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Biochemical EFA status of mothers and their neonates after normal pregnancy

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Summary

The essential fatty acid (EFA) status of neonates was compared with that of their mothers by determining the fatty acid compositions of phospholipids (PL), isolated from umbilical arterial and venous tissue, blood cells (BC) and plasma, from maternal venous plasma and BC, and from non-infarcted placental tissue.

The PL of umbilical arterial tissue (efferent fetal vessels) contained fewer fatty acids of the (n-6) family and more of the (n-9) family than umbilical venous tissue (afferent fetal vessel). The relative amounts of (n-6) and (n-3) fatty acids were less in arterial than in venous plasma. Mead acid, 20:3(n-9), the presence of which indicates a poor EFA status, was 5 times higher in the efferent than in afferent cord vessels. In neonatal plasma and BC it was twice as high as compared with maternal levels. In general, the fatty acid composition of the placenta PL showed a comparable pattern as neonatal venous plasma PL.

These findings demonstrate that the biochemical EFA status of neonates after a normal pregnancy is not optimal. The significant correlations between neonatal and maternal EFAs indicate that the neonatal EFA status depends on the EFA content of the maternal diet.

normal pregnancy; EFA status; mead acid; arachidonic acid; cervonic acid.

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Introduction

During the last trimester of pregnancy, fetal demands for long-chain polyunsaturated fatty acids (LCPs), especially 20:4(n-6) (arachidonic acid, AA) and 22:6(n-3) (cervonic acid, CA) are high [3]. AA and CA are the chain-elongated and desaturated products of the essential fatty acids (EFAs) 18:2(n-6) (linoleic acid, LA) and 18:3(n-3) (α-linolenic acid), respectively (Fig. 1). Since man is unable to synthesize these essential fatty acids, fetal EFAs and their long chain derivatives are ultimately derived from the maternal diet.

LCPs of the (n-3) and (n-6) families comprise 20—25% of the brain fatty acids, largely as constituents of cellular membranes [4,11]. Brain growth mainly takes place before birth and, therefore, it is important that the availability of essential fatty acids and their longer chain, more unsaturated derivatives is guaranteed during the development of the human fetus.

In an earlier study [10] we demonstrated that under normal conditions the EFA status of neonates may be marginal. The phospholipids of the umbilical arteries (efferent fetal vessels) were shown to contain significantly less fatty acids of the linoleic (n-6) and linolenic acid (n-3) families and more of the oleic acid (n-9) family than the umbilical vein (afferent fetal vessel).

The aim of the present investigation is not only to verify our earlier results, which were based on a relatively small set of samples and restricted to cord tissue only, but mainly to extend our knowledge about the fetal EFA status and its relation to the maternal EFA status. Therefore, we also determined the relative fatty acid composition of phospholipids (PL), isolated from umbilical venous and arterial plasma and blood cells (BC), from non-infarcted placental tissue and from maternal venous plasma and BC.

Material and Methods

Study population

The study group comprised 15 healthy women and their neonates (8 girls and 7 boys). The mean age of the mothers was 27 years (range 20—33 years). All mothers gave their informed consent. Umbilical vessel walls and placentas were obtained from 13 persons only. The two other placentas and umbilical vessel walls had to be sent to the pathological-anatomical laboratory for examination, because one mother had a manual placenta removal and the other had an extra placenta. All pregnancies were uncomplicated and the infants were all born *at term* (mean 40 weeks, range 38—41 weeks) after vaginal delivery. The mean birthweight was 3451 g (range 3125—4185 g). There were no differences between the birthweight of boys and girls. The Apgar score 1 min after birth was 9 (range 6—10) and 3 min after birth 10 (range 9—10). Maternal blood was taken under nearly fasting conditions (mothers had not been eating and almost did not drink during labor). Umbilical venous and arterial blood was collected separately, immediately after the cord had been clamped. Shortly after birth, placentas and cord vessels were rinsed with cold saline and frozen at -30°C until further treatment. In a small subset of 5 mothers, venous plasma and BC were also collected approximately ten days before delivery (this blood was taken under

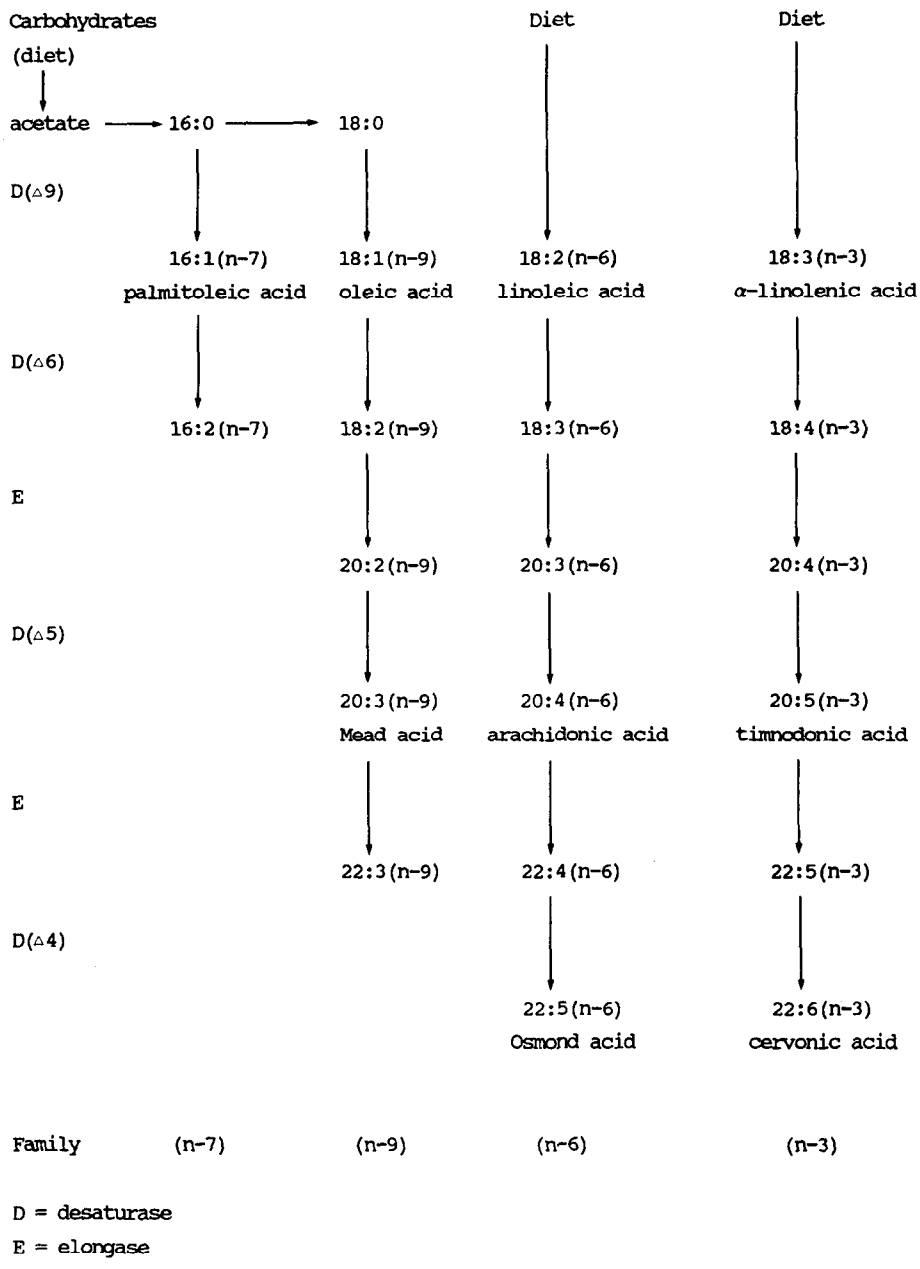

Fig. 1. Main pathway of fatty acid synthesis and interconversion.

conditions of complete fasting). Fatty acid compositions were compared with those just after delivery, to investigate possible fatty acid changes due to labor. There were no significant differences observed.

All material was collected at the Department of Obstetrics and Gynaecology, State University of Groningen, The Netherlands and transported to Maastricht.

Biochemical analyses

The plasma was separated from the blood cells by centrifugation and after plasma collection, the BC were washed with physiological saline. Plasma and BC were frozen and stored at −30°C until lipid extraction.

The placentas were cut into pieces for homogenization. An EDTA/NaCl-solution (Na_2-EDTA-$2H_2O$ 28.64 g, NaCl 7.00 g, H_2O 1000.0 ml) was added to the placentas (w/v = 1/1), after which they were homogenized in a blender. Umbilical cord vessels were homogenized according to the method described earlier [10].

Total lipid extraction was performed as described by Bligh and Dyer [1]. The PL fraction was separated by thin layer chromatography according to van der Vusse et al. [17]. The PL fraction was hydrolyzed and the fatty acids were methylated with boron-trifluoride. The fatty acid composition of the PL was then determined by gas liquid chromatography, as described by Rand et al. [16].

Statistical analyses

Wilcoxon's signed rank test was used for statistical analyses. The relation between the fatty acids of maternal and umbilical venous plasma or umbilical vessel wall phospholipids was studied by linear regression analyses, and Pearson's correlation coefficients were calculated.

Results

Fatty acid composition data (area % of total fatty acids) are given in Tables I and II. For clarity, data are mainly presented for those fatty acids or fatty acid combinations which reflect the EFA status. The full analytical results are available on request.

The results of cord vessel walls (Table I) largely confirm earlier data [10]: the umbilical arteries (UA) contained relatively fewer fatty acids of the essential (n-6) family and more of the non-essential (n-7) and (n-9) families than the umbilical vein (UV). The proportion of the Mead acid (MA) in the UA PL, the presence of which in adults indicates EFA deficiency, was nearly five times that in the UV PL, whereas the proportion of arachidonic acid (AA) was lower. Consequently the ratio of MA:AA, the EFA deficiency index, was eight times that in the vein, indicating a considerable deficiency of EFA in the arterial wall. The cervonic acid deficiency index (CADI), given by the ratio of the proportion of the Osmond acid (OA) to its immediate precursor (Fig. 1), was also raised in UA wall PL compared to that in the vein. In contrast, there was no significant difference in the fatty acid composition of PL from the cells (BC) of UV and UA blood, and, as shown in Table I, this was closer to that of the UV wall than that of the arteries. The differences between UA and UV plasma were also small (Table II) and the proportions of individual acids were, in general, of the

TABLE I

Fatty acid composition (area % of total fatty acids) of umbilical artery (UA) and vein (UV) vessel wall and arterial blood cell (BC) phospholipids (mean ± S.E.M.).

Fatty acid[a]	UA (n = 13)	UV (n = 13)	BC (A) (n = 15)
20:5 (n-3)	nd[d]	nd	0.2 ± 0.03
22:5 (n-3)[b]	0.4 ± 0.22	0.6 ± 0.08*	1.8 ± 0.37
22:6 (n-3) (CA)	4.7 ± 0.19	4.5 ± 0.18	4.7 ± 0.27
Σ (n-3)	5.2 ± 0.28	5.3 ± 0.20	7.0 ± 0.48
18:2 (n-6) (LA)	1.9 ± 0.06	2.8 ± 0.16**	5.9 ± 0.57
20:3 (n-6)	1.5 ± 0.10	2.0 ± 0.06**	2.1 ± 0.16
20:4 (n-6) (AA)	11.4 ± 0.47	15.9 ± 0.50**	14.0 ± 0.71
22:4 (n-6)	3.1 ± 0.20	5.2 ± 0.35**	4.0 ± 0.27
22:5 (n-6) (OA)	3.5 ± 0.15	3.3 ± 0.17	1.4 ± 0.07
Σ (n-6)	21.5 ± 0.76	29.2 ± 0.57**	27.5 ± 0.87
Σ (n-6) LCPs[c]	19.6 ± 0.72	26.4 ± 0.53**	21.6 ± 1.06
20.3 (n-9) (MA)	3.3 ± 0.25	0.7 ± 0.08**	0.6 ± 0.06
22:3 (n-9)	1.5 ± 0.11	0.4 ± 0.06**	0.3 ± 0.05
Σ [(n-7) + (n-9)]	28.1 ± 0.85	19.4 ± 0.36**	19.4 ± 0.85
ΣPUFA	31.5 ± 0.60	36.1 ± 0.55**	35.6 ± 1.06
ΣMUFA	23.4 ± 0.55	17.9 ± 0.27**	18.3 ± 0.85
ΣSAFA	40.3 ± 0.32	41.5 ± 0.35*	41.9 ± 0.64
EFA def. index	0.3 ± 0.03	0.04 ± 0.01**	0.04 ± 0.01
CADI	1.2 ± 0.07	0.7 ± 0.06**	0.4 ± 0.03

[a]SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EFA def. index, 20:3 (n-9)/20:4 (n-6); CADI, cervonic acid deficiency index: 22:5 (n-6)/22:4 (n-6).
[b]Plus 24:2(n-6) as a minor component.
[c](n-6) LCPs = (n-6) long chain polyunsaturated fatty acids.
[d]nd, not detectable.
*Significant difference from arterial values, $0.01 < P < 0.05$; **significant difference from arterial values, $0.001 < P < 0.01$.

same order as in the PL of non-infarcted placental tissue. However, as shown in Table II and Fig. 2, the fatty acid composition of PL from maternal plasma was very different from that in the fetus. The relative amount of linoleic acid (LA) was three times that in cord plasma PL, whereas those of AA, cervonic acid (CA) and its precursor were only half the fetal levels ($P < 0.001$). As expected the proportion of MA, and hence the EFA deficiency index, were low in maternal plasma PL.

On examing results from individual pregnancies, it was clear that the composition of the fetal plasma PL varied with that of the maternal plasma PL. Not only were there significant positive correlations ($P < 0.01$) between mother and fetus for the

TABLE II

Fatty acid composition (area % of total fatty acids) of fetal arterial (PA) and venous plasma (PV), placenta and maternal venous (PM) phospholipids (mean ± S.E.M.). For abbreviations, see Table I.

Fatty acid	PA (n = 15)	PV (n = 15)	Placenta (n = 13)	PM (n = 15)
20:5 (n-3)	0.7 ± 0.23	0.5 ± 0.14	0.1 ± 0.02	0.5 ± 0.04
22:5 (n-3)*	1.1 ± 0.09	1.4 ± 0.17	1.3 ± 0.06	0.7 ± 0.03
22:6 (n-3) (CA)	6.1 ± 0.39	6.7 ± 0.41	4.8 ± 0.26	3.9 ± 0.23
Σ (n-3)	7.9 ± 0.39	8.6 ± 0.40*	6.3 ± 0.28	5.7 ± 0.22
18:2 (n-6) (LA)	7.5 ± 0.42	7.8 ± 0.30	9.5 ± 0.29	23.1 ± 0.55
20:3 (n-6)	4.4 ± 0.29	4.9 ± 0.31*	4.3 ± 0.25	2.9 ± 0.20
20:4 (n-6) (AA)	18.6 ± 0.48	19.8 ± 0.52*	21.1 ± 0.34	9.4 ± 0.46
22:4 (n-6)	1.9 ± 0.36	1.4 ± 0.12	1.6 ± 0.10	0.5 ± 0.03
22:5 (n-6) (OA)	1.4 ± 0.12	1.3 ± 0.13	1.0 ± 0.06	0.7 ± 0.06
Σ (n-6)	34.1 ± 0.76	35.6 ± 0.82	37.6 ± 0.35	36.8 ± 0.66
Σ (n-6) LCPs	26.6 ± 0.81	27.7 ± 0.69	28.1 ± 0.38	13.6 ± 0.58
20:3 (n-9) (MA)	0.9 ± 0.11	0.9 ± 0.13	0.2 ± 0.03	0.5 ± 0.12
Σ [(n-7) + (n-9)]	15.3 ± 0.47	15.2 ± 0.56	12.1 ± 0.26	15.7 ± 0.42
ΣPUFA	43.2 ± 0.90	45.6 ± 0.97*	44.4 ± 0.28	43.5 ± 0.77
ΣMUFA	14.0 ± 0.37	13.7 ± 0.46	11.6 ± 0.26	14.7 ± 0.45
ΣSAFA	42.4 ± 0.87	40.4 ± 0.96	39.4 ± 0.28	41.3 ± 1.17
EFA def. index	0.05 ± 0.01	0.05 ± 0.01	0.01 ± 0.00	0.06 ± 0.01
CADI	1.0 ± 0.16	1.1 ± 0.17	0.6 ± 0.04	1.4 ± 0.13

*Plus 24:2 (n-6) as a minor component.
*Significant difference from arterial values $0.01 < P < 0.05$.

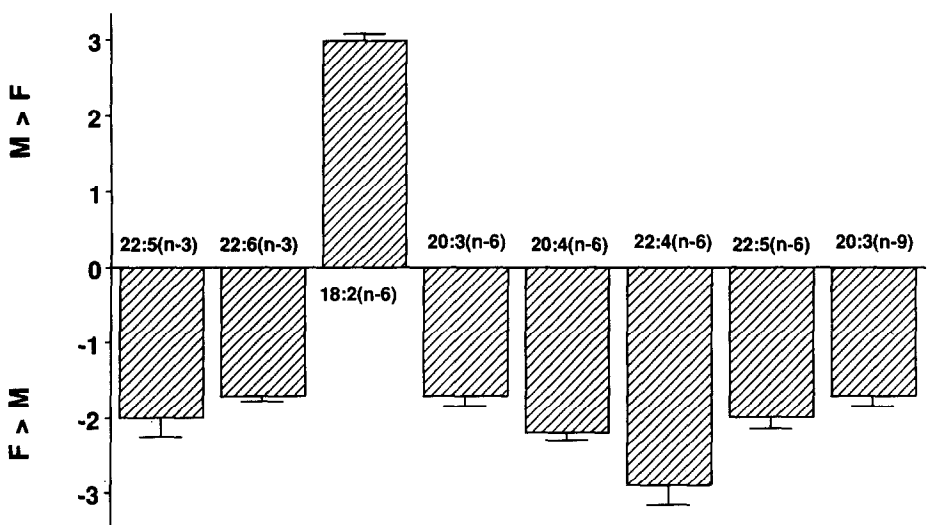

Fig. 2. Ratios between maternal (M) and fetal (F) fatty acid contents in venous plasma phospholipids (mean ± S.E.M.; n = 15).

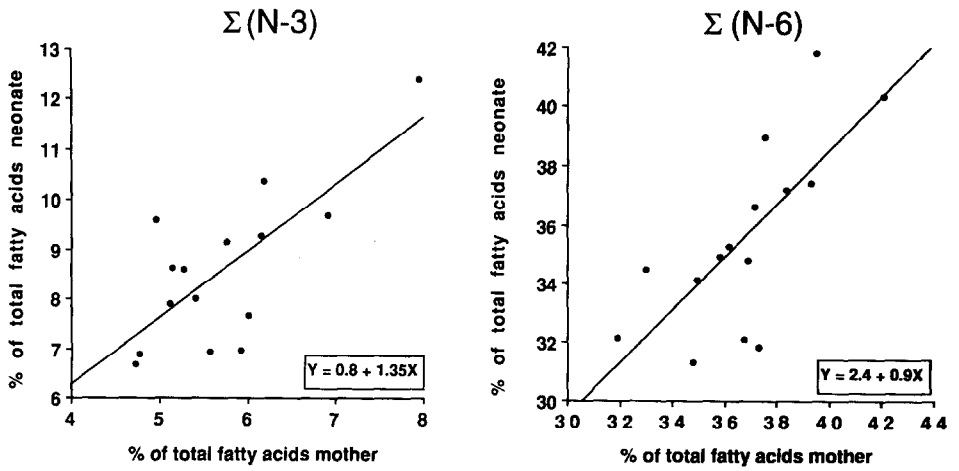

Fig. 3. The relationship between the sum of the (n-3) or (n-6) fatty acids in the venous plasma phospholipids of neonates and that of their mothers. Σ (n-3): $r = 0.74$; $P = 0.002$; $n = 15$; Σ (n-6): $r = 0.73$; $P = 0.002$; $n = 15$.

sum of the (n-3) and (n-6) acids (Fig. 3), but also for some specific saturated and mono-unsaturated acids and for CA, LA and OA.

Discussion

The present investigation confirms and extends the results of our previous study [10], in which evidence was obtained for a marginal EFA status of the newborn. The efferent blood vessel walls have lower proportions of LA and AA and higher relative amounts of 20:3(n-9) (MA) and its elongation product 22:3(n-9) than the afferent blood vessel wall.

Ongari et al. [15] also observed MA in the umbilical artery. MA was rarely observed in adult blood vessels and 22:3(n-9) was not observed at all [10]. Because the various fatty acids compete for the same enzymic desaturation-elongation system (Fig. 1), amounts of linoleic and α-linolenic acid insufficient to occupy the delta-6-desaturase, allows oleic acid to become converted to Mead acid (20:3(n-9), MA). Therefore Mead acid and its elongation product 22:3(n-9) are considered reliable markers of an essential fatty acid deficiency [12].

It should be realized that all our data refer to relative fatty acid compositions; consequently, an increase of one fatty acid inevitably means a decrease of at least one other fatty acid. Such errors can be magnified enormously when ratios are used, if the numerator and denominator are affected in opposite senses. Therefore, all ratios presented have to be seen in this light. For further studies, quantitative and absolute fatty acid composition measurements are required to understand more about placental transfer and fetal uptake of EFAs and their metabolites.

The classical biochemical indicator of a borderline EFA status is the EFA deficiency index, given by the ratio between Mead acid and arachidonic acid (MA/AA ratio). A value of 0.2 or more in human serum PL is considered as the upper limit of normality [8]. The EFA deficiency index in arterial tissue is 0.3 (Table I), which supports the idea that the neonatal EFA status is poor indeed, from the biochemical point of view. The EFA deficiency indices in fetal BC (Table I) and plasma (Table II) do not indicate an EFA deficiency. This seems in contrast to the findings for umbilical arterial tissue, but it should be realized that during each passage of the placenta, the fetal blood will take up new maternal EFAs. Consequently, only small arterio-venous differences are to be expected in the EFA status of circulating blood. This applies to phospholipids in particular, the turn-over rate of which is much lower as compared to free fatty acids. Since the walls of umbilical arteries are exclusively in contact with arterial (efferent) blood, any minor deviation from an optimal fatty acid composition in the blood will be "added up" in the arterial walls and, consequently, this tissue gives a more reliable and longer-term reflection of the average EFA status of the arterial blood than the post-partum blood itself. This consideration, and the fact that the MA content of fetal plasma and BC phospholipids is almost twice as high as the maternal levels (Fig. 2) suggests that the biochemical EFA status of the fetus is not optimal indeed.

The presence of these EFA deficiency indicators do not necessarily reflect a shortage of EFAs, however. An alternative explanation may be that the high proportions of 20:3(n-9) and 22:3(n-9) in umbilical arterial tissue may simply reflect a high desaturase activity in arterial vessel compared to venous vessel walls. However, the ratio between total (n-6) long chain polyunsaturated fatty acids [(n-6) LCPs] and LA (this ratio is an indicator of total desaturase activity) in the umbilical arteries (10.4 $\pm$ 0.29) is not significantly different from that in the umbilical vein (9.9 $\pm$ 0.59), whereas MA is about five times higher in the arteries. This suggests that this higher MA content in umbilical arteries does not simply result from a high desaturase activity. It should be mentioned that the ratio between (n-6) LCPs and LA, as used here, does not represent the true desaturase activity. In further studies this activity needs to be measured more precisely.

Another reason for the poor apparent EFA status could be the specific selection by the placenta for incorporation and transfer of the 20 and 22 carbon chain polyunsaturated fatty acids at the expense of linoleic acid, to satisfy the demand for (n-3) and (n-6) LCPs for fetal brain growth [5,11,14]. Consequently, there is less substrate available for the enzyme delta-6-desaturase and some switching to oleic acid desaturation might be expected. However, this can only partly, explain the elevated levels of MA, because the activity of delta-6-desaturase is also controlled by a feed-back mechanism: higher amounts of arachidonic acid reduce delta-6-desaturation [2].

The relative amounts of 22:5(n-6) are slightly higher in PL of umbilical arterial tissue than in umbilical venous tissue, whereas most other (n-6) fatty acids are significantly lower (Table I). Moreover, the ratio between 22:5(n-6) and its direct precursor 22:4(n-6), which reflects the delta-4-desaturase activity (Fig. 1), is significantly higher in the arteries as compared to the vein ($P < 0.01$, see Table I). This ratio has been used as the Cervonic Acid Deficiency Index (CADI) and, therefore, the present finding may point to a relative shortage of fetal CA also [7,13].

The CADI value for maternal venous plasma PL (1.4 ± 0.13) is significantly higher than that for fetal venous plasma PL (1.1 ± 0.17, $p = 0.03$). Since the adult and fetal CA requirements may differ from one another, this higher maternal CADI value does not necessarily imply that the CA status of the mother is insufficient. It may reflect, nonetheless, an increased maternal CA requirement to improve fetal CA supply.

In conclusion, the biochemical LA status of neonates after a normal pregnancy does not seem to be optimal. In addition, the fetus seems to have a high CA requirement. Since not all the data invariably indicate a marginal EFA status of the fetus, the situation is not that serious. It may become problematic, however, if there is a disturbed maternal-to-fetal fatty acid transfer. Crawford et al. [5] observed the highest concentration of MA and its elongation product 22:3 (n-9) in the umbilical artery of the lowest birthweight baby. Therefore, in further studies the fetal EFA status should be measured after conditions associated with impaired placental circulation (e.g. intrauterine growth retardation, pre-eclampsia, etc.). Since we demonstrated a close association between the maternal and fetal (n-3) and (n-6) fatty acids (Fig. 3), it may be possible to improve the fetal/neonatal EFA status by increasing the maternal EFA intake during pregnancy.

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