High polyunsaturated fatty acid, thromboxane A_2, and alpha-fetoprotein concentrations at the human feto-maternal interface

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Abstract Polyunsaturated fatty acids (PUFA) like arachidonic (C20:4) and docosahexaenoic (C22:6) acids are essential for harmonious fetal development. This study evaluates, at near term, the distributions of free fatty acids (FFA) and their fetal carrier protein, alpha-fetoprotein (AFP) in the maternal (M) and fetal circulation (umbilical arteries (A) and vein (V)), focusing on the feto-maternal interface where maternal intervillous blood (I) contacts the fetal trophoblast. FFA concentrations in intervillous and maternal blood were similar, while those in umbilical arteries and vein were 2- to 4-fold lower ($P < 0.001$). There were more saturated FFA in umbilical vein (41%) and arteries (44%) blood than in maternal (30%) and intervillous (32%) blood ($P < 0.001$). Monounsaturated FFA predominated ($P < 0.001$) in maternal (43%) blood, but not in intervillous (35%), umbilical vein (33%) and arteries (31%) blood. Di-triunsaturated FFA were similar in intervillous and maternal (25%) blood and lower in umbilical vein and arteries (16%) blood ($P < 0.001$). PUFA were low in maternal (2.5%) blood and higher in intervillous and umbilical vein and arteries (9%, $P < 0.001$); consequently, C20:4 (40 μM) and C22:6 (16 μM) were the most abundant in the intervillous space. The AFP concentrations and AFP lectin-reactive isoforms were similar in intervillous and umbilical vein and arteries blood, but immuno-electrophoresis revealed a particular AFP conformation (less immuno-reactive, more anionic) in the intervillous space, suggesting that AFP is heavily loaded with PUFA at the feto-maternal interface. Prostacyclin derived from C20:4 was similar in all compartments but the thromboxane A_2 concentration was 10-fold higher in intervillous blood than in maternal and umbilical vein and arteries blood. Thus the feto-maternal interface has a specific pattern of cell signalling molecules that might critically influence parturition.—**Benassayag, C., T. M. Mignot, M. Haourigui, C. Civel, J. Hassid, B. Carbonne, E. A. Nunez, and F. Ferre.** High polyunsaturated fatty acid, thromboxane A_2, and alpha-fetoprotein concentrations at the human feto-maternal interface. *J. Lipid Res.* 1997. **38**: 276–286.

Supplementary key words free fatty acids • intervillous blood • pregnancy

Several genetic, immunological, hormonal, and environmental factors play a role in physiological events heralding the successful establishment of pregnancy, harmonious fetal development, and parturition determinism. In humans, the absence of a correlation between the evolution of maternal peripheral plasma parameters such as steroid hormones or other effectors of uterine activity and the onset of labor led to the hypothesis that initiation of parturition might result from paracrine/autocrine local interactions between the different tissues of the feto-maternal interface.

The hemomonochorial human placenta, which represents a vital interface between maternal and fetal tissues, evolves during pregnancy to optimize the feto-maternal exchanges, and its dysfunction can lead to various pathological diseases. At term, the chorial villi bathe in the maternal blood in the intervillous space that is delineated both by fetal epithelium, the syncytiotrophoblast, and by maternal decidual epithelium. Placental transfer takes place between maternal and fetal blood streams predominantly at this point (1).

Among the environmental bioactive factors released at the feto-maternal interface, essential fatty acids are known to play a major role in fetal development and

Abbreviations: PUFA, polyunsaturated fatty acids; C20:4, arachidonic acid; C22:6, docosahexaenoic acid; FFA, free fatty acids; C14:0, myristic acid; C16:0, palmitic acid; C18:0, stearic acid; C16:1, palmitoleic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid; AFP, alpha-fetoprotein; M, maternal blood; A, umbilical arteries blood; V, umbilical vein blood; I, maternal intervillous blood; PGI_2, prostacyclin; TXA_2, thromboxane A_2; TXB_2, thromboxane B_2; 6-keto-$PGF_{1\alpha}$; 6-keto prostaglandin $F_{1\alpha}$; ConA, concanavalin A; LCA, *Lens culinaris* agglutinin.

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pregnancy outcome. Thus, fetal demands for free fatty acid (FFA) are high in the late weeks of pregnancy (2–5). In addition to their role as an energy source or as structural elements of cells, fatty acids, especially of the polyunsaturated class (PUFA), are signalling molecules that play a crucial role in mediation of metabolic and endocrine functions of the materno-feto-placental unit.

PUFA are precursors of cellular signals such as eicosanoids. Moreover, they play a key role as regulators of membrane signal transduction pathways (6). Therefore, they are directly or indirectly involved in control of contractile activity of placental vascular and uterine smooth muscles. Epidemiological studies have shown that changes in PUFA nutrition may increase birth weight by prolonging gestation (7). Recent studies also show that unsaturated FFA, depending on their degree of non-saturation and on their classification (n–3 or n–6 families) have a differential impact on proliferation of uterine stromal cells and vascular smooth muscle cells (8). PUFA also play a role as regulators of key enzymes in steroid metabolism (9) and in the way in which hormones, especially estrogens (10–13), progesterone and glucocorticoids (13–17), act. The suppressive effect of PUFA and their metabolites on T-cell proliferation has been also shown and may contribute somewhat to the successful survival of the fetal allograft by acting as one of the local regulation mechanisms during the afferent, central, and efferent phases of maternal cell-mediated immunity (18, 19).

Although these data point out the considerable impact of essential fatty acids on the multifactorial processes involved in pregnancy outcome, little information is available on the essential fatty acid status of mother and fetus during normal and complicated pregnancies.

The purpose of the present study, carried out on women undergoing elective caesarean section at term, was, first, to evaluate the concentration and distribution of the different FFA classes in peripheral maternal and fetal circulations (umbilical arteries and vein), with special emphasis on the feto-maternal interface (intervillous space), where maternal intervillous blood is in direct contact with fetal trophoblast. Second, as FFA are transported in fetal blood bound to a specific oncofetal protein, the alpha-fetoprotein (AFP) (20–27), we determined the AFP concentration and AFP isoforms in the different compartments of the materno-feto-placental unit. Third, as arachidonic acid is the precursor of different eicosanoids, it will be of particular interest to measure the balance between the two major opposing effectors involved in maintaining a healthy vascular bed, i.e., plasma prostacyclin (PGI_2, vasodilator and potent inhibitor of platelet aggregation) and thromboxane A_2 (TXA_2, vasoconstrictor and promoter of platelet aggregation).

TABLE 1. Clinical characteristics of the study population (26 pregnant women and fetuses)

Variables	Mean ± SD	Min	Max
Gestational age (weeks)	39.2 ± 1.1	37.5	41.5
Parity	2.0 ± 1.0	1	5
Gravidity	2.1 ± 0.9	1	4
Birth weight (g)	3412.7 ± 617.9	2440	4930
Birth height (cm)	49.1 ± 2.6	44	57
Placenta weight (g)	649.3 ± 77.5	508	740
Apgar score (at 5 min)	9.2 ± 0.2	7	10
Sex	13M/13F		
Anesthesia	20 rachi/3 general/3 peridural		

METHODS

Subjects

This study was approved by the Consultative Comittee of the Protection of Persons in Biomedical Research, Paris Cochin (France). Samples were collected during elective caesarean section from 26 women (17 Europeans, 9 Africans) with uncomplicated pregnancies (38–40 weeks pregnancy), in good health, normotensive, and not on medication. **Table 1** shows the clinical characteristics of the study population.

Cord blood samples were taken during delivery before clamping the cord with the placenta still attached to the uterus. Maternal peripheral blood at the time of delivery was taken from the antecubital vein, and the blood in the intervillous space was collected from the basal plate of the placenta. Blood samples were collected into tubes containing ethylene diamine tetraacetic (EDTA) in an ice bath. Plasma was separated from the red blood cells by centrifugation (2000 rpm/20 min at 4°C) within 30 min of the sample being taken, and stored at −80°C.

Free fatty acid extraction and analysis

Unlabeled heptadecanoic acid (C17:0) was added to each plasma sample (0.5 ml) as internal standard. Samples were extracted for 30 min with 5 ml organic solvent (ethyl acetate–cyclohexane, vol/vol) and the aqueous phase was removed by freezing (−20°C) (28). The organic extracts were evaporated to dryness, taken up in 0.9 ml chromatography solvent I (benzene–ethanol 95:5) and loaded on Sephadex LH20 (Pharmacia LKB, Uppsala, Sweden) microcolumns (0.5 × 6 cm). Free fatty acids (FFA) were eluted with 3 ml solvent I. The fractions were evaporated to dryness and methylated in

boron trifluoride–methanol (Merck, Darmstadt, Germany).

The methylated FFA were chromatographed on a Chrompack-Packard Chromatograph, (model 439, Chrompack France) using a capillary column (WCOT Fused Silica CP-sil-8 CB, 25 × 0.32 mm) (28).

The response coefficients and concentrations of FFA were determined with a recording chromatography data processor (Chromatopack CRIAB, Packard model 604). The peak area ratio of each FFA from plasma extracts and heptadecanoic acid (C17:0) was compared to the peak area of each FFA standard and C17:0.

The total FFA concentration was obtained by the sum of all methylated derivatives. Blanks from all buffers and solvents were run in parallel, and no interfering exogenous contaminants were detected.

Fatty acid standards were purchased from Sigma Chemical (St. Louis, MO)

Prostanoid assay

Blood samples were collected in standard tubes containing EDTA plus indomethacin (0.04 M). These were immediately centrifuged for 10 min at 3000 rev/min at 4°C. Plasma was then separated and rapidly stored frozen at −80°C until assayed. The concentrations of the stable metabolites of prostacyclin (PGI2) and thromboxane A2 (TXA2), 6-keto prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and thromboxane B_2 (TXB_2), respectively, were measured using a 125-Iodo immunoassay system with Amerlex-MTM magnetic separation (Kits Amersham International pic, Amersham, UK) according to the manufacturer's recommendations. The detection limit was 31 pg/ml. All plasma samples were run at least in duplicate.

Immunoquantification of human alpha-fetoprotein (AFP) and characterization of the AFP isoforms

Human plasma AFP was quantified by the rocket electroimmunodiffusion technique of Laurell (29) using specific polyclonal anti-human AFP antibodies (DAKO, Denmark). The limit of AFP detection was 0.1 nM.

Crossed-immunoelectrophoresis was performed as previously described (26). AFP-lectin affinity was determined by using affino-immunoelectrophoresis (CAIE). The method involves first-dimension electrophoresis of plasma samples in a gel lane (1.2 mm deep 10 g/l agarose gel, Tris barbital lactic acid electrode buffer, pH 8.6, at 14°C, 8V/cm, 120 min) containing 2.5 g/l of concanavalin A (ConA) or 0.4 g/l of *Lens culinaris* agglutin (LCA) (Sigma, France) (30). The reference samples were run in a parallel lane without additives. In the second dimension, proteins were electrophoresed at right angles to the first separation in the same buffer (14°C water cooling, 2 V/cm, 16 h) in agarose gel (10 × 10 cm) containing 70 g/l α-D-methyl-glucopyranoside and 0.5% polyclonal monospecific human AFP antibody (DAKO). The gels were dried and stained with Coomassie brilliant blue.

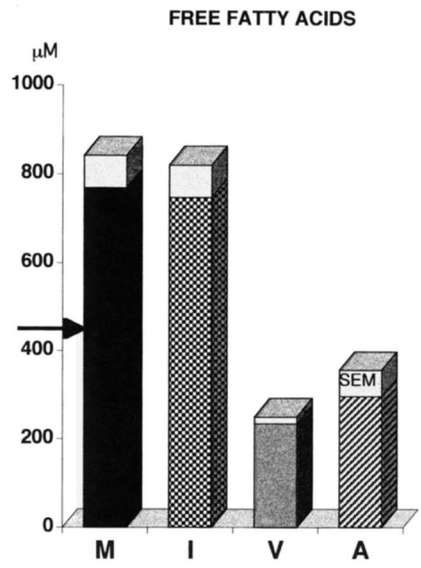

Fig. 1. FFA plasma concentrations in maternal vein (M), intervillous space (I), umbilical vein (V), and umbilical arteries (A) were measured by gas chromatography. Arrow indicates the FFA concentration of plasma from non-pregnant women. Values are means ± SEM from 26 pregnant women at term. ANOVA for repeated measures was used to show a significant compartment effect.

Statistical analysis

Results are expressed as means ± SEM. Data were analyzed by analysis of variance (ANOVA) for repeated measures after verification of their normal distribution. When the F value was significant, Dunnett's *t*-test was used for multiple comparisons of the means.

RESULTS

Free fatty acids concentrations

The FFA concentrations of plasma from pregnant women near term were 2-fold higher than from non-pregnant women (arrow **Fig. 1**). The FFA concentrations of maternal peripheral and intervillous space blood showed no significant differences (Fig. 1), but FFA concentrations in cord blood were significantly lower (3.5-fold, $P < 0.001$) than in maternal (M) or intervillous space (I) blood. The differences between the FFA amounts from umbilical arteries (A) and vein (V) were not significant.

When FFA levels in the umbilical circulation were compared with the maternal level, it was apparent that

TABLE 2. Qualitative and quantitative analysis of free fatty acids in the materno-fetal unit

Free Fatty Acid Class	M	I	V	A
		μM		
Saturated				
C14:0	26 ± 3	23 ± 2.5	12 ± 3	13.8 ± 2.6
C16:0	172 ± 17	168 ± 17	60 ± 5	63.8 ± 6
C18:0	60 ± 4	69 ± 6	27 ± 1.5	29 ± 1.9
Monounsaturated				
C16:1	36 ± 6	31 ± 3.5	10.3 ± 1	13.2 ± 2.4
C18:1	271 ± 24	215 ± 25	61.4 ± 6	65.8 ± 6.7
Di-triunsaturated				
C18:2 + C18:3	161 ± 16	164 ± 17	34.7 ± 2.7	43.2 ± 8.6
Polyunsaturated				
C20:4	12.7 ± 2.1	44.2 ± 5.2	14.8 ± 2.1	14.3 ± 2.4
C22:6	5.8 ± 1.6	19.3 ± 2.8	5.4 ± 1.6	6.6 ± 1.7

The concentrations (μM) of each fatty acid in the maternal vein (M), umbilical vein (V), umbilical arteries (A), and intervillous space (I), measured by gas chromatography, are expressed as means ± SEM from 26 pregnant women at term.

there was a positive correlation between the umbilical vein and the maternal vein ($r = 0.44$, $P < 0.001$) as well as between the umbilical vein and arteries ($r = 0.507$, $p < 0.001$). However, no correlation was found between M and I, nor between I and V or A.

Qualitative analysis of free fatty acids

The absolute concentration of the most familiar fatty acids (**Table 2** and **Fig. 2**) and comparison of the relative percentage of saturated, monounsaturated, and polyunsaturated FFA classes **(Fig. 3)** determined in M, V, A, and I indicated significant differences among the different compartments analyzed.

Saturated free fatty acids. Table 2 shows that palmitic acid (C16:0) was the most representative FFA of the saturated class. Its concentrations were 3-fold higher than those of stearic acid (C18:0) and 5-fold higher than those of myristic acid (C14:0) in all fluids analyzed.

Figure 3 shows that a similar relative percentage of saturated FFA was observed between M (33.8 ± 1.5%) and I (34.8 ± 1.5%). Saturated FFA percentages in umbilical vein and arteries were the same, but they were significantly higher ($P < 0.001$) in V (43.2 ± 1.8%) and A (44.0 ± 1.5%) than in M and I.

Monounsaturated free fatty acids. Oleic acid (C18:1) was the main monounsaturated FFA in all fluids analyzed. Its levels were 6- to 8-fold higher than those of palmitoleic acid (C16:1) (Table 2). Maternal blood presented the highest relative percentage of monounsaturated FFA (42.2 ± 1%) compared to I (32.7 ± 1.2%), V (31.2 ± 1.2%) and A (30.3 ± 1.4%) ($P < 0.001$) (Fig. 3).

Di-triunsaturated free fatty acids. Table 2 shows that the concentrations of linoleic (C18:2) and linolenic acid (C18:3) were the same in M and I, but were 5-fold higher than in V and A ($P < 0.001$). Similar conclusions resulted from comparison of the relative percentages (Fig. 3) of this class of FFA in the different compartments of the materno-feto-placental unit: M (21.9 ± 1.3%) and I (22.4 ± 1.2%) presented a higher relative % of di-tripolyunsaturated FFA than did V (15.6 ± 1%) and A (15.3 ± 1.1%) ($P < 0.001$).

Polyunsaturated free fatty acids (PUFA). Table 2 and Fig. 2 indicate that arachidonic acid (C20:4) and docosahexaenoic acid (C22:6) were present at the same concentration in M, V and A, but were 3- to 4-fold higher ($P < 0.001$) in I than in M, A, and V. Expressed as a relative percentage of FFA (Fig. 3), PUFA were low in

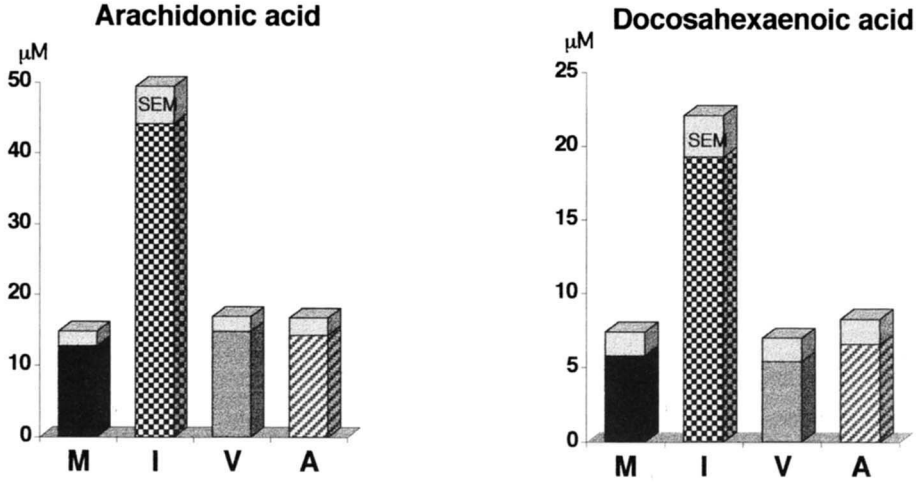

Fig. 2. Plasma polyunsaturated FFA concentrations in maternal vein (M), intervillous space (I), umbilical vein (V), and arteries (A). Values are means ± SEM from 26 pregnant women at term.

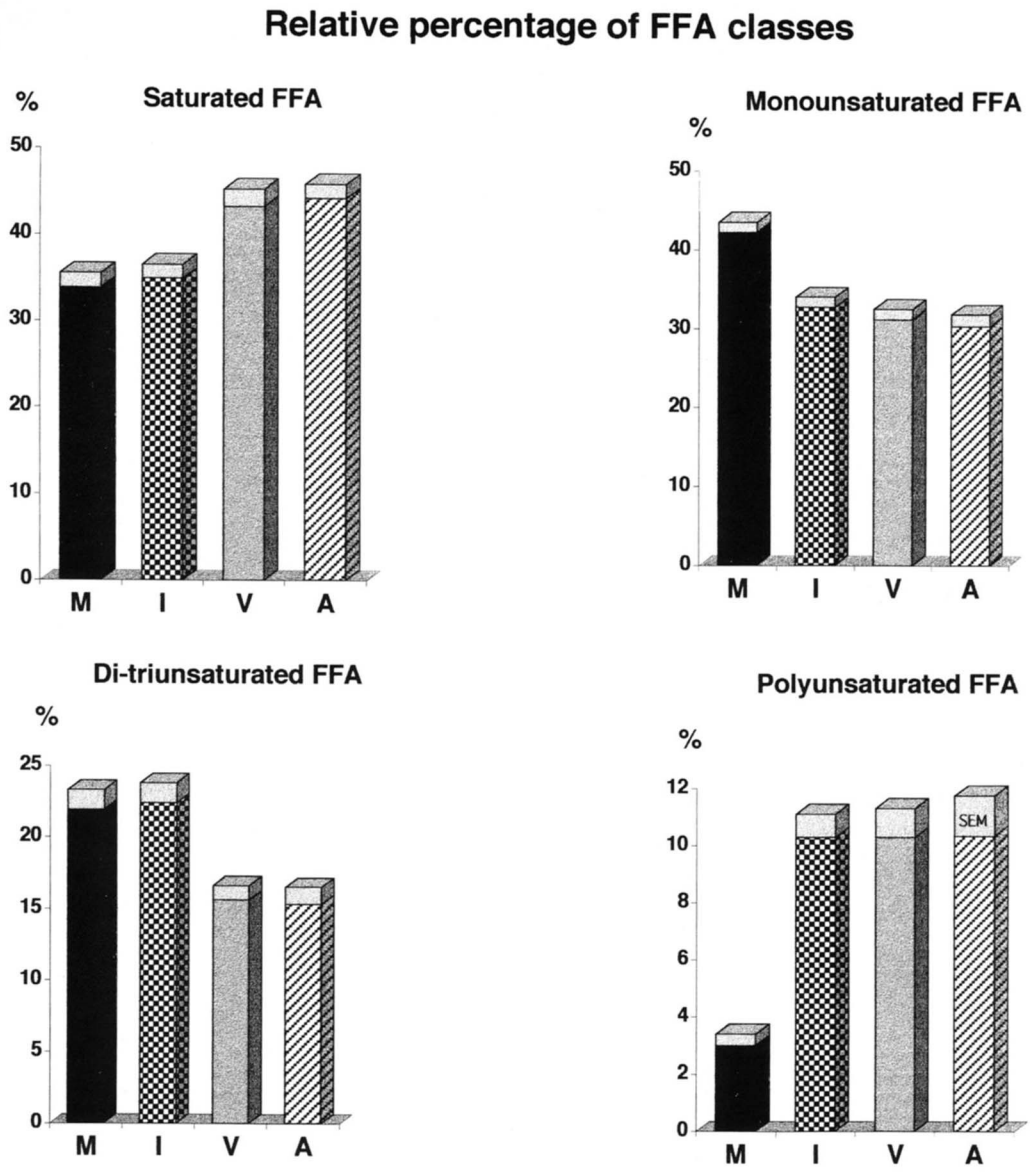

Fig. 3. Relative percentage of the different classes of FFA in the maternal vein (M), intervillous space (I), umbilical vein (V), and arteries (A). Values are means ± SEM from 26 pregnant women at term.

M (3.0 ± 0.4%) but significantly higher (3-fold, $P < 0.001$) in I (10.3 ± 0.8%), A (10.4 ± 1.3%), and V (10.3 ± 1%).

Quantitation of prostacyclin and thromboxane A_2

The plasma concentrations of thromboxane B_2 (TXB_2, the stable metabolite of TXA_2) and 6-keto-$PGF_{1\alpha}$ (the stable metabolite of prostacyclin PGI_2) are shown in **Fig. 4.** There were no significant differences in the concentration of 6-keto-$PGF_{1\alpha}$ among M, V, A, and I. TXB_2 was present at very low concentrations in M, V, and A. By contrast, large amounts of TXB_2 were found in I.

The ratio of TXB_2/6-keto-$PGF_{1\alpha}$, which is an index of the relative activity of the opposing stimuli that modulate vascular tone and platelet aggregation, was markedly elevated in I, i.e., 10-fold higher than in maternal and fetal circulations ($P < 0.001$).

Albumin and alpha-fetoprotein immunoquantification. Characterization of AFP isoforms in cord and intervillous space blood

Albumin concentrations were not significantly different in M (622.2 ± 56.2 μM), I (693.6 ± 66.5 μM), A (775 ± 108.3 μM), and V (776.6 ± 84.4 μM)

Figure 5 indicates that AFP was present at very low

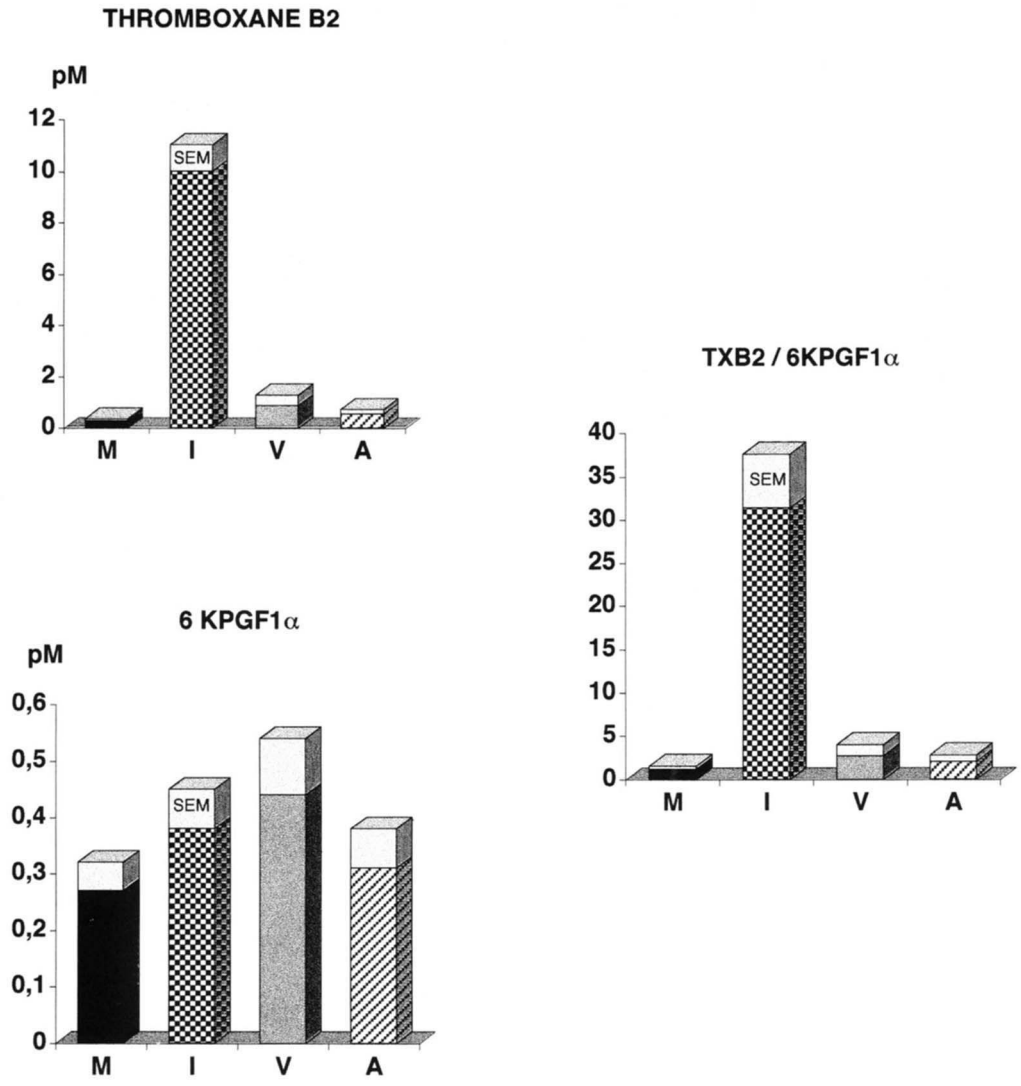

Fig. 4. Means levels (± SEM) of plasma thromboxane B_2 (TXB_2) and 6-keto-prostaglandin $F_{1\alpha}$ ($6KPGF_{1\alpha}$) and their mutual ratio ($TXB_2/6KPGF_{1\alpha}$) in the different compartments of the materno-feto-placental unit.

concentrations in maternal blood (0.1–1 nM). The AFP concentrations were 100- to 500-fold higher in I, V, and A than in M. Although slightly lower, the AFP concentration in I was not significantly different from that detected in V and A. The presence of AFP, which strongly binds PUFA, is correlated with the high percentage of this class of FFA in I, V, and A ($r = 0.33$, $P < 0.001$).

AFP is a glycoprotein. The carbohydrate moiety of AFP enables binding to lectins such as concanavalin A (ConA) and *Lens culinaris* (LCA). Thus, AFP glycoforms can be determined by their differential lectin-binding properties using crossed affino-immuno-electrophoresis. **Figure 6** (upper line) shows the immuno-electrophoretic pattern of AFP from V, A, and I in the absence of lectin. In Fig. 6 (middle line), ConA-AFP complexes (AFP ConA-reactive isoforms) are observed as an immunoprecipitate with clearly retarded mobility. Thus, whatever the AFP source, the fetoprotein was completely reactive with ConA (Fig. 6, middle line) and mainly nonreactive with *Lens* (Fig. 6, lower line); such results are in agreement with previous reports (30).

Identical AFP immunological behaviors were found in the A and V fluids, but the pattern of immunoreactive AFP from I differed. Indeed, the AFP from I migrated slightly faster than the corresponding AFP from A and V, as shown by the superposition of slides in Fig. 6 (upper line I/V). Moreover, this AFP was less immunoreactive towards specific polyclonal anti-human AFP antibodies, and the immunoprecipitating peak was broader and lower, suggesting that the conformational state of

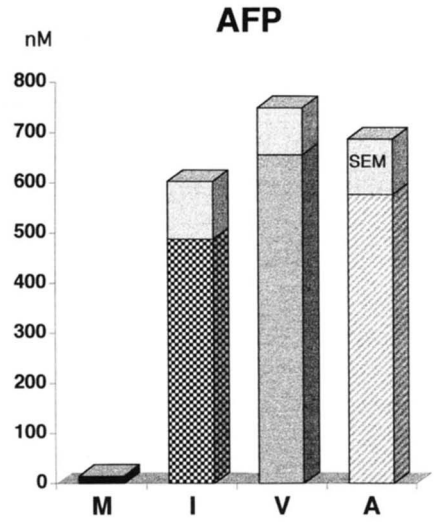

Fig. 5. Immunoquantification of alpha-fetoprotein (AFP) in maternal vein (M), intervillous space (I), umbilical vein (V), and arteries (A). The concentrations of AFP were assessed by rocket immunoelectrophoresis using monospecific human anti-AFP antibodies (0.5%). Values are means ± SEM from 26 pregnant women at term.

AFP from I was different from that of fetal umbilical vein and arteries.

DISCUSSION

Maternal, placental, and fetal lipid metabolic interrelationships represent vital and unique processes that control and determine many aspects of fetal development and parturition determinism. The results presented here provide evidence for major differences at near term in the quantitative and qualitative spectrum of FFA at the materno-fetal interface (intervillous blood) when compared to maternal peripheral circulation and fetal blood (cord blood).

Plasma FFA concentrations in intervillous blood were similar to those measured in maternal peripheral blood, and significantly (2- to 4-fold) higher than in fetal blood ($P < 0.001$). This high concentration of FFA in the intervillous space might reflect nutriment supply to fetal tissues. As previously reported (31, 32), the low plasma cord FFA concentrations might correspond to high FFA requirements of the fetus in the late weeks of pregnancy. In agreement with other authors (31–33), a positive correlation between the FFA levels in umbilical vein and maternal peripheral vein was found ($r = 0.44$, $P < 0.001$). This suggests that, with increasing maternal levels, more FFA appears in cord venous blood from the placenta and more is taken up and used by the fetus.

FFA status specific to maternal and fetal compartments may be observed. Thus, the fetus was characterized by a higher relative amount of saturated FFA (44%) when compared to maternal circulation (34%), which might be expected if the saturated FFA are formed by lipogenesis from glucose or other substrates within the fetal tissues (31). While the relative percentages of monounsaturated and di-triunsaturated FFA in fetal blood were lower ($P < 0.001$) than in maternal peripheral blood, that of the fetal polyunsaturated FFA (PUFA = 10%) was almost 3-fold higher than the maternal levels (3%). Thus, although the FFA concentration was low in the umbilical vein and arteries, arachidonic acid (n–6 C20:4 = 14 μM) and docosahexaenoic acid (n–3 C22:6 = 6 μM) were present at the same concentrations as in the mother (33). Considerable amounts of n–6 and n–3 fatty acids are stored in fetal brain and adipose tissue during the final trimester of gestation. To meet the high fetal need for these fatty acids, it has been suggested that the mother mobilizes these fatty acids from her own stores (34). The lack of Δ5–Δ6 desaturase activities in human placenta microsomes at 18–22 weeks of gestation suggests that fetal C20:4 is either transferred directly from the mother or is synthesized in fetal liver from transferred linoleic acid (3, 5, 35). Moreover, the observation that arterial and venous cord blood contain similar amounts of individual fatty acids suggests that little fatty acid modification occurs in placenta (3, 32).

The intervillous blood corresponding to the materno-fetal interface presents the most striking FFA composition. Indeed, quantitatively as well as qualitatively, the FFA status of the intervillous blood is unlikely to be an average of maternal and fetal FFA, but possesses its own specific characteristics. Thus, the relative percentages of saturated and di-triunsaturated FFA of intervillous blood are similar to those of maternal FFA. However, monounsaturated FFA and PUFA percentages of intervillous blood are very close to those found for fetal plasma; indeed, PUFA are 3-fold higher (10%, $P < 0.001$) than in maternal peripheral plasma. As FFA concentrations in intervillous and maternal peripheral blood are the same, the concentrations of C20:4 and C22:6 in the intervillous chamber (44 and 19 μM) are notably higher than in the maternal vein (13 and 6 μM); moreover, it may be observed that a decrease in the monounsaturated class, especially in oleic acid ($P < 0.001$), counterbalanced this increase in PUFA in the intervillous space.

Such high concentrations of PUFA in the intervillous space raise the question of their origin. The role of the placenta in lipid metabolism and transport is complex. It synthesizes certain lipids and takes up others from the maternal plasma. The major pathway of C20:4 release is by phospholipase A_2 which activity increases at term (33).

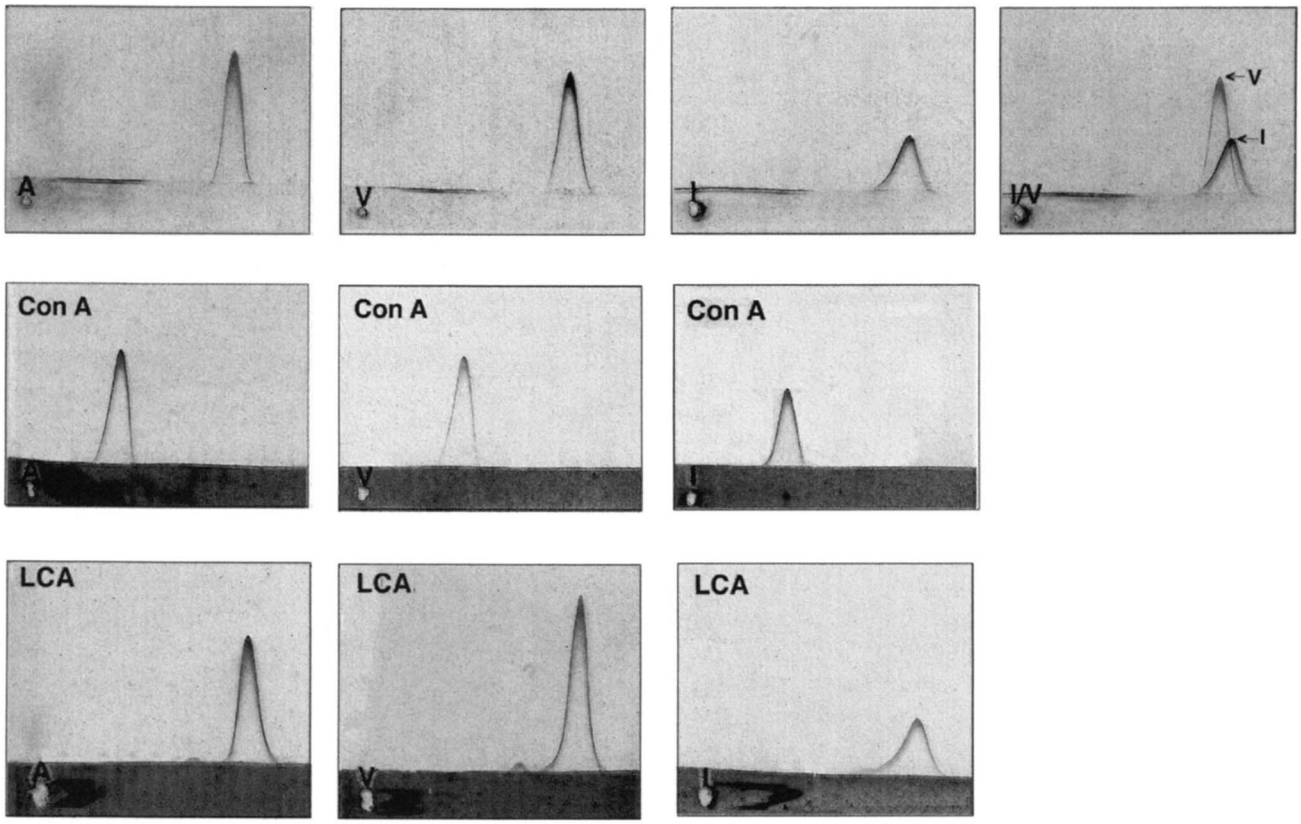

Fig. 6. Crossed affino-immunoelectrophoresis of AFP from umbilical arteries (A), umbilical vein (V), and intervillous space (I) in the absence or presence of lectin. The method involves: first dimension electrophoresis of AFP from V (175 ng), A (185 ng) and I (180 ng) in: -gel 1% agarose in absence of lectin (upper line). -gel 1% agarose in the presence of concanavalin A (ConA) (middle line) -gel 1% agarose in the presence of *Lens culinaris* agglutinin (LCA) (lower line). The second dimension gel contained 1% agarose + 0.5% anti-human AFP antibody (Dako). The gels were dried and stained with Coomassie blue. Two superimposed patterns of human AFP from intervillous space and umbilical arteries bloods are also shown (upper line I/V).

There is increasing evidence that FFA cellular uptake occurs via a process involving a plasma membrane fatty acid binding protein (FABPpm) (35). Fatty acid distribution and uptake into diffusion spaces also appear to be mediated by specific plasma carrier proteins such as albumin and AFP. Several studies show that albumin tends to bind saturated FFA, while AFP binds PUFA to a considerable degree (20, 22–26, 36–38) suggesting that transport and cell delivery of PUFA, especially C20:4 and C22:6, could be the major biological role of AFP.

Our study indicates that a transplacental gradient for AFP exists, as the AFP concentration in intervillous, umbilical vein and artery bloods is 100- to 500-fold higher than in maternal plasma. The AFP is mainly synthesized by the yolk sac, the fetal liver, the gastrointestinal tract and it is generally agreed that the placenta does not produce AFP to any significant degree (39, 40). All these data suggest that the presence of the fetal protein (490 ± 110 nM) detected here in the materno-fetal interface is due to transmembrane passage of this protein from fetal blood through the fetal villi, probably depending on active uptake of the protein by receptor-mediated mechanisms (40, 41).

The study of AFP isoforms by crossed affino-immunoelectrophoresis using lectins such as concanavalin A and *Lens culinaris* did not reveal significant differences in AFP microheterogeneous glycan composition in each compartment; AFP isoforms, were all concanavalin A-reactive and mainly *Lens culinaris*-unreactive. However, the immuno-electrophoretic behavior of AFP in intervillous blood presents special characteristics, AFP from intervillous blood is more anionic than AFP from the fetal vein and arteries and is considerably less immunoreactive towards specific anti-AFP antibodies.

As previously shown in in vitro and in vivo studies performed on rat and human AFP (26, 27), PUFA bound to AFP induced a conformational change in the AFP molecule (AFP holoform), with rearrangement of surface epitopes leading to loss of immunoreactivity of lipidated AFP towards anti-AFP antibodies.

This study emphasized for the first time a special AFP conformation in the intervillous space, which probably

indicates that AFP is highly loaded with PUFA at term. The binding of PUFA to AFP may protect these FFA against catabolism or esterification and could contribute to the uptake and distribution of essential FFA to fetal tissues. Taken together, these results strongly suggest that AFP, which binds polyene fatty acids with high affinity (20–24) may be responsible for the elevated fetal uptake of C20:4 and C22:6 through transplacental exchanges with the mother. Indeed, a good correlation was found between the AFP concentration and the relative percentage of PUFA in the different biological fluids analyzed ($r = 0.32$, $P < 0.001$).

Whatever the analyzed compartment, the amount of FFA was much higher than that of AFP. This may imply accelerated dissociation of FFA upon the interaction of the AFP-fatty acid complex with putative cell receptors, and consequently, enhancement of the concentration of FFA in the vicinity of the cell surface and the amount of FFA cellular uptake (41). This also suggests that FFA are bound by other plasma proteins such as albumin (36) or plasma membrane binding protein (35). The trophoblast could discriminate between fatty acids that had previously been strongly bound to AFP, such as PUFA, and those bound to albumin, such as saturated FFA.

PUFA provides adequate substrates for synthesis of circulating vasoactive factors such as prostaglandins, prostacyclin (PGI_2), and thromboxane A2 (TXA_2). The ratio of TXA_2/PGI_2 is an index of the relative activity of the opposing stimuli that modulate vascular tone and platelet activation (42). This ratio in the plasma of normal pregnant women, in agreement with that of other authors (43), was in the range of 1 to 2. A similar ratio was found in plasma of the umbilical vein and arteries. Such low ratios are compatible with the vasodilated state of maternal and fetal circulation, which progressively increases during pregnancy and is optimal at term. However, a high TXA_2/PGI_2 ratio (10- to 20-fold higher) was observed at the feto-maternal interface in the intervillous blood. This increase was mainly due to higher concentration of TXA_2 than PGI_2 in intervillous blood.

TXA_2 and PGI_2 were both derived from C20:4 through the action of the enzyme cycloxygenase (44). The cause of the imbalance between TXA_2 and PGI_2 in intervillous blood is unknown. The results suggest that TXA_2-type production is promoted in this compartment, while PGI_2 synthesis, which does not seem to be affected, might well be limited by the inhibition of PGI_2 synthetase by high concentrations of hydroperoxide and/or overproduction of lipoxygenase products (42). It is not ruled out that AFP, which has been shown to decrease in vitro PGE_2 synthesis in human placenta (45), may target fatty acid towards one or more specific metabolic pathways. AFP as a carrier of PUFA may also play a role in the preferential distribution of C20:4 to endothelial cells or platelets. But, as C22:6 was increased in intervillous blood, it might be hypothesized that hydroxylated n–3 C22:6 production by lipoxygenase exerts a relatively specific anti-TXA_2 activity, leading to an inhibitory effect on C20:4 or TXA_2-induced vascular contraction and platelet aggregation (44, 46).

Local TXA_2 dominance in intervillous blood could be related to the term of the pregnancy, and could lead to vasospasm and platelet aggregation, causing microthrombosis and vasoconstriction, resulting in separation of the placenta from the uterus. In addition, the trophoblast interacts in a paracrine fashion with the adjacent uterus, thus, the high TXA_2/PGI_2 ratio in intervillous space could be involved in the mechanism initiating myometrial contraction during labor (47). The intervillous space, with its high concentrations of PUFA and TXA_2, might be the compartment responsible for increased production of lipid peroxides and thromboxane in pathological diseases such as preeclampsia and premature labor (42–44, 48).

The higher physiological concentration of unsaturated FFA, at the feto-maternal interface, may have pleiotropic effects. Thus, PUFA may act as endogenous modulators of activities of receptors and enzymes involved in the transduction pathway, of the key enzymes of steroid metabolism, or of the functionality of specific steroid plasma binding proteins (6, 9, 12, 14, 16, 17). FFA could also effect a feedback control of steroid action by modulating the binding of progesterone, glucocorticoids, or estrogens to their specific receptors (10, 11, 13, 15, 17).

Given the known effects of AFP and PUFA on cell growth and immunomodulation (49–53) and the effect of TXA_2 on platelet aggregation, it is possible that these factors, via subtle change of their respective concentrations, play a key role at the feto-maternal interface in immune interactions enabling survival of the fetal allograft, but also in initiation of parturition and finally the expulsion of the neonate.

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