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Placental transfer of essential fatty acids in humans: Venous–arterial difference for docosahexaenoic acid in fetal umbilical erythrocytes

MELISSA RUYLE*, WILLIAM E. CONNOR*†, GREGORY J. ANDERSON*, AND RICHARD I. LOWENSOHN‡

*Division of Endocrinology, Metabolism and Clinical Nutrition, Department of Medicine, and ‡Department of Obstetrics and Gynecology, Oregon Health Sciences University, Portland, OR 97201

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ABSTRACT **Docosahexaenoic acid [22:6(n − 3); 22:6(4,7,10,13,16,19) (DHA)] is required in quantity by the developing nervous system of the fetus. This need could be met through synthesis of DHA from linolenic acid in the fetus or through placental transfer of DHA directly. To study the placental transfer of n − 3 fatty acids, we obtained umbilical and maternal blood samples from 26 healthy women and infants at parturition and measured the fatty acid composition and content of both plasma and erythrocytes. A striking finding was a considerable venous–arterial difference for DHA in the umbilical erythrocytes as a proportion of total fatty acids and in absolute concentration. This difference of 2.2 μg per billion erythrocytes was 6 times larger than the difference in fetal plasma, when the plasma and erythrocyte concentrations were normalized to whole blood. Most other erythrocyte fatty acids showed a similar trend. In umbilical plasma, significant venous–arterial differences were found for 16:0, 16:1, 18:2, and total saturated fatty acids. There was a similar trend for most other plasma fatty acids. Compared with maternal blood, fetal plasma and erythrocytes had higher levels of 20:4 and DHA and lower levels of 18:2 and 18:3(n − 3) fatty acids as a proportion of total fatty acids. These results suggest that erythrocytes play a major role in the necessary transport of the essential fatty acid DHA into the fetus.**

While n − 6 fatty acids were defined as essential fatty acids over 60 years ago (1, 2), it has not been until the last 15 years that evidence has supported the inclusion of n − 3 fatty acids in the list of essential nutrients (3).§ The "essentiality" of n − 6 and n − 3 fatty acids lies not only in the fact that their shorter chain precursors, linoleic [18:2(n − 6); 18:2(9,12)] and linolenic [18:3(n − 3); 18:3(9,12,15)] acids, cannot be synthesized *de novo* by vertebrate animals but also is a result of their distinct and different functional activities (4). Studies in a range of animals, from insects to birds and mammals, have contributed to our understanding of the specific deficits attributed to n − 3 and n − 6 fatty acid deprivation. Fatty acids of the n − 6 series (linoleic and arachidonic acids) are necessary for the integrity of the skin, growth, reproduction, and prostaglandin formation (1, 2, 5). Fatty acids of the n − 3 series—namely linolenic acid and docosahexaenoic acid [22:6(n − 3); 22:6(4,7,10,13,16,19) (DHA)] are necessary for the development and functioning of the retina and central nervous system (6). A triad of symptoms and signs occurs in (n − 3)-deficient rhesus monkeys: polydipsia (7), visual impairment (8, 9), and an abnormal electroretinogram (9). The biochemistry of the neural membranes is abnormal with low DHA content in deficient animals (4).

Since the human fetus cannot synthesize these essential fatty acids, it must derive them from the maternal blood via the placenta. The mechanism and amount of transport of these fatty acids from the maternal to fetal circulation remains unclear. The umbilical arteries bring fetal blood to the placenta, while the umbilical vein returns enriched blood to the fetus. Various compounds cross the placenta via diffusion, facilitated diffusion, and/or active transport. They then must travel to the target organs in the fetus. Several studies have investigated the placental transfer and the subsequent fetal deposition of the essential fatty acids (10–12), but most have assumed fetal plasma to be the chief or only transport vehicle. The potential role of the fetal erythrocytes has not been considered.

In the present experiments, we studied the involvement of both plasma and erythrocytes in the transport of essential fatty acids to fetal tissues. We examined venous–arterial differences in both concentration and percent composition in the plasma and erythrocyte components of venous and arterial cord blood and maternal venous blood in women delivered both by caesarean section and vaginally.

MATERIALS AND METHODS

Patients. Twenty-nine healthy pregnant women and their newborn offspring participated in this study. The protocol was approved by the Committee for Human Research and informed consent was obtained from each mother. All of the infants were delivered in the University Hospital at the Oregon Health Sciences University. Immediately after delivery by either caesarean section (N = 18) or standard vaginal delivery (N = 11), the umbilical cord was doubly clamped and blood was drawn from the venous and arterial circulation of the cord as well as from a maternal antecubital vein. Blood samples were collected into EDTA tubes, gently mixed, and placed on ice until centrifuged at 4°C. The erythrocyte count and the hematocrit were measured on all samples so that the concentrations of erythrocyte fatty acids could be expressed per billion erythrocytes or per ml of whole blood. Plasma fatty acid concentration is expressed per ml of plasma or per ml of whole blood (calculated by use of the hematocrit value).

Sample Analysis. Plasma was removed immediately after centrifugation of ≈1.0 ml of blood and was stored for no longer than 24 hr at −20°C before analysis. The erythrocyte pellet was washed twice with 10 ml of 0.9% saline. Lipids were extracted from erythrocytes by the method of Dodge and Phillips (13); butylated hydroxytoluene was added (10 μg/ml final volume) as an antioxidant. Plasma fatty acids were liberated by incubation with 6% ethanolic KOH and

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Abbreviation: DHA, docosahexaenoic acid.

†To whom reprint requests should be addressed.

§Fatty acid designations give the number of carbons separated by a colon from the number of double bonds followed immediately by information on the location of unsaturation, either by citing all locants or by specifying the locant of the terminal double bond as the quantity n − x, where n is the total number of carbons.

then extracted with hexane. After methylation, erythrocyte and plasma fatty acids were quantitated by capillary gas/liquid chromatography as described (14). An internal standard of heptadecanoic acid was added to both erythrocyte and plasma extracts. Peaks were identified by computerized comparison with standards, which were run daily. Plasma fatty acid concentrations are expressed per ml of plasma; erythrocyte fatty acid concentrations are given per billion erythrocytes.

Statistical Analysis. The data were evaluated by using a paired *t* test for venous–arterial and maternal–umbilical fatty acid comparisons. For 8 of our 29 subjects, we were unable to obtain a complete set of matching plasma and erythrocyte data for maternal and fetal blood samples. This accounts for the various sample sizes in Tables 1 and 2. However, all paired *t*-test calculations were made with matching samples. No statistical differences were seen between fatty acid mass in caesarean section ($N = 18$) vs. standard vaginal delivery ($N = 11$).

RESULTS

The mean concentrations and proportions of individual fatty acids in the erythrocytes of the maternal vein, umbilical vein, and umbilical artery are given in Table 1. Most striking is the statistically significant venous–arterial difference in both the proportion and concentration of DHA in fetal erythrocytes. We also observed a positive umbilical venous–arterial concentration difference for total $n - 3$ fatty acids; this result is largely a reflection of the DHA concentration difference. While there were no significant venous–arterial differences in the proportion of other fatty acids in fetal erythrocytes, we did observe a trend for positive venous–arterial concentration differences for the saturated, monounsaturated, and $n - 6$ polyunsaturated fatty acids.

Venous–arterial differences for the plasma concentrations of palmitic, palmitoleic, linoleic, and total saturated fatty acids (Table 2) reached statistical significance. Almost all of the other fatty acids displayed a similar trend toward positive venous–arterial differences. Results from the two essential fatty acids for which a significant venous–arterial difference was found—namely, DHA (in the erythrocytes) and linoleic acid (in the plasma)—are summarized graphically in Fig. 1.

Both the erythrocyte and plasma fractions of fetal blood contained lower proportions of linoleic and linolenic acids and higher proportions of arachidonic acid and DHA as compared with maternal vein values. The concentration of these fatty acids in the maternal and fetal erythrocytes showed the same trend. In plasma, by contrast, all fatty acids were more concentrated in the maternal circulation than in the fetus.

Table 3 shows the plasma and erythrocyte concentration of arachidonic acid and DHA in the maternal vein, the umbilical vein, and the umbilical artery in units of μg of fatty acid per ml of whole blood. In both plasma and erythrocytes, there was a trend towards a higher concentration of these two long-chain polyunsaturated fatty acids in the umbilical vein compared with the umbilical artery. For DHA in erythrocytes, this difference reached statistical significance. For both DHA and arachidonic acid, the venous–arterial difference was greater in the erythrocytes than in the plasma. While there was a large statistically significant reduction in the plasma concentration of these fatty acids from the maternal to the fetal circulation, there was an increase in concentration in the fetal erythrocytes relative to those of the mother.

DISCUSSION

An important feature of the present study is that it examined the placental transfer of fatty acids at parturition by taking into account the contribution of all blood lipids, i.e., from both plasma and erythrocytes. While fetal uptake of plasma-free fatty acids, as evidenced by significant venous–arterial differences, has been documented (12, 15–18), no studies have addressed the possibility of such differences in fetal

Table 1. Fatty acid content of maternal and fetal erythrocytes

	Composition, % by weight (mean ± SD)			Concentration, μg per 10^9 erythrocytes (mean ± SD)			
		Umbilical			Umbilical		
Fatty acid	Maternal vein ($N = 21$)	Vein ($N = 26$)	Artery ($N = 26$)	Maternal vein ($N = 21$)	Vein ($N = 26$)	Artery ($N = 26$)	Vein–artery difference
Saturated							
16:0	24.6 ± 3.7	25.5 ± 2.7	26.6 ± 2.8	34.7 ± 6.2***	44.4 ± 6.7	44.1 ± 6.2	0.3
18:0	16.5 ± 2.8	16.6 ± 1.4	16.3 ± 1.6	23.4 ± 5.1***	29.2 ± 4.9	27.4 ± 4.8	1.8
Total	45.2 ± 6.6	44.7 ± 3.7	46.4 ± 4.1	62.8 ± 13.1***	77.5 ± 13.1	75.6 ± 12.8	1.9
Monounsaturated							
16:1($n - 7$)	0.7 ± 0.5**	1.2 ± 0.7	1.2 ± 0.6	2.7 ± 7.5	2.1 ± 1.4	2.0 ± 1.3	0.1
18:1($n - 9$)	16.0 ± 2.2***	12.6 ± 1.6	12.8 ± 1.4	22.7 ± 4.1*	22.1 ± 4.2	21.4 ± 4.6	0.7
Total	17.9 ± 2.4***	14.7 ± 1.9	15.7 ± 3.8	25.7 ± 4.5	25.8 ± 5.3	24.8 ± 5.0	1.0
Polyunsaturated							
$n - 6$							
18:2	10.5 ± 2.4***	4.3 ± 1.2	4.4 ± 1.0	14.7 ± 3.0***	7.6 ± 3.2	7.5 ± 3.1	0.1
20:3	1.7 ± 0.5***	2.5 ± 0.5	2.4 ± 0.5	2.3 ± 0.8***	4.4 ± 1.3	4.0 ± 1.2	0.3
20:4	14.1 ± 2.5***	17.9 ± 2.2	17.6 ± 2.5	19.8 ± 4.2***	31.6 ± 7.8	29.4 ± 6.3	2.3
22:4	3.9 ± 1.1	4.1 ± 0.9	3.9 ± 0.9	5.5 ± 1.7**	7.3 ± 2.3	6.5 ± 1.9	0.8
22:5	1.2 ± 0.5**	1.8 ± 0.6	1.7 ± 0.5	1.6 ± 0.7***	2.9 ± 1.2	2.8 ± 1.0	0.1
Total	31.9 ± 4.7	31.0 ± 2.8	30.3 ± 3.8	45.0 ± 8.3**	52.2 ± 12.7	50.9 ± 11.9	1.2
$n - 3$							
18:3	0.2 ± 0.2***	tr	tr	0.3 ± 0.3***	tr	tr	tr
22:5	1.3 ± 0.6***	0.3 ± 0.2	0.4 ± 0.3	1.9 ± 0.8***	0.6 ± 0.4	0.7 ± 0.4	−0.1
22:6	3.7 ± 0.9***	5.6 ± 1.6	4.7 ± 1.7†	5.4 ± 1.9***	10.0 ± 3.7	7.8 ± 2.9	2.2†
Total	5.6 ± 1.5	6.0 ± 1.6	5.3 ± 2.0	7.9 ± 2.6**	10.7 ± 3.9	8.6 ± 3.1	2.2†

Asterisks indicate the degree of significance of the maternal–fetal venous difference: *, $P \leq 0.05$; **, $P \leq 0.02$; ***, $P \leq 0.001$.
tr, Trace.
†Fetal vein–artery difference was significant at $P \leq 0.02$.

Table 2. Fatty acid content of maternal and fetal plasma

Fatty acid	Composition, % by weight (mean ± SD)			Concentration, μg/ml of plasma (mean ± SD)			
		Umbilical			Umbilical		
	Maternal vein (N = 24)	Vein (N = 29)	Artery (N = 29)	Maternal vein (N = 23)	Vein (N = 28)	Artery (N = 28)	Vein–artery difference
Saturated							
16:0	28.7 ± 3.5	29.0 ± 2.9	28.3 ± 2.3	1079.2 ± 395.3***	298.1 ± 110.3	257.9 ± 83.5	40.2†
18:0	6.4 ± 3.7***	9.8 ± 1.9	10.1 ± 2.1	221.6 ± 149.9***	103.1 ± 55.4	92.1 ± 34.1	11.0
Total	38.7 ± 7.7	39.2 ± 7.6	39.9 ± 2.6	1432.1 ± 581.4***	435.3 ± 193.7	373.9 ± 120.3	61.4†
Monounsaturated							
16:1(n − 7)	3.3 ± 1.4***	4.7 ± 1.2	4.6 ± 0.9	127.1 ± 52.9***	47.8 ± 19.2	42.3 ± 14.6	5.5‡
18:1(n − 9)	23.2 ± 3.7*	21.4 ± 3.3	20.8 ± 3.4	879.8 ± 312.4***	224.9 ± 112.2	193.9 ± 70.7	31.0
Total	27.9 ± 4.5	26.7 ± 3.7	26.3 ± 3.9	1062.8 ± 376.8	279.4 ± 126.0	245.5 ± 83.0	33.9
Polyunsaturated							
n − 6							
18:2	23.9 ± 4.9***	11.6 ± 2.0	11.5 ± 1.7	921.7 ± 314.0***	116.0 ± 40.7	104.8 ± 39.6	11.2‡
20:3	1.2 ± 0.6***	2.7 ± 0.9	2.7 ± 0.8	42.8 ± 18.7***	26.5 ± 14.5	24.1 ± 13.5	2.4
20:4	5.3 ± 3.2***	12.7 ± 2.6	13.1 ± 2.3	169.6 ± 64.1***	123.2 ± 43.4	115.9 ± 41.3	7.3
22:4	0.5 ± 0.9	0.6 ± 0.2	0.6 ± 0.4	12.6 ± 18.5	5.4 ± 2.3	5.8 ± 4.0	−0.4
22:5	0.4 ± 0.3***	0.8 ± 0.3	0.9 ± 0.4	13.5 ± 8.9*	8.4 ± 3.9	7.3 ± 3.8	1.1
Total	31.7 ± 3.8**	28.7 ± 4.9	28.9 ± 3.0	1173.4 ± 354.2***	285.1 ± 95.4	269.8 ± 91.2	15.3
n − 3							
18:3	0.5 ± 0.2***	0.2 ± 0.1	0.2 ± 0.2	18.9 ± 11.4***	1.6 ± 0.7	1.5 ± 1.0	0.1
22:5	0.3 ± 0.4	0.2 ± 0.1	0.2 ± 0.1	8.2 ± 9.6**	2.1 ± 1.1	1.7 ± 0.7	0.4
22:6	1.3 ± 0.9***	2.7 ± 0.9	2.7 ± 1.1	41.9 ± 20.9**	26.7 ± 12.1	23.5 ± 7.9	3.2
Total	2.3 ± 1.3**	3.3 ± 1.1	3.1 ± 1.2	77.6 ± 34.7***	32.0 ± 13.2	27.9 ± 9.1	4.1

Asterisks indicate the degree of significance of the maternal–fetal venous difference: *, $P \leq 0.05$; **, $P \leq 0.02$; ***, $P \leq 0.001$.
†Fetal vein–artery difference was significant at $P \leq 0.05$.
‡Fetal vein–artery difference was significant at $P \leq 0.02$.

erythrocytes. Surprisingly, we discovered a significant venous–arterial concentration difference for one important fatty acid in fetal cord erythrocytes. It appears that a significant amount of DHA, the long-chain metabolite of the essential fatty acid linolenic acid, is transported across the placenta and is subsequently incorporated into fetal erythrocytes. The venous–arterial difference in DHA concentration further implies that erythrocytes may play a role in supplying developing fetal tissues with DHA. Despite the fact that the venous–arterial difference for other erythrocyte fatty acids did not reach the conventional level of statistical significance, it is interesting to note that there was a nearly uniform trend towards positive venous–arterial differences for the other erythrocyte fatty acids.

The comprehensive nature of our study is further reflected in the fact that we have calculated changes in fatty acid levels in terms of both composition (% by weight) and concentration. Composition data are limited in that they reveal the relative proportion of fatty acids in a sample without indicating the actual amount present. Concentration measurements, on the other hand, are unobscured by the levels of other fatty acids and express the absolute quantity of the individual fatty acids.

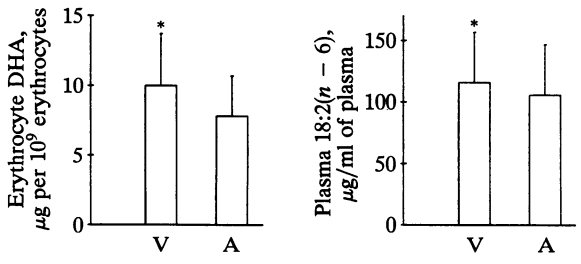

FIG. 1. Concentrations of the two essential fatty acids for which a statistically significant venous–arterial difference was found in umbilical blood fractions. V, umbilical vein; A, umbilical artery; *, $P \leq 0.02$, paired t test.

Most reports of cord venous–arterial differences have found significant differences in the plasma-free fatty acid concentration (12, 15–18). It is estimated that these small but significant venous–arterial differences could provide a substantial portion of the fatty acids required by the fetus for storage and structure (11). Most recently, Hendrickse *et al.* (12) demonstrated a significant venous–arterial mass difference in the plasma free fatty acid fraction for DHA and palmitic, oleic, and linoleic acids. We too found a statistically significant transfer of palmitic, palmitoleic, and linoleic acids in total plasma lipids (Table 2). That we found no significant venous–arterial differences for oleic acid and DHA may be a consequence of procedural limitations in detecting such small differences.

When the data of Hendrickse *et al.* (12) are expressed per unit of whole blood (calculated by using our average fetal hematocrit of 45.7%, N = 26), their plasma venous–arterial difference for DHA in the free fatty acid fraction is 1.1 μg per ml of whole blood. We obtained a similar value—namely, 1.5 μg per ml of whole blood (Table 3)—for DHA in total plasma lipids, including free fatty acids. A comparison of these values with our determination of the venous–arterial difference for DHA in erythrocytes (8.4 μg per ml of blood; Table 3) is striking. It appears that fetal erythrocytes transfer 6 times more DHA to the fetus than does the plasma free fatty acid fraction.

This rate of transfer of DHA from mother to fetus amounts to 4000 mg/day, assuming a fetal cord blood flow of 110 ml/min per kg of body weight at term (19). This rough estimate is greater than the estimated rate of the total accretion of n − 3 fatty acids in third-trimester human fetuses, namely 67 mg/day, which is also a rough estimate (20). A difference between the amount transferred to the fetus and the amount actually laid down in fetal tissue can be partially ascribed to the need to make up for losses to oxidative metabolism. However, the difference between 4000 and 67 mg/day still appears large. Another possible explanation is an increased rate of transfer in the latter part of the

Table 3. Direct comparison of erythrocyte (RBC) and plasma concentrations of arachidonic acid [20:4(n − 6)] and DHA [22:6(n − 3)]

Fatty acid	Fatty acid concentrations, μg/ml of blood				
	Maternal vein	Umbilical		Difference	
		Vein	Artery	Maternal–umbilical vein	Umbilical vein–artery
20:4(n − 6)					
Plasma	114.6 ± 34.3	73.0 ± 25.5	71.4 ± 21.8	43.7**	1.1 ± 13.3
RBC	73.0 ± 15.7	123.1 ± 23.6	118.3 ± 24.8	−50.1**	6.4 ± 21.4
22:6(n − 3)					
Plasma	26.4 ± 10.2	15.2 ± 7.3	13.5 ± 4.2	11.2**	1.5 ± 6.7
RBC	20.4 ± 6.2	41.6 ± 13.8	32.9 ± 14.2	−21.2**	8.4 ± 15.8*

Data are means ± SD. Vein–artery differences are given only for the subjects for whom a paired comparison of plasma and RBC values was possible (N = 19; P = 0.3 for plasma 20:4 vs. RBC 20:4; P = 0.1 for plasma 22:6 vs. RBC 22:6). Asterisks indicate the degree of significance for the differences: *, $P \leq 0.01$; **, $P \leq 0.001$.

third trimester, when growth of the organism is especially rapid (21). Our data, of course, reflect the rate of transfer at the actual time of birth when the greatest accumulation of fatty acids undoubtedly occurs.

A growing body of evidence (4) suggests that n − 3 fatty acids are essential for normal development of the retina and brain. The three major n − 3 fatty acids are 18:3, 20:5, and DHA (22:6). Our data indicate that the transfer of DHA into the fetus was much greater than for the other n − 3 fatty acids. DHA is the n − 3 fatty acid most prevalent in the brain and even in the adipose tissue (20). Carlson and coworkers (22) have shown a relative failure of 18:3(n − 3) to be converted to DHA in the human infant, although in the mouse 18:3(n − 3) is converted to DHA in the liver and transported to the brain (23). It may be that DHA is the key n − 3 fatty acid most needed by the human fetus and infant. The presence of DHA in human milk also supports this idea. It is logical for nature to provide directly via the placenta and via breast milk that fatty acid—i.e., DHA—that is most highly concentrated in the tissues, especially the brain and retina.

Further information about the importance of DHA and its analogous n − 6 fatty acid 20:4 comes from the experiments of Crawford *et al.* (24), who have shown that in both erythrocytes and plasma, the proportions of arachidonic acid and DHA increase in a step-wise progression from maternal liver to placenta to fetal liver to fetal brain. This trend towards fetal enrichment of long-chain polyunsaturates ("biomagnification") has been documented by others who reported an elevated proportion of long-chain polyunsaturated fatty acids [principally 20:4(n − 6) and 22:6(n − 3)] and a concomitant reduction in their metabolic precursors [18:2(n − 6) and 18:3(n − 3)] in the fetal blood as compared with the maternal blood (8, 12, 25). Our results confirm these observations, although we found the actual plasma concentration of all fatty acids to be higher in the maternal circulation.

That fetal erythrocytes should play an important role in the transport of essential fatty acids into the developing fetal tissues is not necessarily surprising. For example, obviously the vast majority of the oxygen and carbon dioxide carried in the blood of the fetus is transported in erythrocytes. Recent evidence has shown that erythrocytes also participate in the transport of amino acids (26) and glucose (27). Further studies are needed to clarify the mode of transfer of long-chain polyunsaturated fatty acids to fetal erythrocytes. It is possible that modification of the fatty acid content in the erythrocyte membrane involves a phosphatidylcholine-specific transfer protein, since the choline-containing phospholipids are located predominantly on the outer erythrocyte membrane (28) and since studies have shown that a phosphatidylcholine-specific transfer protein preferentially stimulates transfer of unsaturated phosphatidylcholine molecules (29). The mechanism of how fetal erythrocytes acquire essential fatty acids warrants further investigation.

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