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Essential Fatty Acids and Their Long-Chain Polyunsaturated Metabolites in Maternal and Cord Plasma Triglycerides during Late Gestation

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Key Words

Essential fatty acids · Long-chain polyunsaturated fatty acids · Placental fatty acid transport · Fetal nutrition in late gestation

Abstract

The fatty acid composition of plasma lipids was determined in 41 pairs of mothers and their term infants at time of birth (38–42 postmenstrual weeks) by high-resolution capillary gas-liquid chromatography. Linoleic and α-linolenic acids were found at smaller concentrations in cord than in maternal triglycerides, in contrast to strikingly higher proportions of their long-chain polyunsaturated metabolites (LC-PUFA), which indicates a preferential maternofetal transport for certain physiologically important LC-PUFA. While no significant gestational age-dependent changes occurred in maternal plasma triglycerides, the values for most of the fetal long-chain n–3 metabolites increased with the duration of gestation, possibly reflecting an increased transplacental fatty acid passage during late pregnancy or a maturation of desaturation in the fetal liver.

Introduction

Fetal tissue growth and development require the provision of essential fatty acids (EFA) transported across the placenta [1]. Daily intrauterine accretion per kilogram of body weight of term-born babies has been estimated as about 400 mg n–6 fatty acids and 50 mg n–3 fatty acids, respectively [2]. The main sources for placental fatty acid transport are considered nonesterified fatty acids (NEFA) derived from triglycerides (TG) of maternal adipose tissue and hepatic lipoproteins (VLDL) [3]. The parent fatty acids linoleic (C18:2n–6) and α-linolenic (C18:3n–3) acids are partially converted into long-chain polyunsaturated fatty acids (LC-PUFA) of the n–6 and n–3 series. LC-PUFA biosynthetic routes comprise alternating chain elongation, hepatic desaturation and peroxisomal β-oxidation [4–6]. LC-PUFA are important for the formation of membrane structures in all tissues and are precursors of prostaglandins and eicosanoids. The rapidly developing brain in the last trimester and the first postnatal months may be especially vulnerable to a poor LC-PUFA status [1, 7, 8]. Marginal EFA status was shown to impair neural development in early infancy [9]. Therefore, elucidation of the physiological regulation of the EFA and LC-PUFA

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status of newborn infants and their gestational age-dependency in late pregnancy is of special interest. Gestational age-dependent fatty acid content of maternal and cord plasma with packed column gas chromatography has been reported [10], but this method has a limited separation power and accuracy. We examined the fatty acid composition of cord and maternal plasma NEFA, TG, phospholipids (PL) and cholesterolesters (CE) at delivery taking advantage of the much higher separating power of capillary gas chromatography.

Subjects and Methods

The study protocol was approved by the local ethical review committee, and informed consent was obtained from the participating mothers. The study population comprised 41 pairs of mothers and their term-born infants (38–42 postmenstrual weeks) (table 1). All children appeared healthy, and there was no indication of any inherited or intrauterine acquired disease or malformation. All participating mothers were free of risk factors for placental dysfunction such as diabetes mellitus, gestational diabetes or hypertension, and were screened for proteinuria and edema.

Blood was drawn at the time of birth from a peripheral vein of the mother and by venipuncture from the placental portion of the umbilical cord immediately after its clamping. Na-EDTA was used as anticoagulant. The samples were centrifuged immediately and stored at –20°C until analysis. Plasma lipids were extracted from 0.25 ml plasma into chloroform/methanol (2:1) after addition of internal standard [11]. PL, TG, CE and NEFA were separated by thin–layer chromatography (TLC) on silica gel plates (Merck, Darmstadt, Germany) with two full developments in petroleum ether/ether/acetic acid (75:25:1, by vol) and then one 5-cm run of chloroform/methanol/water (65:25:4, by vol). Fatty acids were transesterified with methanolic hydrochloric acid, and fatty acid-methyl esters separated by capillary gas-liquid chromatography on a Hewlett-Packard 5890 Series II gas chromatograph, equipped with a 50 m × 0.32 mm (inner diameter) polar cyanopropil silicone-coated column (SGE, Weiterstadt, Germany). Fatty acid determinations were performed at a column–head pressure of 1.3 bar and an initial temperature of 130°C, followed by an increase of 3°C/min to 180°C, consecutively raised to 220°C at 4°C/min. Fatty acid methyl esters were identified by comparison with authentic standards (Nu-Chek-Prep, Minnesota, USA).

Results were evaluated with Minitab for Windows, Release 9.2 (Minitab, Pennsylvania State University, USA) and expressed as percentage values (% w/w) of all detected fatty acids with a chain length of 12–24 carbon atoms. All data are presented as median and interquartile range values because some appeared not to be normally distributed. Maternal and cord plasma results were compared by Mann–Whitney's two-sided rank test. Gestational age dependency was studied by linear least-square regression analysis. The level of statistical significance was set at $p < 0.05$.

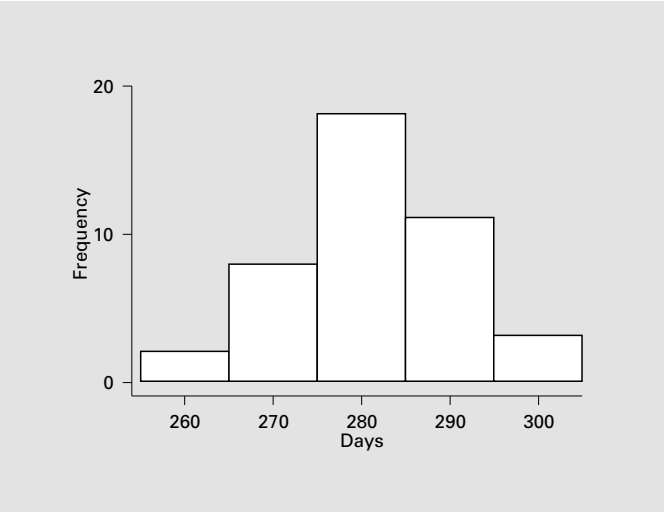

Fig. 1. Distribution of newborns in different age groups (days postmenstruationem).

Table 1. Characteristics of the study population, median (interquartile range)

Maternal age, years	30.0 (7.5)
Maternal weight prior to birth, kg	65.0 (14.0)
Maternal weight at birth, kg	77.0 (12.5)
Maternal length, cm	168.5 (8.0)
Maternal body mass index at birth, kg/m^2	22.6 (4.8)
Duration of pregnancy, days p.m.	282.0 (12.5)
Infants' birth weight, g	3,490.0 (670.0)
Infants' birth length, cm	51.0 (3.0)

Results

Characteristics of our study population are shown in table 1 and figure 1. Total fatty acid concentrations were significantly higher in maternal than in cord plasma TG (table 2). While the median percentages of C18:3n–3 and C18:2n–6 were markedly lower in fetal than in maternal plasma TG, the values for most of the long-chain n–6 and n–3 metabolites were significantly higher in the cord samples. Therefore, although total EFA values were lower in fetal TG, total cord LC-PUFA percentages were distinctively higher than those in maternal plasma, resulting in a greater C20:4n–6/C18:2n–6 and C22:6n–3/C18:3n–3 ratio in cord plasma TG.

Neither total values nor individual fatty acid percentages from maternal plasma NEFA, TG, PL and CE

Table 2. Fatty acid composition of maternal and cord plasma TG (% w/w) and gestational age dependency of cord plasma TG

	Maternal plasma (n = 41)		Cord plasma (n = 41)			Gestational age dependency of cord plasma TG	
	median	ir	median	ir	$p < 0.05$	r	$p < 0.05$
C18:2n–6	11.91	(5.48)	10.05	(3.45)	+	0.243	
C18:3n–6	0.14	(0.08)	0.45	(0.15)	+	0.219	
C20:2n–6	0.28	(0.11)	0.31	(0.18)		0.009	
C20:3n–6	0.22	(0.07)	0.81	(0.43)	+	0.441	+
C20:4n–6	0.75	(0.29)	2.92	(1.11)	+	0.127	
C22:4n–6	0.11	(0.05)	0.57	(0.31)	+	0.204	
Total n–6-LC-PUFA	1.54	(0.41)	5.74	(2.20)	+	–0.119	
Total n–6	13.88	(5.90)	15.82	(4.20)	+	0.135	
C18:3n–3	0.52	(0.19)	0.20	(0.27)	+	0.536	
C20:3n–3	0.05	(0.06)	0.00	(0.00)		–	
C20:5n–3	0.06	(0.04)	0.00	(0.17)		0.242	
C22:5n–3	0.09	(0.04)	0.12	(0.32)		0.564	+
C22:6n–3	0.32	(0.22)	1.33	(1.27)	+	0.371	+
Total n–3-LC-PUFA	0.51	(0.28)	1.59	(1.66)	+	0.453	+
Total n–3	1.09	(0.39)	1.82	(1.79)	+	0.500	+
Total LC-PUFA	2.03	(0.52)	7.38	(2.54)	+	0.119	
n–6-/n–3-LC-PUFA	3.11	(1.62)	3.10	(5.28)		–0.402	+
C18:2n–6/C18:3n–3	22.78	(8.38)	44.80	(15.89)	+	–0.287	
C20:4n–6/C18:2n–6	0.06	(0.02)	0.29	(0.11)	+	0.000	
C22:6n–3/C18:3n–3	0.61	(0.48)	6.32	(4.75)	+	–0.037	
Total (mg/dl)	143.31	(63.73)	24.27	(12.43)	+	0.455	+

ir = interquartile range, r = correlation coefficient.

showed significant gestational age-dependent changes (data not shown). No significant gestational age-dependent changes could be observed in cord plasma NEFA, PL and CE (data not shown), but total fatty acid concentrations in fetal plasma TG significantly increased with advancing gestation (table 2). The median percentage of C18:3n–3 showed no significant gestational age dependency in cord plasma TG, but the values for most of the long-chain n–3 metabolites increased significantly with the duration of gestation. In contrast to n–3 LC-PUFA, the majority of the long-chain n–6 metabolites were not correlated to gestational age, resulting in a negative correlation of the n–6/n–3 LC-PUFA ratio and the duration of pregnancy.

Discussion

In agreement with other studies [12–14], we found the percentage values of C18:3n–3 and C18:2n–6 markedly lower in cord than in maternal plasma TG. In addition to a limited placental transfer, possible higher rates of plasma clearance for storage or metabolism in placental and fetal tissue may have contributed to the low cord plasma levels. While C18:3n–3 and C18:2n–6 can be further desaturated in the ovine placenta [15], human placental tissue shows no activity of both the Δ6- and Δ5-desaturases [16]. Therefore, LC-PUFA in the fetal circulation appear to be synthesized either by the mother or the fetus. Although LC-PUFA biosynthesis is active in the newborn, the biosynthesis capacity appears to be limited [4]. Infants, fed formulas with precursor EFA but lacking LC-PUFA showed a more pronounced decline in LC-PUFA status than breast-fed infants [1, 17]. Thus, the high pro-

portion of LC-PUFA in cord plasma appears to result primarily from a preferential maternal-fetal transfer rather than from an active fetal LC-PUFA synthesis [18]. A preferential LC-PUFA transfer may be explained either by an enhanced placental transport of polyunsaturated fatty acids [19] or by a selective placental utilisation of maternal lipid fractions with a high LC-PUFA content [20].

Gestational age-dependent increase of fetal plasma TG has been reported [10, 21, 22]. Although animal tissues are capable of synthesizing LC-PUFA at an early stage of development [23], deposition of fat in human fetal adipose tissue takes place mainly in the last month of pregnancy [21]. There is evidence in the monkey that the placenta becomes more permeable to the passage of fatty acids from the mother to the fetus as pregnancy advances [24]. This might be explained by an increased blood perfusion of placental tissue in late gestation. If this was the only underlying reason for the gestational age-dependent increase of human fetal plasma LC-PUFA, it remains unclear why a gestational age-dependent increase can only be observed in cord TG but not in other fetal lipid classes like NEFA, CE or PL. Circulating NEFA are mainly derived from lipolysis of TG prior to being transported to the fetus, liberation of fatty acids from adipose tissue is considered to be low in the newborn. Fetal plasma CE are predominantly synthesized by circulating lecithin–cholesterol acyltransferase (LCAT) that transfers fatty acids from phosphatidylcholine to cholesterol. Low LCAT activity in the fetal liver may be the cause for the constancy of fetal CE-LC-PUFA during late gestation. PL are main components of biological membranes and are steadily incorporated into fetal lipid structures as pregnancy advances, this might prevent a gestational age-dependent accumulation of PL-LC-PUFA in the fetal plasma. Circulating TG are derived from both exogenous lipids after transplacental passage and endogenous lipids as hepatic VLDL [3]. In addition to an increased placental fatty acid transfer in late gestation, it seems that endogenous synthesis of fatty acids by the fetus appears to account for the fetal TG-LC-PUFA accumulation [16]. Maintenance of plasma TG levels in term and premature infants during the first postnatal day shows that at least during late gestation the fetal liver is capable of VLDL secretion [25]. Moreover, desaturation and chain elongation of both, C18:2n–6 and C18:3n–3 respectively, is possible in the fetal liver, but the substrate specifity of the the Δ6-desaturase is much higher for C18:3n–3 than for C18:2n–6 [26], possibly reflecting the gestational age-related gradual increase of TG-LC-PUFA of the n–3 series in contrast to stable levels of most of the n–6 LC-PUFA in cord TG during late gestation. Hence, the gestational age-related gradual increase of n–3 LC-PUFA in fetal TG may be caused by a combination of increased transplacental fatty acid passage in late pregnancy and a maturation of the desaturase activity in the fetal liver during late gestation.

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