patient-oriented research

Gestational and hormonal regulation of human placental lipoprotein lipase

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Abstract The fetal demand for FFA increases as gestation proceeds, and LPL represents one potential mechanism for increasing placental lipid transport. We examined LPL activity and protein expression in first trimester and term human placenta. The LPL activity was 3-fold higher in term ($n = 7$; $P < 0.05$) compared with first trimester ($n = 6$) placentas. The LPL expression appeared lower in microvillous membrane from first trimester ($n = 2$) compared with term ($n = 2$) placentas. We incubated isolated placental villous fragments with a variety of effectors [GW 1929, estradiol, insulin, cortisol, epinephrine, insulin-like growth factor-1 (IGF-1), and tumor necrosis factor-α] for 1, 3, and 24 h to investigate potential regulatory mechanisms. Decreased LPL activity was observed after 24 h of incubation with estradiol (1 μg/ml), insulin, cortisol, and IGF-1 ($n = 12$; $P < 0.05$). We observed an increase in LPL activity after 3 h of incubation with estradiol (20 ng/ml) or hyperglycemic medium plus insulin ($n = 7$; $P < 0.05$). To conclude, we suggest that the gestational increase in placental LPL activity represents an important mechanism to enhance placental FFA transport in late pregnancy. Hormonal regulation of placental LPL activity by insulin, cortisol, IGF-1, and estradiol may be involved in gestational changes and in alterations in LPL activity in pregnancies complicated by altered fetal growth.—Magnusson-Olsson, A. L., B. Hamark, A. Ericsson, M. Wennergren, T. Jansson, and T. L. Powell. **Gestational and hormonal regulation of human placental lipoprotein lipase.** ***J. Lipid Res.*** **2006.** 47: **2551–2561.**

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The placental transfer of fatty acids to the fetus is important for normal fetal development and growth, as they are essential components of cell membranes, are used as energy sources, and act as precursors to cellular signaling molecules. The fetus is able to synthesize saturated and monounsaturated FAs from glucose and ketone bodies (1) but depends completely on placental transport for its supply of the essential fatty acids (EFAs) linoleic and α-linolenic acid. The source of the EFA-derivative long-chain PUFAs arachidonic acid (ARA) and docosahexaonic acid (DHA) is primarily of maternal origin, as the placenta lacks or has only minor activity of the enzymes (δ5- and δ6-desaturase) for converting EFAs into long-chain PUFAs (2–5). However, infants born premature have been suggested to be able to synthesize both ARA and DHA at an age when they should be developmentally dependent on the placenta (6, 7). Because of the high content of ARA and DHA in the brain and retina, these fatty acids are critical to normal neurological and vascular development (8, 9).

The placental transfer of FFAs is likely to be increased during the second half of pregnancy in association with rapid fetal fat deposition. Maternal circulating triglycerides (TGs) and FFAs are increased during the third trimester, providing a source of FFAs for milk production, placental uptake, and possibly transfer to the fetus (10). A gestational increase in estrogen, which stimulates the production of VLDLs, together with a decreased removal of lipoprotein TGs by LPL and/or hepatic lipase, has been suggested to be responsible for this hyperlipidemia (11). It has been shown in pregnant rats that a concurrent increase in LPL activity in mammary gland and placenta (10) redistributes the utilization of TGs to milk production and supplying the fetus.

The source of fatty acids transported to the fetus is either TG-rich maternal lipoproteins, such as chylomi-

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Abbreviations: ARA, arachidonic acid; C-FABP, cardiac fatty acid binding protein; DHA, docosahexaonic acid; EFA, essential fatty acid; FABP, fatty acid binding protein; hCG, human chorionic gonadotropin; IDDM, insulin-dependent diabetes mellitus; IGF, insulin-like growth factor; IUGR, intrauterine growth restriction; L-FABP, liver fatty acid binding protein; MVM, microvillous membrane; PPAR, peroxisome proliferator-activated receptor; TBP, TATA box binding protein; TG, triglyceride; TNF, tumor necrosis factor.

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crons and VLDL, or FFAs bound to albumin. The maternal plasma FFAs (12, 13) have been suggested to represent the main class of lipids crossing the placenta (8, 14). The pathway for FFA transport to the fetus is thought to be driven by the maternal-fetal gradient and thereby depends on the maternal FFA concentration (15). The relative contribution of maternal FFAs versus TG-derived FFAs for total placental transport is currently unclear; however, isotope studies in guinea pigs indicate that the fatty acids in the fetal circulation are obtained from maternal TG rather than FFA (16), underscoring the role of LPL. In the same study, the authors stated that LPL activity was a predictor of fetal guinea pig weight. Also, isolated placental trophoblasts have been shown to have a 10-fold preference in uptake of FAs derived from TGs rather than FFAs bound to albumin, indicating a mechanism that could favor the fetal delivery of EFAs and energy acquired from the diet via chylomicrons (17). Additionally, LPL seems to prefer hydrolyzing fatty acids that are more unsaturated, such as long-chain PUFAs from circulating TGs (18).

As mentioned above, several studies indicate that TGs are not transported intact over the placenta (8, 14, 19). Therefore, LPL, localized in the placental microvillous membrane (MVM), is a critical TG hydrolase and represents the initial step in transplacental transfer of TG-derived FFAs by cleaving TGs in maternal circulating lipoproteins, allowing the FFAs to be taken up by the placenta and transported across the MVM. Recent data from our group showed that LPL is produced by placental syncytiotrophoblasts and cytotrophoblasts (A. L. Magnusson-Olsson, unpublished data), as confirmed by real-time PCR and LPL activity measurements. Similar findings were published recently showing that LPL mRNA was found in both syncytiotrophoblasts and cytotrophoblasts, as confirmed by in situ hybridization (20). These data are in agreement with previous findings in adipose, heart, skeletal muscle and lactating gland that LPL is produced and secreted by the underlying parenchymal cells (21). Along with LPL, endothelial lipase was also found in syncytiotrophoblasts and cytotrophoblasts (20). The role of endothelial lipase in the placenta is not clear, but in other endothelial lipase-producing endothelial cells, the preferred substrate is the TG-poor HDL (22, 23). A release of TG-derived FAs by endothelial lipase to the fetus, therefore, may not be of major significance compared with LPL. However, a recent study indicated that endothelial lipase is able to hydrolyze unsaturated FAs such as ARA from HDL phosphatidylcholine and therefore may be able to supply cells with unsaturated FAs (24).

Once cleaved from TG, the FFAs are taken up by the syncytial cell, and when the FFAs have reached the syncytial cytoplasm, fatty acid binding proteins (FABPs) guide them to various sites of esterification or β-oxidation or transfer them directly to the fetus. At least two FABP isoforms have been detected in the syncytial cytoplasm: liver fatty acid binding protein (L-FABP) and cardiac fatty acid binding protein (C-FABP) (25). To become available for the fetus, the fatty acids are thought to cross the basal membrane either through diffusion or with the assistance of FABPs (26).

Placental LPL activity appears to be positively correlated with fetal size and/or fat depots of the fetus. We have shown previously an increased activity of MVM LPL in placentas from pregnancies complicated by maternal insulin-dependent diabetes mellitus (IDDM) (27) associated with accelerated fetal growth and large fat depots (28) and a decreased activity in pregnancies delivering preterm fetuses with intrauterine growth restriction (IUGR), a condition typically characterized by reduced fetal fat depots (27, 29). In the same study, we demonstrated increased placental L-FABP expression in gestational diabetes mellitus and IDDM (27). Apart from altered LPL activity, changes in glucose metabolism in mother and/or fetus seen in both diabetic and IUGR pregnancies could also contribute to the altered fat depots attributable to an altered substrate availability for fatty acid synthesis. For example, the fetal supply of glucose in IDDM pregnancies is increased as a result of an increased placental transport of glucose (30). The IUGR fetus is often hypoglycemic in utero (31–33), indicating that less glucose is available for fatty acid synthesis. Szabo, Grimaldi, and Jung (34) 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 (35), which may be altered in these pregnancy complications (36, 37).

LPL is anchored to the MVM via heparan sulfate proteoglycans and can be displaced by heparin. The heparin-released LPL is often referred to as "functional LPL" (38). In other tissues, such as adipose tissue, muscle tissue, and mammary gland, LPL activity can be regulated in a variety of ways: by altered gene expression, intracellular transport and glycosylation, dimerization, alteration of the active site, and degradation (21, 39). Rapid alterations in LPL activity occur mostly at the posttranscriptional level in rat adipose tissue, as no changes are seen at the mRNA or protein level (40). In rat adipose tissue, LPL activity appears to be reversibly inhibited between meals (41). LPL activity in adipose and skeletal muscle tissue seems to be regulated in a reciprocal manner by fasting/feeding, insulin, and epinephrine (42). For example, the effect of insulin in the fed state is to increase LPL activity in adipose tissue and decrease LPL activity in skeletal muscle. Catecholamines have the opposite effect: increased LPL activity in skeletal muscle and decreased LPL activity in adipose tissue. Estrogens have been shown to decrease LPL activity in adipose tissue and postheparin plasma in humans (43–45) but to increase LPL activity in cardiac and diaphragmatic muscle in rats (46). Also, high glucose alone and high glucose together with insulin have been suggested to decrease total LPL activity in postheparin plasma (47). Peroxisome proliferator-activated receptor γ (PPARγ) stimulates LPL mRNA (48), whereas cortisol (49), insulin-like growth factor-1 (IGF-1) (50), and tumor necrosis factor-α (TNF-α) (51) all decrease LPL activity in adipose tissue. The

mechanisms for the regulation of LPL activity in the placenta remain unclear.

In this study, we established a technique for measuring LPL activity in fresh human placental villous tissue. We studied LPL activity and the expression of LPL, C-FABP, and L-FABP in first trimester placentas and compared them with term placenta levels. We further investigated possible regulators of placental LPL by studying the effect of a variety of hormones, effectors, and high versus low glucose on heparin-releasable LPL activity in fresh placental villous tissue from term placentas. The hormones and effectors were chosen either because they are known to be altered in pregnancies complicated by altered fetal growth, such as in IUGR and IDDM, or because they have been shown to regulate LPL activity in other tissues.

METHODS

Materials

Monoclonal antibody 5D2 against the C terminus 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 with LPL from other species but not with related lipases (52). 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). F12 Kaighn's medium and the penicillin/streptomycin solution were purchased from the American Type Culture Collection (LGC Nordic Borås, Sweden). The ATP lite® kit was purchased from Perkin-Elmer Life Sciences (Boston, MA), and the human chorionic gonadotropin (hCG) ELISA kit was from IBL (Hamburg, Germany).

Buffers

DMEM containing 5.6 mM glucose was used. Tyrode's buffer was made up containing 135 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 10 mM HEPES, and 5.6 mM glucose. As DMEM contains high concentrations of amino acids, it was diluted with Tyrode's buffer (1:3) to achieve more physiological concentrations. To achieve hypoglycemic medium with a final concentration of 1.3 mM, glucose was excluded from the Tyrode's buffer and was thereafter mixed with DMEM. For the hyperglycemic medium with a final concentration of 15 mM, Tyrode's buffer with 18.1 mM glucose was used. To the DMEM/Tyrode's buffer, 10% antibiotic cocktail (F12 medium of Kaighn's modification supplemented with 10% calf serum, 50 units of penicillin, and 50 μg/ml streptomycin) was added. Buffer D was prepared and contained 250 mM sucrose, 10 mM HEPES/Tris, 1.6 μM antipain, 0.7 μM pepstatin A, 0.5 μg/ml aprotinin, and 1 mM EDTA.

Tissue

Human placental tissue collection was approved by the Göteborg University Committee for Research Ethics and conducted with informed consent. First trimester tissue (6–13 weeks of gestation) was collected from 14 women who underwent elective terminations at Sahlgren's University Hospital, Göteborg. All patients were healthy, and none had evidence of complications during the index pregnancy or had complications in previous pregnancies. Trophoblast tissue was identified and rinsed in saline. Tissue from normal healthy placentas at term (n = 39; 37–41 weeks of gestation) were obtained immediately after cesarean section or vaginal delivery, and the newborns were all appropriately grown for gestational age. Villous tissue was dissected from the placentas and washed in saline, and all samples were transported to the laboratory in DMEM/Tyrode's.

Validation of villous fragments after incubation

hCG produced by the villous fragments and released into the incubation medium was analyzed. Villous fragments (50 mg) in triplicate were incubated (37°C, 5% CO_2) for 24 h in 5 ml of DMEM/Tyrode's + antibiotics on a six-well microtiter plate. After 1 h, the first 5 ml sample was collected. Thereafter, new incubation medium was added. For time points 3, 6, and 24 h, the medium was changed 1 h before sampling. The samples were frozen and stored at −80°C. The incubation medium was lyophilized by freeze-drying and resuspended in 100 μl of DMEM/Tyrode's. To detect the amount of hCG produced by the villous fragments at each time point, a hCG ELISA kit was used (IBL). ATP content at each time point was determined in intact villous tissue transferred to Eppendorf tubes containing 0.5 ml of buffer D. The samples were frozen and stored at −80°C until use. The ATP content of tissues was determined after release from the tissue using a luminescence ATP lite® detection assay system (Perkin-Elmer) according to the manufacturer's instructions.

Microvillous plasma membrane preparation

MVM vesicles from term and first trimester samples were prepared according to methods described previously (53) with some modifications. Briefly, the tissue was processed within 30 min of delivery. The chorionic plate and decidua were removed, and ~100 g of villous tissue was cut and washed in buffered saline. After differential centrifugation (at 4°C) steps in buffer D with protease inhibitors, MVM was separated by Mg^{2+} precipitation. The first trimester vesicle isolation protocol was scaled down to accommodate the small volume of villous tissue available from a single patient. 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 (54).

Hormones and effectors

Placental villous tissue was dissected, and 50 mg (six to eight pieces) was placed in each well on a six-well microtiter plate containing 5 ml of DMEM/Tyrode's + antibiotics (control) and the hormones or effectors to be studied. Plates with villous tissue were incubated (37°C, 5% CO_2) for 1, 3, and 24 h. Insulin and cortisol stock solutions were freshly prepared for every experiment. The other hormones and effectors in stock solutions were kept in a −20°C freezer until use. The PPARγ ligand (GW 1929) was diluted in DMSO to a stock solution and was further diluted to a final concentration of 10 μg/ml. Estradiol was dissolved in ethanol and used at a final concentration of 2, 20, and 100 ng/ml and 1 μg/ml. For the estradiol used for the 3 h incubation, one stock solution was made. Because in initial experiments we saw that a 3 h incubation with ethanol slightly decreased LPL activity, the 2, 20, and 100 ng/ml concentrations have separate controls with corresponding amounts of ethanol (see Fig. 4A). The concentration of ethanol in the 1 μg/ml estradiol sample was

similar to the amount used for the 2 ng/ml assay and was shown not to interfere with LPL activity after 24 h of incubation (data not shown). Insulin (bovine) was first dissolved in an insulin stock solution containing 0.1% BSA and 1.6% glycerol and was further diluted to 145 or 290 ng/ml. The water-soluble form of cortisol (hydrocortisone-cyclodextrin complex) was used and diluted directly in DMEM/Tyrode's medium (340 ng/ml). Epinephrine was dissolved in a 0.5 M HCl stock solution and further diluted to a concentration of 500 pg/ml. IGF-1 and TNF-α were made up in sterile PBS with 0.1% BSA (final concentrations: IGF-1, 250 ng/ml; TNF-α, 20 ng/ml). Samples were tested with corresponding amounts of solvents for all experiments. Except for ethanol, as described above, the solvents did not affect LPL activity (data not shown). We further incubated villous tissue with hypoglycemic (1.3 mM), hyperglycemic (15 mM), or normoglycemic medium for 3 h and combined this with two different concentrations of insulin (145 and 290 ng/ml). We also tested insulin with and without cortisol.

Determination of LPL activity

Measurements in first trimester and term placentas. To study the LPL activity in placental villous tissue, a slightly modified version of a previously published protocol was used. Here, we measured LPL activity in fresh intact villous tissue, in contrast to measuring LPL activity in isolated MVM vesicles that had been frozen before measurement (27, 55). Briefly, 10 μl of glycerol tri[9,10(n)-^{3}H]oleate was added to an Intralipid solution (1% in 10 mM Tris-HCl). To incorporate trioleate into Intralipid, the mixture was sonicated on ice for 30 s. The incubation buffer was based on 0.2 M pH 8 sodium hydrogen phosphate buffer containing 500 μg/ml BSA (dissolved in 10 mM Tris-HCl, pH 7.4, and 10 μg/μl), 7.5% fetal calf serum, 1 mM glycerol tri[9,10(n)-^{3}H]oleate, and 0.5 μl/ml Intralipid. Fifty milligrams of tissue from first trimester and term placentas was placed in 2 ml of LPL activity assay buffer with the addition of 36 IU/ml heparin to release the LPL into the buffer. The tissue and heparin-containing assay buffer was shaken for 30 min at room temperature to optimize the LPL release. Thereafter, samples (200 μl) of the LPL-containing assay buffer (in triplicate) were removed to test tubes. Samples were incubated in a water bath (37°C) for 30 min. The reaction was discontinued by adding 3.25 ml of stop solution containing methanol, chloroform, and heptane [1.40:1.25:1.00 (v/v)] and [1-^{14}C]oleic acid (2,400 dpm/ml). Subsequently, 1 ml of borate-carbonate (pH 10.5) was added, and the samples were vortexed vigorously for 30 s and centrifuged at 4°C at 2,200 rpm for 20 min. The upper aqueous phase, containing ^{14}C-labeled oleic acid, 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 LPL was expressed as pmol [9,10(n)-^{3}H]oleate released/min/mg tissue (wet weight).

Measurement after 1, 3, and 24 h of incubation. A minor modification was made before measuring LPL activity after hormone incubations. After incubation with and without hormones for 1, 3, and 24 h in DMEM/Tyrode's buffer at 37°C, heparin only was added (final concentration, 36 IU/ml) to the DMEM/Tyrode's incubation buffer. Thereafter, the plates were placed on a shaker for 30 min at room temperature. Samples (100 μl) from the LPL-containing incubation buffer were mixed with 100 μl of LPL activity assay buffer (as described above but with no heparin, doubled concentrations of BSA, glycerol tri[9,10(n)-^{3}H]oleate incorporated into Intralipid, and fetal calf serum) and subsequently incubated in a water bath for 30 min at 37°C. The remainder of the assay was performed as described previously.

SDS-PAGE and Western blotting

Western blotting was performed according to a previously published protocol (27) with some modifications. For comparison of protein expression of LPL in first trimester MVM vesicles with normal term MVM, vesicle samples (10 μg of total protein) were separated by 10% SDS-PAGE. Rat cardiac muscle homogenate was used as a positive control. Placental homogenates from first trimester and term samples with rat liver/rat cardiac muscle as positive controls were loaded (40 μg of total protein) on a 12% SDS-PAGE apparatus to determine the protein expression of L-FABP and C-FABP. The nitrocellulose membranes were incubated with respective primary antibodies [LPL-5D2 (1:250), L-FABP (1:100), and C-FABP (1:300)], washed, and incubated with peroxidase-labeled horse anti-mouse IgG (1:1,000). After repeated washings, the immunolabeling was made visible using ECL detection (Amersham). The analysis included scanning of films followed by densitometry by means of IP Lab gel software (Signal Analysis Corp., Vienna, VA). LPL densities in MVM samples from first trimester placentas were compared with those in MVM samples from normal term placentas.

Real-time RT-PCR

Total RNA was extracted using RNA STAT-60 (Tel-Test, Inc.) according to the manufacturer's protocol, measured using the SPECTRA max Plus microplate reader (Molecular Devices Corp., Sunnyvale, CA), and registered by Soft Max PRO 3.1 (Molecular Devices). Reverse transcription was performed using a SuperScript™ II Reverse Transcriptase kit (Invitrogen, San Diego, CA), random hexamer primers, and deoxynucleoside triphosphates (dATP, dCTP, dTTP, and dGTP; Roche Diagnostics GmbH, Mannheim, Germany) as described previously (56) on a Minicycler™ (MJ Research, Inc., Watertown, MA). Relative quantification of LPL mRNA expression was carried out on a LightCycler (Roche Diagnostics) using SYBR® Green I. TATA box binding protein (TBP) was selected as an endogenous control, as it has been shown to be stable in the placenta (57), to correct for potential variation in RNA loading or efficiency in the amplification reaction. Primers for LPL and TBP were designed according to previously published works [LPL upper primer, 5′-GAGATTTCTCTGTATGGCACC-3′, and lower primer, 5′-CTGCAAATGAGACACTTTCTC-3′ (20); TBP upper primer, 5′-CACCACAGCTCTTCCACTCA-3′, and lower primer, 5′-GCGGTACAATCCCAGAACTC-3′ (57)] and purchased from Cybergene AB (Novum Research Park, Huddinge, Sweden). Two microliters of cDNA diluted 1:4 was added to 18 μl of reaction mixture consisting of LightCycler FastStart reaction mix (10×; Taq DNA polymerase, reaction buffer, deoxynucleoside triphosphate mix, Fast Start Enzyme), $MgCl_2$ (LPL, 3 μM; TBP, 2 μM), and upper and lower primers at a final concentration of 0.5 μM each. Thermal cycling was initiated with denaturation at 95°C for 10 min followed by 40 amplification cycles including denaturation for 15 s at 95°C, annealing for 4 s at 58°C, and 1 s/25 bp elongation (LPL, 10 s; TBP, 5 s) at 72°C. Amplification was immediately followed by melting, 15 s at 95°C and 1 min at 63°C, and a stepwise temperature increase of 0.1°C/s to 95°C. This melting curve served as a specificity control. Finally, samples were cooled at 35°C for 30 s. The genes were analyzed with LightCycler Probe Design Software version 1.0 (Roche Diagnostics). A standard curve was obtained by serial template dilutions of cDNA from 1:1 to 1:64 for each gene. Relative quantification was carried out by calculation based on the crossing point values. Differences in RNA quality and quantity between samples were accounted for

using the ratio between target and TBP cDNA. Samples from LPL and TBP were separated on a 1.5% agarose/0.5× Tris-borate-EDTA gel containing ethidium bromide to ensure that the correct products were amplified.

Statistical analysis

To evaluate the data statistically, Student's *t*-test, repeated-measures ANOVA, and Dunnett's test were used, and the results are given as means ± SEM. Statistical significance was considered as $P < 0.05$.

RESULTS

Validation of the in vitro model

LPL activity in freshly obtained villous tissue was measured by a modified protocol used previously to measure LPL activity in isolated MVM vesicles (27, 55). Our procedure is similar to measurements done in freshly isolated adipose cells (58). It is well known that heparin binds to the heparan sulfate proteoglycans and thereby displaces LPL (38). Therefore, heparin was used to release LPL from the membrane surface of villous tissue, and subsequently, the ability of the released LPL to cleave oleic acid from trioleate was determined. In addition to trophoblast cells, the placenta contains a great quantity of macrophages that have been suggested to be responsible for a larger part of the LPL activity in the placenta (17). The role of the placental macrophage LPL is believed to be minimal but was not assessed in this study. Preliminary experiments to determine adequate amounts of villous tissue, sufficient time for heparin release, and optimization of the hydrolysis reaction and extractions were conducted but are not presented.

Assay efficiency

The oleic acid extraction efficiency was 77 ± 0.02% (n = 324 assay tubes), and the intra-assay coefficient of variation was 0.43 ± 0.03%. The interassay efficiency had a coefficient of variation of 6.87%.

hCG production and ATP content

To assess the viability of villous tissue after the 24 h incubation, we measured hCG production and tissue ATP content. hCG production from the syncytiotrophoblast reaches its peak near the end of the first trimester but remains at high concentrations in serum throughout pregnancy (59). We have shown previously that villous explants maintain their functional and morphological integrity in cell culture medium for at least 4 h (60). Also, placental explant cultures similar to ours have been shown to remain viable for at least 18 h in other studies (61–63). There was a significantly higher production of hCG (ng/mg wet weight/h tissue) by fragments when comparing the 1st h of incubation with the 6th and 24th h (n = 4; $P < 0.05$) (**Fig. 1A**). There was

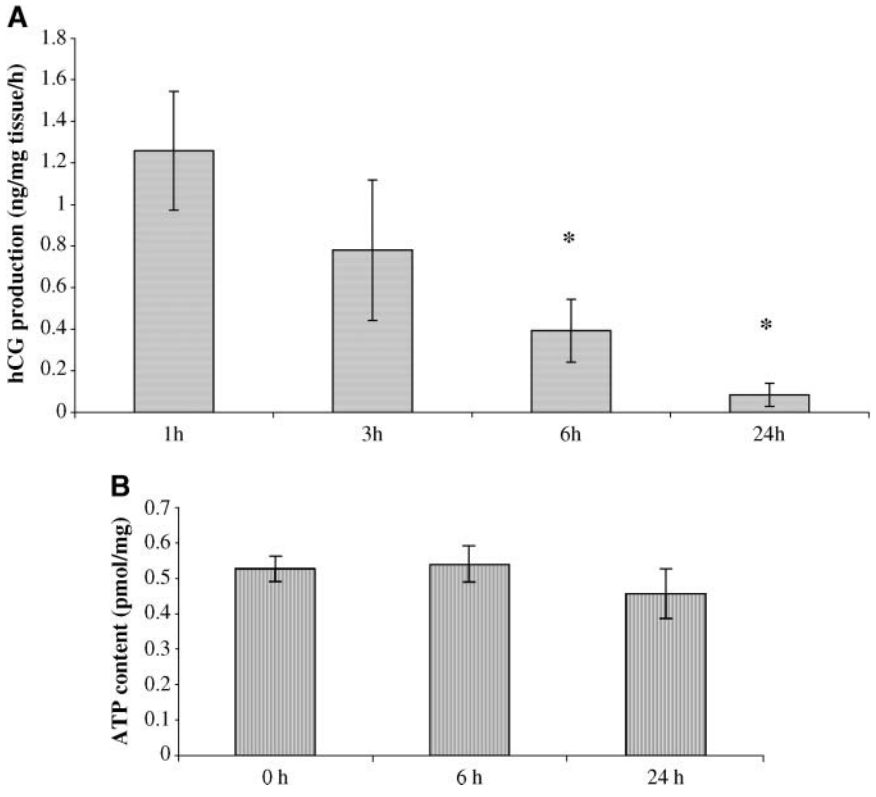

Fig. 1. Human chorionic gonadotropin (hCG) production (A) and ATP content (B) of placental villous fragments (n = 4) after incubation for up to 24 h in DMEM/Tyrode's medium at 37°C. One hour before each sampling, the culture medium was changed. * $P < 0.05$ compared with 1 h of incubation, by repeated-measures ANOVA. Results are given as means ± SEM.

no significant alteration in the ATP content (pmol/mg wet weight) of villous tissue when comparing the three time points (n = 4) (Fig. 1B).

In addition, control LPL activity levels did not change between 1 h (1.51 ± 0.18 pmol oleate/min/mg) and 24 h (1.92 ± 0.22 pmol oleate/min/mg). Together, these data indicate that the tissue remains viable under these conditions and gives support to the method used.

LPL activity and expression

Gestational changes. When analyzing first trimester LPL activity and mRNA expression, placentas before and after 10 gestational weeks were grouped. The LPL activity in term placental villous tissue (mean gestational age, 38 ± 0.1 weeks; n = 7) was 3-fold higher compared with that in both first trimester groups [mean gestational age for 8–9 weeks, 9.2 ± 0.3 weeks (n = 3); for 10–11 weeks, 10.5 ± 0.2 weeks (n = 3)] ($P < 0.05$ by ANOVA) (**Fig. 2A**). The human placental LPL protein was identified as primarily a 37 kDa band in MVM vesicles, and the expression in first trimester placentas (n = 2) appeared to be lower than that in normal term placentas (n = 2), although the difference was not quantified because of the low sample number (Fig. 2B).

When analyzing the expression of LPL mRNA in the first trimester by PCR, placentas at 6–9 weeks of gestation (mean gestational age, 8.1 ± 0.7 weeks; n = 4) and 10–13 weeks of gestation (mean gestational age, 11.6 ± 0.7 weeks; n = 4) were subgrouped. The mRNA expression was 3-fold higher in first trimester placentas at 10–13 weeks of gestation compared with both the term control group (n = 4) and the 6–9 weeks of gestation group ($P < 0.05$ by ANOVA) (Fig. 2C). Standard curves for the two genes (LPL and the housekeeping gene TBP) were obtained by plotting log dilution (x axis) against crosspoint values (y axis). The correlation factor for linear regression analysis for the genes was between 0.993 and 0.994. The PCR products were found as single bands as expected at 135 bp (TBP) and 225 bp (LPL).

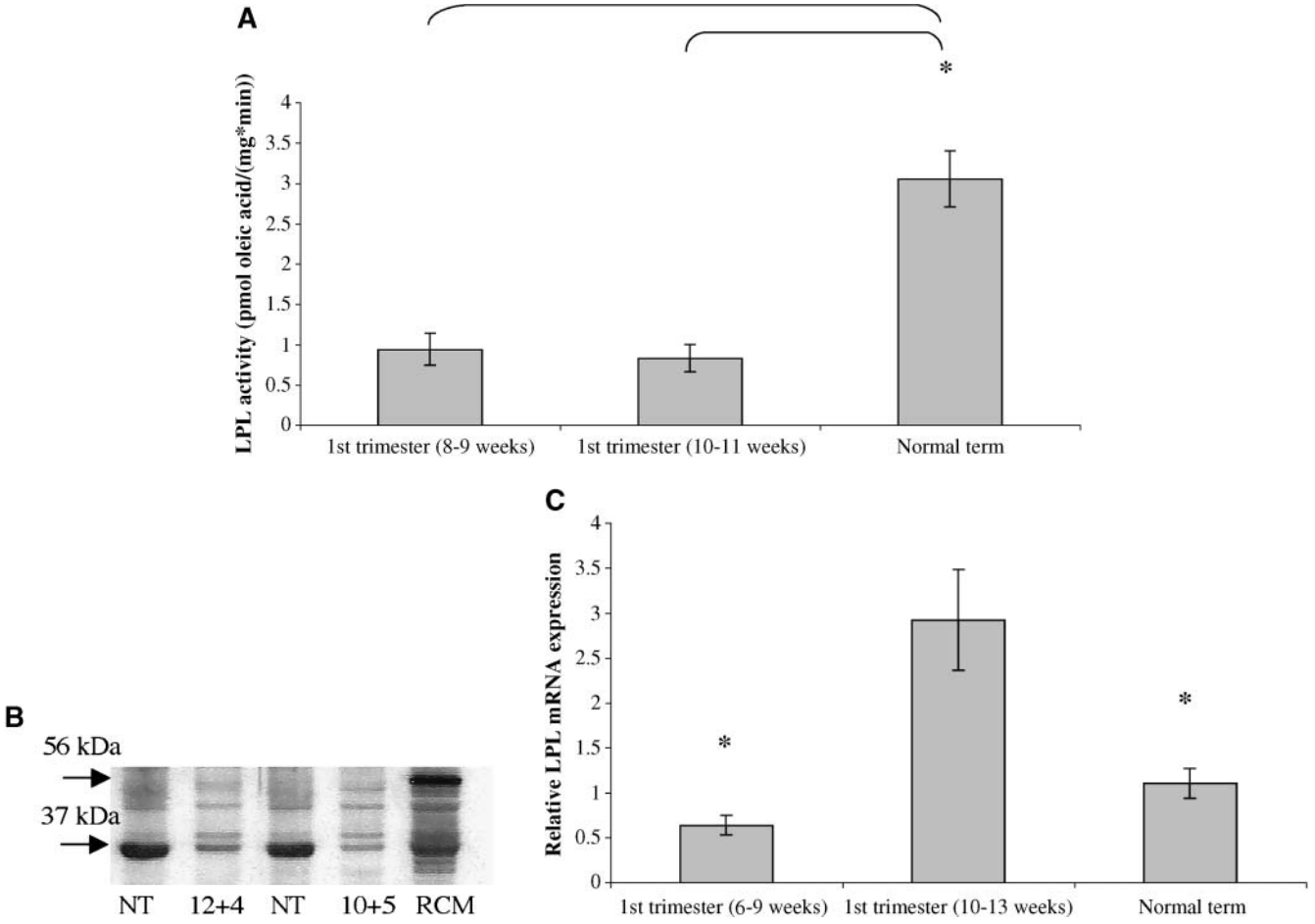

Fig. 2. A: LPL activity [pmol oleic acid/min/mg tissue (wet weight)] in placental villous fragments from first trimester and term pregnancies (n = 7). First trimester placentas are grouped as 8–9 (mean, 9.2 ± 0.3 weeks; n = 3) and 10–11 (mean, 10.5 ± 0.2 weeks; n = 3) gestational weeks. * $P < 0.05$ compared with both first trimester groups, by ANOVA. B: Protein expression of LPL in microvillous membrane (MVM) in first trimester (n = 2; 12+4 and 10+5 indicate gestational weeks + days) and normal term (NT; n = 2) pregnancies. Rat cardiac muscle (RCM) homogenate was used as a positive control. C: LPL mRNA expression in first trimester and term placentas. First trimester placentas are grouped as 6–9 (mean, 8.1 ± 0.7 weeks; n = 4) and 10–13 (mean, 11.6 ± 0.7 weeks; n = 4) gestational weeks. * $P < 0.05$ compared with the first trimester 10–13 week group, by ANOVA. Results are given as means ± SEM.

Hormone incubations. Incubation of villous tissue with GW 1929 (10 μg/ml), estradiol (1 μg/ml), insulin (290 ng/ml), cortisol (340 ng/ml), epinephrine (500 pg/ml), IGF-1 (340 ng/ml), and TNF-α (20 ng/ml) for 1 or 3 h did not result in any significant alterations in LPL activity using this explant model (data not shown). LPL activity decreased (n = 12; $P < 0.05$) when villous tissue was incubated for 24 h with four of the tested hormones: estradiol (−60%), insulin (−44%), cortisol (−36%), and IGF-1 (−36%) (**Fig. 3**).

In a separate set of experiments, we incubated villous tissue for 3 h with a range of doses of estradiol and studied the effect on LPL. We also tested the combined effect of altered glucose concentration with and without insulin, insulin alone, and cortisol. Incubation with estradiol (20 ng/ml) increased LPL activity by 70% (**Fig. 4A**) (n = 7; $P < 0.05$). A 60% increase in LPL activity was seen after incubation with insulin (145 ng/ml) together with hyperglycemic (15 mM) medium (n = 7; $P < 0.05$) (Fig. 4B). No change was seen after incubation with cortisol + 290 ng/ml insulin, hyperglycemic medium + 290 ng/ml insulin, or hypoglycemic (1.3 mM) medium (data not shown). No significant alterations in protein expression after 3 h of incubation were detected in any of the groups studied (data not shown).

L-FABP and C-FABP protein expression

As in our previous studies (27), the human placental L-FABP and C-FABP proteins were both identified at 15 kDa (data not shown). The term FABP expression data were arbitrarily assigned a density value of 1. No significant differences were detected in either L-FABP [term, 1.00 ± 0.16 (n = 4); first trimester, 1.03 ± 0.27 (n = 4)] or C-FABP [term, 1.00 ± 0.29 (n = 4); first trimester, 0.99 ± 0.29 (n = 4)].

DISCUSSION

In this study, we investigated changes in LPL activity and expression during normal gestation and whether placental LPL is hormonally regulated. To the best of our knowledge, this report represents the first study of LPL in villous explants, a model that may provide a useful procedure for the analysis of lipid metabolism in small amounts of placental villous tissue. Our results indicated a 3-fold higher LPL activity in term villous tissue compared with first trimester tissue, but with mRNA expression that is 3-fold lower in term compared with first trimester placentas at 10–13 weeks of gestation. We have demonstrated that treating villous tissue for 3 h with estradiol (20 ng/ml) or high glucose together with insulin increases LPL activity. In addition LPL activity was decreased by 24 h of incubation with estradiol (1 μg/ml), IGF-1, insulin, and cortisol.

Because of an enhanced maternal adipose tissue lipolysis and an estradiol-induced increase in lipoprotein concentration, maternal plasma TG concentrations are markedly increased in both humans and experimental animals in late gestation (10, 11). LPL activity in villous fragments immediately after delivery was 3-fold higher at term compared with during the first trimester. Examination of LPL protein expression in isolated MVM from term and first trimester samples correlated with activity by visual evaluation. However, relatively few placenta samples collected from first trimester terminations provide sufficient material for preparing plasma membranes; therefore, the sample size in the first trimester was too low to allow for statistical evaluation. We used isolated MVM rather than homogenates of tissue to ensure that we were detecting LPL on the membrane surface, where hydrolysis takes place, rather than total LPL expression in all placental cell types. Interestingly, we found that the LPL mRNA expression in the first trimester (10–13 gestational

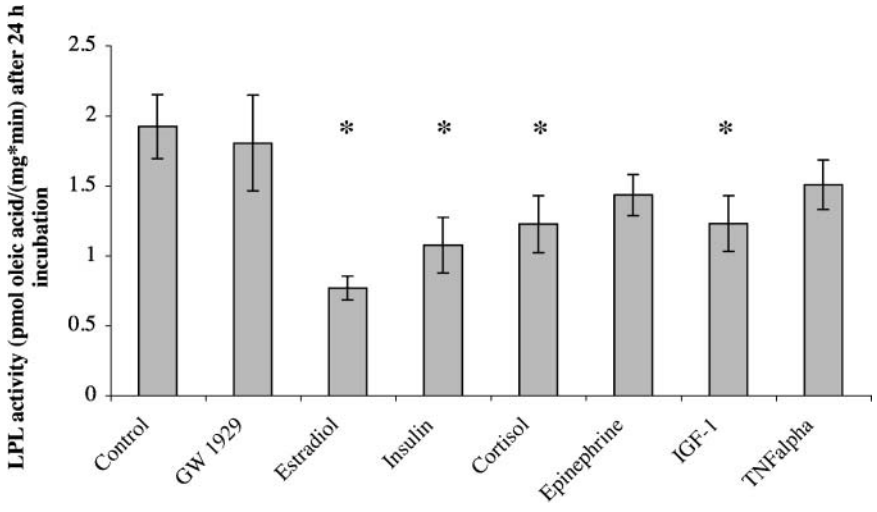

Fig. 3. LPL activity [pmol oleic acid/min/mg tissue (wet weight)] in placental villous fragments from term pregnancies after incubation with GW 1929 (10 μg/ml), estradiol (1 μg/ml), insulin (290 ng/ml), cortisol (340 ng/ml), epinephrine (500 pg/ml), insulin-like growth factor-1 (IGF-1; 250 ng/ml); or tumor necrosis factor-α (TNF-α; 20 ng/ml) for 24 h (n = 12). * $P < 0.05$ compared with the control group, by repeated-measures ANOVA. Results are given as means ± SEM.

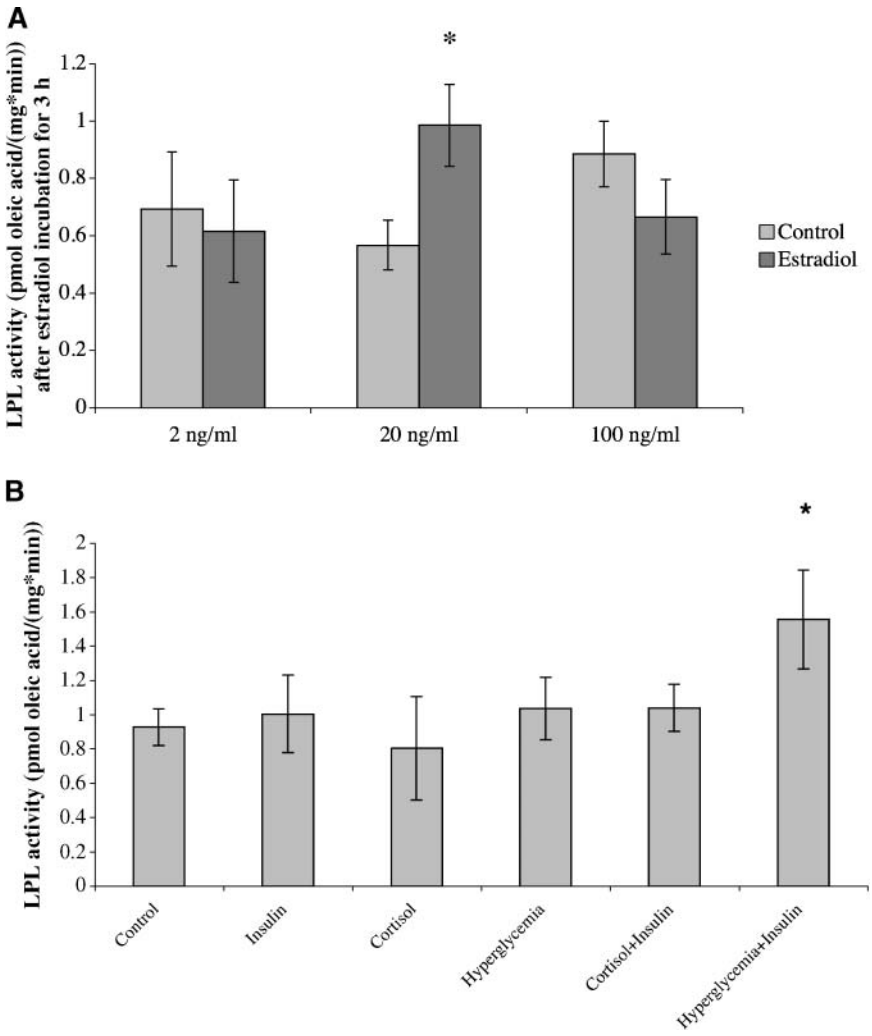

Fig. 4. A: LPL activity [pmol oleic acid/min/mg tissue (wet weight)] in placental villous fragments from term pregnancies after incubation with estradiol at concentrations of 2, 20, and 100 ng/ml for 3 h (n = 7). Controls contained a corresponding amount of solvent. * $P < 0.05$ compared with the control group, by *t*-test. B: LPL activity in placental villous fragments from term pregnancies after incubation with insulin (145 ng/ml), cortisol (340 ng/ml), hyperglycemic medium (15 mM), cortisol + insulin (145 ng/ml), or hyperglycemic medium + insulin (145 ng/ml) for 3 h (n = 7). * $P < 0.05$ compared with the control group, by repeated-measures ANOVA. Results are given as means ± SEM.

weeks) was 3-fold higher than in term tissue. Also, the mRNA expression in 6–9 week placentas was 3-fold lower than in 10–13 week placentas. The rationale for grouping the first trimester group was the increased blood flow that occurs at approximately week 10 in human pregnancy. The maternal arterial supply to the placenta has been shown to be extremely restricted until 10–12 weeks of gestation (64, 65). These two first trimester groups had a distinct 3-fold difference in mRNA expression, indicating that week 10 is critical for LPL mRNA production. The onset of maternal placental blood flow seems to upregulate mRNA expression, and macronutrients, hormones, and/or cytokines in the maternal plasma might be responsible for this upregulation. The discrepancy between the findings in first trimester LPL protein and mRNA expression could be explained by the fact that different tissue fractions were used. To measure protein expression, we used isolated MVM, whereas to measure mRNA, we used intact placental villous tissue. The placenta contains macrophages that produce a large amount of LPL (17). Consequently, part of the mRNA seen in the first trimester may be attributable to production by macrophages.

Maternal plasma estradiol increases 10-fold from the first trimester to term (11), indicating that the increase in placental LPL might be partly attributable to estradiol. Previous studies assessing total postheparin LPL activity have shown decreased maternal LPL activity in late gestation (10, 11). In pregnant rats, LPL activity in adipose tissue and liver decreases toward term and increases in placental and mammary gland tissue (10). Therefore, it would appear that the increases in mammary and placental tissue do not equal the decreases in adipose and liver, which results in a net decrease in total postheparin

LPL activity. These data, together with our current findings, indicate that placental LPL plays an important role in the redistribution of TGs in late pregnancy that facilitates the transfer of lipid to the fetus.

L-FABP and C-FABP are two cytosolic FABPs expressed in the syncytiotrophoblast, and we demonstrated previously that the protein expression of L-FABP is increased in gestational diabetes mellitus and IDDM pregnancies (27). In contrast to LPL activity and protein expression, we found no change in L-FABP and C-FABP protein expression between the first trimester and term. These findings underscore the role of LPL as an important factor controlling transplacental FFA transfer during human gestation.

We previously reported an increased placental LPL activity in large for gestational age babies of IDDM pregnancies and decreased LPL activity in placentas from preterm IUGR pregnancies but not term IUGR pregnancies (27), suggesting that placental LPL activity is related to fetal size and/or fat depots at birth. To test the hypothesis that maternal, fetal, and/or placental hormones may be involved in the regulation of LPL activity in the placenta and that this could alter lipid transport capacity in pregnancies with growth complications, we developed a convenient method for studying LPL activity and regulation in human villous tissue. Hormones tested in this study are known to be altered in IUGR and/or IDDM pregnancies in either fetal or maternal plasma or placenta. For example, estradiol in maternal plasma is lower in IUGR (66) and higher in IDDM (67) pregnancies. Insulin and IGF-1 concentrations in fetal plasma are lower in IUGR (68, 69) and higher in IDDM (70, 71) pregnancies. Cortisol and TNF-α levels are higher in placenta from IUGR pregnancies (72–74). The IUGR mother has an increased insulin sensitivity (75) and lower concentrations of IGF-1 (76). A change in LPL activity after incubation with any of these hormones could potentially explain the altered activity seen in IUGR and IDDM pregnancies. In addition, the hormones and effectors used in this study have been shown to alter LPL activity and/or expression in other tissues. Insulin is known to have stimulatory effects on LPL in adipose tissue (42), and LPL in adipose tissue and skeletal muscle is known to be regulated in a reciprocal manner, such as by fasting, feeding, and insulin (42). The PPARs (α, β, and γ) regulate lipid and glucose metabolism in many tissues and are all expressed in the human placenta (77). Epinephrine is a potential regulator in that it inhibits LPL activity in adipose tissue and stimulates LPL in skeletal muscle (42). Estrogen has a biphasic effect on LPL in adipose tissue, with high doses inhibiting and low doses stimulating LPL (78). IGF-1, cortisol, and TNF-α are known to have inhibitory effects on LPL in adipose tissue (49–51). In our experimental model, we found no change in LPL activity after 1 or 3 h of incubation with insulin alone, PPARγ ligand, TNF-α, cortisol, epinephrine, or IGF-1. With extended exposures, we found that LPL activity decreased after 24 h of incubation with estradiol (1 μg/ml), insulin, cortisol, and IGF-1. The effects of estradiol, insulin, cortisol, and IGF-1 are consistent with known effects in other tissues.

Estrogens increase over gestation and are suggested to be responsible for the enhancement in VLDL production during gestation (11). In our study, after 24 h of incubation, a high dose of estradiol inhibited LPL activity by 60%. The rationale for testing a high concentration was the fact that the majority of the newly synthesized estrogen comes from the placenta. Therefore, the levels of estrogen found in the intervillous space are likely to be many times higher than those found in the circulation. In addition, a biphasic response of LPL to estrogen has been seen in adipose tissue (78). Therefore, we were interested in demonstrating two aspects of LPL regulation: the potential for a biphasic response to a hormone, which can be quite high in the immediate proximity, and the fact that placental LPL has a regulatory pattern similar to that seen in adipose tissue. We continued to test lower doses of estradiol and found a 70% increase in LPL activity after 3 h of incubation with 20 ng/ml estradiol, which is a physiologically relevant concentration at term (11). Interestingly, in IUGR pregnancies at term, estradiol is reduced (66), and in IDDM pregnancies, estradiol is increased (67), in maternal plasma. We speculate that alterations in maternal plasma concentrations of estrogen in pregnancies complicated by altered fetal growth may affect placental LPL activity and fetal fat acquisition.

Total postheparin plasma LPL activity did not change with increased insulin alone in healthy nonobese men but decreased with high glucose alone and high glucose together with insulin (47), suggesting that LPL activity in the fed state is lower than in the postabsorptive state. Glucose has also been shown to increase the gene expression of LPL via PPARα in macrophages (79). We hypothesized that glucose alone or in combination with insulin could influence LPL activity in the placenta. Although insulin alone and in combination with cortisol did not alter LPL, we detected a 60% increase after 3 h of incubation with hyperglycemic and hyperinsulinemic medium. IDDM in pregnancy results in periods of both hyperglycemia (80) and hyperinsulinemia (80) in maternal and fetal plasma. We have shown previously that placental LPL activity is increased in MVM from IDDM mothers (27), suggesting a role for the glycemic state and insulin in regulating placental LPL. The fetus in IDDM pregnancies is often hyperinsulinemic and may contribute to the regulation of placental LPL activity. Interestingly, IUGR is a condition often associated with fetal hypoglycemia and hypoinsulinemia, in which the LPL activity in MVM was decreased (27). Therefore, it is possible that the altered placental LPL activity observed in these pregnancy complications is caused by changes in insulin and glucose concentrations.

In summary, we have developed a procedure in which small amounts of placental villous tissue can be analyzed for LPL activity at any stage of human gestation. We have demonstrated a marked increase in LPL activity from the first trimester to term that appears to correspond to changes in maternal TG and estradiol concentrations and may be important for supplying the fetus with increas-

ing amounts of lipid as gestation proceeds toward term. Placental LPL activity was increased by physiological concentrations of estradiol and by a combination of hyperglycemia and hyperinsulinemia. We speculate that these effectors contribute to the gestational increase in LPL activity and to the alterations in LPL activity seen in IUGR and IDDM.

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