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Triglyceride Hydrolase Activities and Expression of Fatty Acid Binding Proteins in the Human Placenta in Pregnancies Complicated by Intrauterine Growth Restriction and Diabetes

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Triglyceride (TG) hydrolases in the placental microvillous plasma membrane (MVM) release fatty acids from circulating lipoproteins and represent the critical initial step in transplacental fatty acid transfer. We investigated the activity of two TG hydrolases in MVM isolated from placentas of appropriately grown for gestational age pregnancies and pregnancies complicated by intrauterine growth restriction (IUGR), insulin-dependent diabetes mellitus (IDDM) or gestational diabetes mellitus (GDM). In addition, we measured protein expression of lipoprotein lipase (LPL) in MVM and two fatty acid binding proteins (L- and C-FABP) in placental homogenates. The TG hydrolase activities were assessed by measuring hydrolysis of ^{3}H-trioleic acid incorporated into intralipid micelles after incubation with MVM. The placenta-specific TG hydrolase activity (optimum at pH 6) did not differ in the patient groups studied. MVM LPL activity (optimum at pH 8) was reduced by 47% in preterm IUGR (n = 8, $P < 0.05$), compared with gestational age-matched controls. The LPL activity in placentas of IDDM pregnancies was increased by 39% (n = 8, $P < 0.05$), compared with controls. No significant differences were observed in cases of GDM. We found no alteration in protein expression of LPL or C-FABP. The expression of L-FABP was increased by 112% (n = 8, $P < 0.05$) in IDDM and 64% (n = 8, $P < 0.05$) in GDM. These results indicate that alterations in MVM LPL activity and expression of L-FABP may contribute to the altered lipid deposition and metabolism in IUGR and diabetic pregnancies. (*J Clin Endocrinol Metab* 89: 4607–4614, 2004)

ADEQUATE PLACENTAL TRANSPORT of fatty acids (FAs) to the fetus is crucial for normal fetal development and growth due to their importance as cell membrane components, energy sources, and precursors to cellular signaling molecules. The essential fatty acids (EFAs), linoleic acid (LA) and α-linolenic acid (αLA), and their metabolites, the long-chain polyunsaturated fatty acids (LCPUFAs), arachidonic acid (AA) and docosahexanoic acid (DHA), play a particularly important role in fetal development due to the high AA and DHA content of the brain and retina (1, 2). AA is also the major precursor of eicosanoids such as prostaglandins, prostacyclins, leukotrienes, and thromboxanes, which are involved in the control of blood pressure, smooth muscle contraction, and blood clotting. An insufficient supply of LCPUFAs could therefore lead to neural and vascular complications. The placenta lacks the enzymes Δ5 and Δ6 desaturase (3–6) for conversion of EFAs to LCPUFAs, and the fetus has only limited desaturase activity (4). Therefore, the primary source of LCPUFAs available to the fetus is of maternal origin in the form of circulating free fatty acids (FFAs) bound to albumin or as moieties of very low-density lipoproteins (VLDLs) and dietary-derived chylomicrons. FAs from VLDLs and chylomicrons must be released by triglyceride (TG) hydrolases before uptake by the placenta because TG is not transported intact over the placenta (1, 6, 7). These hydrolases are therefore an important initial step in transplacental delivery of FAs to the fetus. The importance of the TG hydrolases is underscored by a study of Bonet *et al.* (8) in which they showed a preference (10-fold higher) in uptake of FAs from TGs, compared with uptake of albumin-bound FFAs by isolated placental trophoblasts.

The transporting epithelium in the human placenta is the syncytiotrophoblast, a syncytial cell that is polarized as other epithelia by having a brush border or microvillous plasma membrane (MVM), which is bathed in maternal blood and a basal plasma membrane facing the fetal capillary. This unique epithelium provides vectorial transport of all nutrients, ions, and water to the fetus. In terms of lipid transport, several key features have been described. Two unique TG hydrolases have been described in the MVM of the syncytiotrophoblast (9). One of these has been confirmed to be lipoprotein lipase (LPL) with an activity optimum at pH 8.0, releasable by heparin, activated by addition of serum, and inhibited by high salt concentrations. The other hydrolase has an activity optimum at pH 6.0, is not released by heparin,

Abbreviations: AA, Arachidonic acid; AGA, appropriately grown for gestational age; C-FABP, cardiac FABP; DHA, docosahexanoic acid; EFA, essential FA; FA, fatty acid; FABP, FA binding protein; FFA, free FA; FHR, fetal heart rate; GDM, gestational diabetes mellitus; IDDM, insulin-dependent diabetes mellitus; IUGR, intrauterine growth restriction; LA, linoleic acid; αLA, α-linolenic acid; LCPUFA, long-chain polyunsaturated FA; L-FABP, liver FABP; LPL, lipoprotein lipase; MVM, microvillous plasma membrane; PI, pulsatility index; TG, triglyceride; VLDL, very low-density lipoprotein.

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and is inhibited by serum and high salt concentrations. This appears to be a placenta-specific hydrolase activity but has not been identified at the molecular level. Once FFAs are released from VLDLs, either they are bound to a placental plasma membrane FA binding protein in the MVM and translocated into the cell or they diffuse freely into the syncytial cell. In the syncytiotrophoblast cytoplasm, two forms of FA binding proteins (FABPs) have been identified; cardiac (C-FABP) and liver (L-FABP) (10). These cytosolic FABPs have been suggested to be responsible for transfer of FAs to the sites of esterification and β-oxidation or directly to the fetal circulation via the placental basal membrane. C-FABP has been suggested to bind only long-chain FAs, whereas L-FABP binds heterogeneous ligands, *i.e.* bile salts and eicosanoids (11). The TGs and phospholipids are stored as droplets in the placental cytosol and intracellular lipolysis by a hormone sensitive lipase is likely to be required before transfer to the fetus. The FFAs are believed to reach the fetal circulation by facilitated diffusion across the basal membrane. In the fetal blood under saturated albumin, which has a capacity to bind additional FFAs to its high affinity binding sites, facilitates diffusion of FFAs to the fetal side. When reaching the fetal liver, FFA is removed from albumin, esterified to TG and phospholipids, and used in production of fetal VLDL.

Intrauterine growth restriction (IUGR) is characterized by the failure of the fetus to reach its genetic growth potential. Several placental transport functions have been shown to be altered in IUGR fetuses such as a decreased capacity to transport nonessential amino acids through system A and essential amino acids through systems β (taurine) and L (leucine), decreased Na^+/ H^+ exchanger activity, and decreased Na^+/K^+ ATPase activity (12–17). Other transport functions, *i.e.* Ca^{2+} ATPase, appear to be up-regulated (18). The opposite end of the growth spectrum is represented by fetal overgrowth, which is common in pregnancies complicated by insulin-dependent diabetes mellitus (IDDM) as well as gestational diabetes mellitus (GDM). Placental transport functions in these pregnancies are also altered with an increased capacity to transport glucose, increased system A and system L and Ca^{2+} ATPase activity, but no change in Na^+ K^+ ATPase (18–22).

The asymmetric IUGR newborn has reduced fat depots, compared with normally grown babies (23). The mechanism behind this is not well understood. Previous studies have shown some alterations in lipid status in the mother, fetus, and placenta in IUGR pregnancies, *i.e.* a decrease in the conversion of LA and αLA into AA and DHA (24, 25), altered phospholipid composition in the umbilical artery and placenta (26–28), and/or abnormal maternal lipoprotein metabolism (29).

In pregnancies complicated by maternal diabetes, placental lipid transfer may be increased due to an increased maternal-fetal concentration gradient, especially of FFAs and TGs (30, 31). This may explain the increase in body fat mass in the newborns of diabetic women (32). In diabetes both TGs and phospholipids are accumulated in the placental unit. This indicates an enhanced uptake, hydrolysis, and reesterification activity (33) in the placenta and could be viewed as a physiological brake to diminish the excessive transfer of FFAs and TGs to the fetus (34). However, the increased placental storage may not be sufficient to prevent excess transfer because higher birth weights seen in diabetes are correlated to the extent of maternal hyperlipoproteinemia (35).

In this study we investigated the activities of the placenta-specific TG-hydrolase and LPL in the placental MVM isolated from pregnancies complicated by IUGR, GDM, and IDDM and compared them with appropriately grown-for-gestational-age (AGA) pregnancies. Protein expression of LPL, L-FABP, and C-FABP was studied by Western blot in cases of IUGR and diabetes.

Materials and Methods

Materials

Monoclonal antibody 5D2 against the C terminal of LPL was kindly provided by Dr. John Brunzell (University of Washington, Seattle). The 5D2 antibody was originally produced against bovine LPL but cross-reacts strongly with human LPL and LPL from other species but not with related lipases (36). The monoclonal L-FABP (Abcam Ltd., Cambridge, UK) cross-reacts with human and rat L-FABP. C-FABP (clone 6B6, Research Diagnostics, Inc., Flanders, NJ) is derived from hybridization of Sp 2/0 myeloma cells with spleen cells of BALB/c mice immunized with human FABP. All chemicals were purchased from Sigma (St. Louis, MO), except for [1-^{14}C] oleic acid and glycerol tri[9,10(n)-^{3}H] oleate (Amersham Pharmacia Biotech, Buckinghamshire, UK), and Intralipid (KABI, Uppsala, Sweden).

Tissue

Human placental tissue collection was approved by the Göteborg University Committee for Research Ethics and conducted with informed consent. Tissue from AGA term placentas (37–41 wk), AGA preterm placentas (25–36 wk), and placentas from pregnancies complicated with IUGR (term and preterm), IDDM, or GDM were obtained immediately after vaginal delivery or cesarean section and placed on ice.

Microvillous plasma membrane preparation

MVM vesicles were prepared according to methods previously described (37) with some modifications. Briefly, the tissue was processed within 30 min of delivery. The chorionic plate and decidua were removed and approximately 100 g villous tissue were cut and washed in buffered saline. After differential centrifugation (at 4 C) steps in buffer D (250 mM sucrose, 10 mM HEPES/Tris, 1.6 μM antipain, 0.7 μM pepstatin A, 0.5 μg/ml aprotinin, 1 mM EDTA), MVM was separated by Mg^{2+} precipitation. MVM vesicles were snap frozen in liquid nitrogen and stored at −80 C. To determine the purity of the membrane vesicles, alkaline phosphatase activity was used as a marker for MVM. All MVM vesicles used in this study had at least a 10-fold enrichment of alkaline phosphatase. Protein concentrations were determined by the Bradford method (38).

Determination of TG hydrolase activities

Activities of TG hydrolases were determined as previously described (9, 39) with some modifications. To incorporate triolein into micelles, 10 μl glycerol tri[9,10(n)-^{3}H] oleate was dried down under nitrogen, 490 μl 10 mM Tris-HCl containing 1% Intralipid was added, and the mixture was sonicated on ice for 30 sec. The hydrolase reaction was started when MVM vesicles (150 μg total protein) were added to each of the two different reaction mixtures, one with pH 6 and the other pH 8. The incubation buffers were based on 0.2 M sodium hydrogen phosphate buffers (pH 6 and 8) containing 500 μg/ml BSA [dissolved in 10 mM Tris-HCl (pH 7.4), 10 μg/μl], 1 mM glycerol tri[9,10(n)-^{3}H] oleate, and 0.5 μl/ml Intralipid. The pH 8 incubation buffer also contained 7.5% fetal calf serum. The samples were incubated in a water bath at 37 C for 30 min. The reaction was discontinued by adding 3.25 ml stop solution containing methanol, chloroform, and heptane [1.40:1.25:1.00 (vol/vol)]

and [1-^{14}C] oleic acid (2400 dpm/ml), serving as an internal standard. One milliliter borate-carbonate (pH 10.5) was added, and the samples were vortexed vigorously for 30 sec and centrifuged at 4 C at 2200 rpm for 20 min. The upper aqueous phase containing labeled oleate was transferred to a scintillation vial for counting. By measuring recovery of the internal standard ([1-^{14}C] oleic acid in the stop solution) the efficiency of extraction could be determined and values adjusted accordingly. In all assays individual standard curves were made to adjust for interassay variations. The activity of the TG hydrolases was expressed as picomoles [9,10(n)-^{3}H] oleate released per minute per milligram protein. Different protein concentrations and time points were tested to optimize the activities for each TG hydrolase.

SDS-PAGE and Western blotting

Western blotting was performed according to a previously published protocol (16). To determine protein expression of LPL, MVM vesicle samples were loaded (20 μg total protein) on a 10% SDS-PAGE. Placental homogenates with rat liver/rat cardiac muscle as positive controls were loaded (40 μg total protein) on a 12% SDS-PAGE to determine expression of C-FABP and L-FABP. The nitrocellulose membranes were incubated with primary antibody [LPL-5D2 (1:250), C-FABP (1:300) and L-FABP (1:100)], washed, and incubated with peroxidase-labeled horse-anti-mouse IgG (1:1000). After repeated washings the immunolabeling was made visible using enhanced chemiluminescence detection (Amersham). The analysis included scanning of films followed by densitometry by means of IP Lab gel software (Signal Analysis Corp., Vienna, VA). To normalize density readings between gels, two samples from term AGA were loaded on each gel. To facilitate comparisons between groups, all densitometry readings were divided by the mean density of the term AGA group. Enzyme densities in samples from placentas complicated by IUGR, IDDM, or GDM were compared with samples from AGA placentas.

Statistical analysis

To evaluate the data statistically, Student's *t* test, ANOVA, and Dunnett's test were used, and results are given as mean ± SEM. Significance was considered at $P < 0.05$.

Results

Clinical data

Clinical data of pregnancies from which vesicles were prepared are shown in Table 1. The IUGR groups were well matched with AGA groups with respect to gestational age. The AGA term placentas used as controls for IUGR term were all delivered by cesarean section, whereas the IUGR term group included both cesarean section and vaginal deliveries. However, the AGA control group for diabetes included both cesarean section and vaginal deliveries. We saw no alteration in TG hydrolase activities with respect to mode of delivery. For IDDM and GDM, only full-term (>37 wk) placentas were used (except for one 36-wk IDDM placenta). Birth weight was reduced by 40% ($P < 0.05$, n = 8) in the preterm IUGR and by 38% ($P < 0.05$, n = 8) in the term IUGR groups. In the IDDM group, birth weight was increased by 35% ($P < 0.05$, n = 8), and in the GDM group, the birth weight was 23% higher ($P < 0.05$, n = 6) than in AGA controls. The term AGA group included only uncomplicated pregnancies. Apart from premature delivery, preterm AGA did not have any other major complications. Both preterm and term IUGR groups included one patient diagnosed with preeclampsia. IUGR pregnancies were classified for clinical severity according to Pardi *et al.* (40). In this classification, group 1 corresponds to normal fetal heart rate (FHR) pattern and normal pulsatility index (PI) in the umbilical artery, group 2 is characterized by normal FHR + abnormal PI, and in group 3 both FHR and PI are abnormal. In the study of Pardi *et al.* (40), the majority of the fetuses in group 3 showed acidemia and hypoxemia in fetal blood samples obtained by cordocentesis. In our study, all preterm IUGR pregnancies were associated with abnormal PI (groups 2 and 3 and Table 1). Abnormal cardiotocography (in two of eight subjects), oligohydramnios (three of eight), and pathological umbilical

TABLE 1. Selected clinical data

	Preterm (25–36 wk)		Term (37–41 wk)	
	AGA	IUGR	AGA	IUGR
n	8	8	8	8
Gestational age (wk)	33.4 ± 1.54	32.5 ± 1.25	38.7 ± 0.34	39.1 ± 0.45
Placental weight (g)	470 ± 73	293 ± 36[a]	781 ± 60	408 ± 43[a]
Birth weight (g)	1959 ± 319	1171 ± 145[a]	3733 ± 273	2317 ± 127[a]
Mode of delivery (c/v)	5/3	7/1	8/0	4/4
Clinical severity[b]				
Group 1		0		6
Group 2		5		2
Group 3		3		0
Preeclampsia		1		1
Ponderal index	2.24 ± 0.06	2.42 ± 0.21	2.61 ± 0.06	2.22 ± 0.14[a]

	AGA	IDDM	GDM
n	8	8	8
Gestational age (wk)	39.5 ± 0.45	37.9 ± 0.5	39.3 ± 0.56
Placental weight (g)	608 ± 33	942 ± 48[a]	870 ± 50[a]
Birth weight (g)	3336 ± 295	4500 ± 121[a]	4353 ± 187[a]
Mode of delivery (c/v)	5/3	5/3	6/2
Ponderal index	2.71 ± 0.22	2.91 ± 0.14	2.88 ± 0.09

Data are presented as mean ± SEM. c/v, Cesarean/vaginal; ponderal index, 100 × birth weight/length^{3}.

[a] $P < 0.05$ compared to AGA.

[b] Classification of clinical severity of IUGR according to Pardi *et al.* (40). Group 1, Normal FHR pattern and PI in the umbilical artery; group 2, normal FHR and abnormal PI; group 3, abnormal FHR and abnormal PI.

blood flow patterns (two of eight) were evident also in the IUGR term group, although less frequent than in the preterm IUGR group. Ponderal index was 15% lower ($P < 0.05$, n = 8) in term IUGR, compared with AGA term group, suggesting asymmetrical growth. The IDDM group included six patients diagnosed White D and two White C (41). In the GDM group, three patients were treated with insulin (treatment started between 3 and 9 wk before delivery), and the remaining five were treated with diet only.

Activity of TG hydrolases

The TG hydrolase activity was related to protein added to the incubation media, and 150 μg protein gave about a 2-fold higher activity (per μg protein) than 75 μg at both pHs [+75% (n = 2) at pH 6; +140% (n = 2) at pH 8]. A time course showed that the activities were highest per milligram protein per minute at 30 min incubation, compared with 60 min [+40% (n = 2) at pH 6; +160% (n = 2) at pH 8], confirming prior descriptive studies of TG hydrolase in MVM from human placenta (9, 39). The oleic acid extraction efficiency was 80.4 ± 0.2% (n = 300) and had an intraassay coefficient of variation of 0.5 ± 0.02%. The interassay efficiency had a coefficient of variation of 4.8%. Activity of the placenta-specific pH 6.0 TG hydrolase did not differ significantly in any of the groups studied (Fig. 1), compared with AGA. The activity of the placenta-specific pH 6.0 TG hydrolase was about 5-fold higher than the activity of LPL (Fig. 2). However, when MVM vesicles were incubated with 7.5% fetal calf serum, the pH 6 activity was inhibited by 76% (n = 2), giving an activity level similar to that seen at pH 8.

The activity of LPL was decreased by 47% ($P < 0.05$; n = 8) in MVM from preterm IUGR placenta, compared with preterm AGA (Fig. 2A), and the activity decreased by 27% ($P < 0.05$; n = 8) between preterm and term AGA. The LPL activity was increased by 39% ($P < 0.05$; n = 8) in MVM from the IDDM placentas, compared with AGA (Fig. 2B). There was no significant difference between GDM and AGA groups. Also, there was no significant difference in TG activity within the GDM group when analyzing placentas from insulin-treated placentas and those from diet-treated patients, either for pH 6 TG hydrolase or LPL (data not shown).

Protein expression of LPL, L-FABP, and C-FABP

The LPL protein was identified as a major band at 37 kDa and a minor at 56 kDa (Fig. 3) in MVM and rat cardiac muscle. The minor 56-kDa band was present only in term groups. The human placental C-FABP and L-FABP were both identified at 15 kDa (Fig. 4, A and B), and their positive controls (rat cardiac muscle and rat liver) were observed at a slightly lower molecular weight. After densitometry analysis of signal intensity, the term AGA group was assigned a relative density value of 1 to facilitate comparisons between groups. No significant differences among IUGR, IDDM, or GDM placentas were observed when compared with AGA for LPL, which was analyzed both as a single band and as a combination of the two bands (Table 2 shows the results of the major 37-kDa band). The ratio between the 56-kDa (inactive) and 37-kDa (active) band was analyzed and found to be similar when comparing the different groups. No significant

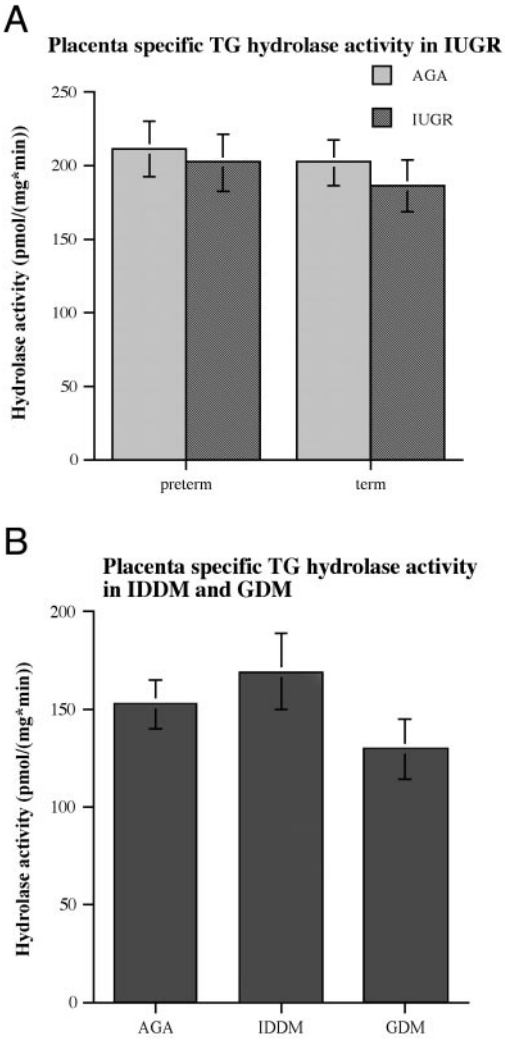

FIG. 1. A, Hydrolase activity of placenta-specific TG hydrolase in MVM in preterm and term IUGR placentas, compared with AGA (n = 8 in each group). B, Hydrolase activity of placenta-specific TG hydrolase in IDDM (n = 8), GDM (n = 6), and AGA MVM (n = 8), ANOVA.

differences were observed in expression of C-FABP in any of the groups (Table 2). Similarly, protein expression of L-FABP in placental homogenates was unaltered in IUGR (Fig. 5A). However, the protein expression of L-FABP was increased by 112% ($P < 0.05$, n = 8) when comparing IDDM with AGA placentas (Fig. 5B). Also, there was an increase of 64% ($P < 0.05$, n = 8) in the GDM group (Fig. 5B), compared with AGA. When comparing placentas from insulin-treated patients with placentas from patients treated with diet only, no significant alteration in L-FABP expression was observed (data not shown).

Discussion

The human fetus is dependent on the supply of EFAs and their LCPUFA derivatives, in addition to many other nutrients, from the maternal circulation. The process of exchange occurs across the placenta. As a consequence, impaired placental function, as in pregnancies complicated by IUGR,

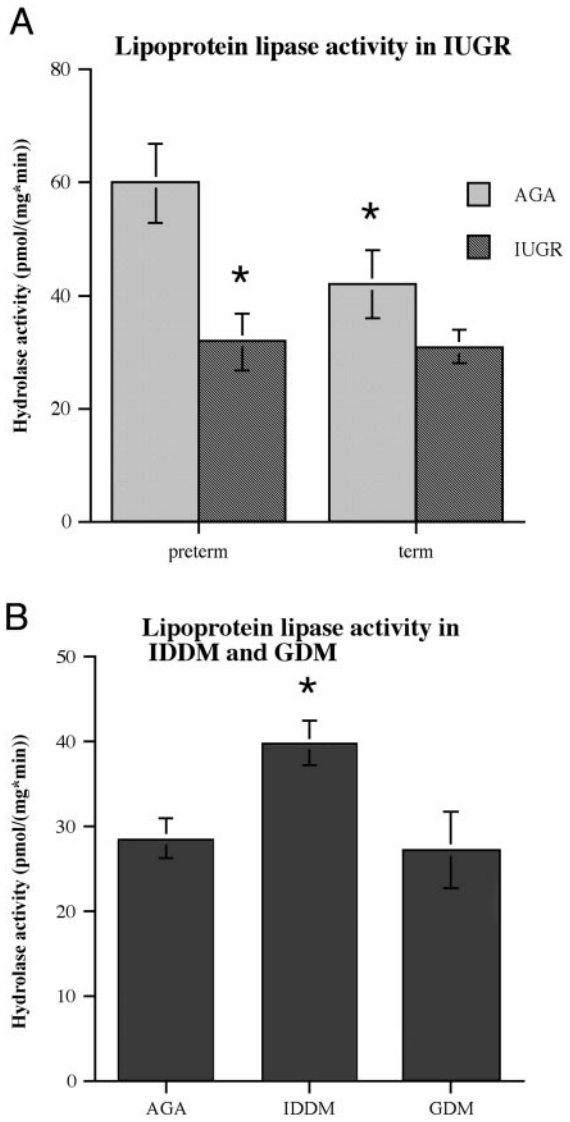

FIG. 2. A, Hydrolase activity of LPL in MVM in preterm and term IUGR placentas, compared with AGA (n = 8 in each group). *, $P < 0.05$, compared with preterm AGA, ANOVA. B, Hydrolase activity of LPL in IDDM (n = 8), GDM (n = 6), and AGA MVM (n = 8). *, $P < 0.05$, compared with AGA, ANOVA.

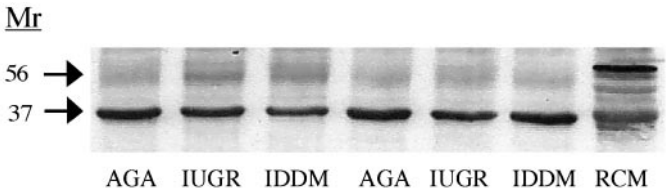

FIG. 3. Western blot demonstrating protein expression of LPL in MVM from AGA, IUGR, and IDDM pregnancies. Rat cardiac muscle (RCM) was used as positive control.

IDDM, and GDM, can markedly affect normal growth and development of the fetus. Szabo *et al.* (42) suggested that placental maternal-to-fetal transport of FFAs in humans contributes significantly to fetal lipid synthesis and fetal fat storage, and these processes are dependent on the maternal serum FFAs (43). The importance of maternal TG as a lipid

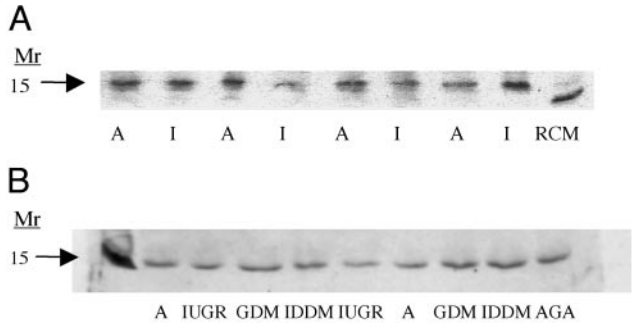

FIG. 4. A, Western blot demonstrating protein expression of C-FABP in AGA (A) and IUGR (I) homogenates of term placenta. Rat cardiac muscle (RCM) was used as positive control. B, Protein expression of L-FABP in homogenates of AGA (A), IUGR term, IDDM, and GDM placenta.

TABLE 2. Relative protein expression of LPL and C-FABP

	LPL (in MVM) (n = 6)	C-FABP (in homogenates) (n = 8)
AGA term	1.00 ± 0.05	1.00 ± 0.07
AGA preterm	0.81 ± 0.19	1.22 ± 0.09
IUGR term	1.09 ± 0.12	1.08 ± 0.17
IUGR preterm	0.63 ± 0.13	1.11 ± 0.21
IDDM	1.03 ± 0.08	1.19 ± 0.15
GDM	1.13 ± 0.19	1.22 ± 0.13

Data are presented as mean ± SEM. Protein expression of IUGR, IDDM, and GDM groups were not statistically different from AGA group. The relative density in Western blots of the term AGA group was arbitrarily assigned a value of 1.

source for the fetus has further been strengthened by a study in which a 10-fold preference in placental uptake of FAs derived from TGs instead of FFAs was observed in isolated trophoblast cells (8). Animal studies have shown that poor fetal nutrition, such as deficiencies in EFAs and preformed LCPUFAs, can impact structure and physiology of many organs and additionally may result in reduced cognitive ability and retinal development (1, 6, 7). The role of TG hydrolase activities at the placental MVM boundary has not been well documented, yet these activities are of critical importance in the metabolism of maternally circulating TGs and mobilization of FFAs available for placental transport. FAs are principally stored esterified as TGs and are transported in the maternal circulation as moieties of VLDLs and diet-derived chylomicrons. Because TG is not transported intact across the placenta (1), FAs are liberated from VLDLs and chylomicron TG moieties by lipid hydrolase activities at the MVM surface. Consequently, in clinical conditions of IUGR, IDDM, and GDM, the placentas of which show disrupted lipid metabolism, altered MVM lipid hydrolase activities would impact on EFA and preformed LCPUFA flux to the fetus.

Although no significant change in the placental-specific (pH 6 optima) hydrolase activity was observed in this study, a clear and distinct 47% decrease in LPL activity was demonstrated for MVM obtained from IUGR preterm placentas. Furthermore, Western blot analysis showed no significant difference in protein expression between IUGR and AGA pre- and term placental groups. It has previously been demonstrated that growth-restricted infants exhibit a decreased

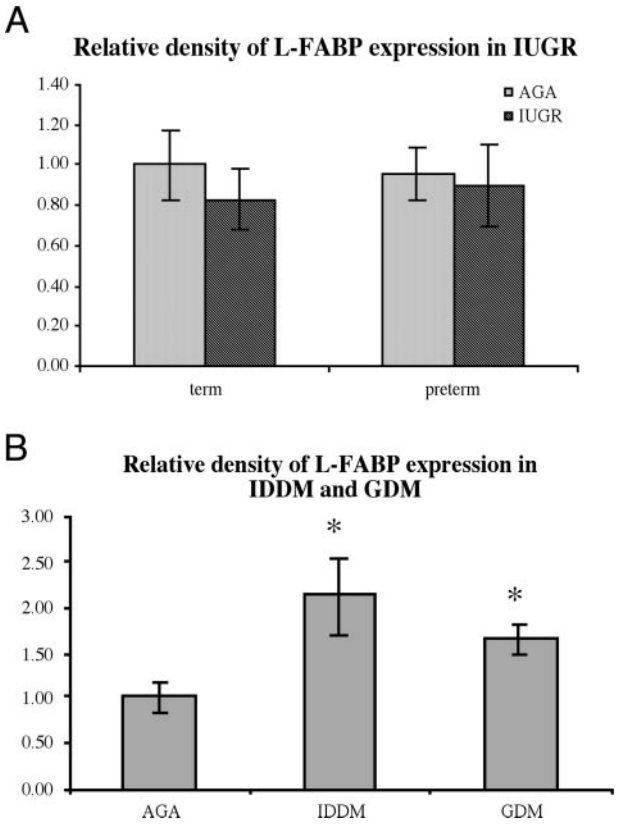

FIG. 5. A, Relative protein expression of L-FABP after densitometry readings in placental homogenates obtained from IUGR term (n = 8) and IUGR preterm placentas (n = 8), compared with AGA (n = 8) in term and preterm group, respectively. AGA term group was given an arbitrary value of 1 to facilitate comparisons between groups. B, L-FABP protein expression in placental homogenates from IDDM (n = 8) and GDM (n = 8), compared with AGA (n = 8). *, $P < 0.05$, compared with AGA, ANOVA.

body fat mass, secondary to intrauterine malnourishment (44). This cannot be explained solely by a decreased placental glucose transport capacity to the fetus (45). However, the decreased LPL activity demonstrated for the IUGR preterm group would impact the concentration of liberated FFAs at the MVM surface and consequently the availability of FFAs for placental transport. Indeed, Cetin *et al.* (24) found significantly lower fetal/maternal ratios in IUGR pregnancies for the relative amounts of DHA and AA, compared with normal pregnancies. The placenta does not account for any substantial conversion of LA and αLA to DHA or AA (46). Therefore, the potential restriction of FFA flux into the fetal circulation due to suboptimal LPL activity may then help account for the decrease in both fetal body fat mass and specific LCPUFAs (24). The levels of protein expression for both C-FABP and L-FABP were not significantly altered for the IUGR and control groups, strongly suggesting that they are not limiting FA flux in these pregnancies.

By contrast, LPL activity of the MVM from pregnancies complicated by IDDM showed a 39% increase. In the same way as for IUGR, no significant difference of protein expression of LPL or C-FABP was shown in either IDDM of GDM. However, a 112% increase in the protein expression of L-FABP in IDDM was demonstrated. Excessive lipid accumulation of the placenta and fetus is associated with diabetic pregnancies (33). In the present study, we demonstrate a potential link in the etiology of this excessive lipid accumulation attributed to diabetic pregnancies. Significantly elevated LPL activity liberates increased FAs from TG moieties of VLDLs and chylomicrons at the MVM surface. This creates the situation of promoting FA transfer by increasing the maternal-fetal gradient, which may then contribute to increased body fat mass in newborns of diabetic women. Coupled to this is the observed increase in FA binding capacity of placental samples for uptake of the liberated FA, as provisioned by the increased expression of L-FABP. The increased expression of L-FABP could, in part, be the response to increased FA availability as a result of stimulated LPL activity; the L-FABP gene has been shown to be up-regulated by FAs (47). However, the effect of high glucose levels in contributing to the excessive accumulation of lipid in the fetus cannot be ignored. Maternal hyperglycemia will sustain lipid accumulation by contributing to the placental transfer of glucose, which itself serves as a lipogenic substrate (48). The increased placental expression of L-FABP in GDM pregnancies, without an alteration in MVM hydrolase activity, cannot be readily explained, and its significance requires further investigation. However, elevated insulin levels will affect expression of L-FABP (49) and may explain why an increase is observed in GDM. Moreover, the lower level of increase, compared with IDDM (64 *vs.* 112%, respectively), may be accounted for by the lack of increase in LPL activity for GDM, compared with IDDM.

The LPL protein in MVM was found as a major band at a molecular weight of 37 kDa and a minor at 56 kDa, which corresponds well with the characteristics of the 5D2 antibody (50). In rat cardiac muscle, the 5D2 antibody bound to the 37- and 56-kDa protein. The 37-kDa protein represents the C terminal of LPL, which, after cleaving from the intact LPL molecule at 56 kDa, becomes active (50). The antibody used was originally produced against bovine LPL but has been reported to strongly cross-react with human LPL and LPL from other species (36). Although we found a reduction of LPL activity in IUGR and increased activity in IDDM, we did not find any differences in LPL protein expression, although a tendency toward lower protein expression was seen in the IUGR preterm group. We analyzed the two bands separately, as a combination of the two bands, and we also measured the ratio between the active and inactive forms without observing any significant differences. The altered activity of LPL may to be due to modulation of its activity at the posttranscriptional level because no change in protein expression was detected. The posttranscriptional regulation of LPL may be related to LPL's ability to associate to, or dissociate from, its dimeric active state. Cells that synthesize LPL contain quantities of inactive lipase in the monomeric state (51). LPL activity has been shown to increase by insulin and thyroxin in white adipose tissue (human and rodent) without changes in levels of protein expression (52). However, the possible mechanism behind any potential posttranslational control in placenta, whether by increased LPL association/dissociation or some other process, is not known and is outside the scope of the current study.

In our study the activity of LPL decreased in late gestation,

a time during which fetal fat accumulation increases. This apparent discrepancy may be explained by an increased activity or expression of placental plasma membrane FA binding protein and lipoprotein receptors toward the end of pregnancy and/or an increased TG hydrolysis of the placental TG store providing more FFAs available for the fetus. An important feature to remember is that the surface area in the term placenta is larger than the preterm placenta, which is not taken into account in our study using MVM vesicles. Therefore, the total placental LPL activity probably is higher at term than at preterm, even though the activity per unit membrane protein is decreased. Another possibility is that the placenta-specific TG hydrolase becomes more important toward term. No significant decrease in activity was observed in this hydrolase. The activity of the placenta-specific hydrolase was inhibited by fetal calf serum, giving an activity level essentially the same as LPL. The presence of fetal calf serum would better resemble the *in vivo* situation because both these TG hydrolases are situated in the placental microvillous membrane, in direct contact with maternal blood. No differences were observed in activity of this hydrolase when comparing the different groups.

Our values for the TG hydrolase activities are about 2-fold higher than previously published data (9). The difference might be explained by a small modification in our protocol. To obtain micelles, into which the labeled trioleic acid could incorporate, we used an Intralipid-emulsion (instead of preparing micelles from phosphatidylcholine and phosphatidylserine), which has an effect of enhancing sensitivity of the assay system. Also, we used more MVM protein in each assay.

In conclusion, we found altered placental LPL MVM activity and placental L-FABP expression in IUGR and diabetic pregnancies. This modulation may be linked to the etiology of the disrupted lipid metabolism in these pregnancies. Further investigation into the cause of these modulations my help improve treatment regimens and the health outlook of both the fetus and mother.

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