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Composition and permeability of syncytiotrophoblast plasma membranes in pregnancies complicated by intrauterine growth restriction

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Abstract

The objective of this study was to determine placental membrane permeabilities to water, urea and mannitol in intrauterine growth restriction (IUGR) and compare them to normal gestational age matched controls. Further, we wished to investigate whether potential changes in permeability were related to changes in membrane fluidity, cholesterol or phospholipid fatty acid content of the membranes. Syncytiotrophoblast microvillous (MVM) and basal membranes (BM) were isolated from normal and IUGR placentas at term. Passive permeability to water, urea, and mannitol showed no significant alterations in IUGR compared to controls. Cholesterol content in BM, but not in MVM, was lower in placentas from pregnancies complicated by IUGR. However, membrane fluidity did not change in these pregnancies. The phospholipid fatty acid composition of the plasma membranes isolated from all placentas showed a predominance of unsaturated fatty acid species in the BM and saturated species in the MVM. In the MVM from IUGR, mead acid (20:3), behenic acid (22:0) and nervonic acid (24:1) constituted higher percentages of the total when compared to normally grown controls. In the BM from IUGR, mead acid (20:3) was increased relative to the total phospholipid fatty acid content. In conclusion, the syncytiotrophoblast membranes exhibit only minor changes in passive permeability and composition when the pregnancy is complicated by IUGR.

Keywords: Placenta; Membrane; Permeability; Human; Essential fatty acid; Intrauterine growth restriction

1. Introduction

Normal fetal growth and development is dependent on sufficient transport of nutrients from the maternal to fetal circulation. The syncytiotrophoblast layer constitutes the primary barrier for transplacental transport in the human. There are two possible pathways for solutes to permeate the syncytiotrophoblast layer: a transcellular route requiring permeation of both the microvillous and basal plasma membranes or through water filled trans-trophoblastic channels. It has been assumed that hydrophilic molecules are transported across the placenta almost exclusively by means of water filled channels [1]. Permeabilities to hydrophilic molecules of the term human placenta have been studied in vivo [2–5]

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and using in vitro perfused tissue [6,7]. Syncytiotrophoblast plasma membranes isolated from term placenta have also been used to define the permeabilities of these plasma membranes to a range of non-electrolyte solutes [8] and water [9]. The data from isolated plasma membranes suggest that a transcellular route may account for a significant portion of the in vivo permeability for small hydrophilic solutes in the human placenta [8].

Alterations in plasma membrane composition affect membrane order, fluidity and lipid-protein interactions. A number of studies have shown that the activity of membrane bound enzymes and passive permeability to small solutes are altered with changes in membrane composition due to dietary influences [10–12]. Likewise, clinical conditions such as cystic fibrosis [13,14], diabetes [15–17], cigarette smoking and alcohol consumption [18] are associated with alterations in membrane fatty acid composition, permeability and enzymatic activity. Altered placental membrane lipid composition in pregnancy would affect transcellular transport but not permeation through water filled channels. Assuming that the transcellular component is a significant proportion of total transport of biologically important substances such as water, glucose and amino acids, then alterations in membrane composition and passive permeability might be involved in a variety of pregnancy complications. Passive permeability characteristics of the human placenta in abnormal pregnancies are largely unknown.

Pregnancies complicated by intrauterine growth restriction (IUGR) are those in which the fetus fails to reach its genetic growth potential. A current theory based on epidemiological data suggests that maternal nutritional state may play an important role in this condition and that adult health may be predetermined by fetal nutritional status in utero [19]. Developing fetuses are dependent on the placenta for transport of essential fatty acids (EFA) and their derivatives, long chain polyunsaturated fatty acids (LCPUFA). These fatty acids are major components of cellular membranes, precursors to eicosanoids and are critical for organogenesis and proper growth. Developmental deficiencies in EFA have been linked to neurological [20,21] and visual disorders [22]. Maternal undernutrition affects the free fatty acid composition of placental homogenates and is correlated with low birth weight [23]. Other studies have shown that an altered placental fatty acid composition may be related to IUGR [24], pre-eclampsia [25] and preterm labor [26]. However, the phospholipid fatty acid composition of the isolated syncytiotrophoblast plasma membranes has not been previously reported. Changes in EGA, LCPUFA or cholesterol content in these membranes would affect the fluidity as well as the passive permeation of important fetal nutrients. We tested the hypothesis that pregnancies complicated by IUGR have an altered syncytiotrophoblast plasma membrane permeability and composition.

2. Materials and methods

2.1. Membrane isolation and characterization

In order to study placental transcellular permeability and determine the lipid composition of the syncytiotrophoblast plasma membranes, we simultaneously isolated microvillous (MVM) and basal (BM) membranes from human placentas obtained from pregnancies complicated by IUGR and from healthy, gestational age matched controls. Human placental tissue was obtained immediately following delivery and processed according to protocols approved by the local ethical committee for Göteborg University and Sahlgrenska University Hospital, Göteborg, Sweden. Selected clinical characteristics for the patient population from which placentas were obtained are given in Table 1. IUGR was defined as a birth weight two standard deviations below the population mean for gestational age and sex. The normal term group included only uncomplicated pregnancies.

MVM and BM were prepared simultaneously from placentas as described previously [27]. Approximately 100 g of villous tissue was dissected away from the chorionic plate, decidua and fetal membranes. The tissue was washed in buffered saline and homogenized (at 4°C, in buffer D: 250 mM sucrose, 10 mM HEPES-Tris pH 7.4, 1.6 μM antipain, 0.7 μM pepstatin A, 0.5 μM aprotinin, 1 mM EDTA). Differential centrifugation steps were conducted (two runs at $10\,000 \times g$ for 15 min followed by $125\,000 \times g$ for 30 min, at 4°C) to remove cellular debris. From the crude membrane fraction the MVM

Table 1
Selected clinical data

	Normal term	IUGR term
n	9	10
Maternal age (years)	33.6 ± 1.5	31.9 ± 1.7
Gestational age (weeks)	38.6 ± 0.4	38.1 ± 0.2
Smokers (*n*)	1	2
Route of delivery		
vaginal	1	4
cesarean	8	6
Placental weight (g)	649 ± 46	405 ± 26*
Birth weight (g)	3474 ± 117	2230 ± 73*

Mean ± S.E.M. *$P < 0.05$, Student's *t*-test.

were separated by Mg^{2+} precipitation and BM were further purified on a discontinuous sucrose step gradient (140 000 × *g* for 60 min at 4°C). A final wash in buffer D and centrifugation (125 000 × *g* for 30 min, at 4°C) of both membrane samples was conducted. Samples were resuspended in buffer D and snap frozen in liquid nitrogen and stored at −80°C until use.

Using alkaline phosphatase activity [28] as a marker for MVM and forskolin (30 μM) stimulated adenylate cyclase activity as a BM marker, this preparation produced plasma membranes with a high yield (~25%) and enrichment (MVM ~20-fold and BM ~12-fold). Enrichment of the membranes isolated from term IUGR placentas was not significantly different from controls. In addition, only minimal contamination by intracellular and non-syncytial plasma membranes could be demonstrated (data not shown). Measurement of vesicular surface area-to-volume ratio (S/V) was carried out using two fluorescent probes, diphenylhexatriene (DPH) and 9-aminoacridine, as reported in detail elsewhere [9]. The fraction of closed vesicles was determined by 2,4,6-trinitrobenzene sulfonic acid quenching [9]. These date are summarized in Table 2.

2.2. Permeability measurements

All permeability measurements were performed at 37°C using a Hi-Tech SF 51 stopped flow apparatus (Salisbury, Wiltshire, UK) with a 50 W quartz halogen lamp in series with a single grating monochromator (excitation 500 ± 5 nm) as a light source. Osmotic water permeability (Pf) was measured by subjecting membrane vesicles loaded with 150 mM raffinose in 5 mM HEPES-Tris pH 7.4 to a 100 mOsm/kg outwardly directed osmotic gradient. The subsequent vesicle shrinkage was monitored (2500 data points per experiment) by measuring the intensity of scattered light and single exponential curves were fitted to the experimental data. Pf was calculated by comparing single exponential time constants to simulated curves as previously described [9]. Permeabilities to urea and mannitol (Ps) were measured using published protocols [29]. Briefly, membrane vesicles were subjected to 100 mOsm/kg inwardly directed solute gradients under isoosmotic conditions. Vesicle swelling, due to water entry following the permeation of solute, was monitored by the decrease in the intensity of scattered light. Using the initial rate of vesicle swelling and the amplitude of swelling in experiments in which an inwardly directed osmotic gradient of 100 mOsm/kg was used, single exponential curves were simulated. Ps was calculated using the single exponential time constant (τ) according to the relation: $Ps = 1/\{(S/V)\tau\}$.

2.3. Membrane fluidity and composition

Membrane fluidity was assessed by steady state DPH anisotropy at 37°C [30]. After hexane extraction the membrane lipid fraction cholesterol content was assayed using *o*-phthalaldehyde [31].

Table 2
Vesicle purity and enrichment

	Normal term MVM	IUGR term MVM	Normal term BM	IUGR term BM
Alkaline phosphatase enrichment	21.5 ± 2.7	20.21 ± 7.9	2.9 ± 0.3	3.8 ± 1.4
Adenylate cyclase enrichment	–	–	13.2 ± 3.1	11.7 ± 3.2
S/V ($cm^{-1} \times 10^5$)	5.4 ± 1.3	5.2 ± 0.9	5.0 ± 0.7	5.0 ± 0.1

Mean ± S.E.M. $n = 6$–10. S/V was determined by diphenylhexatriene and 9-aminoacridine fluorescence using a value for placental MVM obtained by electron microscopy as a reference.

2.4. Determination of fatty acid composition of membrane phospholipids

Lyophilized samples of MVM and BM were extracted three times with chloroform:methanol 2:1 (v/v) containing 0.01% butylated hydroxytoluene (BHT). The lipids were fractionated on a single SEP-PAK aminopropyl cartridge (Waters Corp., Massachusetts, USA) by the method described by Kaluzny et al. [32]. The fraction of phospholipids was transmethylated in methanolic HCl (3 N) at 90°C over 4 h. The fatty acid methyl esters were extracted with *n*-hexane and thereafter washed with water until neutral, dried with $MgSO_4$ and blown to dryness with nitrogen. The fatty acid methyl esters were separated by capillary gas-liquid chromatography in a Hewlett-Packard 6890 gas chromatograph equipped with a 30 m × 0.25 mm SP-2380 column, film thickness 20 mm. Helium at 2.0 ml/min was used as carrier gas and a splitless injector was used. The injector and detector temperatures were 300 and 250°C, respectively. The column oven temperature was programmed from 50°C to 230°C at a heating rate of 20°C/min up to 180°C thereafter 2°C/min. The separation was recorded with HP GC Chem Station software. As internal standard C 21:1 was used and the fatty acid methyl esters identified by comparison with retention times of pure reference substances.

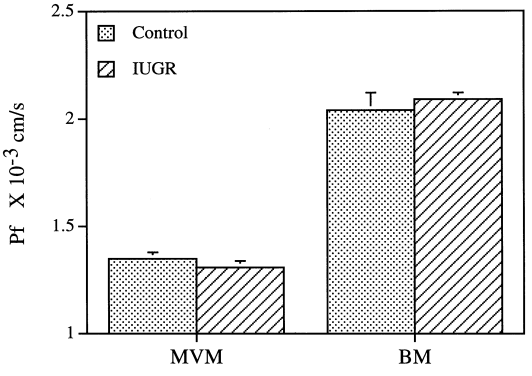

Fig. 1. Water permeability (Pf) of syncytiotrophoblast MVM and BM in IUGR compared to control. Bars represent mean ± S.E.M., $n = 6$ for each group. Pf for MVM was significantly different from BM in both groups. IUGR and controls were not significantly different from each other.

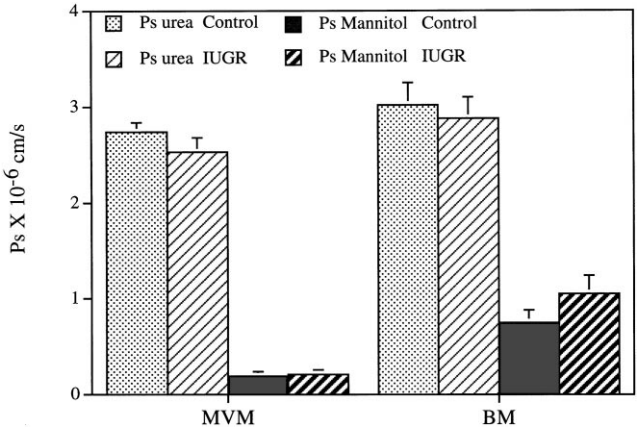

Fig. 2. Syncytiotrophoblast plasma membrane permeability (Ps) to urea and mannitol in IUGR compared to control. Ps urea was not different between MVM and BM in either group. Ps mannitol for MVM was significantly lower than BM in both groups. IUGR and controls were not significantly different from each other. Bars represent mean ± S.E.M., $n = 6$ for each group.

2.5. Statistics

Results are given as mean ± S.E.M. Student's *t*-test or paired *t*-test were used to evaluate data statistically. A P value < 0.05 was considered significant.

2.6. Materials

All materials were purchased from Sigma Chemical Co. unless otherwise stated.

3. Results

3.1. Membrane permeability

Passive permeability to water (Fig. 1) was not altered in MVM or BM from IUGR placentas ($n = 6$) compared to normally grown controls ($n = 6$). BM water permeability was nearly two-fold higher than in MVM as has been previously shown [8]. Urea and mannitol permeability for MVM and BM in control placentas ($n = 6$) and IUGR ($n = 6$) are presented in Fig. 2. No significant differences could be demonstrated in either urea or mannitol permeabilities in membranes isolated from the IUGR placentas compared to control. The general pattern of higher solute

permeability in the BM compared to MVM was consistent with previous data [9].

3.2. Membrane composition and fluidity

Cholesterol content was approximately two-fold higher in MVM as compared to BM (Fig. 3). The MVM from IUGR placentas ($n=4$) were not different from MVM controls ($n=6$). However, BM obtained from the IUGR placentas ($n=4$) contained approximately 30% less cholesterol ($P<0.05$) than normally grown controls ($n=6$).

Steady state DPH anisotropy is inversely related to membrane fluidity. Anisotropy measurements are presented in Fig. 4. Membranes isolated from IUGR placentas showed no alterations in DPH anisotropy.

The proportion (mol %) of 18 phospholipid fatty acid species in MVM and BM are shown in Table 3. The predominant fatty acid in both apical and basal membrane surfaces was palmitic acid (16:0) with 35.9 ± 0.8 mol % in MVM and 31.7 ± 0.3 mol % in BM ($P<0.01$, MVM vs. BM by paired t-test, $n=8$). Arachidonic acid (20:4) was the second most prevalent fatty acid species in both membranes with 18.9 ± 0.7 mol % in MVM and 23.7 ± 0.7 mol % in BM ($P<0.001$ by paired t-test, $n=8$). Comparisons between MVM and BM for each of the 18 fatty acid species showed statistical differences in 12 of the 18 tested. The unsaturated fatty acids predominated in the BM ($53.0\pm0.4\%$) while the saturated forms predominated in the MVM ($55.7\pm0.7\%$).

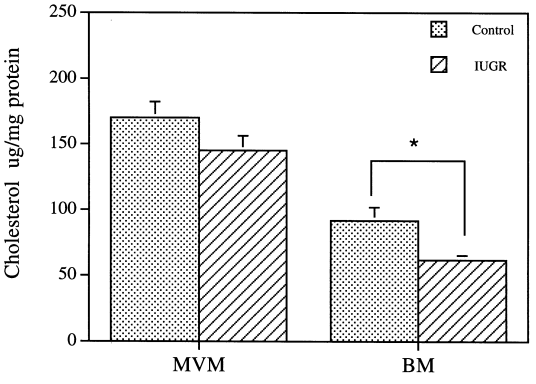

Fig. 3. Cholesterol content of syncytiotrophoblast MVM and BM. MVM was significantly different from BM in both groups. BM IUGR was significantly lower than term control BM. Bars represent mean ± S.E.M., $n=4$ for IUGR group, $n=6$ for control group. *$P<0.05$.

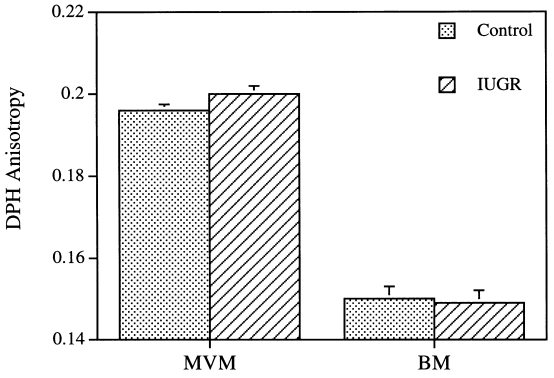

Fig. 4. DPH anisotropy (1/fluidity) in placental MVM and BM. MVM were significantly different from BM in all groups. IUGR and controls were not significantly different from each other. Bars represent mean ± S.E.M., $n=4$ for IUGR group, $n=6$ for control group.

The general patterns described above persisted in the MVM and BM isolated from IUGR pregnancies ($n=5$, Table 3). Several phospholipid fatty acid species showed significant alterations in the IUGR group when compared to the control group. The MVM of IUGR placentas had significantly higher percentages of mead acid (20:3), behenic acid (22:0) and nervonic acid (24:1). The BM of IUGR placentas had a significantly higher percentage of mead acid (20:3).

4. Discussion

Previous studies of syncytiotrophoblast membrane permeability at term [9] comparing MVM and BM suggested that the BM was a more fluid membrane with generally greater passive permeability to water and small solutes and that a lower cholesterol content was one feature of this difference. However, changes in passive permeability of syncytial membrane composition in pregnancies complicated by IUGR have never been addressed. We have recently reported changes in water and solute permeability throughout the course of normal gestation [33]. In order to avoid any confounding factors of gestational alterations we have included only term (37–41 weeks) samples in both the control and IUGR groups.

IUGR at term was not associated with significant

Table 3
Fatty acid composition of phospholipids

FA mol %	Normal term		IUGR	
	MVM $n=8$	BM $n=8$	MVM $n=5$	BM $n=5$
12:0	0.09 ± 0.01	0.09 ± 0.01	0.10 ± 0.0	0.10 ± 0.0
14:0	0.68 ± 0.04	0.56 ± 0.06*	0.72 ± 0.07	0.64 ± 0.05
14:1	0.01 ± 0.01	0.01 ± 0.01	0.04 ± 0.02	0.04 ± 0.02
16:0	35.9 ± 0.8	31.7 ± 0.3**	33.9 ± 0.7	30.7 ± 0.8^{+}
16:1	0.45 ± 0.03	0.63 ± 0.06**	0.58 ± 0.06	0.82 ± 0.07^{+++}
18:0	10.0 ± 0.3	9.4 ± 0.3*	9.4 ± 0.4	9.6 ± 0.3
18:1	8.1 ± 0.2	9.2 ± 0.2***	7.5 ± 0.3	8.8 ± 0.3^{++}
18:2	7.0 ± 0.3	10.1 ± 0.4***	6.0 ± 0.5	9.1 ± 0.5^{+++}
20:0	0.9 ± 0.08	0.3 ± 0.02***	0.9 ± 0.05	0.3 ± 0.02^{+++}
18:3	0.09 ± 0.01	0.10 ± 0.0***	0.10 ± 0.0	0.10 ± 0.0
20:2	0.30 ± 0.02	0.34 ± 0.03	0.26 ± 0.02	0.36 ± 0.02^{+++}
20:3	0.13 ± 0.02	0.11 ± 0.01	0.22 ± 0.02$^{\diamond\diamond}$	0.24 ± 0.02$^{\phi\phi\phi}$
22:0	4.8 ± 0.5	5.9 ± 0.6	7.5 ± 0.5$^{\diamond\diamond}$	5.5 ± 0.3^{++}
20:4	18.9 ± 0.7	23.7 ± 0.7***	18.7 ± 0.8	25.3 ± 1.5^{+++}
24:0	3.7 ± 0.3	0.9 ± 0.06***	3.9 ± 0.5	1.0 ± 0.4^{+++}
24:1	2.6 ± 0.3	1.6 ± 0.1***	3.4 ± 0.2$^{\diamond}$	1.2 ± 0.2^{+++}
22:6	6.5 ± 0.5	5.4 ± 0.4***	6.9 ± 0.4	6.2 ± 0.5^{++}
18:1/18:2	1.2 ± 0.06	0.9 ± 0.03***	1.3 ± 0.07	1.0 ± 0.06^{+++}
20:3/20:4	0.01 ± 0.0	0.01 ± 0.0	0.01 ± 0.0	0.01 ± 0.0

*$P<0.05$, **$P<0.01$, ***$P<0.001$, normal term MVM vs. BM, paired t-test. $^{+}P<0.05$, $^{++}P<0.01$, $^{+++}P<0.001$, IUGR MVM vs. BM, paired t-test. $^{\diamond}P<0.05$, $^{\diamond\diamond}P<0.01$, IUGR MVM vs. normal term MVM, Student's t-test. $^{\phi\phi\phi}P<0.001$, IUGR BM vs. normal term BM, Student's t-test.

alterations in the permeability of syncytiotrophoblast membranes to water or small solutes. In sheep, IUGR did not affect placental urea permeability when normalized to placental DNA, however when normalized to fetal weight a reduction could be demonstrated [34]. This is a result of the often markedly increased fetal weight-placental weight ratio in experimental models of IUGR in sheep, which is not a prominent feature in the human condition. A decreased relative placental exchange area could have consequences for passive permeation across the placenta in IUGR even with preserved permeability characteristics of the transcellular pathway. However, in term IUGR of unknown origin, total trophoblastic surface area normalized to fetal weight is somewhat increased rather than decreased [35]. Thus, our data suggest that transplacental permeation of water and small hydrophilic solutes is unaffected in term IUGR.

An increased membrane fluidity generally results in a higher water and solute permeability of the lipid bilayer [36]. Factors like cholesterol and sphingomyelin content, protein-to-lipid ratio, fatty acyl chain length and degree of unsaturation in the membrane are major determinants of fluidity [37]. Addition of cholesterol to artificial bilayers and biological membranes typically lowers fluidity. The substantially higher cholesterol content and lower fluidity of the MVM as compared to the BM in both groups are in line with findings in other transporting epithelia [37] and are the likely basis for the generally lower permeabilities of the MVM compared to BM. A 30% reduction in the cholesterol content in the BM of the IUGR placentas was observed. This did not, however, result in an altered membrane fluidity.

The physiological functions of EFA and their long chain derivatives (LCPUFA) include their important role as cytoskeletal components as well as their biological mediator role in cell signaling. Many fatty acids are essential nutrients due to the lack of synthetic pathway enzymes in animal cells. The essential nature of these nutrients is magnified during development due to the low rate of fetal synthesis by desaturation and elongation [38]. Therefore a wide range

of fatty acid species must be transported across the placenta. The fatty acids of greatest interest include linoleic acid (18:2), arachidonic acid (20:4), and docosahexaenoic acid (22:6). These are critical components in the development of the brain as well as other organ systems and serve as precursors to prostaglandins, thromboxanes and leukotrienes. Long chain fatty acids are accumulated in the fetus during gestation, with increased transport during the third trimester when fetal demand for LCPUFA is increased. Studies of the mechanisms for transplacental transport of EFA/LCPUFA show that a fatty acid binding protein which preferentially binds LCPUFA is located on the maternal facing MVM of the syncytiotrophoblast [39]. Other fatty acid binding proteins which bind both EFA and LCPUFA are located on both membrane surfaces [40]. Intracellular transport of fatty acids is thought to be mediated by cytoplasmic fatty acid binding proteins of which the placenta has two forms [41].

To our knowledge this is the first study to report the distribution of phospholipid fatty acids in the syncytiotrophoblast membranes which comprise the epithelial barrier to transport across the human placenta. The predominant fatty acid in both membrane surfaces was palmitic acid (16:0) which is consistent with most epithelial plasma membranes [42]. The second most abundant fatty acid was arachidonic acid (20:4), which is an essential nutrient to the fetus. The accumulation of arachidonic acid in fetal plasma during gestation is well documented [43,44] but the accumulation of arachidonic acid in the fetal facing membrane of the syncytiotrophoblast has not been previously reported. Of the 12 fatty acid species which have unique distributions in the trophoblast plasma membranes, the straight chain saturated forms were found in higher proportion in the apical surface while the unsaturated forms were in greater abundance in the basal membrane. This distribution is compatible with the finding that BM have a greater fluidity and passive permeability to water and small solutes than MVM.

The pattern of saturated fatty acids dominating in the MVM and unsaturated forms dominating in the BM was preserved in the syncytial membranes isolated from IUGR pregnancies. This would support the findings in this study showing little or no change in membrane fluidity or passive permeability to water, urea and mannitol. The small increase in palmitoleic acid (16:1) in the IUGR membranes may be an indicator of fatty acid deficiency in maternal plasma in the IUGR pregnancies, since elevated palmitoleic acid (16:1) in plasma is considered an early indicator of fatty acid deficiency. This is further supported by the significant increase in mead acid (20:3) which is also considered an indicator of essential fatty acid deficiency. The isolated syncytiotrophoblast basal membrane showed reductions in linoleic acid (18:2) similar in magnitude to those seen in placental homogenates [24] but the changes were not statistically significant. No changes were found in arachidonic acid (20:4) or docosahexaenoic acid (22:6) in either membrane. The importance of arachidonic acid (20:4) as a substrate for eicosanoid synthesis might explain why the concentration of this acid was preserved despite a decrease in linoleic acid (18:2). The reductions of the important LCPUFA precursors oleic acid (18:1) and linoleic acid (18:2) in BM might indicate a reduced fetal delivery of these essential fatty acids. However, without measurements of maternal and fetal plasma fatty acids we can only speculate that these alterations have limited the supply of essential nutrients to the IUGR fetuses.

A number of studies have shown a relationship between fatty acid status in the mother, fetus or placenta and IUGR [23,24,43,45–47]. In studies of fetal and maternal plasma fatty acid content the data are contradictory. In one study little or no change was seen in the cord blood of IUGR fetuses compared to controls; however, the maternal plasma in these pregnancies was shown to have reduced stearic acid (18:0) [43]. Vilbergsson et al. [47] found alterations in both maternal and fetal plasma fatty acid content in pregnancies complicated by IUGR. In maternal blood there was a reduction in linoleic acid (18:2), arachidonic acid (20:4) and docosahexaenoic acid (22:6) while IUGR fetuses had reduced mead acid (20:3), arachidonic acid (20:4) and docosahexaenoic acid (22:6) and elevated oleic acid (18:1) and linoleic acid (18:2) content compared to control. Leaf et al. [45] demonstrated a relationship between 20:3 and 20:4 and head circumference in both term and preterm infants. The differing results of these studies may in part be due to different types of analysis conducted. The patient population may also vary

between studies since IUGR fetuses can be defined by different criteria.

Studies of the fatty acid composition of umbilical artery and vein walls in IUGR showed mead acid (20:3) content of both vessels was correlated with abdominal circumference and birth weight. Since the umbilical vessel walls are derived from essential fatty acids in the fetal blood, these data suggest the growth restricted fetuses were lacking in these essential nutrients [48].

The data from the present study suggest that changes in membrane fluidity or passive permeability do not contribute to the development of IUGR. Whether the small but significant changes we noted in essential fatty acids might interfere with metabolism and subsequently alter fetal growth in utero remains unclear. In a recent report, a regime of intravenous infusion of essential fatty acids (linoleic, 18:2 and linolenic, 18:3) to pregnant women carrying growth retarded fetuses resulted in significant increases in biparietal diameter and birth weight compared to mothers who received only amino acids and glucose by intravenous supplementation [49]. This suggests that essential fatty acids and their long chain derivatives are involved. The mechanisms by which essential fatty acids alter fetal growth in the pathophysiology of IUGR requires further attention.

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References

[1] H. Schneider, The role of the placenta in nutrition of the human fetus, Am. J. Obstet. Gynecol. 164 (1991) 967–973.
[2] D. Willis, J. O'Grady, J. Faber, K. Thornburg, Diffusion permeability of cyanocobalamin in human placenta, Am. J. Physiol. 250 (1986) R459–R464.
[3] M. Bain, D. Copas, M. Landon, T. Stacey, In vivo permeability of the human placenta to inulin and mannitol, J. Physiol. 399 (1988) 313–319.
[4] K. Thornburg, K. Burry, A. King Adams, E. Kirk, J. Faber, Permeability of placenta to inulin, Am. J. Obstet. Gycecol. 158 (1988) 1165–1169.
[5] M. Bain, D. Copas, A. Taylor, M. Landon, T. Stacey, Permeability of the human placenta in vivo to four non-metabolized hydrophilic molecules, J. Physiol. 431 (1990) 505–513.
[6] H. Schneider, R. Sodha, M. Prögler, M. Young, Permeability of the human placenta for hydrophilic substances studied in the isolated dually in vitro perfused lobe, Contr. Gynecol. Obstet. 13 (1985) 98–103.
[7] N. Illsley, S. Hall, T. Penfold, Diffusional permeability of the human placenta, Contr. Gynecol. Obstet. 13 (1985) 92–97.
[8] T. Jansson, T. Powell, N. Illsley, Non-electrolyte solute permeabilities of human placental microvillous and basal membranes, J. Physiol. 468 (1993) 261–274.
[9] T. Jansson, N.P. Illsley, Osmotic water permeabilities of human placental microvillous and basal membranes, J. Membr. Biol. 132 (1993) 147–155.
[10] S. Alam, B. Alam, Effect of essential fatty acid deficiency on acyl group composition, membrane fluidity and adenylate cylase activity in plasma membranes of rat submandibular salivary glands, J. Nutr. 116 (1986) 1620–1630.
[11] M. Clandinin, S. Cheema, C. Field, M. Garg, J. Venkatraman, T. Clandinin, Dietary fat: exogenous determination of membrane structure and cell function, FASEB J. 5 (1991) 2761–2769.
[12] E. Haeffner, O. Privett, Influences of dietary fatty acids on membrane properties and enzyme activities of liver mitochondria of normal and hypophysectomized rats, Lipids 10 (1975) 75–81.
[13] L. Sigström, B. Strandvik, Erythrocyte sodium-potassium transport in cystic fibrosis, Pediatr. Res. 31 (1992) 425–427.
[14] M. Roulet, P. Frascarolo, I. Rappaz, M. Pilet, Essential fatty acid deficiency in well nourished young cystic fibrosis patients, Eur. J. Pediatr. 156 (1997) 952–956.
[15] M. Keelan, K. Walker, R. Rajotte, T. Clandinin, A. Thomson, Diets alter jejunal morphology and brush border membrane composition in streptozotocin-diabetic rats, Can. J. Physiol. Pharmacol. 65 (1986) 210–218.
[16] V. Salomaa, I. Ahola, J. Tuomilehto, A. Aro, P. Pietinen, H. Korhonen, I. Penttilä, Fatty acid composition of serum cholesterol esters in different degrees of glucose intolerance: a population based study, Metabolism 39 (1990) 1285–1291.
[17] B. Singh, J. Lauzon, J. Venkatraman, A. Thomson, M. Clandinin, Effect of high/low dietary linoleic acid levels on

the function and fatty acid composition of T-lymphocytes of normal and diabetic rats, Diabetes Res. 8 (1988) 129–134.
[18] J. Simon, J. Fong, J. Bernert, W. Browner, Relation of smoking and alcohol consumption to serum fatty acids, Am. J. Epidemiol. 144 (1996) 325–334.
[19] D. Barker, The fetal origins of coronary heart disease, Acta Paediatr. 422 (Suppl.) (1997) 78–82.
[20] P. Wainwright, Do essential fatty acids play a role in brain and behavioral development?, Neurosci. Biobehav. Rev. 16 (1992) 193–205.
[21] F. Cockburn, The minds of our children, in: F. Cockburn (Ed.), Advances in Perinatal Medicine, Parthenon, New York, 1997, pp. 53–60.
[22] M. Suh, A. Wierzbicki, E. Lien, M. Clandinin, Relationship between dietary supply of long chain fatty acids and membrane composition of long and very long chain essential fatty acids in developing fat photoreceptors, Lipids 31 (1996) 61–64.
[23] J. Araya, C. Soto, A. Aquilera, C. Bosco, R. Monlina, Modification of the lipid profile of human placenta by moderate undernutrition, Rev. Med. Chili 122 (1995) 503–509.
[24] P. Percy, G. Vilbergsson, A. Percy, J. Månsson, M. Wennergren, L. Svennerholm, The fatty acid composition of placenta in intrauterine growth retardation, Biochim. Biophys. Acta 1084 (1991) 173–177.
[25] N. Cester, R. Staffolani, R. Rabinin, R. Magnanelli, E. Salvolini, R. Galassi, L. Mazzanti, C. Romanini, Pregnancy induced hypertension: a role for peroxidation in microvillous plasma membranes, Mol. Cell. Biochem. 131 (1994) 151–155.
[26] M. Reece, J. McGregor, K.G. Allen, M. Harris, Maternal and perinatal long-chain fatty acids: possible roles in preterm birth, Am. J. Obstet. Gynecol. 176 (1997) 907–914.
[27] N.P. Illsley, Z.Q. Wang, A. Gray, M.C. Sellers, M.M. Jacobs, Simultaneous preparation of paired, syncytial, microvillous and basal membranes from human placenta, Biochim. Biophys. Acta 1029 (1990) 218–226.
[28] G.N. Bowers, R.B. McComb, A continuous spectrophotometric method for measuring the activity of serum alkaline phosphatase, Clin. Chem. 12 (1966) 70–89.
[29] T. Jansson, M. Wennergren, N.P. Illsley, Glucose transporter protein expression in human placenta throughout gestation and in intrauterine growth retardation, J. Clin. Endocrinol. Metab. 77 (1993) 1554–1562.
[30] N.I. Illsley, H.Y. Lin, A.S. Verkman, Lipid domain structure correlated with membrane protein function in placental microvillous vesicles, Biochemistry 26 (1987) 446–454.
[31] L. Rudel, M. Morris, Determination of cholesterol using *o*-phthalaldehyde, J. Lipid Res. 14 (1973) 364–366.
[32] M. Kaluzny, L. Duncan, M. Merritt, D. Epps, Rapid separation of lipid classes in high yield and purity using bonded phase columns, J. Lipid Res. 26 (1985) 135–140.
[33] T. Jansson, T. Powell, N. Illsley, Gestational development of water and non-electrolyte permeability of human syncytiotrophoblast plasma membranes, Placenta 20 (1999) 155–160.
[34] J. Kulkanek, G. Meschia, E. Makowski, F. Battaglia, Changes in DNA content and urea permeability of the sheep placenta, Am. J. Physiol. 226 (1974) 1257–1263.
[35] F. Teasdale, G. Jean-Jacques, Intrauterine growth retardation: Morphometry of the microvillous membrane of the human placenta, Placenta 9 (1988) 47–55.
[36] B. Cohen, The permeability of liposomes to nonelectrolytes. II. The effect of nystatin and gramicidin A, J. Membr. Biol. 20 (1975) 235–268.
[37] C. Le Grimellec, G. Friedlander, E. El Yandouzi, P. Zlatkine, M. Giocondi, Membrane fluidity and transport properties in epithelia, Kidney Int. 42 (1992) 825–836.
[38] R. Kirum, Fatty acid metabolism in the fetus, Semin. Perinatol. 13 (1989) 202–210.
[39] F. Campbell, M. Gordon, A. Dutta-Roy, Preferential uptake of long chain polyunsaturated fatty acids by isolated human placental membranes, Mol. Cell. Biochem. 155 (1996) 77–83.
[40] F. Campbell, P. Bush, J. Veekamp, A. Dutta-Roy, Detection and cellular localization of plasma membrane associated and cytoplasmic fatty acid binding protein in human placenta, Placenta 19 (1998) 409–415.
[41] T. Das, G. Sa, M. Mukherjea, Characterization of cardiac fatty acid-binding protein from human placenta: Comparison with placental hepatic types, Eur. J. Biochem. 211 (1993) 725–730.
[42] P. Cullis, M. Hope, Physical properties and functional roles of lipids in membranes, in: D. Vance, J. Vance (Eds.), Biochemistry of Lipids, Lipoproteins and Membranes, Vol. 20, Elsevier, Amsterdam,1991, pp. 1–40.
[43] R. Matorras, L. Perteagudo, A. Nieto, P. Sanjurjo, Intrauterine growth retardation and plasma fatty acids in the mother and fetus, Eur. J. Obstet. Gynecol. Reprod. Biol. 57 (1994) 189–193.
[44] T. Berghaus, H. Demmelmair, B. Koletsko, Fatty acid composition of lipid classes in maternal and cord plasma at birth, Eur. J. Pediatr. 157 (1998) 763–768.
[45] A. Leaf, M. Leighfield, K. Costeloe, M. Crawford, Long chain polyunsaturated fatty acids and fetal growth, Early Hum. Dev. 30 (1992) 183–191.
[46] N. Sattar, I. Greer, P. Galloway, C. Packard, J. Shepherd, T. Kelly, A. Mathers, Lipid and lipoprotein concentrations in pregnancies complicated by intrauterine growth retardation, J. Clin. Endocrin. Metab. 84 (1999) 128–130.
[47] G. Vilbergsson, M. Wennergren, G. Samsione, A. Percy, J. Månsson, L. Svennerholm, Essential fatty acid status is altered in pregnancies complicated by intrauterine growth retardation, World Rev. Nutr. Diet 76 (1994) 105–109.
[48] C. Felton, T. Chang, D. Crook, M. Marsh, S. Robson, J. Spencer, Umbilical vessel wall fatty acids after normal and retarded fetal growth, Arch. Dis. Child. 70 (1994) F36–F39.
[49] L. Zhang, The effects of essential fatty acids preparation in the treatment of intrauterine growth retardation, Am. J. Perinatol. 14 (1997) 535–537.