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Short Communication

Umbilical venous–arterial plasma composition differences suggest differential incorporation of fatty acids in NEFA and cholesteryl ester pools

Rohan M. Lewis[1]*, Mark A. Hanson[1] and Graham C. Burdge[2]

[1]*Institute of Developmental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK*
[2]*Institute of Human Nutrition, Faculty of Medicine, University of Southampton, Southampton, UK*

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Abstract

The developing fetus requires an adequate supply of fatty acids, in particular PUFA, for optimal growth and development. Little is known about the transfer of fatty acids by the placenta into the fetal circulation. However, the molecular form in which fatty acids are transferred into the fetal circulation may influence their metabolism and hence their availability to specific tissues. The aim of the present study was to determine which lipid pools in the fetal circulation become enriched in fatty acids from the placenta by comparing the fatty acid compositions of individual lipid pools between umbilical venous (UV) and umbilical arterial (UA) plasma. Plasma from the UV and UA was collected after delivery from ten uncomplicated pregnancies, and the fatty acid composition of each lipid class was determined by GC. Total NEFA concentration in the UV was twofold higher than in the UA ($P<0{\cdot}05$) due to enrichment in 16 : 0, 16 : 1*n*-7, 18 : 1*n*-9, 18 : 1*n*-7, 18 : 2*n*-6, 20 : 3*n*-6, 20 : 4*n*-6, 24 : 0 and 22 : 6*n*-3. Total cholesteryl ester concentration was twofold higher in the UV than in the UA ($P<0{\cdot}05$) due to enrichment in 16 : 0, 16 : 1*n*-7, 18 : 0, 18 : 1*n*-9, 18 : 1*n*-7, 18 : 2*n*-6 and 20 : 4*n*-6. There were no significant UV–UA differences in the total concentration or composition of TAG or phosphatidylcholine. The present study demonstrates differential enrichment across the placenta of fatty acids into specific lipid pools in the fetal circulation. Such partitioning may facilitate supply of individual fatty acids to specific fetal tissues.

Key words: Fetal nutrition: Placenta: NEFA: Cholesteryl esters: PUFA

Placental transfer of fatty acids from the maternal to fetal circulation is important for the growth of fetal tissues[1]. During the late second and third trimesters, there is substantial accumulation of fatty acids into cell membranes to support growth and development, for example, accumulation of 22 : 6*n*-3 into membrane phospholipids in the central nervous system[2]. Fatty acids also accumulate in adipose tissue to provide insulation and a reserve pool of specific fatty acids, including 22 : 6*n*-3, to support development following transition from placental supply to suckling[2] and to protect brain development[3].

Transfer of lipids across the placenta involves transport across the syncytiotrophoblast and the fetal capillary endothelium. The placental syncytiotrophoblast can assimilate fatty acids from the NEFA pool and from esterified lipids in lipoproteins in the maternal circulation[4]. Esterified fatty acids are hydrolysed by placental lipoprotein lipase[5] or, in the case of LDL particles, taken into the syncytiotrophoblast layer by endocytosis[6]. NEFA and fatty acids released by hydrolysis of esterified lipids are assimilated via a specific fatty acid-binding protein which binds PUFA preferentially[7]. Within the trophoblast layer, fatty acids are either incorporated into transient TAG or phospholipid pools, or transported directly to the basal membrane[4]. The mechanisms by which fatty acids are transported out of the syncytiotrophoblast and across the fetal capillary endothelium into the fetal circulation are less well understood.

The molecular form in which fatty acids enter the fetal circulation is uncertain. Perfusion of human placenta *ex vivo* with radiolabelled fatty acids complexed with albumin showed differential secretion of individual species into the perfusate from the fetal side such that secretion of 22 : 6*n*-3 > 20 : 4*n*-6 > 18 : 3*n*-3 > 18 : 2*n*-6[8,9]. However, this technique did not allow investigation of the molecular form of the secreted fatty acids. NEFA concentration increases between the umbilical artery (UA) and umbilical vein (UV), which suggests secretion of fatty acids in a non-esterified form followed by binding to albumin[10]. Placental secretion

Abbreviations: CE, cholesteryl ester; UA, umbilical artery; UV, umbilical vein.

* **Corresponding author:** Dr R. M. Lewis, fax +44 2380795255, email rml2@soton.ac.uk

of lipoprotein particles containing apoB100 into the UV by the placenta has been reported[11]. It is, therefore, possible that fatty acids may also be secreted in esterified form associated with apoB100. In addition, cholesterol has been shown to efflux from human placental UV endothelial cells linked to HDL particles[12]. Since NEFA and lipoproteins are metabolised differently, partitioning of fatty acids between lipid classes may be critical for their supply to individual fetal tissues and hence influence fetal development.

In order to understand how the placenta transfers fatty acids to the fetus, we investigated the relative enrichment across the placenta of individual fatty acids into different lipid pools in fetal plasma by measuring the difference in fatty acid composition of individual lipid classes between UA and UV blood.

Methods

Sample collection and fatty acid analysis

Placentas were collected with written informed consent, with approval from the Southampton and Southwest Hampshire Local Research Ethical Committee. Plasma from the UV and UA was collected after delivery from ten uncomplicated pregnancies. Blood was collected from arteries and veins at the base of the umbilical cord, into tubes containing EDTA and centrifuged to separate the plasma which was collected and stored at −80°C.

Dipentadecanoyl phosphatidylcholine, triheptadecanoin, heneicosaenoic acid and cholesteryl heptadecanoate were added to plasma (0·8 ml) as internal standards (Sigma, Welwyn Garden City, Herts, UK). Total plasma lipids were extracted with chloroform–methanol (2:1, v/v)[13]. Individual lipid classes were separated by solid-phase extraction using 100 mg aminopropylsilica BondElut cartridges (Varian, Crawley, West Sussex, UK)[14]. Purified lipid classes were transmethylated by incubation with methanol containing 2% (v/v) H_2SO_4 at 50°C for 2 h[14]. Fatty acid methyl esters were recovered by extraction with hexane. Fatty acid methyl esters were resolved by GC using a 6890 gas chromatograph (Agilent Technologies, Wokingham, Berks, UK) equipped using a BPX-70 fused silica capillary column (30 m × 0·25 μm × 0·25 mm) (SGE Europe Limited, Milton Keynes, Bucks, UK) and flame ionisation detection[15]. Fatty

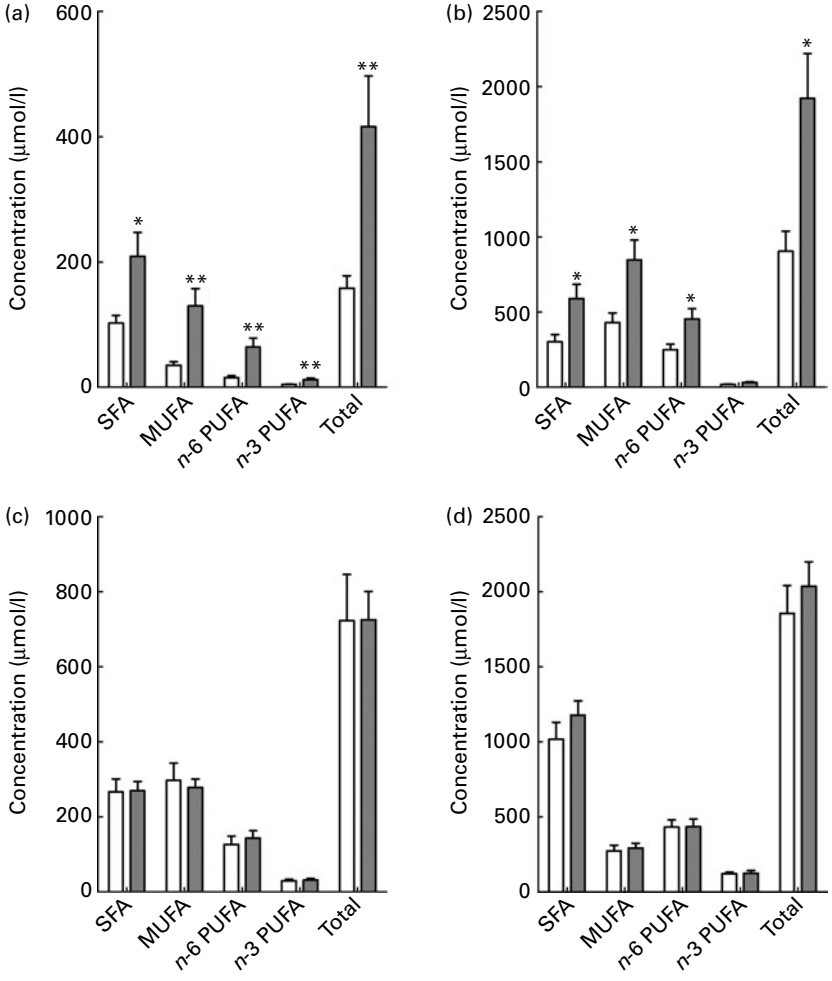

Fig. 1. Concentrations of fatty acid classes in (a) NEFA, (b) cholesteryl esters, (c) TAG and (d) phosphatidylcholine in plasma from the umbilical artery (UA; □) and vein (UV; ■). Values are means, with their standard errors represented by vertical bars (n 10). Mean values were significantly different between the UA and UV by Student's paired t test: * $P<0·05$, ** $P<0·01$.

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acid methyl esters were identified by their retention times relative to standards (Sigma), and peak areas were quantified using Chemstation software (Agilent Technologies). The concentrations of individual fatty acids were calculated relative to the peak area of the appropriate internal standard.

Statistical analysis

Data are presented as means with their standard errors. Differences between UV and UA concentrations were assessed using Student's paired samples *t* test and significance was assumed when $P<0{\cdot}05$. A total of ten subjects provided 85 % statistical power of detecting a 30 % difference in NEFA total concentration and in 22 : 6*n*-3 concentration in the NEFA fraction between the UA and UV with a probability of $P<0{\cdot}05$.

Results

Total NEFA concentration in the UV was 2·6-fold higher than in the UA due to increased concentrations of SFA and MUFA, and *n*-6 and *n*-3 PUFA (Fig. 1(a)), specifically 16 : 0, 16 : 1*n*-7, 18 : 1*n*-9, 18 : 1*n*-7, 18 : 2*n*-6, 20 : 3*n*-6, 20 : 4*n*-6, 24 : 0 and 22 : 6*n*-3 (Table 1). Total cholesteryl ester (CE) concentration was 2·1-fold higher in the UV than in the UA due to increased concentrations of SFA, MUFA and *n*-6, but not *n*-3, PUFA (Fig. 1(b)), specifically, 16 : 0, 16 : 1*n*-7, 18 : 0, 18 : 1*n*-9, 18 : 1*n*-7, 18 : 2*n*-6 and 20 : 4*n*-6 (Table 1). There was no significant UV−UA difference in total TAG (Fig. 1(c)) or phosphatidylcholine (Fig. 1(d)) concentration or in the amounts of individual fatty acids in these fractions (Table 1).

Discussion

The present study shows for the first time that UV plasma is enriched in both NEFA and CE compared with UA plasma and that this is associated with a differential increase in specific fatty acids within these pools. We interpret this as evidence for differential placental transfer of these lipid classes. To our knowledge, only one previous study has reported individual fatty acid concentrations in blood from umbilical vessels[16]. NEFA concentrations in that study were higher in UV plasma than that in UA plasma, although the overall increment and absolute concentrations were lower than those reported here. Since umbilical cord NEFA concentration is positively related to that of the mother[17], such differences between studies may reflect variations in maternal lipid metabolism. In addition, the previous study has reported a more limited range of individual fatty acids and observed differences in 16 : 0 and 18 : 0 not observed in the present study.

The present findings show for the first time that UV plasma CE concentration is higher than that in UA plasma, and that the fatty acids which are enriched in UV CE differ from those which increase in NEFA. For example, 22 : 6*n*-3 was enriched in UV NEFA but not in UV CE. Previous studies have shown that umbilical cord plasma is enriched in the proportions of specific fatty acids, in particular 22 : 6*n*-3, compared with maternal plasma, an effect termed 'biomagnification'[9,17–19]. This may reflect selective uptake of individual fatty acids from the maternal circulation and selective conversion to acyl-CoA within the placenta[7,20]. The present results

Table 1. Concentrations of fatty acids in NEFA, phosphatidylcholine, TAG and cholesteryl esters in plasma from the umbilical artery (UA) and umbilical vein (UV)

(Mean values with their standard errors, *n* 10)

	Fatty acid concentration (μmol/l)															
	NEFA				Cholesteryl esters				Phosphatidylcholine				TAG			
	UA		UV		UA		UV		UA		UV		UA		UV	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
16 : 0	45·3	6·7	135·3**	26·7	252·3	39·8	492·8*	75·5	664·3	242·4	792·9	63·5	215·0	24·5	224·4	18·5
18 : 0	47·6	7·5	67·3	12·2	34·2	4·6	72·2	21·2	336·6	37·0	372·6	31·3	39·2	8·1	34·5	3·0
20 : 0	0·3	0·1	0·4	0·1	0·8	0·2	1·5	0·4	2·4	0·4	2·4	0·3	3·9	1·3	2·7	1·3
22 : 0	2·9	0·5	3·4	1·0	0·8	0·4	2·6	0·4	4·3	1·6	3·6	1·5	3·4	1·8	4·0	0·8
24 : 0	0·4	0·1	2·4	0·9	2·3	0·4	5·7	1·5	9·1	2·6	7·8	1·5	5·7	3·3	4·3	2·3
16 : 1*n*-7	2·8	0·6	18·4*	3·8	73·4	14·7	170·2*	26·7	21·1	2·5	25·8	3·2	24·2	9·6	20·1	7·7
18 : 1*n*-7	1·7	0·4	9·1	2·2	39·1	6·2	89·1*	12·6	185·5	23·4	202·3	22·3	30·2	5·9	31·8	4·4
18 : 1*n*-9	24·1	3·8	99·5*	21·0	271·9	39·2	586·5*	92·5	60·2	8·7	58·3	7·3	240·4	34·5	223·3	17·8
20 : 1*n*-9	0·2	0·1	1·1	0·4	ND		ND		3·8	1·2	4·0	1·3	1·7	0·7	3·1	0·6
24 : 1*n*-9	0·2	0·1	1·4	0·7	ND		ND		4·7	2·2	1·2	0·2	ND		ND	
20 : 2*n*-9	1·7	0·2	1·7	0·5	6·1	3·7	4·5	1·9	9·1	2·5	7·5	1·5	2·1	0·7	2·6	0·3
18 : 2*n*-6	9·5	1·7	49·8*	10·6	207·3	30·6	409·5*	58·2	164·8	22·3	181·4	21·5	93·7	17·0	103·6	8·3
18 : 3*n*-6	3·4	0·6	5·7	1·6	6·7	1·4	13·5	3·4	1·1	0·3	2·2	0·7	4·8	2·2	5·5	4·3
20 : 3*n*-6	0·6	0·1	5·2*	2·3	3·2	0·9	2·7	0·8	50·5	13·2	47·0	13·8	7·7	3·7	5·8	0·9
20 : 4*n*-6	2·3	0·3	3·3*	0·4	15·2	2·5	27·9*	5·1	214·8	29·1	203·6	28·5	20·4	5·8	29·5	6·6
18 : 3*n*-3	0·1	0·1	3·8	1·8	1·7	0·8	4·8	1·4	ND		ND		0·5	0·2	3·1	1·6
20 : 5*n*-3	0·6	0·2	0·7	0·2	ND		ND		ND		ND		ND		ND	
22 : 5*n*-3	1·2	0·3	1·4	0·4	1·5	0·2	3·2	0·6	9·5	0·8	8·9	1·3	6·2	1·4	5·7	0·7
22 : 6*n*-3	1·1	0·2	6·1***	1·0	9·0	2·5	21·5	4·5	112·6	9·7	115·7	15·7	22·8	2·8	23·0	2·3

ND, not determined.

Mean values were significantly different between the UA and UV by Student's paired *t* test: * $P<0{\cdot}05$, ** $P<0{\cdot}01$, *** $P<0{\cdot}001$.

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suggest that this process also involves selective incorporation of fatty acids into different lipid classes and hence those supplied to fetal tissues. The relative transfer of 18 : 2*n*-6 to 22 : 6*n*-3 which we report is the reverse of that obtained by *ex vivo* placental perfusion, but is consistent with previous measurements of UV plasma fatty acid composition[17]. One possible explanation is that since umbilical cord NEFA concentration is related to that of the mother, and that the pattern of fatty acids in the maternal circulation resembles that of the UV plasma[17], the supply of fatty acids from the mother overrides any selectivity of uptake and transfer by the placenta. If so, these findings emphasise the importance of maternal lipid metabolism and diet in the supply of fatty acids to the fetus.

The precise mechanism by which UV plasma is enriched in CE cannot be determined from these findings; however, there are two possible mechanisms – secretion of LDL-like apoB100 particles or export of cholesterol to circulating HDL[12]. HDL-CE is the result of the uptake of cholesterol from the plasma membrane followed by conversion to CE by lecithin cholesterol acyltransferase activity using circulating phospholipids as a substrate. Therefore, this may be less likely as a mechanism for the secretion of fatty acids in the form of CE into the circulation. However, release of lipoprotein particles from the placenta into the fetal circulation may provide a mechanism for the secretion of preformed CE. Irrespective of the mechanism of CE enrichment, secretion of fatty acids into the lipoprotein pool may allow targeting of specific fatty acids to individual tissues. For example, NEFA and lipoprotein pools have been suggested to be involved differentially in the supply of *n*-6 and *n*-3 PUFA to the brain[21]. Furthermore, in rats, supply of 22 : 6*n*-3 to the developing brain is preferentially in the form of TAG[22]. If this is also important in humans, then the molecular form in which fatty acids, in particular 22 : 6*n*-3, enter the fetal circulation and their availability for processing by the liver may determine their subsequent metabolism and hence availability to developing tissues.

Because of its central role in fetal brain development, we estimated the placental transfer of 22 : 6*n*-3. Using an estimate of umbilical flow rate of 0·108 litres/kg fetal weight per min[23], placental transfer of 22 : 6*n*-3 for a 3·2 kg term fetus with a UV–UA difference of 5·0 μmol/l would be 2·4 mmol/d (817 mg/d). Our estimate of 22 : 6*n*-3 supply is in good agreement, albeit slightly higher, with the previous estimate of placental 22 : 6*n*-3 transfer of 200 mg/d in the literature[16]. This difference may reflect the different estimates of placental blood flow and differences in maternal plasma lipid concentrations. Accumulation of 22 : 6*n*-3 in the fetal brain, adipose tissue and liver during the last trimester is reported to be about 0·4 g/week in late gestation[24]. Our estimate exceeds this value (5·7 g/week). However, the fetal requirement for 22 : 6*n*-3 also includes demands, albeit to different extents dependent on tissue, for growth and development, and losses due to turnover and β-oxidation. Thus, it is reasonable that the total demand of the fetus for 22 : 6*n*-3 greatly exceeds that of liver, brain and adipose tissue, and so our estimate of placental transfer may be of an appropriate magnitude to meet fetal requirements.

Umbilical cord, usually UV, plasma has often been used as a marker of maternal fatty acid supply to the fetus[25–30]. The present findings suggest that measurements of fatty acid concentrations in the umbilical cord should be interpreted cautiously. There was no difference between the UV and UA in the fatty acid composition or total concentration of phosphatidylcholine and TAG. Thus, one possible interpretation is that the composition of these lipids reflects primarily fetal hepatic synthesis and turnover by peripheral tissues rather than the supply of fatty acids across the placenta. In contrast, the fatty acid composition of CE and NEFA in the UV appears to reflect both placental fatty acid supply and fetal metabolism.

Together, these findings suggest that the placenta may play a critical role in regulating the supply of fatty acids to fetal tissues. It would be of interest to investigate the extent to which variations in placental fatty acid secretion influence the development of the fetus, including the effects of common complications of pregnancy including obesity, diabetes mellitus and pre-eclampsia on this process.

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R. M. L. undertook the sample collection, and G. C. B. undertook the analysis. R. M. L., G. C. B. and M. A. H. contributed to the design of the study and preparation of the manuscript. None of the authors had a personal or financial interest to declare. M. A. H. is supported by the British Heart Foundation.

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