>
<td>2 h</td>
<td>8 h</td>
<td></td>
</tr>
<tr>
<td>PA (16:0)</td>
<td>1,775 ± 143 a</td>
<td>1,895 ± 104 a</td>
<td>1,779 ± 197 a</td>
<td>1,515 ± 120 a</td>
<td>N.S.</td>
</tr>
<tr>
<td>OA (18:1, n-9)</td>
<td>1,679 ± 132 a</td>
<td>1,737 ± 127 a</td>
<td>1,683 ± 235 a</td>
<td>1,395 ± 120 a</td>
<td>N.S.</td>
</tr>
<tr>
<td>LA (18:2, n-6)</td>
<td>1,302 ± 95 a</td>
<td>1,303 ± 80 a</td>
<td>1,227 ± 149 a</td>
<td>967 ± 82 a</td>
<td>N.S.</td>
</tr>
<tr>
<td>LNA (18:3, n-3)</td>
<td>66.5 ± 4.8 a</td>
<td>63.5 ± 5.3 a</td>
<td>58.2 ± 8.5 ab</td>
<td>42.8 ± 3.9 b</td>
<td>0.0489</td>
</tr>
<tr>
<td>DHA (22:6, n-3)</td>
<td>129 ± 9 a</td>
<td>142 ± 26 a</td>
<td>138 ± 8 a</td>
<td>159 ± 7 a</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Different lowercase letters indicate significant differences between hours for a specific fatty acid of which ANOVA $p$ value is shown at the end of the row. Since there was no difference in the fatty acid profile in the rats after receiving the different tracers values for each fatty acid correspond to the mean ± S.E.M. values of all the rats studied at the corresponding time point (n: 4x5).
TABLE 6. Fatty acid concentrations in fetal plasma at different times after the oral administration of tracer amounts of $^{14}$C-palmitic acid (PA, 16:0), $^{14}$C-oleic acid (OA, 18:1n-9), $^{14}$C-linoleic acid (LA, 18:2n-6), $^{14}$C-$\alpha$-linolenic acid (LNA, 18:3n-3) or $^{14}$C-docosahexaenoic acid (DHA, 22:6n-3) to 20 d pregnant rats.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>0.5 h</th>
<th>1 h</th>
<th>2 h</th>
<th>8 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA (16:0)</td>
<td>317</td>
<td>290</td>
<td>322</td>
<td>337</td>
</tr>
<tr>
<td>OA (18:1, n-9)</td>
<td>262</td>
<td>240</td>
<td>315</td>
<td>303</td>
</tr>
<tr>
<td>LA (18:2, n-6)</td>
<td>49.2</td>
<td>55.1</td>
<td>67.8</td>
<td>81.9</td>
</tr>
<tr>
<td>LNA (18:3, n-3)</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>DHA (22:6, n-3)</td>
<td>17.8</td>
<td>20.3</td>
<td>28.2</td>
<td>30.6</td>
</tr>
</tbody>
</table>

Fatty acid analyses were carried out in triplicate on a pool of all the fetuses of rats receiving the same treatment and at the same time. Mean values are shown.
TABLE 7. Specific activities of fatty acids in maternal plasma at different times after the oral administration of tracer amounts of \(^{14}\)C-palmitic acid (PA, 16:0), \(^{14}\)C-oleic acid (OA, 18:1n-9), \(^{14}\)C-linoleic acid (LA, 18:2n-6), \(^{14}\)C-\(\alpha\)-linolenic acid (LNA, 18:3n-3) or \(^{14}\)C-docosahexaenoic acid (DHA, 22:6n-3) to 20 d pregnant rats.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>dpm(^1)/mg of the corresponding fatty acid</th>
<th>(p^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\frac{1}{2}) h</td>
<td>1 h</td>
</tr>
<tr>
<td>PA (16:0)</td>
<td>146,457 ± 11,073 (^{ab,A})</td>
<td>332,206 ± 77,962 (^{a,A})</td>
</tr>
<tr>
<td>OA (18:1, n-9)</td>
<td>366,888 ± 8,186 (^{ab,A})</td>
<td>515,463 ± 105,478 (^{a,AB})</td>
</tr>
<tr>
<td>LA (18:2, n-6)</td>
<td>310,274 ± 39,911 (^{ab,A})</td>
<td>519,660 ± 86,526 (^{a,AB})</td>
</tr>
<tr>
<td>LNA (18:3, n-3)</td>
<td>11,427,323 ± 583,180 (^{a,B})</td>
<td>10,373,401 ± 1,377,278 (^{ab,C})</td>
</tr>
<tr>
<td>DHA (22:6, n-3)</td>
<td>3,283,278 ± 434,269 (^{a,B})</td>
<td>1,063,653 ± 274,102 (^{b,C})</td>
</tr>
</tbody>
</table>

Mean ± S.E.M. from 4 rats per fatty acid and time point. \(^1\)Corrected as explained in the text. Different lowercase letters indicate significant differences between hours for a specific fatty acid of which its ANOVA \(p\) value is shown by \(p^2\); different uppercase letters indicate significant differences between radioactive fatty acids at the same time of which its ANOVA \(p\) value is shown by \(p^3\).
TABLE 8. Specific activities of fatty acids in fetal plasma at different times after the oral administration of tracer amounts of $^{14}$C-palmitic acid (PA, 16:0), $^{14}$C-oleic acid (OA, 18:1n-9), $^{14}$C-linoleic acid (LA, 18:2n-6), $^{14}$C-$\alpha$-linolenic acid (LNA, 18:3n-3) or $^{14}$C-docosahexaenoic acid (DHA, 22:6n-3) to 20 d pregnant rats.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>dpm$^1$/mg of the corresponding fatty acid</th>
<th></th>
<th></th>
<th></th>
<th>$p^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>½ h</td>
<td>1 h</td>
<td>2 h</td>
<td>8 h</td>
<td></td>
</tr>
<tr>
<td>PA (16:0)</td>
<td>8,158 ± 366$^{a,A}$</td>
<td>10,697 ± 875$^{a,A}$</td>
<td>17,543 ± 2,824$^{a,A}$</td>
<td>36,890 ± 6,146$^{b,A}$</td>
<td>0.0003</td>
</tr>
<tr>
<td>OA (18:1, n-9)</td>
<td>15,900 ± 1,617$^{a,A}$</td>
<td>27,186 ± 10,727$^{a,A}$</td>
<td>36,003 ± 335$^{a,A}$</td>
<td>49,499 ± 15,883$^{a,A}$</td>
<td>N.S</td>
</tr>
<tr>
<td>LA (18:2, n-6)</td>
<td>132,298 ± 11,341$^{a,b,A}$</td>
<td>125,697 ± 22,675$^{a,A}$</td>
<td>126,846 ± 10,704$^{a,A}$</td>
<td>205,770 ± 22,188$^{b,A}$</td>
<td>0.0198</td>
</tr>
<tr>
<td>LNA (18:3, n-3)</td>
<td>6,848,167 ± 252,434$^{a,b}$</td>
<td>8,370,042 ± 2,111,376$^{a,B}$</td>
<td>13,523,417 ± 2,985,295$^{a,B}$</td>
<td>29,152,833 ± 1,449,825$^{b,B}$</td>
<td>0.0000</td>
</tr>
<tr>
<td>DHA (22:6, n-3)</td>
<td>323,783 ± 35,228$^{a,A}$</td>
<td>772,830 ± 143,719$^{b,B}$</td>
<td></td>
<td></td>
<td>0.0230</td>
</tr>
</tbody>
</table>

Mean ± S.E.M. from 4 rats per fatty acid and time point. $^1$Corrected as explained in the text. Different lowercase letters indicate significant differences between hours for a specific fatty acid of which its ANOVA $p$ value is shown by $p^2$; different uppercase letters indicate significant differences between radioactive fatty acids at the same time of which its ANOVA $p$ value is shown by $p^3$. 

$p^3$ 0.0000 0.0000 0.0000 0.0000
TABLE 9. Ratio of fatty acid specific activities between fetal and maternal plasma lipids at different times after the oral administration of tracer amounts of $^{14}$C-palmitic acid (PA, 16:0), $^{14}$C-oleic acid (OA, 18:1n-9), $^{14}$C-linoleic acid (LA, 18:2n-6), $^{14}$C-α-linolenic acid (LNA, 18:3n-3) or $^{14}$C-docosahexaenoic acid (DHA, 22:6n-3) to 20 d pregnant rats.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Ratio of specific activities</th>
<th>$p^1$</th>
<th>$p^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>½ h</td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>PA (16:0)</td>
<td>0.057 ± 0.005 $^{a,A}$</td>
<td>0.038 ± 0.009 $^{a,A}$</td>
<td>0.062 ± 0.010 $^{a,A}$</td>
</tr>
<tr>
<td>OA (18:1, n-9)</td>
<td>0.044 ± 0.005 $^{a,A}$</td>
<td>0.053 ± 0.021 $^{a,AB}$</td>
<td>0.170 ± 0.045 $^{ab,A}$</td>
</tr>
<tr>
<td>LA (18:2, n-6)</td>
<td>0.435 ± 0.034 $^{a,B}$</td>
<td>0.255 ± 0.046 $^{a,B}$</td>
<td>0.334 ± 0.044 $^{a,A}$</td>
</tr>
<tr>
<td>LNA (18:3, n-3)</td>
<td>0.607 ± 0.054 $^{a,C}$</td>
<td>0.773 ± 0.088 $^{a,C}$</td>
<td>1.79 ± 0.38 $^{a,B}$</td>
</tr>
<tr>
<td>DHA (22:6, n-3)</td>
<td>0.110 ± 0.029 $^{a,AB}$</td>
<td>0.791 ± 0.159 $^{b,A}$</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± S.E.M. from 4 rats per fatty acid and time point. Different lowercase letters indicate significant differences between hours for a specific fatty acid of which its ANOVA $p$ value is shown by $p^1$; different uppercase letters indicate significant differences between radioactive fatty acids at the same time of which its ANOVA $p$ value is shown by $p^2$. 
FIGURE 1

- PA (16:0)
- OA (18:1, n-9)
- LA (18:2 n-6)

- LNA (18:3, n-3)
- DHA (22:6, n-3)

Legend:
- 0.5h
- 1h
- 2h
- 8h
FIGURE 2

PA (16:0)

OA (18:1, n-9)

LA (18:2 n-6)

LNA (18:3, n-3)

DHA (22:6, n-3)

0, 5, 1, 2, 8h
FIGURE 3

- PA (16:0)
- OA (18:1, n-9)
- LA (18:2 n-6)
- LNA (18:3, n-3)
- DHA (22:6, n-3)

Legend:
- 0.5h
- 1h
- 2h
- 8h

Data represent means ± SEM; different letters indicate significant differences at P < 0.05.
FIGURE 4

- PA (16:0)
- OA (18:1, n-9)
- LA (18:2 n-6)
- LNA (18:3, n-3)
- DHA (22:6, n-3)

Different letters indicate significant differences (p < 0.05).
FIGURE 5.