Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status

BY MONIQUE D. M. AL[1], ADRIANA C. VAN HOUWELINGEN[1], ARNOLD D. M. KESTER[2], TOM H. M. HASAART[3], ANDRÉ E. P. DE JONG[4] AND GERARD HORNSTRA[1]

[1] *Department of Human Biology, University of Limburg, Maastricht, The Netherlands*
[2] *Department of Methodology and Statistics, University of Limburg, Maastricht, The Netherlands*
[3] *Department of Obstetrics and Gynecology, University of Limburg, Maastricht, The Netherlands*
[4] *Analytical Biochemical Laboratory, Assen, The Netherlands*

Although essential fatty acids (EFA) and their longer chain, more unsaturated derivatives play a major role during pregnancy, hardly any information is available with respect to the course of the maternal EFA status during an uncomplicated pregnancy and its relationship to the neonatal EFA status. Therefore, a longitudinal study was started in which 110 pregnant women gave repeated blood samples from the 10th week of gestation until delivery. After birth a blood sample from the umbilical vein and a maternal venous blood sample were collected as well, and 6 months after delivery a final blood sample from the mother was taken. The absolute (mg/l) and relative (% total fatty acids) amounts of the fatty acids in plasma phospholipids were determined. The total amounts of fatty acids increased significantly during pregnancy. This pattern was similar for the individual fatty acids and fatty acid families. The relative amount of linoleic acid (18:2n-6) did not change during pregnancy, whereas the relative amount of arachidonic acid (20:4n-6) decreased. Despite maternal mobilization of docosahexaenoic acid (22:6n-3, DHA), suggested by a temporary increase in the DHA status until 18 weeks gestation, the DHA status steadily declined thereafter. This pattern was associated with a progressive increase in the DHA deficiency index in maternal blood throughout pregnancy and resulted in a sub-optimal neonatal DHA status. The overall maternal EFA status also declined steadily during pregnancy. Therefore, the question arises whether the mother, under the prevailing dietary conditions, is able to meet the high fetal requirement for EFA.

Essential fatty acids: Docosahexaenoic acid: Pregnancy: Human

Essential fatty acids (EFA) and their longer chain, more unsaturated derivatives play a major role during pregnancy. They provide the precursors for prostaglandins and leukotrienes and they are important constituents of all cell membranes, especially those in the brain and in the nervous and vascular systems. During the third trimester of pregnancy, rapid synthesis of brain tissue occurs (Dobbing, 1972) and the quantitative accretion in the human brain of the long-chain polyunsaturated fatty acids arachidonic acid (20:4n-6, AA) and docosahexaenoic acid (22:6n-3, DHA) increases steadily with gestation (Clandinin *et al.* 1980; Martinez, 1991). DHA plays an important role in the visual process and the amount of DHA in the human retina also increases with maturation (Martinez *et al.* 1988). Evidence is growing that DHA is essential for normal development of brain and retina (Neuringer *et al.* 1986; Uauy *et al.* 1992; Makrides *et al.* 1993).

Recently it has been suggested that fish oil, rich in fatty acids of the *n*-3 family, might reduce the occurrence of obstetric complications such as preterm delivery (Olsen *et al.* 1992) and pregnancy-induced hypertension (Dyerberg & Bang, 1985; Andersen *et al.* 1989;

Secher & Olsen, 1990; Baker & Broughton-Pipkin, 1991; Popeski et al. 1991). Other fatty acids also seem to be important in pregnancy; decreased maternal levels of linoleic acid (18:2n-6, LA) have been observed in pregnancy-induced hypertension (Schouw et al. 1991; Wang et al. 1991) and intra-uterine growth retardation (Vilbergsson et al. 1991). Therefore, fatty acids like LA, AA and DHA may be of great importance in the diet of pregnant women. However, most studies that relate pregnancy complications to EFA status are based on retrospective case–control designs, using material collected in late pregnancy or postpartum. Therefore, these studies do not allow any conclusion with respect to a possible cause-and-effect relationship. Such a conclusion would require prospective longitudinal monitoring of the maternal EFA status and comparison of the maternal EFA courses during normal and complicated pregnancies. Hardly any information is presently available with respect to the course of the maternal EFA status during uncomplicated pregnancy. Therefore, we performed a longitudinal study to investigate the EFA status of women during normal pregnancy. In addition, the relationship between maternal and neonatal EFA status was investigated.

SUBJECTS, MATERIALS AND METHODS
Study design

At their first antenatal medical visit, pregnant women were asked to participate in the study. The selection criteria for entering the study were a gestational age less than 16 weeks, singleton pregnancy, Caucasian race, diastolic blood pressure below 90 mmHg, and the absence of any metabolic, cardiovascular, neurological or renal disorder. In total, 140 women entered the study. Excluded were women who developed pregnancy-induced hypertension (n 11) or gestational diabetes (n 2), women whose babies were delivered before 37 weeks (n 8) or after 42 weeks (n 5) gestation, and women whose babies were not of an appropriate size for gestational age ($<$ percentile 2·3 (n 3) and $>$ percentile 97·7 (n 1), based on the Dutch intrauterine growth curves, making allowance for sex and parity (Kloosterman, 1970)). Finally, 110 women with an uncomplicated pregnancy met the inclusion criteria. Their relevant clinical information is summarized in Table 1. Maternal venous blood samples were collected into EDTA-tubes at 10 weeks gestation (n 45), at 14 weeks (n 98), at 18 weeks (n 101), at 22 weeks (n 97), at 26 weeks (n 98), at 30 weeks (n 105), at 32 weeks (n 103), at 34 weeks (n 100), at 36 weeks (n 101), at 38 weeks (n 86) and at 40 weeks (n 39). Immediately after delivery a blood sample from the umbilical vein (n 98) and a maternal venous blood sample (n 95) were also collected. At least 6 months postpartum, forty-three women gave a blood sample once more. Of these forty-three women, only four were still breastfeeding their child at the time of blood sampling. The protocol was approved by the Ethics Committee of the University of Limburg and written consent was obtained from each participant.

Biochemical analyses

Plasma was separated from erythrocytes by centrifugation and stored at $-80°$ until fatty acid analysis. All plasma samples of a given subject were analysed simultaneously. To check whether the fatty acid profiles changed with different lengths of storage time at $-80°$, Pearson correlation coefficients were calculated between the relative amounts of fatty acid in maternal or umbilical plasma phospholipid and storage time at $-80°$. Since no important significant correlations were observed, an effect of storage time on the fatty acid profile can be excluded.

Fatty acid composition analysis of the phospholipid fraction was performed using 100 μl plasma samples. A total lipid extract was prepared using a modified Folch extraction

Table 1. *Characteristics of the study population* (n *110*)

	n	Mean	Range
Maternal age (years)		29·5	19–43
Maternal weight at entry (kg)*		63·9	43–120
Maternal height (m)*		1·66	1·52–1·80
Parity at entry 0	47		
1	46		
2	14		
3	3		
Gestational age at delivery (weeks)		40·4	37–42
Birthweight (g)		3403	2130–4410
Apgar score† (5 min)		10	6–10
Sex			
Male	62		
Female	48		

* *n* 108.

† The Apgar score judges the condition of a child shortly after birth and varies between 0 and 10. It is calculated by giving 0, 1 or 2 points for each of the five following items: heart rate, respiration, muscle tone, reaction to reflexes and colour of skin. An Apgar score, after 5 min, of < 4 means a poor prognosis, between 4 and 7 an intermediate prognosis and ≥ 7 an excellent prognosis.

(Folch *et al.* 1957; Hoving *et al.* 1988). Before lipid extraction, 50 μl internal standard (IS) solution was added. The IS solution consisted of 62 mg 1,2,dinonadecanoyl-*sn*-glycero-3-phosphocholine (PC(19:0)$_2$), 40 mg 10-heptadecenoic acid (17:1) to check carry-over of free fatty acids during the phospholipid separation procedure (see below), and 100 mg butylated hydroxytoluene dissolved in 100 ml methanol. Aminopropyl bonded silica columns (500 mg) were used to separate phospholipids from the total lipid extract (Kaluzny *et al.* 1985). The phospholipids were saponified and the fatty acids transmethylated to the corresponding fatty acid methyl esters (FAME) by reaction with BF$_3$ in methanol (140 g/l) at 100° for 1 h (Morrisson & Smith, 1964). The FAME were separated and quantified using a HP 5890 II gas chromatograph (Hewlett Packard, Amstelveen, The Netherlands) fitted with a 50 m CP Sil5 CB non-polar capillary column with 0·25 mm i.d. and 0·12 μm film thickness (Chrompack, Middelburg, The Netherlands) and a split ratio of 1:40. The injection temperature was 250° and the detector temperature 300°. The starting temperature of the column was 160°. After 2 min the temperature increased to 220° at a rate of 2°/min and finally to 280° at a rate of 6°/min. The flow rate of the carrier gas (N$_2$) was 35 ml/min. A standard FAME mixture was used to identify the FAME by means of the retention times. A control pooled plasma sample was analysed as part of every run to check the quality of the methods. The coefficient of variation of the amounts (mmol/l) measured for the main fatty acids of interest varied between 2·1 and 3·7% (de Jong *et al.* 1993).

Quantification of FAME was based on the amount of 19:0 internal standard FAME recovered. The results were expressed in absolute amounts (mg/l plasma) as well as in relative amounts (% total fatty acids).

EFA status variables

Two indices were calculated to describe the EFA status of the mother and her newborn child. The DHA deficiency index (DHADI; Al *et al.* 1990; Hornstra *et al.* 1992), defined as the ratio between 22:5*n*-6 and 22:4*n*-6, was used because a deficit of 22:6*n*-3 is

accompanied by an increase in the conversion of 22:4n-6 to 22:5n-6, resulting in higher DHADI values (Neuringer et al. 1986). The other index was the EFA status (Hornstra et al. 1992), defined as the ratio between the sum of the n-3 and n-6 fatty acids and the sum of the n-7 and n-9 fatty acids. In general, if the supply of n-3 and n-6 fatty acids is sufficient, the desaturation of oleic acid to the long-chain polyunsaturated fatty acids of the n-9 family is limited, leading to higher EFA status values. The EFA status was used instead of the classic triene:tetraene ratio (an indicator of EFA deficiency; Holman, 1960), because this ratio does not account for changes in Δ-5-desaturase activity (Hornstra, 1992).

Statistical analyses

During the gestational period (10–40 weeks), not all blood samples were taken at exactly the intended days, for practical reasons. Since we wanted to analyse values at specified gestational ages, the values of the fatty acid amounts and percentages at those days were estimated by linear interpolation between the days when blood had been sampled. For blood samples taken within 1 week before or after the intended sampling day, interpolation was considered unnecessary. Because of remaining missing values, means were estimated with adjustment for missing values using the repeated measures analysis program BMDP 5V (Jennrich & Schluchter, 1986; Dixon, 1990). This program adjusts the means using the correlations between measurement times.

Using a model with a quadratic dependence of the fatty acid variable on time, we tested whether the temporal trend was possibly increasing or decreasing and whether there was an upward or downward curve to the trend. Data from blood samples at 10 weeks gestation, immediately postpartum (PP), 6 months after delivery (AD), and from the umbilical vein (UV) were analysed in a similar model. The values from weeks 22–32 were included in this analysis to take full advantage of the missing values adjustment. Comparisons of PP, AD, UV, and week 10 were done by the Wald test (Dixon, 1990).

Pearson correlations were used to correlate maternal with neonatal values at delivery and to relate the variables gestational age, birthweight, head circumference and placental weight and size with maternal values early in pregnancy (weeks 10, 14 and 18) and maternal and neonatal fatty acid values at delivery. Correlation coefficients were calculated for DHADI and EFA status and for the absolute and relative amounts of total saturated fatty acids (ΣSAFA), total monounsaturated fatty acids (ΣMUFA), total n-3 (Σn-3), total n-6 (Σn-6), 18:2n-6, 20:4n-6 and 22:6n-3. Multiple regression was performed to adjust for the potential confounding variables parity, maternal height, weight and age, smoking habits and educational level of the mother and sex of the neonate for the relationship between gestational age and fatty acid values. When studying the association between size of the newborn, placental weight and placental size and fatty acid levels, gestational age was also included as a potential confounder.

When groups were compared, Student's t test was performed.

RESULTS

Plasma fatty acids of chain lengths ranging from 14 to 24 C atoms were identified; however, only the three major EFA, namely LA (18:2n-6), AA (20:4n-6) and DHA (22:6n-3), Σn-6, Σn-3, ΣSAFA and ΣMUFA and the two indices DHADI and EFA status are discussed in the present paper. The full results are available on request.

The results are presented mostly in Figures, showing the mean amounts (with their standard errors) of fatty acids in maternal venous plasma phospholipids (PL) during gestation, in maternal (M) and umbilical (U) venous plasma PL immediately after delivery, and in maternal venous plasma PL at least 6 months postpartum.

The maternal fatty acid patterns during pregnancy

The average total amount of fatty acid (TF) in maternal venous plasma PL increased significantly ($P < 0.0001$) during pregnancy (Fig. 1), but the rise in TF became less pronounced towards the end of gestation ($P < 0.0001$). The mean amount of TF increased by 51 % from 1238·11 mg/l at week 10 to 1867·84 mg/l at week 40 of gestation. All the fatty acid families, when expressed as mg/l plasma, showed a similar course: the Σn-6 increased by 44 %, the Σn-3 by 41 %, the ΣSAFA by 57 % and the ΣMUFA by 65 % from week 10 to week 40 (results not shown). Among the individual fatty acids LA, AA and DHA (Fig. 1), the highest increment was for DHA (52 %), followed by LA (48 %) and AA (23 %). Six months after delivery, TF in maternal plasma PL had returned to levels comparable with those at the 14th week of pregnancy. The same applied to Σn-6, ΣSAFA, ΣMUFA, LA and AA. The mean absolute amounts of DHA and Σn-3 had declined to levels significantly ($P < 0.01$) lower than those at 10 weeks gestation.

The relative amounts of the fatty acids did show a different pattern compared with the absolute amounts. For ΣSAFA and ΣMUFA in maternal plasma PL a significant ($P < 0.0001$) increase was observed throughout pregnancy (Fig. 2). After a slight increase at the end of the first trimester the Σn-3 decreased significantly ($P < 0.0001$) during pregnancy and levelled off at the end of gestation. The mean relative amounts of Σn-6 steadily diminished ($P < 0.0001$) throughout pregnancy (Fig. 2). This latter observation was mainly due to the maternal AA status which declined from 9·6 % at week 10 to 7·8 % at week 40 (Fig. 3). The maternal DHA status also diminished, but only after a temporary increase until 18 weeks of gestation. In contrast, the maternal LA status did not change throughout pregnancy (Fig. 3).

While 6 months after delivery the Σn-6, ΣMUFA (Fig. 2) and the relative amounts of LA and AA in maternal plasma PL (Fig. 3) had returned to early pregnancy levels, the ΣSAFA levels were comparable with those midway through gestation (Fig. 2). After delivery the maternal DHA status decreased further to 2·8 % 6 months postpartum, which was significantly ($P < 0.0001$) lower than at 10 weeks gestation (Fig. 3). A similar pattern was observed for the Σn-3 (Fig. 2) which declined to 4·5 % 6 months postpartum.

The DHADI increased significantly ($P < 0.0001$) throughout gestation (Fig. 4) and levelled off towards the end of pregnancy ($P < 0.0001$). Six months after delivery the DHADI was equal to the DHADI at 10 weeks gestation. The EFA status declined continuously throughout the duration of pregnancy. Six months after birth the maternal EFA status had returned to early pregnancy levels.

Comparison between maternal and neonatal fatty acid values

The mean amount of TF in umbilical plasma PL was substantially lower ($P < 0.0001$) than all maternal values (Fig. 1). This was observed for all four fatty acid families: for ΣSAFA and ΣMUFA the mean amount (mg/l) in umbilical plasma PL was only about 35 % that in maternal plasma PL at delivery; for Σn-6 and Σn-3 these proportions were 32 and 47 % respectively (results not shown). The mean amount of LA (mg/l) in umbilical plasma PL (Fig. 1) was only 13 % of the maternal value at delivery, while for AA and DHA the percentages were 68 and 56 % respectively ($P < 0.0001$ for all three fatty acids). The neonatal value for DHA was not significantly different from the maternal level 6 months after delivery (Fig. 1).

In contrast to the absolute amounts of AA and DHA, the mean relative amounts of AA and DHA in umbilical plasma PL were significantly ($P < 0.0001$) higher than all maternal values (Fig. 3). However, the mean relative amount of LA in umbilical venous plasma PL was significantly ($P < 0.0001$) lower than that of the mother (Fig. 3). This resulted in a
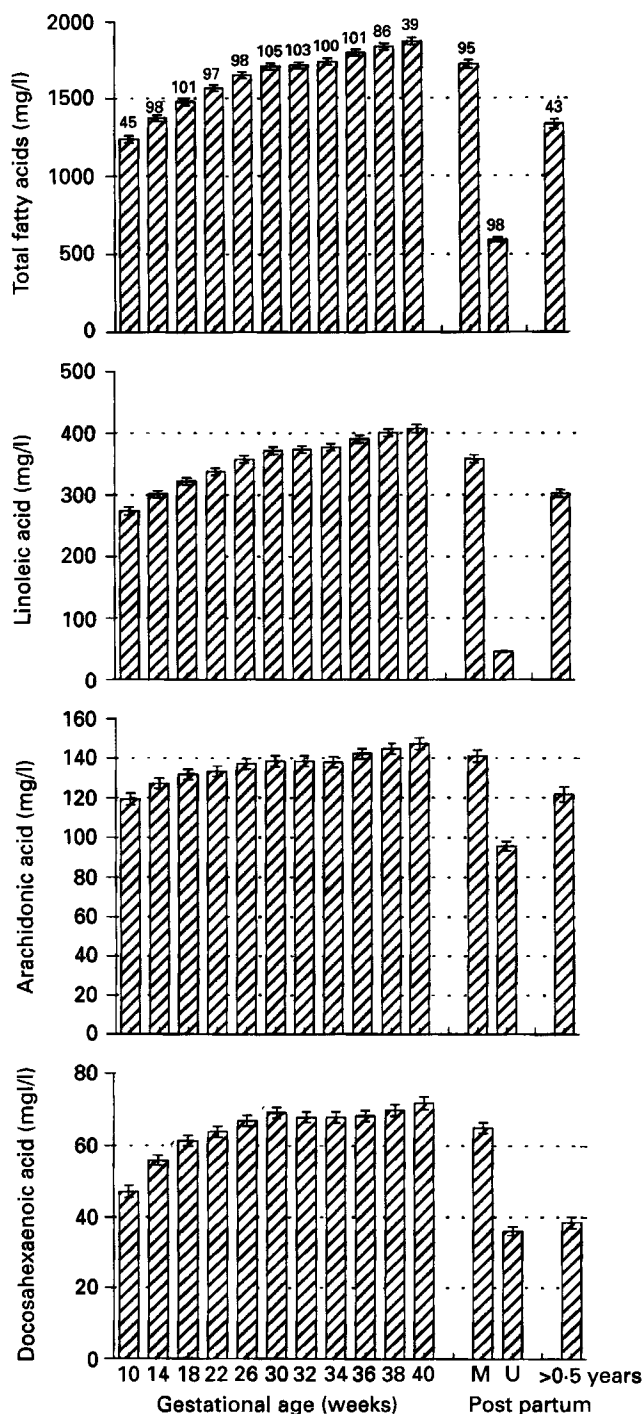
Fig. 1. Absolute fatty acid composition (mg/l) of phospholipids from maternal venous plasma throughout gestation, maternal (M) and umbilical (U) venous plasma at delivery, and maternal venous plasma 6 months after delivery (> 0·5 years). Values are means with their standard errors represented by vertical bars. Results are for 110 subjects after correction for missing values; the number of subjects in the original data set is given above each histogram bar.
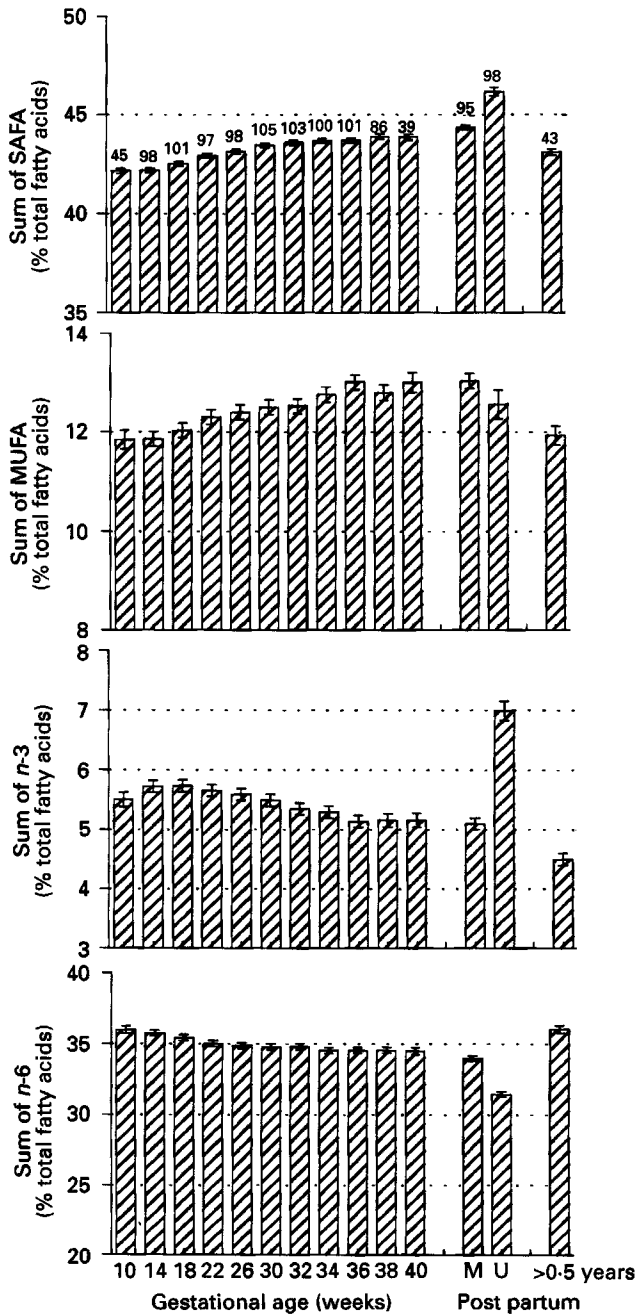
Fig. 2. Relative fatty acid composition (% total fatty acids) of phospholipids from maternal venous plasma throughout gestation, maternal (M) and umbilical (U) venous plasma at delivery, and maternal venous plasma 6 months after delivery (> 0·5 years). Values are means with their standard errors represented by vertical bars. Results are for 110 subjects after correction for missing values; the number of subjects in the original data set is given above each histogram bar. SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; n-3, fatty acids of the n-3 family; n-6, fatty acids of the n-6 family.
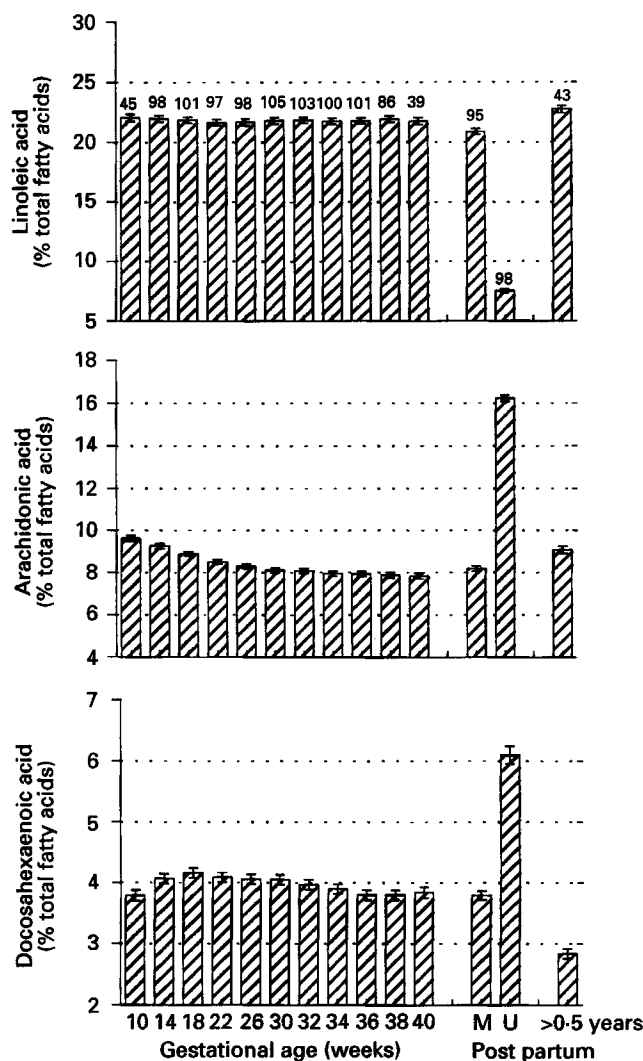
Fig. 3. Relative fatty acid composition (% total fatty acids) of phospholipids from maternal venous plasma throughout gestation, maternal (M) and umbilical (U) venous plasma at delivery, and venous plasma 6 months after delivery (> 0·5 years). Values are means with their standard errors represented by vertical bars. Results are for 110 subjects after correction for missing values; the number of subjects in the original data set is given above each histogram bar.

significantly lower Σn-6 in umbilical plasma PL compared with maternal plasma PL, despite the higher neonatal AA levels (Fig. 2). The amounts of Σn-3 and ΣSAFA in umbilical plasma PL were significantly ($P < 0.0001$) higher than the maternal values. For ΣMUFA no significant difference between mother and child was observed.

The mean DHADI value in umbilical venous plasma PL was significantly ($P < 0.0001$) lower than the maternal DHADI immediately after delivery, but significantly ($P < 0.0001$) higher than the maternal DHADI 6 months postpartum and at 10 weeks gestation (Fig. 4).

The neonatal EFA status did not differ significantly from the maternal value at delivery, but was significantly ($P \leqslant 0.005$) lower than the EFA status in maternal plasma PL 6 months after birth and during early pregnancy (Fig. 4).
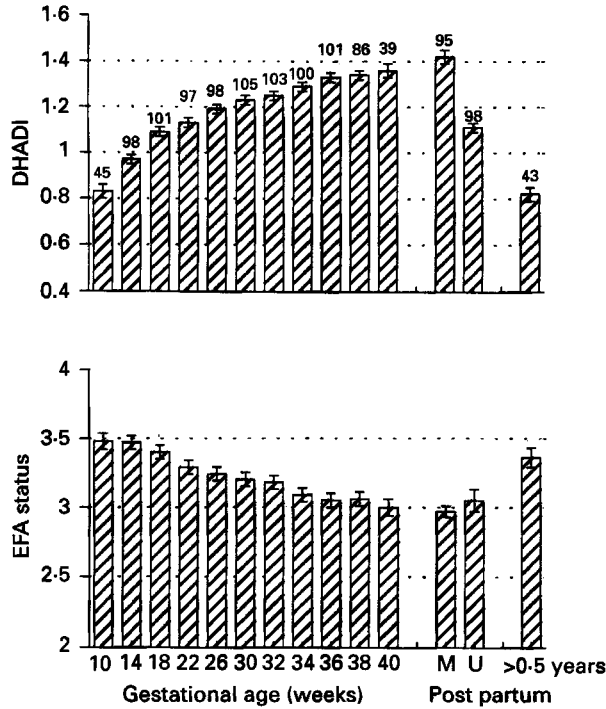
Fig. 4. Essential fatty acid status indices calculated from phospholipids of maternal venous plasma throughout gestation, maternal (M) and umbilical (U) venous plasma at delivery, and maternal venous plasma 6 months after delivery (> 0·5 years). Values are means with their standard errors represented by vertical bars. Results are for 110 subjects after correction for missing values; the number of subjects in the original data set is given above each histogram bar. DHADI, docosahexaenoic acid deficiency index (22:5n-6/22:4n-6); EFA status, essential fatty acid status (Σn-3 + Σn-6)/(Σn-7 + Σn-9).

Table 2. *Pearson correlation coefficients between maternal and umbilical venous plasma phospholipid fatty acids* (n 90)

Fatty acid	r	P
TF (mg/l)	0·04	NS
ΣSAFA (% TF)	0·45	< 0·0001
ΣMUFA (% TF)	0·45	< 0·0001
Σn-6 (% TF)	0·60	< 0·0001
Σn-3 (% TF)	0·60	< 0·0001
18:2n-6 (% TF)	0·52	< 0·0001
20:4n-6 (% TF)	0·56	< 0·0001
22:6n-3 (% TF)	0·60	< 0·0001

TF, sum of total fatty acids; ΣSAFA, sum of saturated fatty acids; ΣMUFA, sum of monounsaturated fatty acids; Σn-6, sum of fatty acids of the n-6 family; Σn-3, sum of fatty acids of the n-3 family.

In Table 2 Pearson correlation coefficients between postpartum maternal and umbilical venous plasma values are shown for TF, and for the relative amounts of ΣSAFA, ΣMUFA, Σn-6, Σn-3, LA, AA and DHA. No significant correlation was observed for TF, but all the other fatty acids and fatty acid combinations did show a highly significant positive correlation.

Relationship of maternal and neonatal fatty acid composition in plasma PL with some clinical variables and smoking habits of the mother

Because of the large number of comparisons made, only those with $P \leqslant 0.01$ are reported For maternal values, a significant positive correlation was only observed between the amount of AA (% TF) in maternal plasma PL at 10 weeks gestation and birth weight ($r +0.39$, $P = 0.007$, n 45). This correlation did not hold for the other time points.

The amount of LA (% TF) in umbilical plasma PL was negatively correlated with gestational age ($r -0.37$, $P = 0.0001$, n 98). The relative, as well as the absolute, amounts of DHA and Σn-3 in umbilical plasma PL correlated positively with gestational age (% TF: $r +0.38$, $P = 0.0001$ for DHA and Σn-3; mg/l: $r +0.42$, $P < 0.0001$ for DHA and $r +0.43$, $P < 0.0001$ for Σn-3). Significant positive correlations were also observed between placental weight and the absolute amounts in umbilical plasma PL for AA ($r +0.27$, $P = 0.007$), DHA ($r +0.29$, $P = 0.005$), Σn-3 ($r +0.29$, $P = 0.005$) and ΣSAFA ($r +0.28$, $P = 0.006$, n 96). These associations did not change when correcting for the potential confounders parity, maternal height, weight and age, smoking habits and educational level of the mother, sex of the neonate and gestational age.

After adjustment for parity, a significant negative correlation was found between the relative amount of LA in umbilical plasma PL and head circumference (partial $r -0.36$, $P = 0.001$). Introducing gestational age in the multiple regression analysis did not alter the above conclusion.

No differences were observed between smokers (n 32) and non-smokers (n 78) for the various fatty acids and fatty acid families in maternal and neonatal plasma PL at delivery. The mean absolute amounts of DHA and Σn-3 in umbilical plasma PL were significantly higher in girls than in boys (DHA: 40 (SE 2.0) *v.* 33 (SE 1.1), $P = 0.009$; Σn-3: 46 (SE 2.3) *v.* 38 (SE 1.5), $P = 0.008$). No significant differences in gestational age were observed between boys and girls.

DISCUSSION

To our knowledge, this is the first report about the EFA composition of maternal plasma PL measured longitudinally throughout normal pregnancy, in an absolute (mg/l) and in a relative (% TF) manner. The decision to use the PL fraction was based on the fact that PL are structural lipids, which are the richest source of polyunsaturated fatty acids (PUFA). Moreover, changes in PUFA profile are most pronounced in PL (Holman, 1986) reflecting the EFA status best.

To correct for the unavoidable missing values, an analysis model was chosen which estimated the means with adjustment for the missing data. The uncorrected data, however, showed a similar course to that of the corrected data. Moreover, the correction factor was never more than 2.5%.

The increases in absolute amounts of fatty acids in maternal plasma PL throughout normal pregnancy (Fig. 1) were a direct consequence of the enhanced PL concentration during gestation (Desoye *et al.* 1987). The increase in TF during pregnancy could not be calculated, because prepregnancy levels were not available. As an approximation, maternal blood samples, taken 6 months after delivery, were analysed. In these samples the amounts of most of the fatty acids were comparable with their values at the end of the first trimester. Eighteen women provided information at 8 weeks gestation and the fatty acid levels in the plasma PL of these samples were lower than at 10 weeks. This strongly indicates that the absolute maternal fatty acid levels measured 6 months after delivery do not yet reflect true prepregnancy levels which are still appreciably lower.

During pregnancy the increment observed for DHA in maternal plasma PL was the highest for all PUFA, and compared with prepregnancy levels (which are most probably

lower than the levels 6 months after delivery, see above) the absolute amounts doubled during gestation. This probably reflects the special requirement for DHA by the developing brain and retina (Clandinin *et al.* 1980; Martinez, 1989; Uauy *et al.* 1992; Makrides *et al.* 1993).

In theory, the improved absolute maternal DHA status could result from a change in dietary habits, an increase in fish consumption in particular. However, a dietary study in which 176 Dutch pregnant women had to fill out a food-frequency questionnaire before 13, at 22 and at 32 weeks of pregnancy showed no significant changes in the amount and type of fat consumed by these women throughout gestation (Al, 1994). Another explanation could be an increase in the activity of the enzymes involved in the synthesis of DHA from its precursors. However, this process occurs at a very low rate only (Voss *et al.* 1992) and, therefore, it seems more likely that the improved maternal DHA status during pregnancy is caused by an increased mobilization from maternal stores, although a metabolic re-routing (from energy substrate to structural use) cannot be excluded.

When expressed in relative terms, the maternal DHA increase lasted until 18 weeks gestation. Thereafter a gradual decrease was seen, which may reflect the maternal inability to mobilize adequate amounts for optimal fetal development. These changes in the maternal DHA status during pregnancy may be normal physiological phenomena. However, the continuous increase of the DHADI may point to a progressive deterioration of the maternal DHA status, suggesting that under the prevailing dietary conditions the maternal capacity to meet the high fetal requirement for DHA (Innis, 1991) is working at its limits or may even be inadequate. The significantly higher DHADI values in umbilical plasma compared with the maternal non-pregnant values also indicate that the mother is unable to provide an optimum fetal DHA status. Whether or not the fetus will benefit from a higher maternal DHA status can only be established by controlled dietary intervention studies.

The AA status (% TF) in umbilical venous plasma PL was, like DHA, significantly higher than the maternal relative amount, despite the decrease of the maternal AA status throughout pregnancy. This might point to a high neonatal need for AA which is the most abundant fatty acid of the *n*-6 family in neural tissue and the retina (Svennerholm, 1968; Fliesler & Anderson, 1983; Sastry, 1985).

No changes were observed for the maternal LA status throughout pregnancy. The neonatal relative amounts of LA were almost three times lower than the maternal plasma values. Results from a recent study demonstrated that the low fetal amounts of LA originate early in pregnancy (van Houwelingen *et al.* 1992). In spite of the low LA levels in umbilical venous plasma PL, the neonatal EFA status did not differ from the maternal EFA status at delivery. However, since the neonatal EFA status was significantly lower than the maternal EFA status 6 months postpartum and at 10 weeks gestation, the question arises whether the neonatal EFA status is indeed optimal.

The absolute amounts of the fatty acids in umbilical plasma were significantly lower compared with maternal values, because the total phospholipid concentration was significantly lower in cord blood than in maternal blood at delivery (see Fig. 1). The significantly higher relative amounts of AA and DHA could be due to *de novo* synthesis by the fetus, but since the capacity of the placenta and fetal liver to synthesize the long-chain PUFA (LCP) AA and DHA from their parent EFA LA and α-linolenic acid (18:3*n*-3, α-LA) is very limited (Chambaz *et al.* 1985), other mechanisms must be responsible for the different fatty acid profiles for LA, AA and DHA between maternal and umbilical plasma PL. These could be caused by preferential transport of LCP across the placenta, but selective sequestering of AA and DHA, at the expense of LA, into PL on the fetal side of the placenta (Kuhn & Crawford, 1986) may also be responsible. As a consequence, AA and

DHA are compartmentalized into a lipid fraction that does not recross the placental barrier (Coleman, 1989) and therefore remains available for the developing tissues, like brain and retina. A possible important role in the transport of LCP in the fetal circulation may be reserved for α-fetoprotein (AFP). This protein is mainly synthesized by the fetal liver and the yolk sac. Although the biological role of AFP is not completely elucidated, it binds PUFA, mainly AA and DHA, with high affinity. Because these fatty acids are important components of structural lipids, the transport and cell delivery of LCP could be a major physiological activity of AFP (Subbiah, 1991).

The placenta is, for the greater part, a fetal organ which has a fatty acid composition more similar to that of fetal plasma than of maternal plasma (Chambaz *et al.* 1985; Al *et al.* 1990). This might explain why significant correlations were observed for placental weight with some fetal fatty acid values, but not with maternal fatty acid levels.

A significant positive correlation between DHA and Σn-3 in umbilical plasma PL and gestational age was observed. In a preterm population (n 22) Leaf *et al.* (1992) also observed a significant positive correlation between 22:6n-3 in neonatal plasma choline-phosphoglyceride and gestational age. Negative correlations were demonstrated in the present study for LA (% TF) with gestational age and with head circumference. These associations were not reported by Leaf *et al.* (1992), although they found positive correlations for birth weight and head circumference with the sum of 20:3n-6 + 20:4n-6 and with 22:6n-3. These latter two associations, however, were not observed in our term population, where all infants were born between 37 and 42 weeks gestation, while in the preterm population the range in gestational age was 12 weeks. This wider range in gestational age could partly explain the difference in result. Moreover, it is known that head circumference of the newborn, for instance, also depends on such factors as the head circumferences of the parents, duration of expulsion, the way of delivery and method of recording head circumference. Corrections for these possible confounders were not made.

At the moment, the modification of the fatty acid composition of infant milk formulas to ensure an optimal administration of AA and DHA after birth is under discussion. On the basis of the results described in the present paper it can be concluded that lipid nutrition of high quality during gestation is also important, especially with respect to DHA. The correlations between the maternal and umbilical relative amounts for LA, AA and DHA are strong and suggest that the fetal EFA status can be influenced by the mother.

This study has yielded data both on the course of the maternal EFA profiles throughout normal pregnancy and on the EFA status of umbilical venous plasma and maternal plasma, 6 months after delivery. In addition, the fatty acid composition was determined as mg/l and as %TF. These results will serve as reference values in a further comparison between uncomplicated and complicated pregnancies which is presently being executed.

REFERENCES

Al, M. D. M. (1994). Essential fatty acids, pregnancy, and pregnancy outcome: relationship between mother and child. PhD Thesis, University of Limburg, The Netherlands.

Al, M. D. M., Hornstra, G., van der Schouw, Y. T., Bulstra-Ramakers, M. T. E. W. & Huisjes, H. J. (1990). Biochemical essential fatty acid status of mothers and their neonates after normal pregnancy. *Early Human Development* **24**, 239–248.

Andersen, H. J., Andersen, L. F. & Fuchs, A. R. (1989). Diet, pre-eclampsia and intra-uterine growth retardation. *Lancet* i, 1146.

Baker, P. & Broughton-Pipkin, F. (1991). Fish-oil and pre-eclampsia (letter). *British Journal of Obstetrics and Gynaecology* **98**, 499–500.

Chambaz, J., Ravel, D., Manier, M.-C., Pepin, D., Mulliez, N. & Bereziat, G. (1985). Essential fatty acids interconversion in the human fetal liver. *Biology of the Neonate* **47**, 136–140.

Clandinin, M. T., Chappell, J. E., Leong, S., Heim, T., Swyer, P. R. & Chance, G. W. (1980). Intrauterine fatty acid accretion rates in human brain: implications for fatty acid requirements. *Early Human Development* 4, 121–129.

Coleman, R. A. (1989). The role of the placenta in lipid metabolism and transport. *Seminars in Perinatology* 13, 180–191.

de Jong, A. E. P., van den Berg, T. S., Nijmeijer-Couprie, A., Goedhart, J. P. & Oosting, E. (1993). Long-term aspects of phospholipidic analysis of fatty acid methylesters by use of nonpolar capillary gas chromatography. *American Journal of Clinical Nutrition* 57, Suppl., 813S.

Desoye, G., Schweditsch, M. O., Pfeiffer, K. P., Zecher, R. & Kostner, G. M. (1987). Correlation of hormones with lipid and lipoprotein levels during normal pregnancy and post-partum. *Journal of Clinical Endocrinology and Metabolism* 64, 704–712.

Dixon, W. J. (1990). *BMDP Statistical Software Manual*, vol. 2. Berkeley: University of California Press.

Dobbing, J. (1972). Vulnerable periods of brain development. In *Lipids, Malnutrition and the Developing Brain*, pp. 9–29 [K. Elliott and J. Knight, editors]. Amsterdam: Elsevier.

Dyerberg, J. & Bang, H. O. (1985). Pre-eclampsia and prostaglandins (letter). *Lancet* i, 1267.

Fliesler, S. J. & Anderson, R. E. (1983). Chemistry and metabolism of lipids in the vertebrate retina. *Progress in Lipid Research* 22, 79–131.

Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 226, 497–509.

Holman, R. T. (1960). The ratio of trienoic:tetraenoic acids in tissue lipids as a measure of essential fatty acid requirement. *Journal of Nutrition* 70, 405–410.

Holman, R. T. (1986). Control of poly-unsaturated fatty acids in tissue lipids. *Journal of the American College of Nutrition* 5, 183–211.

Hornstra, G. (1992). Essential fatty acids, pregnancy and pregnancy complications: a round table discussion. In *Essential Fatty Acids and Eicosanoids. Invited Papers from the Third International Congress*, pp. 177–182 [A. Sinclair and R. Gibson, editors]. Champaign, IL: American Oil Chemists Society.

Hornstra, G., Al, M. D. M., Gerrard, J. M. & Simonis, M. M. G. (1992). Essential fatty acid status of neonates born to Inuit mothers: comparison with caucasian neonates and effect of diet. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 45, 125–130.

Hoving, E. B., Jansen, G., Volmer, M., Van Doormaal, J. J. & Muskiet, F. A. J. (1988). Profiling of plasma cholesterol ester and triglyceride fatty acids as their methyl esters by capillary gas chromatography, preceded by a rapid aminopropyl-silica column chromatographic separation of lipid classes. *Journal of Chromatography* 434, 395–409.

Innis, S. M. (1991). Essential fatty acids in growth and development. *Progress in Lipid Research* 30, 39–103.

Jennrich, R. I. & Schluchter, M. D. (1986). Unbalanced repeated-measures models with structured covariance matrices. *Biometrics* 42, 805–820.

Kaluzny, M. A., Duncan, L. A., Merritt, M. V. & Epps, D. E. (1985). Rapid separation of lipid classes in high yield and purity using bonded phase columns. *Journal of Lipid Research* 26, 135–140.

Kloosterman, G. J. (1970). On intrauterine growth. *International Journal of Gynaecology and Obstetrics* 8, 895–912.

Kuhn, D. C. & Crawford, M. (1986). Placental essential fatty acid transport and prostaglandin synthesis. *Progress in Lipid Research* 25, 345–353.

Leaf, A. A., Leighfield, M. J., Costeloe, K. L. & Crawford, M. A. (1992). Long chain polyunsaturated fatty acids and fetal growth. *Early Human Development* 30, 183–191.

Makrides, M., Simmer, K., Goggin, M. & Gibson, R. A. (1993). Erythrocyte docosahexaenoic acid correlates with the visual response of healthy, term infants. *Pediatric Research* 34, 425–427.

Martinez, M. (1989). Dietary poly-unsaturated fatty acids in relation to neural development in humans. *Progress in Lipid Research* 28, 123–133.

Martinez, M. (1991). Developmental profiles of polyunsaturated fatty acids in the brain of normal infants and patients with peroxisomal diseases: severe deficiency of docosahexaenoic acid in Zellweger's and Pseudo-Zellweger's syndromes. *World Review of Nutrition and Dietetics* 66, 87–102.

Martinez, M., Ballabriga, A. & Gil-Gibernau, J. J. (1988). Lipids of the developing human retina. I. Total fatty acids, plasmalogens, and fatty acid composition of ethanolamine and choline phosphoglycerides. *Journal of Neuroscience Research* 20, 484–490.

Morrisson, W. R. & Smith, L. M. (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride–methanol. *Journal of Lipid Research* 5, 600–608.

Neuringer, M., Connor, W. E., Lin, D. S., Barstad, L. & Luck, S. (1986). Biochemical and functional effects of prenatal and postnatal ω-3 fatty acid deficiency on retina and brain in rhesus monkeys. *Proceedings of the National Academy of Sciences USA* 83, 4021–4025.

Olsen, S. F., Dalby-Sørensen, J. D., Secher, N. J., Hedegaard, M., Hendriksen, T. B., Hansen, H. S. & Grant, A. (1992). Randomised controlled trial of effect of fish-oil supplementation on pregnancy duration. *Lancet* 339, 1003–1007.

Popeski, D., Ebbeling, L. R., Brown, P. B., Hornstra, G. & Gerrard, J. M. (1991). Blood pressure during

pregnancy in Canadian Inuit: community differences related to diet. *Canadian Medical Association Journal* **145**, 445–454.

Sastry, P. S. (1985). Lipids of nervous tissue: composition and metabolism. *Progress in Lipid Research* **24**, 69–176.

Secher, N. J. & Olsen, S. F. (1990). Fish-oil and pre-eclampsia (commentaries). *British Journal of Obstetrics and Gynaecology* **97**, 1077–1079.

Subbiah, M. T. R. (1991). Newly recognized lipid carrier proteins in fetal life. *Proceedings of the Society for Experimental Biology and Medicine* **198**, 459–499.

Svennerholm, L. (1968). Distribution and fatty acid composition of phosphoglycerides in normal human brain. *Journal of Lipid Research* **9**, 570–579.

Uauy, R., Birch, E., Birch, D. & Peirano, P. (1992). Visual and brain function measurements in studies of n-3 fatty acid requirements of infants. *Journal of Pediatrics* **120**, S168–S180.

van der Schouw, Y. T., Al, M. D. M., Hornstra, G., Bulstra-Ramakers, M. T. E. W. & Huisjes, H. J. (1991). Fatty acid composition of serum lipids of mothers and their babies after normal and hypertensive pregnancies. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **44**, 247–252.

van Houwelingen, A. C., Puls, J. & Hornstra, G. (1992). Essential fatty acid status during early human development. *Early Human Development* **31**, 97–111.

Vilbergsson, G., Samsioe, G., Wennergren, M. & Karlsson, K. (1991). Essential fatty acids in pregnancies complicated by intrauterine growth retardation. *International Journal of Gynaecology and Obstetrics* **36**, 277–286.

Voss, A., Reinhart, M. & Sprecher, H. (1992). Differences in the interconversion between 20- and 22-carbon (n-3) and (n-6) polyunsaturated fatty acids in rat liver. *Biochimica et Biophysica Acta* **1127**, 33–40.

Wang, Y., Kay, H. H. & Killam, A. P. (1991). Decreased levels of polyunsaturated fatty acids in pre-eclampsia. *American Journal of Obstetrics and Gynecology* **164**, 812–818.