In vivo investigation of the placental transfer of ^{13}C-labeled fatty acids in humans

Elvira Larqué,* Hans Demmelmair,* Britta Berger,† Uwe Hasbargen,† and Berthold Koletzko[1,*]

Division of Metabolism and Nutrition,* Kinderklinik and Kinderpoliklinik, Dr. von Hauner Children's Hospital, Munich, Germany; and Klinik für Frauenheilkunde und Geburtshilfe,† Ludwig-Maximilians-University, Munich, Germany

Abstract **Placental fatty acid transfer in humans in vivo was studied using stable isotopes. Four pregnant women undergoing cesarean section received 4 h before delivery an oral dose of [^{13}C]palmitic acid (PA), [^{13}C]oleic acid (OA), [^{13}C]linoleic acid (LA), and [^{13}C]docosahexaenoic acid (DHA). Maternal blood samples were collected at −4 h (basal), −3 h , −2 h, −1 h, 0 h, and +1 h relative to time of cesarean section. At the time of birth, venous cord blood and placental tissue were collected. Fatty acid composition was determined by gas-liquid chromatography and isotopic enrichment by gas chromatography-combustion-isotope ratio mass spectrometry. ^{13}C-enrichment of fatty acids in the nonesterified fatty acids (NEFA) of cord plasma tended to be higher than in NEFA of placenta, with statistically significant differences for the nonesterified OA and DHA ([^{13}C]PA, 0.024 ± 0.011 vs. 0.001 ± 0.001; [^{13}C]OA, 0.042 ± 0.008 vs. 0.005 ± 0.003; [^{13}C]LA, 0.038 ± 0.010 vs. 0.008 ± 0.002; [^{13}C]DHA, 0.059 ± 0.009 vs. 0.010 ± 0.003). The ratio of tracer fatty acid concentrations of placenta to maternal plasma was significantly higher for [^{13}C]DHA than for the other fatty acids ([^{13}C]PA, 7.1 ± 1%; [^{13}C]OA, 3.8 ± 0.4%; [^{13}C]LA, 9.2 ± 1.3%; [^{13}C]DHA, 25.9 ± 3.4%). These results suggest that only a part of the placental NEFA participated in fatty acid transfer, and that the placenta showed a preferential accretion of DHA relative to the other fatty acids.**—Larqué, E., H. Demmelmair, B. Berger, U. Hasbargen, and B. Koletzko. **In vivo investigation of the placental transfer of [^{13}C]labeled fatty acids in humans. *J. Lipid Res.* 2003.** 44: **49–55.**

Supplementary key words fatty acids • long chain polyunsaturated fatty acids • placenta • pregnancy • stable isotopes

The rapid growth and development of the fetus depends on a sufficient supply of nutrients across the placenta. An adequate supply of long chain polyunsaturated fatty acids (LC-PUFA), such as arachidonic acid (C20:4n-6, AA) and docosahexaenoic acid (C22:6n-3, DHA), is critical for the early development of the brain and visual function (1, 2). Numerous studies have reported that, in contrast to essential fatty acids (EFA), LC-PUFA percentages in the lipids of cord plasma are higher than in maternal plasma at the time of birth (3, 4). Since the ability of the fetus and the human placenta to desaturate and elongate fatty acids is limited (5), a preferential materno-fetal transfer of LCPUFA has been suggested. However, the underlying processes for placental fatty acid transfer have not been elucidated.

Fatty acids are released from maternal triglycerides (TG) by lipoprotein lipase on the maternal surface of the placenta (6–8) and the liberated fatty acids, as well as nonesterified fatty acids (NEFA) from the circulation, can be utilized by the placenta. The placental transfer of fatty acids is considered a complex process that involves their binding to membrane proteins and cytoplasmatic transport proteins (8). Fatty acids may be esterified in the placenta and cross the tissue in either direction (8). Recently, in vitro studies have identified a placental plasma membrane fatty acid binding protein (p-FABPpm), which showed a higher affinity and binding capacity for DHA and AA compared with linoleic acid (LA, C18:2n-6) and oleic acid (OA, C18:1n-9) (9). Thus, p-FABPpm facilitates the observed preferential transfer of LC-PUFA. Kuhn and Crawford (10) reported that LA and AA were passed to the developing fetus in different lipid fractions during placental perfusion experiments, suggesting that a compartmentalization of fatty acids by the placenta might contribute to the selective transfer of some fatty acids.

In placental perfusion studies, nonesterified LA and palmitic acid (PA, C16:0) cross the placenta at similar rates (11, 12), and LA shows a relative preference for the transfer from the maternal to the fetal circulation compared with AA (10). From human placental perfusion studies, transfer selectivity of the placenta from the mater-

Manuscript received 7 February 2002 and in revised form 17 July 2002.

Published, JLR Papers in Press, October 1, 2002.
DOI 10.1194/jlr.M200067-JLR200

Abbreviations: AA, arachidonic acid, C20:4n-6; APE, atom percent excess; DHA, docosahexaenoic acid, C22:6n-3; LA, linoleic acid, C18:2n-6; LC-PUFA, long-chain polyunsaturated fatty acids; NEFA, nonesterified fatty acids; PA, palmitic acid C16:0; PL, phospholipids; OA, oleic acid, C18:1n-9; TG, triglycerides.

[1] To whom correspondence should be addressed.
e-mail: berthold.koletzko@kk-i.med.uni-muenchen.de

nal to the fetal circulation has been reported to decrease from DHA to α-linolenic acid (ALA) to LA, to OA, and to AA (13). However, substrates may be metabolized within the placenta itself and the fatty acid release from albumin limits the rate of transfer of fatty acids across the tissue (14). Therefore, data derived from perfusion experiments may not reflect in vivo transfer of fatty acids.

Fatty acids labeled with stable isotopes are safe and can be used in pregnant women (15), allowing the study of human placental transport under in vivo conditions. Since the distribution of individual fatty acids in plasma and placenta might influence their selective materno-fetal transfer (10), we aimed at studying the placental transfer of [^{13}C]PA, [^{13}C]OA, [^{13}C]LA, and [^{13}C]DHA given orally to pregnant women before planned cesarean section.

MATERIALS AND METHODS

Subjects

Four pregnant women undergoing elective cesarean section at 39 ± 0.6 weeks (mean ± SD) of gestation in the Dept. of Gynecology and Obstetrics (University of Munich, Grosshadern, Germany) participated in the study. The study protocol was approved by the Ethical Committee of the Ludwig-Maximilians University. Written informed consent was obtained from all participating women after careful explanation of the study. The women were aged 34 ± 2 years, had an average weight at delivery of 83 ± 20 kg and a height of 167 ± 11 cm. All subjects habitually consumed an omnivorous diet and none reported any health problems or pregnancy complications.

Four hours before delivery, the subjects received one oral dose of uniformly (98%) ^{13}C-labeled fatty acids (Martek, Bioscience, Columbia, MD) with 0.5 mg/kg body weight of [^{13}C]PA, 0.5 mg/kg of [^{13}C]OA, 0.5 mg/kg of [^{13}C]LA, and 0.1 mg/kg of [^{13}C]DHA. The tracers were supplied as free fatty acids on a sugar cube to the pregnant women, who were fasted for at least 12 h and did not eat during the study period.

Blood and placenta sampling

Blood samples were collected from the mothers before tracer intake (−4 h relative to cesarean section), and at −3 h, −2 h, −1 h, 0 h, and +1 h. Blood samples (3 ml) were taken by venipuncture and immediately transferred into EDTA-containing tubes. Venous cord blood was sampled immediately after clamping the cord. Blood cells and plasma were separated within 1 h by centrifugation at 1,000 *g* for 5 min. A plasma aliquot of at least 200 μl was frozen immediately at −80°C for later analysis.

TABLE 1. Fatty acid composition (%w/w, mean ± SE) of maternal plasma, venous cord plasma and placental tissue at the time of birth

	Maternal Plasma	Cord Plasma	Placental Tissue
Phospholipids			
PA	31.77 ± 0.32	27.51 ± 0.65[b]	24.77 ± 0.23
OA	9.82 ± 0.17	6.68 ± 0.17[b]	8.34 ± 0.39
LA	18.26 ± 1.30	6.77 ± 0.29[b]	8.39 ± 0.33
DHA	4.68 ± 0.47	5.62 ± 0.42	5.25 ± 0.21
Total PUFA	39.04 ± 0.43	38.41 ± 0.65	45.98 ± 0.27
Total LC-PUFA	20.40 ± 1.37	31.43 ± 0.90[b]	37.28 ± 0.51
Total FA (mg/dl)	154.16 ± 14.69	58.06 ± 12.15[b]	5.36 ± 0.25[a]
Triglycerides			
PA	34.72 ± 1.26	32.40 ± 3.57	28.07 ± 1.75
OA	33.70 ± 2.16	25.31 ± 2.90[b]	13.37 ± 0.76
LA	12.51 ± 0.37	10.98 ± 0.74	8.46 ± 0.63
DHA	0.85 ± 0.18	3.48 ± 0.80[b]	4.84 ± 0.48
Total PUFA	16.56 ± 0.28	23.99 ± 3.34[b]	37.78 ± 2.60
Total LC-PUFA	3.13 ± 0.42	12.18 ± 1.37[b]	28.58 ± 2.00
Total FA (mg/dl)	109.23 ± 7.22	11.15 ± 5.70[b]	0.18 ± 0.02[a]
Cholesterol esters			
PA	13.54 ± 0.68	22.04 ± 1.24[b]	20.28 ± 0.54
OA	19.85 ± 0.75	24.33 ± 1.55[b]	16.61 ± 0.87
LA	46.61 ± 2.37	18.35 ± 0.69[b]	27.05 ± 1.99
DHA	0.80 ± 0.12	1.18 ± 0.20	1.86 ± 0.13
Total PUFA	57.43 ± 1.81	37.25 ± 0.68[b]	44.88 ± 0.72
Total LC-PUFA	9.44 ± 1.03	18.09 ± 0.47[b]	16.67 ± 1.29
Total FA (mg/dl)	75.67 ± 9.15	19.20 ± 3.03	0.11 ± 0.01[a]
Nonesterified fatty acids			
PA	31.28 ± 0.75	27.59 ± 1.15	25.02 ± 1.28
OA	27.59 ± 2.21	13.22 ± 1.13[b]	9.56 ± 0.61
LA	10.08 ± 0.42	9.79 ± 0.06	8.71 ± 0.37
DHA	1.03 ± 0.32	4.19 ± 0.29	4.86 ± 0.47
Total PUFA	15.56 ± 1.48	35.09 ± 0.45	44.79 ± 1.42
Total LC-PUFA	4.72 ± 1.55	24.88 ± 0.40[b]	35.63 ± 1.30
Total FA (mg/dl)	25.86 ± 11.36	6.52 ± 2.00[b]	0.54 ± 0.20[a]

PA, palmitic acid (C16:0); OA, oleic acid (C18:1n-9); LA, linoleic acid (C18:2n-6); DHA, docosahexaenoic acid (C22:6n-3); PUFA, polyunsaturated fatty acids; LC-PUFA, long-chain polyunsaturated fatty acids (≥ 20 C atoms, ≥ two double bounds); FA, fatty acids. FA proportions of the placenta were not considered in the statistical analysis because they are data from a tissue and not plasma.

[a] In placenta, total FA is expressed in mg/g wet weight.

[b] Significant differences between FA proportions in maternal and cord plasma ($P < 0.05$).

The total placenta was weighed immediately after delivery. Samples of 1 × 1 × 1 cm of placenta cotyledons were cut with a sharp knife. Placenta samples were subjected to three washings with a 0.9% NaCl solution until blood was eliminated as far as possible, frozen in liquid nitrogen, and stored at −80°C until later analysis.

Analysis of plasma and placenta fatty acids

After addition of internal standard, total lipids from 0.1 ml of plasma were extracted into hexane-isopropanol (4:1, v/v) (16). Lipids from placenta tissue were extracted into chloroform-methanol (2:1, v/v) (17). For application on silica gel plates (MERCK, Darmstadt, Germany), the residue was taken up in chloroform-methanol (1:1, v/v). Phospholipids (PL), TG, cholesterol esters (CE), and NEFA were isolated by development of the plates in n-heptane-diisopropylether-glacial acetic acid (60:40:3, v/v/v) (18). Synthesis of fatty acid methyl esters was performed with 3M methanolic HCl (Supelco, Bellafonte, PA) at 85°C for 45 min. Derivatives were extracted into hexane and stored at −20°C until gas chromatographic analysis.

Gas chromatography was performed on a Hewlett-Packard 5890 series II gas chromatograph (Hewlett-Packard, Waldbronn, Germany) equipped with a BPX70 column (SGE, Weiterstadt, Germany) with 60 m length and 0.32 mm inner diameter (19). The ^{13}C-enrichment of individual fatty acid methyl esters was measured by gas chromatography-combustion-isotope ratio mass spectrometry (Hewlett-Packard GC interfaced to Finnigan MAT delta S mass spectrometer, Bremen, Germany) (19).

Expression of the results

From the $^{13}C/^{12}C$ ratio of the samples measured by gas chromatography-combustion-isotope ratio mass spectrometry, the $\delta^{13}C$ relative to the international PDB (Pee Dee Belemnite) standard and the ^{13}C atom percentage excess (APE) were calculated (20), representing the fatty acid enrichment evaluated. The concentrations of the labeled fatty acids (μmol/l or μmol/g) were calculated by multiplying the absolute concentrations of the fatty acids by their APE values.

As an estimation of the mean enrichment of tracer in the maternal plasma during all the experiment, we calculated the area under the curve from the μmol/l tracer of the individual fatty acids in the maternal plasma according the trapezoidal rule. The transfer ratio of the labeled fatty acids from the mother to the fetus was estimated by the percentage of tracer concentration (μmol ^{13}C/l) in cord blood relative to the area under the tracer concentration curve in the maternal plasma (Table 4).

The distribution of [^{13}C]tracer between placental tissue and maternal plasma at the time of delivery (in **Fig. 3**) was calculated as:

$$\%\ \text{Ratio}\ \frac{\text{Placenta}}{\text{Plasma}} = \frac{\text{fatty acid amount in placenta (nmol/g)} \cdot \text{APE [}^{13}\text{C]placenta}}{\text{fatty acid concentration in maternal plasma (μmol/ml)} \cdot \text{APE [}^{13}\text{C]plasma}} \times 100 \quad \textit{(Eq. 1)}$$

Statistics

The results are expressed as mean ± SEM. Statistically significant differences were assessed using a Student's *t*-test. The significance level was set a $P < 0.05$. All analyses were performed with the statistical software SPSS, version 10.0 (SPSS, Chicago, IL).

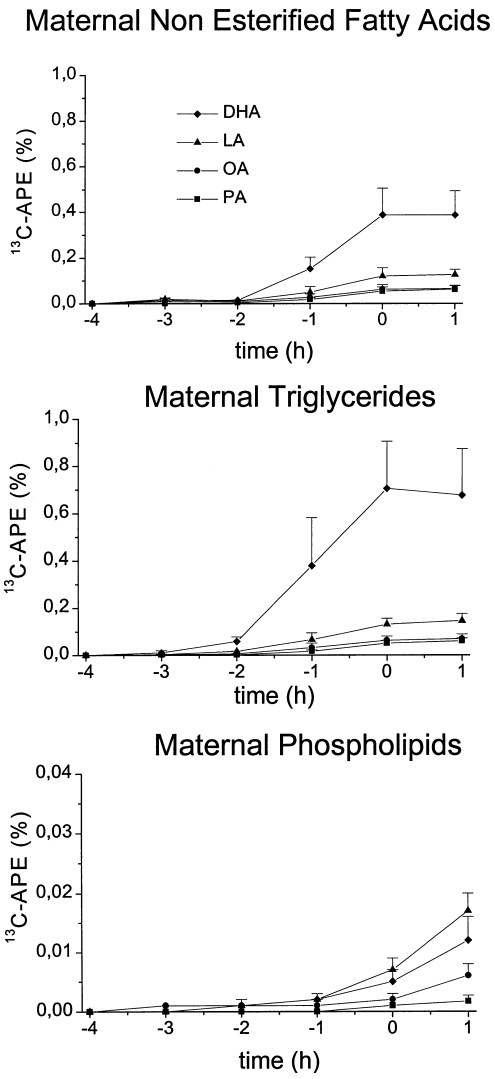

Fig. 1. Enrichment [[^{13}C]atom percent excess (APE) %] of fatty acids in maternal plasma during the time of study (diamond, docosahexaenoic acid (DHA); triangle, linoleic acid (LA); circle, oleic acid (OA); square, palmitic acid C16:0 (PA)]. Results are expressed as mean ± SEM.

RESULTS

The absolute concentration (mg/l) of the fatty acids studied at delivery was significantly higher ($P < 0.05$) in maternal plasma PL (PA: 490 ± 54, OA: 152 ± 17; LA: 281 ± 42; DHA: 72 ± 14) than in cord plasma PL (PA: 160 ± 36; OA: 39 ± 7; LA: 40 ±11; DHA: 33 ± 9). **Table 1** shows the percentage values of these selected fatty acids, as well as some fatty acid indices, in the lipid fractions of maternal plasma, cord plasma and placental tissue. Only total LC-PUFA proportions were higher in every lipid fraction in cord plasma, than in maternal plasma; in TG fraction, the DHA percentage was higher in cord plasma than in maternal plasma (Table 1). For the placental tissue, high DHA proportions were found in PL, TG, and NEFA, but not in the CE fraction. PL were the most abundant lipid fraction in the placenta (Table 1).

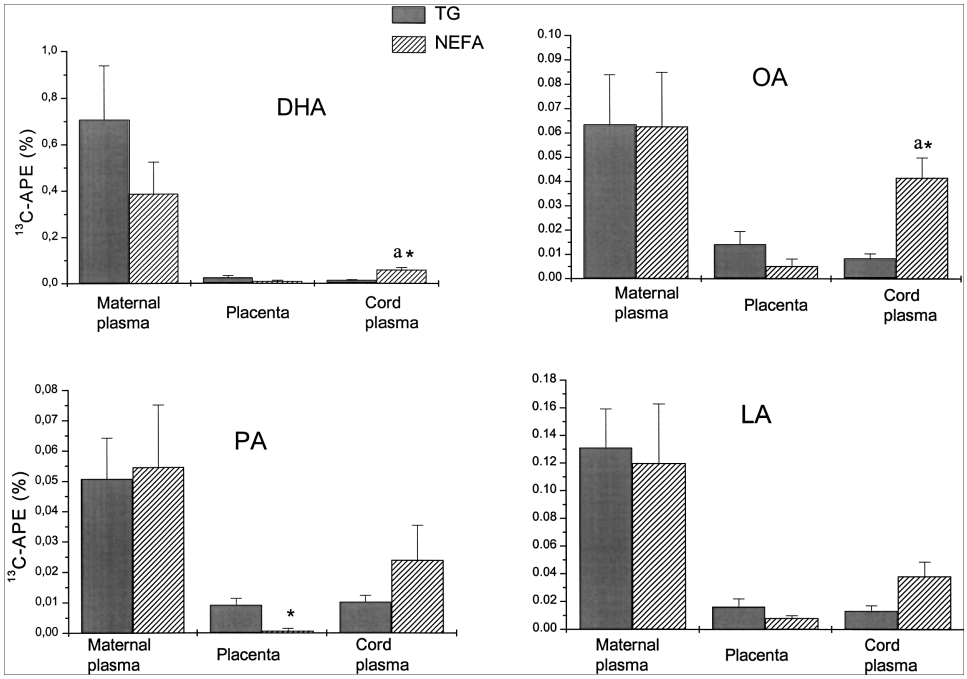

Fig. 2. APE values of tracer fatty acids in triglycerides and nonesterified fatty acids (NEFA) in maternal plasma, placenta and cord plasma at the time of delivery [(black bar, triglycerides (TG); striped bar, NEFA]. Results are expressed as mean ± SEM. [a] Statistically significant differences ($P < 0.05$) between nonesterified fatty acids enrichment of placenta and cord plasma. *Statistically significant differences ($P < 0.05$) between nonesterified fatty acids and triglycerides.

Figure 1 shows the evolution of ^{13}C-enrichment of the labeled fatty acids in maternal plasma during the experimental time. The enrichment of the [^{13}C]fatty acids in maternal plasma was maximal at the time of delivery in NEFA and TG. One hour after delivery (time +1), the ^{13}C-enrichments of the fatty acids continued in a similar range, showing that at delivery a relative stable steady state of tracer enrichment in these fractions was achieved. The high enrichment in the [^{13}C]DHA observed in these fractions, relative to the other fatty acids, was only due to the higher dose intake of the DHA tracer relative to the DHA concentration in plasma. ^{13}C-enrichments were hardly detected in maternal PL, and no incorporation into the CE fraction was detected during the time of this study.

The enrichment of the [^{13}C]fatty acids in TG and NEFA fractions of maternal plasma, placenta, and cord plasma at the time of delivery is shown in **Fig. 2**. We detected a different distribution of the ^{13}C-enrichment between placenta and cord plasma lipids. ^{13}C-enrichments tended to be higher in maternal plasma TG than in placenta TG or in cord plasma TG, reflecting a maternofetal flux. In contrast, the enrichments of the labeled fatty acids in the NEFA fraction were higher in cord plasma than in placenta (maternal NEFA> cord plasma NEFA> placenta NEFA), although the differences were only statistically significant for the nonesterified OA and DHA. In cord plasma, the APE values in NEFA were significantly higher than in the TG fraction for [^{13}C]DHA and [^{13}C]OA. In placenta, [^{13}C]PA was significantly more incorporated into TG than into NEFA. A similar trend was also observed for the other fatty acids, but did not reach statistical significance (Fig. 2).

Table 2 shows the concentrations of tracer in the different lipid fractions of plasma and placenta at the time of delivery. In the placenta, most of the tracer fatty acids were esterified into placenta PL.

In order to estimate the distribution of the labeled fatty acids within the different lipid fractions of cord plasma, we calculated the percentage of the μmol/l of tracer in all lipid fractions (**Table 3**). The results indicate that [^{13}C]PA, [^{13}C]OA, and [^{13}C]LA are mainly found in NEFA and TG of cord plasma, while [^{13}C]DHA was distributed in similar proportions among all fractions, although the differences were not statistically significant. This different distribution of DHA, as compared with the other fatty acids, might reflect differences in the transfer mechanisms of DHA, and is considered by some authors as an effect of compartmentalization of the lipids by the placenta (Table 3).

We calculated the percentage of μmol/l tracer fatty acids in cord plasma relative to the area under the tracer concentration curve in maternal plasma to estimate the ratio of placental transfer of labeled fatty acids from mother to fetus (**Table 4**). Due to a large variability in the results, there were no statistically significant differences. No preferential transfer of any of the fatty acids studied was evident (Table 4); however, the ratio of the fatty acid concentration in placental tissue to that in maternal plasma is significantly higher for [^{13}C]DHA than for the other fatty acids (**Fig. 3**). Furthermore, the placenta/plasma ratio for [^{13}C]LA was significantly higher than that for [^{13}C]OA (Fig. 3).

TABLE 2. Concentration of tracer in maternal plasma (μmol ^{13}C/l), placenta (nmol ^{13}C/g) and cord plasma (μmol ^{13}C/l) in the lipid fractions at the time of delivery

Phospholipids	PA	OA	LA	DHA
Maternal plasma	0.18 ± 0.11	0.23 ± 0.12	1.39 ± 0.42	0.23 ± 0.05
Placenta	0.45 ± 0.27	0.36 ± 0.11	0.97 ± 0.42	0.86 ± 0.20
Cord plasma	0.16 ± 0.06	0.06 ± 0.03	0.03 ± 0.01	0.04 ± 0.01
Triglycerides				
Maternal plasma	11.67 ± 2.14	14.36 ± 3.67	11.42 ± 2.17	3.58 ± 0.66
Placenta	0.29 ± 0.06	0.23 ± 0.09	0.18 ± 0.07	0.16 ± 0.07
Cord plasma	0.20 ± 0.03	0.12 ± 0.02	0.09 ± 0.02	0.03 ± 0.01
NEFA				
Maternal plasma	2.22 ± 0.39	2.35 ± 0.49	1.57 ± 0.21	0.43 ± 0.13
Placenta	0.05 ± 0.05	0.12 ± 0.05	0.25 ± 0.07	0.16 ± 0.04
Cord plasma	0.33 ± 0.10	0.27 ± 0.07	0.15 ± 0.04	0.04 ± 0.01

Results are expressed as mean ± SEM.

DISCUSSION

The materno-fetal transfer of fatty acids is considered to depend in part on concentration gradients across the placenta (21) that is enhanced by maternal gestational hyperlipidemia (3). This study confirms higher concentrations of individual fatty acids in maternal than in cord plasma, compatible with a gradient driven fatty acid flux from the mother to the fetus. The observed percentages of DHA and total LC-PUFA were within the range previously reported by others authors (3, 4) and higher in cord than in maternal plasma, which is in agreement with a preferential transfer of LC-PUFA to the neonatal circulation.

Four hours after tracer ingestion, there is some enrichment of [^{13}C]fatty acids in the maternal plasma TG and NEFA, while little has been esterified into PL and CE at this time (Fig. 1). However, since the placenta is reported to utilize almost exclusively fatty acids from maternal plasma TG and NEFA fractions for transfer to the fetus (8), we consider that this experimental design allows satisfactory evaluation of the transfer of the labeled fatty acids.

The ^{13}C-enrichment of cord blood NEFA shows a trend to be higher than the [^{13}C]enrichment in placental NEFA (Fig. 2) with statistically significant differences for NEFA OA and DHA, which lead us to conclude that there are different compartments of human placental NEFA. In agreement with this hypothesis, Hummel et al.(22) reported indications for the existence of two different pools of free fatty acids in rat placenta. They studied the placental transfer of [^{14}C]PA administrated intravenously at intervals from 0.3 min to 300 min to pregnant rats. They described one compartment, accounting for only 5% of the total placental free fatty acids, which represented maternal free fatty acids passing across the placenta. The second compartment comprised 95% of the placental free fatty acids and appeared not to take part in materno-fetal fatty acid transport (22). If only a small part of placental NEFA is involved in the transfer, this could explain the relatively low enrichment of total NEFA in the placenta in the present study, since the enrichment measured by us is obtained from the mixture of both NEFA pools. Another possibility that could explain the lower enrichment in placental NEFA than in cord plasma NEFA would be a methodological error, since part of the placental PL might have been hydrolyzed by phospholipase A2 of the placenta (23) and have diluted the tracer in the NEFA fraction during the time span between delivery and sample freezing. However, the placental samples we studied were obtained and frozen in liquid nitrogen immediately after cesarean section, thus we assume that lipolysis should be minimal (24).

The lipid fraction in which the fatty acids are present in the placenta might influence their rate of transfer (Table 2). It is known that transient esterification is an intermediate step in the placental fatty acid transport of some of the NEFA. Szabo et al. (25) estimated that about 20% of isotope labeled palmitate was esterified in placental TG after 2 h of incubation of human placental tissue. Cultured human term trophoblast cells incubated with radiolabeled oleate for 24 h released to the medium high proportions of NEFA, while labeled PL and TG were retained in the cells (26). Thus, NEFA bound to cytosolic fatty acid binding proteins (8) are transferred into cord plasma faster

TABLE 3. Distribution of [^{13}C]labeled fatty acids within the lipid fractions of cord plasma

	PA	OA	LA	DHA
		%		
PL	24 ± 8	13 ± 6	15 ± 7	37 ± 13
TTG	30 ± 5	29 ± 7	33 ± 7	26 ± 7
NEFA	46 ± 12	59 ± 7	52 ± 1	36 ± 7

PL, phospholipids; TG, triglycerides; NEFA, nonesterified fatty acids. Results are expressed as mean ± SEM. No significant differences.

TABLE 4. Estimation of tracer fatty acids transfer from the mother to the neonate

	PA	OA	LA	DHA
	μmol/l tracer cord plasma/AUC maternal plasma (%)			
PL	1.63 ± 1.45	0.36 ± 0.43	0.38 ± 0.34	1.17 ± 0.63
TG	1.92 ± 1.30	0.81 ± 0.46	0.70 ± 0.49	0.70 ± 0.53
NEFA	3.03 ± 2.54	1.97 ± 1.48	1.13 ± 0.90	1.14 ± 0.89
Total[a]	6.59 ± 4.94	3.14 ± 2.08	2.22 ± 1.83	3.01 ± 1.59

Results are expressed as mean ± SEM. No significant differences.
[a] Total, sum of labeled fatty acids transfer in phospholipids + triglycerides + nonesterified fatty acids.

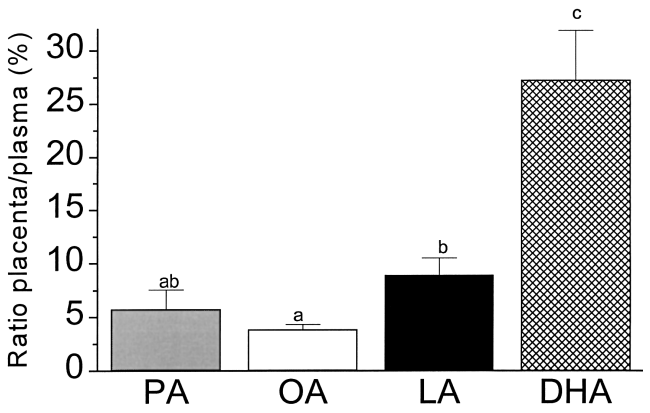

Fig. 3. Ratio between placental and maternal plasma concentration of [^{13}C]fatty acids at the time of delivery. Results are expressed as mean ± SEM. Means with no letters in common are significantly different, $P < 0.05$.

than PL, CE, or TG. The synthesis of apolipoprotein B in rat placenta (27), and the identification of small amounts of lipoproteins very similar to LDL and VLDL in human term placentas (with most of the cellular PL and TG present in the preparation) (28), suggests a probable mechanism of exportation of the esterified fatty acids to the fetal side, which takes longer time than the transfer of NEFA.

Kuhn and Crawford (10) reported that perfused human placenta transferred [^{14}C]AA mostly into fetal phosphoglycerides, which do not recross the placental barrier and thus may be retained in the fetal circulation, while [^{14}C]LA was found mostly in NEFA, which may cross the placenta in either direction. A different compartmentalization of individual fatty acids by the placenta might be a powerful mechanism for selective transfer of fatty acids. In the present study, [^{13}C]DHA was esterified in similar proportions into PL, TG, and NEFA of cord lipids (Table 3), while the other tracers were mainly incorporated into NEFA and TG, although the differences were not statistically significant. The relative esterification of [^{13}C]DHA in cord PL in this study (37%) was lower than that reported for [^{14}C]AA by Kuhn and Crawford (59.4%) (10). Differences in the distribution between DHA and AA were also described in BeWo cells where almost 37% of [^{14}C]DHA was esterified into PL relative to the 60% for [^{14}C]AA (29). The role of AA as precursor of eicosanoids might influence its selective accumulation in placental membrane PL and might explain differences to the placental transfer of DHA.

The concept of a preferential and selective transfer of LC-PUFA emerged from observations that maternal plasma lipids at birth contained higher percentage levels of the precursors ALA and LA than cord blood lipids of their infants, but percentage values for LC-PUFA were clearly and significantly higher in infants than in their mothers (3, 4). In contrast, some studies performed in human perfused placentas showed a higher rate of placental transfer for LA that for AA (10, 13). Haggarty et al. (30) recently reported that the fatty acid composition of the perfusate markedly modifies placental fatty acid transfer. While the selectivity for individual PUFA was DHA>ALA>LA>AA when the placenta was perfused with fatty acids in the same ratios as found in circulating TG of pregnant women in the last trimester of pregnancy, the preference changed to DHA>AA>ALA>LA when the maternal perfusate contained fatty acids in the same ratios as found in circulating NEFA.

In the present in vivo study, the tracer free fatty acids were administrated orally 4 h before delivery, and no preferential transfer of individual fatty acids across the placenta was detected (Table 4). However, we demonstrate a preferential sequestration of DHA into the human placenta relative to essential fatty acids and NEFA (Fig. 3). This result is in agreement with the preferential binding of maternal DHA by the FABPpm of the placenta described by other authors (8, 9). The DHA uptake in placental tissue might play an important role for the DHA transfer. Haggarty et al. (13) did not detect any preferential accumulation of [^{14}C]DHA in the perfused human placenta, but they found a preferential materno-fetal transfer of DHA. It appears possible that DHA transfer is a slow stepwise process, with initial placental uptake of DHA and later release into the fetal circulation. Our experiment may have detected mostly the first part of this process occurring until 4 h after tracer administration. Since we could not detected enrichment of tracer in PL and CE in the maternal side, it is unlikely that the time was sufficient to investigate the transfer of labeled fatty acids involving these components. Further studies with longer time intervals between tracer administration and delivery might contribute to describing the transfer process. It is likely that the results obtained 8 h, 12 h, or 24 h after administration of the labeled isotopes would be considerably different, but longer time frame studies also imply dilution of tracer by dietary intake and the fatty acids transferred quickly to the fetus as NEFA might already have been incorporated into fetal tissues.

In conclusion, the results of the present study demonstrate that stable isotope labeled fatty acids administrated 4 h before delivery to pregnant women appear in maternal TG and NEFA, but hardly in PL. The ^{13}C-enrichment of cord blood NEFA tended to be higher than the ^{13}C-enrichment in placental NEFA, which we interpret to reflect the existence of different pools of placental NEFA. Placental tissue shows a preferential incorporation of DHA relative to LA, OA, and PA at the studied time point, which may reflect a metabolic mechanism for a preferential materno-fetal DHA transfer.

E.L. was the recipient of a fellowship provided by the Humboldt Foundation (Germany). This work was partially supported by the German Research Council-Deutsche Forschungsgemeinschaft (Ko 912/5-1 and 5-2). Furthermore, the studies reported herein have been carried out partially with financial support from the Commission of the European

Communities specific RTD program Quality of Life and Management of Living Resources, QLK1-2001-0138, "Influence of Dietary Fatty Acids on the Pathophysiology of Intrauterine Foetal Growth and Neonatal Development" (PeriLip). This paper does not necessarily reflect its views and in no way anticipates the Commission's future policy in this area.

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