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ORIGINAL COMMUNICATION

Relationship between plasma fatty acid profile and antioxidant vitamins during normal pregnancy

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Objective: To study the changes of plasma fatty acids and lipophilic vitamins during normal pregnancy.
Design: Plasma fatty acid profile and the concentration of carotenoids, tocopherols and retinol were measured in healthy women at the first and third trimesters of pregnancy, at delivery, and in cord blood plasma.
Results: Maternal plasma cholesterol and triglycerides increased from the first to the third trimester of gestation, while free fatty acids progressively increased from the first trimester through the third trimester to delivery, suggesting an enhanced lipolytic activity. Plasma levels of α- and γ-tocopherols, lycopene and β-carotene also progressively increased with gestation, but values in cord blood plasma were lower than in mothers at delivery. Retinol levels declined with gestational time and values in cord blood plasma were even lower. The proportion of total saturated fatty acids increased with gestation, and it further increased in cord blood plasma. Total n-9 fatty acids remained stable throughout pregnancy, and slightly declined in cord blood plasma, the change mainly corresponding to oleic acid. Total n-6 fatty acids declined with gestation and further decreased in cord blood plasma, and a similar trend was found for linoleic acid. However, arachidonic acid declined in women at the third trimester and at delivery as compared to the first trimester, but was enhanced in cord blood plasma. The proportion of total n-3 fatty acids remained stable throughout pregnancy at the expense of decreased α-linolenic acid at delivery but enhanced eicosapentaenoic acid, with small changes in docosahexaenoic acid. The proportion of these n-3 fatty acids was similar in cord blood plasma and maternal plasma at delivery.
Conclusions: Owing to the different placental transfer mechanisms and fetal capability to metabolize some of the transferred fatty acids and lipophilic vitamins, the fetus preserves the essential compounds to assure their appropriate availability to sustain its normal development and to protect itself from the oxidative stress of extrauterine life.
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Introduction

The dietary essential fatty acids (EFA), linoleic (18:2 (n-6), LA) and α-linolenic acids (18:3(n-3), ALA), and their long-chain polyunsaturated derivatives (LC-PUFA), are vitally important structural elements of cell membranes and are therefore of pivotal importance for the formation of new tissue. Some of these LC-PUFA are precursors of prostaglandins, playing important roles in pregnancy and delivery (Hornstra *et al*, 1995). LC-PUFA occur in high concentrations in the central nervous system (Elliot & Knight, 1972), and the brain content of LC-PUFA—arachidonic acid (20:4 (n-6), AA) and docosahexaenoic acid (22:6(n-3), DHA)—increases progressively during brain organogenesis (Crawford *et al*, 1976). Although a maternal-fetal gradient in most polyunsaturated fatty acids (PUFA) has been reported (Friedman *et al*, 1978; Al *et al*, 1990, 1995; Otto *et al*, 1997; Min *et al*, 2001), the percentage of ALA is almost undetectable in fetal plasma and that of LA is nearly half of that in the mother. However,

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the proportions of AA and DHA are normally higher in the fetus (Cetin *et al*, 2002). These findings suggest that although the fatty acid mix delivered to the fetus is largely determined by the fatty acid composition of the maternal blood, the placenta is able to preferentially transfer AA and DHA to the fetus by a combination of several mechanisms, as has recently been reviewed (Haggarty, 2002). Furthermore, although LC-PUFA synthesis from EFA precursors has been demonstrated to occur in preterm infants as early as 26-week gestation (Uauy *et al*, 2000), other reports have estimated that the contribution of endogenous synthesis to the total plasma LC-PUFA pool in term neonates is small (Demmelmair *et al*, 1995; Szitanyi *et al*, 1999).

The nutritional status of the mother during gestation has been related to fetal growth, and supplementation with LC-PUFA-rich oils during the last trimester of pregnancy, to increase levels in neonates, has been advised (Van Houwelingen *et al*, 1995; Connor *et al*, 1996). However, the competitive desaturation of the n-3 and n-6 series by Δ6- and Δ5-desaturases plays an important role in the desaturating and elongating pathways of the parent EFA (Uauy-Dagach & Mena, 1995). Excessive dietary intake of certain LC-PUFA has also been found to decrease the formation of others (for a recent review, see Herrera, 2002).

Hyperlipidemia, characteristic of normal pregnancy during late gestation, is associated with enhanced oxidative stress (Uotila *et al*, 1991; Morris *et al*, 1998; Toescu *et al*, 2002), although this effect seems to be counteracted by increased oxidative resistance of LDL (De Vriese *et al*, 2001). The latter probably occurs due to the enhanced level of vitamin E, although other antioxidant vitamins, like β-carotene and vitamin A, remain, respectively, stable or decreased during normal pregnancy (De Vriese *et al*, 2001). Plasma levels of α- and γ-tocopherols have consistently been shown to be reduced in the cord blood of normal newborns and in preterm infants compared to either maternal or normal adult levels (Muller, 1994; Yeum *et al*, 1998; Kiely *et al*, 1999). The transfer of vitamin E in perfused normal term human placenta was found at a rate of only 10% of L-glucose (Schenker *et al*, 1998), and a lack of a significant improvement of the vitamin E status in neonates after a short-term supplementation of pregnant women before delivery has also been reported (Leger *et al*, 1998). This strongly suggests that the transfer of vitamin E through the placental barrier is low, despite the known risk of oxidant damage occurring in newborns (Johnson, 1998). On the other hand, vitamin A is an essential micronutrient for the development and growth of the fetus (Clagett-Dame & DeLuca, 2002), and low cord and maternal serum retinol have been associated with poor vitamin A status, which in turn may affect fetal growth (Gazala *et al*, 2003).

The aim of this study was to determine the plasma fatty acid profile and concentration of tocopherols, carotenoids and retinol during normal pregnancy. Furthermore, since in most previous studies, one time point was used to collect maternal blood, being either before delivery (Kiely *et al*, 1999; Min *et al*, 2001) or during labor (Al *et al*, 1990; Yeum *et al*, 1998; Berghaus *et al*, 2000), it is not known whether delivery itself modifies any of the studied variables. Thus, the objective of this study was to measure these variables in the first and third trimesters of pregnancy and at delivery, as well as in cord blood samples.

Methods

Study sample

Healthy women ($n=52$) aged 31.4 ± 0.6 y were recruited at the first prenatal visit in the outpatient clinic of Hospital San Paolo, Milan. The Local Ethical Committee approved the study protocol and, although informed consent was obtained from each participant, there were a substantial number of losses during the study. Maternal anthropometric data were collected, together with smoking habits, and an ultrasound exam performed for the dating of the pregnancy. Average body mass index (BMI) was 21.6 (kg/m^2) and seven of the 52 women were smokers. These characteristics reflected the normal pregnant population of the area, and no significant differences were observed in terms of maternal age, prepregnancy weight, height, weight gain in pregnancy, nutritional status, social demographic characteristics and smoking habits between the women who continued the study compared to those who did not. Exclusion criteria were: maternal diseases known to affect pregnancy, previous pregnancies with adverse gestational outcomes and maternal alcohol consumption. All women underwent uncomplicated pregnancies and gave birth at 39.2 ± 0.2 weeks to healthy newborns, with normal birth weights (3206.0 ± 81.4 g). None of the women were taking nutritional supplements that contained specific fatty acids or lipid-soluble vitamins. A nutritional questionnaire was given in order to analyze the nutritional intake (Fidanza *et al*, 1995) corresponding to the month before the interview. A fasting venous blood sample was taken from each participant at the first trimester of gestation (9.6 ± 0.4 weeks gestation). Nutritional questionnaires and venous blood samples were also collected at the third trimester of gestation (35.5 ± 0.3 weeks gestation) from some of the women ($n=32$). Venous blood samples were also collected from some of the mothers ($n=13$) at delivery. Cord blood was obtained immediately postpartum from the umbilical vein after clamping of the cord ($n=21$). Blood was drawn into vacutainers containing EDTA, centrifuged ($1000\times$ g at 4°C, 15 min) within 15 min of collection, and plasma stored at −80°C until analyzed.

Analytical methods

α- and γ-Tocopherol, retinol, lycopene and β-carotene were analyzed simultaneously by an isocratic reverse-phase HPLC method (Elinder & Walldius, 1992), with some modifications. Retinyl acetate and tocopherol acetate (Sigma Chemical Co., St Louis, MO, USA) were added as internal standards for the analysis of carotenoids, retinol and tocopherol,

respectively. Vitamins were measured with a Nova Pak (150 × 3.9 mm) reversed-phase column (Waters) at 37°C, attached to a multiwavelength ultraviolet detector (164 Diodo Array, from Beckman). The recovery was always over 94%, and the coefficient of variation in all cases was less than 10%. Plasma cholesterol, triglycerides and free fatty acid (FFA) concentrations were determined by commercial kits (Menarini Diagnostic, Florence, Italy, for cholesterol and triglycerides, and Wako Chemicals GmbH, Neuss, Germany, for FFA). Lipids were extracted from 0.20 ml of plasma into chloroform/methanol (2:1) (Folch *et al*, 1957). Fatty acids were transesterified with acetyl chloride, and fatty acid-methyl esters separated and analyzed on a Perkin-Elmer gas chromatograph (Autosystem; Norwalk, CT, USA), as previously reported (Amusquivar *et al*, 2000). Fatty acids results were expressed as a percentage (% w/w) of all detected fatty acids, with a chain length of 12–24 carbon atoms in the sample.

Statistical analysis

Results are expressed as means ± s.e.m. Data from smoker and nonsmoker women were pooled in the same group, since no significant differences were detected for any of the studied variables between them. Differences in plasma variables of the mothers between the first and third trimesters and delivery were evaluated by one-way ANOVA. When statistically significant differences appeared ($P<0.05$), the differences between each pair of groups were assessed by Tukey's multiple range comparison test. Although repeated-measures ANOVA for the 13 participants that were studied at all time points were also determined, they did not change the results. Thus, only the one-way Anova applied to the overall study is shown. Student's *t*-test was used to compare values between cord blood and either third trimester or delivery. Before statistical comparisons, γ-tocopherol, lycopene and β-carotene, given their skewed distribution, were log transformed. Correlations were tested using Spearman's analysis. All statistical analyses were performed using a computer software package (Statgraphics Plus, version 5.0, Statistical Graphics Corp.).

Results

There were no significant differences in the data obtained from the evaluation of the nutritional intake at the first and third trimesters of gestation, although there was a trend for total intake to increase, equally distributed in carbohydrates, lipids, proteins and vitamins (data not shown).

Table 1 shows concentrations of cholesterol, triglycerides, FFA, tocopherols, retinol, lycopene and β-carotene, and tocopherols and retinol adjusted for lipids in maternal plasma levels at the first and third trimesters of gestation as well as at delivery and in cord blood plasma. Maternal plasma levels of both cholesterol and triglycerides increased from the first to the third trimester of pregnancy, the change

Table 1 Plasma levels of cholesterol, triglycerides, free fatty acids and liposoluble vitamins in women throughout pregnancy and in umbilical cord blood[a,b]

	Maternal			
	First trimester (n = *52*)	*Third trimester* (n = *32*)	*Delivery* (n = *13*)	*Cord* (n = *21*)
Cholesterol (mg/dl)	141.9 ± 3.9[a]	246.2 ± 9.7[b]	245.0 ± 13.3[b]	49.5 ± 3.9***†††
Triglyceride (mg/dl)	86.9 ± 5.1[a]	222.5 ± 11.9[b]	227.9 ± 11.5 [b]	37.8 ± 3.9***†††
Free fatty acids (μmol/l)	270.0 ± 15.6[a]	342.8 ± 20.2[a]	671.6 ± 73.9[b]	214.0 ± 24.5***†††
Absolute values (μmol/L)				
α-Tocopherol	17.72 ± 0.72[a]	28.01 ± 1.46[b]	35.43 ± 3.13[c]	5.70 ± 0.45***†††
γ-Tocopherol	0.879 ± 0.054[a]	1.408 ± 0.131[b]	1.674 ± 0.150[b]	0.189 ± 0.032***†††
Vitamin E	18.60 ± 0.75[a]	29.41 ± 1.56[b]	37.10 ± 3.25[c]	6.17 ± 0.51***†††
Retinol	1.220 ± 0.052[a]	0.985 ± 0.055[b]	1.018 ± 0.086[ab]	0.609 ± 0.041***†††
Lycopene	0.170 ± 0.010[a]	0.234 ± 0.030[a]	0.421 ± 0.044[b]	0.050 ± 0.018***†††
β-Carotene	0.386 ± 0.037[a]	0.483 ± 0.060[ab]	0.782 ± 0.193[b]	0.078 ± 0.037***†††
Adjusted values (μmol/mmol lip)[c]				
α-Tocopherol	3.76 ± 0.11[a]	3.25 ± 0.16[b]	3.91 ± 0.21[a]	3.45 ± 0.19
γ-Tocopherol	0.190 ± 0.012	0.161 ± 0.015	0.187 ± 0.015	0.104 ± 0.015*†††
Vitamin E	3.95 ± 0.12[a]	3.42 ± 0.17[b]	4.10 ± 0.22[a]	3.67 ± 0.18
Retinol	0.263 ± 0.010[a]	0.117 ± 0.008[b]	0.119 ± 0.012[b]	0.385 ± 0.032***†††

[a]Values are expressed as means ± s.e.m., *n* = number/group.
[b]Tukey's test was used to determine differences between the three groups of maternal plasma after one-way ANOVA. Different letters in a row indicate significant differences ($P<0.05$). No superscript letters in a row indicate no significant differences. Statistical comparison between maternal plasma at third trimester and cord blood plasma is shown by asterisk (*$P<0.05$; **$P<0.01$; ***$P<0.001$) and between maternal plasma at delivery and cord blood plasma, by the † symbol ($^{\dagger}P<0.05$; $^{\dagger\dagger}P<0.01$; $^{\dagger\dagger\dagger}P<0{,}001$), and was determined by the Student's *t*-test (n.s. = no significant differences ($P>0.05$).
[c]Expressed as the ratio of plasma concentration/ lipids (cholesterol + triglycerides)

for triglycerides being greater than that for cholesterol. No difference was found in these two variables among women studied at delivery as compared to those studied at the third trimester, and values for both cholesterol and triglycerides in cord blood plasma were significantly lower than those found in the plasma of the mothers. Plasma FFA levels were not significantly higher in the third trimester compared to the first, but they greatly increased at delivery, where values were significantly higher than in either of the other two groups. FFA levels in umbilical cord were significantly lower than in both the third trimester and delivery mothers. Plasma levels of α-tocopherol progressively increased from the first to the third trimester of gestation and at delivery. However, values in cord blood plasma were lower than in mothers, there being statistical differences between these points. A similar trend although with less striking changes was found for the γ-tocopherol levels, values being much lower than those of α-tocopherol and differences between delivery and third trimester not reaching statistical significance. Plasma levels of vitamin E (corresponding to the sum of α- and γ-tocopherol values) changed similarly to those of α-tocopherol, with values progressively increasing from the first to the third trimester of gestation and at delivery, but with much lower values found in cord blood plasma. Retinol levels decreased from the first trimester of gestation to the third trimester, with the values kept stable at delivery. However, they were significantly lower in the cord blood plasma than in the mothers. The plasma levels of both lycopene and β-carotene progressively increased from the first to the third trimester of gestation and to delivery, differences being only significant between the third trimester and delivery. These two variables appeared significantly lower in cord blood plasma than in the mothers. Major differences among the groups in α- and γ-tocopherol as well as vitamin E values were smaller when corrected by plasma lipids (cholesterol + triglycerides). However, values of both α-tocopherol and vitamin E at delivery remained higher than at the third trimester, while those of γ-tocopherol in cord plasma remained lower than those found in the mothers. Maternal plasma retinol values corrected by plasma triglycerides were found to be lower at the third trimester of gestation and at delivery than those found at the first trimester, whereas values in cord blood plasma were higher than in maternal plasma at both the third trimester of gestation and at delivery (Table 1).

As shown in Table 2, there was a progressive increase in the proportion of total saturated fatty acids in maternal plasma from the first trimester through to delivery, and with even higher values in cord blood plasma. Total n-9 fatty acids, mainly corresponding to oleic acid (18:1 (n-9)), were found stable in the first and third trimesters of gestation and at delivery, but significantly lower in cord than in the mothers' plasma (Table 2). Total n-6 fatty acids declined from the first to the third trimester and at delivery, and further decreased in cord blood plasma (Table 2). The most abundant n-6 fatty acid was LA, and although its proportion remained stable in maternal plasma throughout gestation, it was significantly lower in cord blood plasma. Dihomo γ-linolenic acid (20:3 (n-6)) was lower in the third trimester and at delivery than at the first trimester, whereas values in cord blood plasma did not differ from those found in the mother at delivery. The percentage of AA decreased from the first to the third trimester of gestation, values remaining low at delivery, whereas in cord blood plasma they were significantly higher than found at either the third trimester or at delivery. Despite the stability of the percentage of total n-3 fatty acids

Table 2 Plasma fatty acids composition (g/100 g fatty acids) in mothers at first, third trimester and at delivery and in cord blood[a,b]

	Maternal			
	First trimester (n = *52*)	*Third trimester* (n = *32*)	*Delivery* (n = *13*)	*Cord* (n = *21*)
Total saturated	34.66 ± 0.94[a]	38.39 ± 0.71[b]	40.57 ± 0.93[b]	52.98 ± 1.63***†††
Total n-9	25.9 ± 0.6	26.1 ± 0.6	24.4 ± 0.6	22.2 ± 1.3***
Total n-6	33.6 ± 0.7[a]	29.7 ± 0.8[b]	28.7 ± 0.9[b]	17.6 ± 0.8***†††
18:2 (n-6)	24.7 ± 0.6	24.1 ± 0.7	23.3 ± 1.0	9.8 ± 0.8***†††
18:3 (n-6)	0.093 ± 0.025	0.076 ± 0.018	0.127 ± 0.052	0.148 ± 0.061
20:2 (n-6)	0.027 ± 0.014[a]	0.117 ± 0.035[b]	0.055 ± 0.040[ab]	0.000 ± 0.000***
20:3 (n-6)	1.53 ± 0.15[a]	0.76 ± 0.13[b]	0.21 ± 0.14[b]	0.27 ± 0.13***
20:4 (n-6)	7.16 ± 0.21[a]	4.69 ± 0.18[b]	4.97 ± 0.35[b]	7.35 ± 0.44***†††
22:2 (n-6)	0.086 ± 0.059	0.000 ± 0.000	0.000 ± 0.000	0.041 ± 0.041
Total n-3	3.36 ± 0.13	3.03 ± 0.14	3.18 ± 0.39	3.66 ± 0.34
18:3 (n-3)	0.093 ± 0.023[a]	0.379 ± 0.080[b]	0.079 ± 0.054[a]	0.085 ± 0.065***
20:5 (n-3)	0.217 ± 0.080[a]	0.475 ± 0.139[a]	1.137 ± 0.417[b]	1.122 ± 0.281*
22:6 (n-3)	3.05 ± 0.13[a]	2.18 ± 0.10[b]	1.96 ± 0.13[b]	2.45 ± 0.21

[a]Values are expressed as means ± s.e.m., *n* = number/group.
[b]Tukey's test was used to determine differences between the three groups of maternal plasma after one-way ANOVA. Different letters in a row indicate significant differences ($P<0.05$). No superscript letters in a row indicate no significant differences. Statistical comparison between maternal plasma at third trimester and cord blood plasma is shown by asterisk (*$P<0.05$; **$P<0.01$; ***$P<0.001$) and between maternal plasma at delivery and cord blood plasma, by the † symbol ($^{\dagger}P<0.05$; $^{\dagger\dagger}P<0.01$; $^{\dagger\dagger\dagger}P<0.001$), and was determined by the Student's *t*-test.

among the groups (Table 2), major differences were found in individual fatty acids. Thus, the percentage of ALA was higher at the third trimester of gestation than in any of the other groups studied, including the cord blood plasma, whose values did not differ from those of the mothers at delivery. The percentage of eicosapentaenoic acid (20:5 (n-3), EPA) did not differ between the first and third trimesters of gestation but significantly increased at delivery, with values remaining at this same level in cord blood plasma. However, the percentage of DHA decreased from the first to the third trimester of gestation, while values remained stable at delivery and in cord blood plasma.

A positive linear correlation was found when all individual values of total saturated fatty acids in maternal plasma were estimated against vitamin E levels ($r=0.4172$, $P<0.001$), whereas a negative correlation was found when the values of total polyunsaturated fatty acids were calculated against vitamin E levels ($r=-0.3372$, $P<0.01$)

Discussion

Besides confirming previous findings in the maternal hyperlipidemia, increment in plasma lipophilic antioxidant vitamins during late pregnancy and changes in fatty acid profile reveal some new aspects that deserve to be discussed. The most significant corresponds to major differences found in some of the studied variables at delivery as compared to the third trimester. This includes plasma FFA levels, which almost double at delivery, and would indicate a further increase in adipose tissue lipolytic acitivity over the activity already known to be enhanced during the third trimester of pregnancy (Elliott, 1975). Interestingly, plasma levels of those lipophilic vitamins known to be stored in adipose tissue, α-tocopherol (Kardinaal *et al*, 1993; Burton *et al*, 1998), lycopene and β-carotene (Brody, 1994) as opposed to those that are not, retinol (Olson, 2001) and γ-tocopherol (Handelman *et al*, 1994), also showed a significant increase in women at delivery as compared to values at the third trimester of pregnancy. This suggests that the proposed enhanced breakdown of fat deposits taking place at the time of delivery could also be responsible for such increases in those antioxidant lipophilic vitamins in plasma. Due to the low placental transfer of these compounds (Leger *et al*, 1998; Schenker *et al*, 1998), it may be proposed that this enhanced concentration of antioxidant lipophilic vitamins in maternal plasma at the time of delivery assures their appropriate availability for the fetus at the time of birth, when the risk for oxidant damage increases and the demand for antioxidant protection is essential (Johnson, 1998).

In this study plasma levels of retinol, α-tocopherol, γ-tocopherol, lycopene and β-carotene were lower in cord blood than in the mothers, which agrees with previous findings (Yeum *et al*, 1998; Kiely *et al*, 1999). The role of retinol and its metabolites in reproduction and embryonic development have been clearly established (for a recent review, see Clagett-Dame & DeLuca, 2002). Thus, the decline in plasma retinol levels during late pregnancy found here may reflect its enhanced utilization in favor of the fetus, as suggested by the enhanced retinol/lipid ratio seen in cord blood plasma. Transfer of retinol has been reported in human placenta (Torma & Vahlquist, 1986; Dancis *et al*, 1992), and the capacity of retinoic acid synthesis and catabolism by the embryo have been clearly established (Clagett-Dame & DeLuca, 2002). Furthermore, numerous genes are known to be regulated by all-*trans* retinoic acid during development (Clagett-Dame & Plum, 1997; McCaffery & Dräger, 2000). Thus, since retinol is the only vitamin A form that supports reproduction and embryonic development in full, preservation of fetal retinol levels at the expense of a decline on the maternal side is of pivotal importance for appropriate pregnancy outcome.

In agreement with previous reports (Godel, 1989; Dison *et al*, 1993) tocopherol levels in cord blood plasma correlated with cholesterol and triglyceride levels, allowing the lipid-adjusted α-tocopherol and the total tocopherols (α- plus γ-tocopherol, vitamin E) to agree between cord blood plasma and the mothers. As shown in other studies (Kiely *et al*, 1999), and distinct from what occurs with α-tocopherol, lipid-adjusted values of γ-tocopherol were lower in cord blood plasma than in the mothers. Despite its abundance in the diet, tissue content and plasma levels of γ-tocopherol are normally much lower than α-tocopherol (Mino *et al*, 1985). This is mainly due to the presence of α-tocopherol transfer protein (α-TTP) in liver (Sato *et al*, 1991), which preferentially facilitates the incorporation of α-tocopherol, but not of γ-tocopherol or other forms of vitamin E, into very low density lipoproteins (VLDL), which are released into the circulation (Traber & Arai, 1999). The presence of α-TTP in uterus has recently been demonstrated in mice (Kaempf-Rotzoll *et al*, 2002), playing an important role in supplying the placenta and the fetus with α-tocopherol throughout pregnancy. Although little is known about lipid-soluble vitamin placental transfer (Moriss *et al*, 1994), placental transfer of γ-tocopherol may depend, among other factors, on maternal plasma concentration. Thus, lower levels than α-tocopherol in maternal plasma would also reflect an even lower placental transfer capacity for γ-tocopherol, therefore explaining its decreased lipid-adjusted value in cord blood plasma. Although the functional importance of γ-tocopherol has recently been recognized to be greater than previously thought (Jiang *et al*, 2001), its low concentration in cord plasma would indicate a limited role in adaptations to extrauterine life in newborns.

Increments in lipophylic antioxidant vitamins during late pregnancy could be associated with an increase in polyunsaturated fatty acids. In fact, although this study found that the percentage of total n-3 remained unchanged between the first and the third trimesters of gestation, and that total n-6 fatty acids decline at the third trimester of gestation, mainly due to the decline in AA, which agrees with previous reports (Crastes de Paulet *et al*, 1992), this is

not the case if these values are corrected by the actual fatty acid concentration. We have previously shown that when expressed as PUFA concentration per plasma volume, its amount in the different lipoprotein lipid fractions was higher in pregnant than in nonpregnant women (Herrera, 2002). Since among the different PUFA LA (18:2(n-6)) shows the highest proportion, and it was found here that its percentage value did not change between the first- and third-trimesters in pregnant women, it is expected that its absolute concentration is enhanced during late pregnancy when corrected by the increase in plasma lipids (mainly triglycerides) that takes place at this stage. The inverse linear correlation found here between α-tocopherol and total polyunsaturated fatty acids would suggest a relationship between these two variables. Proportional declines of DHA (22:6(n-3)) and AA (20:4(n-6)) in the mother during late gestation contrast with their stable (in the case of DHA) or even increased (in AA) values found in the fetus. This agrees with the reported selective transfer of these LC-PUFA by the placenta, carried out by multiple mechanisms yielding the 'biomagnification' of these fatty acids within the fetal circulation (Haggarty, 2002). The synthesis of these fatty acids from EFA precursors by the fetus cannot be ruled out as contributing to the high proportion of AA and DHA in fetal circulation. The capacity for the metabolic elongation and desaturation of LA and ALA to form AA and DHA, respectively, has been consistently demonstrated to occur during the first days of life in humans, including very premature preterm neonates (Demmelmair *et al*, 1995; Carnielli *et al*, 1996; Salem Jr *et al*, 1996; Sauerwald *et al*, 1997; Szitanyi *et al*, 1999; Uauy *et al*, 2000), and it has also been shown to take place in fetal baboons (Su *et al*, 1999, 2001). Placental transfer of EPA (20:5(n-3)) has not been reported despite its growth inhibitor action (Sellmayer *et al*, 1996), its inhibitory effect on human placental membrane binding of EFA (Dutta-Roy, 2000) and its effect in reducing the availability of AA and its metabolites by a competition effect on pathways of EFA metabolism (Dutta-Roy, 1994), all of this denoting an important and active functional activity. The synthesis of EPA (20:5(n-3)) from its EFA precursor (ALA, 18:3(n-3)) and/or by the retroconversion of DHA (22:6(n-3) has been shown to take place in fetal rhesus monkeys (Greiner *et al*, 1996), and thus the possibility exists that a similar mechanism is acting in the human fetus, explaining the similarity of its concentration in cord and maternal plasma seen here.

In agreement with previous reports (Crastes de Paulet *et al*, 1992), the greatest proportion in maternal plasma fatty acids corresponded to the saturated fatty acids. The enhanced proportion of these fatty acids in cord blood plasma in contrast to the limited placental transfer for saturated fatty acids as compared to PUFA (Campbell *et al*, 1996; Haggarty *et al*, 1997) would indicate an active lipogenesis in the fetus, as demonstrated in previous studies (Dunlop & Court, 1978). Similar reasoning could be used to justify the high proportion of oleic acid in cord blood plasma, although slightly lower than in the mothers during late gestation. Placental transfer of oleic acid is also lower than that of PUFA (Campbell *et al*, 1996; Haggarty *et al*, 1997), and therefore its proportional abundance in the fetus may reflect an active desaturation of stearic acid.

Although the present work has the limitation of the high number of subject losses during the study, and studies of larger sample size and carried out in multiple populations are still needed, the studied population was sufficiently heterogenous and representative of healthy pregnant women. We therefore propose that under normal conditions and besides specific placental transfer mechanisms, both the enhanced lipolytic activity and the circulating level of antioxidant vitamins at delivery may actively contribute to the availability of both LC-PUFA and these vitamins to the fetus in preparation for extrauterine life.

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