Placental triglyceride accumulation in maternal type 1 diabetes is associated with increased lipase gene expression

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Abstract Maternal diabetes can cause fetal macrosomia and increased risk of obesity, diabetes, and cardiovascular disease in adulthood of the offspring. Although increased transplacental lipid transport could be involved, the impact of maternal type 1 diabetes on molecular mechanisms for lipid transport in placenta is largely unknown. To examine whether maternal type 1 diabetes affects placental lipid metabolism, we measured lipids and mRNA expression of lipase-encoding genes in placentas from women with type 1 diabetes (n = 27) and a control group (n = 21). The placental triglyceride (TG) concentration and mRNA expression of endothelial lipase (EL) and hormone-sensitive lipase (HSL) were increased in placentas from women with diabetes. The differences were more pronounced in women with diabetes and suboptimal metabolic control than in women with diabetes and good metabolic control. Placental mRNA expression of lipoprotein lipase and lysosomal lipase were similar in women with diabetes and the control group. Immunohistochemistry showed EL protein in syncytiotrophoblasts facing the maternal blood and endothelial cells facing the fetal blood in placentas from both normal women and women with diabetes.▪ These results suggest that maternal type 1 diabetes is associated with TG accumulation and increased EL and HSL gene expression in placenta and that optimal metabolic control reduces these effects.— Lindegaard, M. L. S., P. Damm, E. R. Mathiesen, and L. B. Nielsen. **Placental triglyceride accumulation in maternal type 1 diabetes is associated with increased lipase gene expression.** *J. Lipid Res.* 2006. **47:** 2581–2588.

Supplementary key words free fatty acids • endothelial lipase • hormone sensitive lipase

Patients with diabetes mellitus display increased levels of blood glucose and abnormal lipid metabolism (1). Moreover, maternal diabetes affects lipid metabolism in the offspring (2), and infants of mothers with diabetes often display dyslipidemia and macrosomia (3, 4). The Pedersen hypothesis suggests that fetal hyperinsulinemia (secondary to maternal hyperglycemia) causes fetal growth and results in macrosomia (5). However, infants of mothers with diabetes also have increased fat deposition, and several studies have supported the theory that increased lipid transport from the mother contributes to fetal macrosomia (6, 7). It has become increasingly clear that the intrauterine milieu in pregnant women with diabetes can have profound effects on their offspring later in life, with increased susceptibility to obesity, diabetes, and cardiovascular disease, all associated with dyslipidemia. This phenomenon is referred to as "fetal programming" (8, 9).

Lipid transport to the fetus across the placenta involves the active transport of FFAs. FFAs can be derived from FFAs bound to albumin or from lipoprotein-associated triglycerides (TGs) and phospholipids. Albumin-bound FFAs in the maternal blood are probably transferred directly to the placental membrane fatty acid binding protein. FFA release from lipoprotein-associated TGs and phospholipids requires lipase activity. Both LPL and endothelial lipase (EL) are highly expressed in placenta and have the capacity to hydrolyze lipoprotein TGs and phospholipids (10–12). In the cytosol of the syncytiotrophoblast, FFAs may be reesterified and deposited as TGs or phospholipids and hydrolyzed for later release into the fetal circulation (13). Lysosomal acid lipase (LAL) and hormone-sensitive lipase (HSL) are intracellular lipases that are capable of hydrolyzing TGs and cholesteryl esters; both are expressed in human placental tissue (14, 15).

Previous studies detected lipase activities in human placenta, and some have addressed the impact of maternal diabetes (16–18). However, the results have been divergent and the interpretation complicated by uncertainties about the identity of the lipase activities measured. Also in these studies, the numbers of patients with diabetes have

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Abbreviations: EL, endothelial lipase; HbA1c, hemoglobin A1c; HSL, hormone-sensitive lipase; LAL, lysosomal acid lipase; TG, triglyceride.
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been fairly small (n < 10), and no or very little information was given about the metabolic control of the mothers with diabetes during pregnancy.

Other groups have presented studies suggesting that diabetes might increase the expression of lipase genes (e.g., HSL and LPL) in various tissues (19–23). Thus, we hypothesized that maternal diabetes affects the expression of lipase genes in placenta. To test this idea and to examine whether variation in the expression of lipase genes might be associated with placental lipid accumulation, fetal plasma lipid concentration, or perhaps fetal weight, we examined placentas and fetal plasma lipids in a well-defined group of diabetic subjects and healthy controls.

MATERIALS AND METHODS

Collection of blood and tissue samples

Thirty-six women with type 1 diabetes and 24 healthy women (controls) were included in a prospective manner from the outpatient clinic at the Department of Obstetrics, Rigshospitalet, after having received verbal and written information on the study. All participating mothers gave informed written consent. All women visited the obstetric outpatient clinic at Rigshospitalet for prenatal care on a regular basis during their pregnancy. The women with diabetes were recruited during one of these visits at 34 gestational weeks. The women in the control group were either contacted and informed by phone at home or recruited while attending an information meeting before having an elective cesarean section.

All healthy pregnant women in our department are screened for gestational diabetes by a risk factor-based procedure (24). Exclusion criteria for this study were delivery before 34 weeks of gestation, diabetic nephropathy, preeclampsia, and other medically treated illnesses than diabetes. The study protocol was approved by the local ethics committee (Ref. No. KF 01-048/01). All baseline data of mother and child were obtained from medical records.

Maternal venous blood for lipid and hemoglobin A1c (HbA1c) measurements was drawn before elective delivery by induction of labor, elective cesarean section, or when entering the obstetric ward in spontaneous labor. The reason for collecting the blood samples at this time was to keep the discomfort for the women included in the study to a minimum. This way, in the majority of women with diabetes, the blood was drawn from an intravenous line established routinely, so they did not have to suffer the discomfort of an additional venous puncture. All measurements were determined after delivery. The umbilical cord was doubly clamped immediately after delivery, and venous blood was drawn into EDTA-containing tubes (1.5 mg/ml). The tubes were immediately placed on ice, centrifuged, and stored at −20°C for 1–3 days and then at −80°C until analysis. Transplacental biopsies (0.2–0.6 g) from the center (close to insertion of the umbilical cord), the periphery, and the part in between were snap-frozen in liquid nitrogen and stored at −80°C. From six patients, we only obtained placental biopsies from the middle part. Only samples that were frozen within 40 min after delivery were included. Placental biopsies from two women with type 1 diabetes and two normal women were fixed in 4% paraformaldehyde, pH 7.0 (Bie and Berntsen, Rodovre, Denmark), for immunohistochemistry. Twelve women were excluded from the study on the following grounds: from six women (five from the diabetes group and one from the control group), only fetal venous blood samples and no placenta samples were collected;

four placentas (three from the diabetes group and one from the control group) were collected after 40 min after delivery; in one woman with diabetes, glycosylated HbA1c was not measured; and one woman from the control group had a HbA1c value of 6.8%, which is well above the normal range for pregnant women (25). We did not find any correlation between lipase gene expression and time from placental birth to snap-freezing of samples.

Plasma and tissue biochemistry

Plasma total cholesterol and TGs, HDL, and LDL cholesterol were measured with a Modular Analytics module P (Roche, Hvidovre, Denmark) using enzymatic assays (cholesterol, catalog No. 11491458; HDL, catalog No. 03045935; LDL, catalog No. 03038777; TGs, catalog No. 11730711). HbA1c was measured by high-performance liquid chromatography (26). With this assay, the reference interval of a general population sample is 4.1–6.4%.

Placental lipids were extracted from biopsies taken from the part between the center and the periphery with chloroform-methanol (27). After washing and drying (28), lipids were re-dissolved in isopropanol with 1% Triton X (v/v) before quantification of cholesterol, TGs, and phospholipids in duplicate with enzymatic assays [cholesterol, CHOD-PAP (Roche); TGs, GPO-Trinder (No. 337-40A and 337-10B; Sigma, Vallensbæk Strand, Denmark); phospholipids, phospholipid B (Wako Chemicals GmbH, Neuss, Germany)]. The coefficients of variation of duplicate measurements were ≤3%.

mRNA quantification

Total RNA was isolated with TriZol (Invitrogen, Taastrup, Denmark). Each of three biopsies (from the center, middle, and periphery of the placenta) from one placenta was homogenized in 1 ml Trizol/100 mg frozen tissue. Equal amounts of the three homogenates were then pooled, and RNA isolation was carried out according to the manufacturer's protocol. The RNA integrity was assured with an RNA Name LabChip® (Agilent Technologies Denmark A/S, Naerum, Denmark), and RNA concentration was assessed from the absorbance at 260 nm. First-strand cDNA was synthesized from 1 µg of total RNA with Moloney murine leukemia virus reverse transcriptase (40 units; Roche A/S, Avedore, Denmark) and random hexamer primers in 1 µl reactions. The primers for EL (hEL-51, 5′-GTATGCAGGCAACTTCGTGA-3′; hEL-31, 5′-TGATCCCAAGACATCGTTGA-3′), LPL (hLPL-53, 5′-GAGATTTCTCTGTATGGCACC-3′; hLPL-33, 5′-CTGCAAATGA-GACACTTTCTC-3′), HSL (hHSL-51, 5′-GGAAGGCACTTTCT-GAGTGG-3′; hHSL-31, 5′-GTGATTCCGAGCACTGGTTT-3′), LAL (hLAL-51, 5′-CTGGTTTTGACGTGTGGATG-3′; hLAL-31, 5′-GCCTTGAGAATGACCCACAT-3′), and HL (hHL-51, 5′-TCCCCTGTGTTTCTCCATTC-3′; hHL-31, 5′-TAACGTGTCCG-GATGATTGA-3′) and β-actin (29) were obtained from Sigma-Genosys (Pampisford, UK). hEL-51 and hEL-31 span intron 4 in the human EL gene (*LIPG*), hLPL-53 and hLPL-33 span introns 7 and 8 in the human LPL gene (*LPL*), hLAL-51 and hLAL-31 span intron 4 in the human LAL gene (*LIPA*), hHL-53 and hHL-31 span intron 1 in the human HL gene (*LIPC*), and hHSL-51 and hHSL-31 are located in exon 1 of the human HSL gene (*LIPE*).

The specificity of each PCR was confirmed by DNA sequencing of the upper and lower strands of RT-PCR transcripts. Real-time PCR analyses were done with a LightCycler and a FAST START DNAmaster SYBR Green kit (Roche A/S). The PCRs (20 µl) contained 2 µl of SYBR Green I mixture, 2–3 mM MgCl$_2$, 10 pmol of each primer, cDNA synthesized from 2 ng of total RNA, and PCR-grade water. The relationship between the time point of the log-linear increase in fluorescence and the concentration of a mRNA transcript was determined in each run by analyzing the dilution series of cDNA made from 20, 2, 0.2, and 0.02 ng of total

TABLE 1. Baseline data of mothers with and without diabetes and their newborn infants

| | | Diabetes | |
| | Controls (n = 21) | Good Metabolic Control (n = 13) | Suboptimal Metabolic Control (n = 14) |
Variable			
Hemoglobin A1c (%)	5.4 ± 0.2	5.7 ± 0.3[a]	6.5 ± 0.3[b]
Prepregnancy body mass index (kg/m²)	23.4 ± 4	24.0 ± 4	24.5 ± 3
Gestational age at delivery (days)	272 ± 7	264 ± 7[a]	267 ± 8
Birth weight (g)	3,522 ± 389	3,530 ± 510	3,890 ± 321[b]
Length at birth (cm)	51.1 ± 2	51.4 ± 3	53.1 ± 2[b]
Placental weight (g)	732 ± 168	696 ± 190 (11)	750 ± 178 (13)
Delivery mode (V/VC/CSP/CSE)	5/1/15/0	5/1/2/5	5/2/3/4

V, vaginal delivery; VC, vacuum extraction; CSP, planned cesarean section; CSE, emergency cesarean section. Values are means ± SD. Results were analyzed with ANOVA followed by Dunnett's multiple comparisons test. Values in parentheses are n if different from the values indicated in the column heads.
[a] $P < 0.01$ compared with the control group.
[b] $P < 0.05$ compared with the control group.

RNA from a pool of human placentas. For genes that appeared to be differentially expressed in placentas from normal women and women with diabetes, the results were reassessed by analyzing all samples in separate runs. Coefficients of variation for the real-time PCR assays were assessed from variation between duplicate analyses or by measuring the same sample 4–12 times on each of 3 different days and were 4–11%. The mRNA copy numbers of EL, LPL, HSL, and LAL in human placenta were estimated by parallel real-time PCR analysis of placental cDNA and dilution series of purified PCR transcripts (30).

Immunohistochemistry

To raise an EL antiserum, a 21 amino acid peptide corresponding to the loop-shaped lid region of EL was used for immunization of randomly bred white Danish rabbits as described previously (10). Blood was collected from an ear vein, and the serum was stored at −20°C. For LPL immunohistochemistry, serum IgG from a rabbit that had been immunized with LPL purified from bovine milk (31) was isolated on a protein A column (IgG from rabbit 4, A_{280} = 0.79; a kind gift from Gunilla Olivecrona, Department of Medical Biosciences, Physiological Chemistry, Umeå University, Umeå, Sweden).

Placental biopsies were fixed for 12–16 h in 4% paraformaldehyde, pH 7.0 (Bie and Berntsen), embedded in paraffin, cut into 2–3 µm thick sections, mounted on SuperFrost®Plus slides, and baked at 60°C for 30 min. Paraffin was removed with xylene for 10 min, and the sections were rehydrated through a series of alcohol solutions before being treated with 1 mM citric acid, pH 6.0, at 100°C for 15 min and with 1% H_2O_2 for 10 min. After washing with distilled water, sections were incubated at room temperature with 5% porcine serum (No. P0399; DAKO, Glostrup, Denmark) in TBS (0.05 M Tris, 0.15 M NaCl, and 0.01% Triton X-100, pH 7.6) for 20 min, and EL antiserum (1:500) or anti-LPL IgG (1:1,000) was diluted with 5% porcine serum in TBS for 1 h before being washed three times for 5 min in TBS. Bound antibodies were visualized with the EnVision™+ System/HRP, Rabbit (DAB+) (Cytomation, No. K4011; DAKO). Finally, after washing in distilled water, sections were dehydrated through a series of alcohol solutions and mounted in Pertex (No. 00801; HistoLab Products Ab, Göteborg, Sweden).

Statistics

Data are presented as means with SD. Comparisons between groups were performed with ANOVA or the Kruskal-Wallis test. ANOVAs were followed by Dunnett's multiple comparisons test and/or the test for linear trend between mean and column number when appropriate.

RESULTS

Basic characteristics of the study groups

To study a homogenous group of women with diabetes, only women with type 1 diabetes and not with gestational or type 2 diabetes were included. The women with type 1 diabetes were divided into two groups according to their HbA1c level before delivery. Arbitrarily, women with a HbA1c below the median (6.2%) were considered in good metabolic control (5.2–6.1%) and women with HbA1c at or above the median were considered in suboptimal metabolic control (6.2–7.0%). In the control group, the HbA1c ranged from 5.0% to 5.9%. All pregnant women in our department are screened for gestational diabetes by a risk factor-based procedure (24). The gestational age was slightly lower, whereas the weight and length of the infants were greater, in the groups with diabetes than in the controls (**Table 1**).

TABLE 2. Plasma lipids in mothers with and without diabetes and their newborn infants

| | | Diabetes | |
Lipids	Controls (n = 21)	Good Metabolic Control (n = 13)	Suboptimal Metabolic Control (n = 14)
Maternal blood			
Total cholesterol (mmol/l)	7.2 ± 1.3	6.6 ± 1.6 (11)	6.6 ± 1.1 (11)
Total TGs (mmol/l)	2.8 ± 0.8	2.5 ± 0.7 (11)	2.6 ± 0.8 (11)
LDL cholesterol (mmol/l)	4.4 ± 1.2	4.2 ± 1.7 (11)	3.8 ± 0.8 (11)
Fetal cord blood			
Total cholesterol (mmol/l)	1.49 ± 0.4 (20)	1.83 ± 0.5 (11)	1.74 ± 0.4
Total TGs (mmol/l)	0.33 ± 0.1 (20)	0.49 ± 0.3 (11)	0.43 ± 0.4
Fetal TGs/maternal TGs	0.13 ± 0.06 (20)[a]	0.21 ± 0.07 (9)	0.19 ± 0.17 (11)

TG, triglyceride. Values are means ± SD. Results were analyzed with the Kruskal-Wallis test. Values in parentheses are n in the group when different from the total number in the study.
[a] $P < 0.05$.

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Maternal, fetal, and placental lipids

Maternal plasma total cholesterol, total TGs, and lipoprotein cholesterol concentrations were similar in the groups with diabetes and the control group (**Table 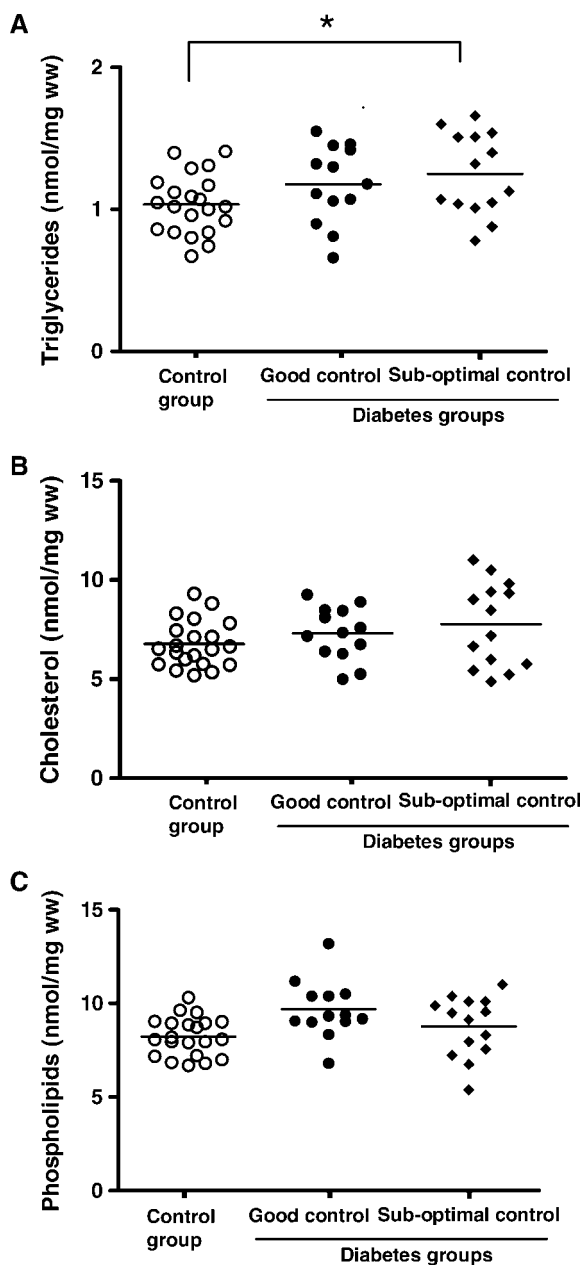2**). Also, fetal plasma total cholesterol and total TGs did not differ significantly when comparing the control group with each of the two groups with diabetes. Nevertheless, the ratio of fetal to maternal plasma TG concentrations was significantly higher in pregnancies of women with diabetes (Table 2).

Maternal diabetes had a significant effect on placental TG content, which increased with increasing HbA1c (**Fig. 1A**), but did not affect placental cholesterol or phospholipid concentrations (Fig. 1B, C).

Impact of maternal diabetes on the expression of lipase genes in placenta

Initially, we assessed the placental expression of the five major TG lipase genes by comparing their mRNA copy numbers in transplacental biopsies (one from the center of the placenta close to insertion of the umbilical cord, four from the periphery, and four from the region in between) from each of three normal placentas. There were no differences in the expression of any of the lipases between the sites where the biopsies were collected (data not shown). LAL had the highest mRNA copy number, followed by LPL, EL, and HSL. HL mRNA was not detectable in placenta (**Fig. 2**).

Maternal diabetes conferred an increased placental EL mRNA expression as well as HSL mRNA expression (**Fig. 3A, C**). EL mRNA expression was 26% higher ($P < 0.03$) and HSL mRNA expression was 41% higher ($P < 0.04$) in placentas from women with type 1 diabetes and suboptimal metabolic control compared with the controls. There was no effect of maternal diabetes on the placental expression of mRNA encoding either LPL (Fig. 3B) or LAL (Fig. 3D). However, we found a positive association between fetal plasma TG level and the expression of LPL mRNA in placenta when combining placentas from healthy women and women with diabetes ($r = 0.33$, $P < 0.03$) (**Fig. 4**). We did not find any correlations between lipase gene expression and infant birth weight or gestational age (data not shown).

Fig. 1. Impact of maternal type 1 diabetes on placental lipid accumulation. Lipids were extracted from placental biopsies collected from normal women (controls; n = 21) and women with type 1 diabetes with good (n = 13) or suboptimal (n = 14) metabolic control. Quantifications of triglycerides (TGs; A), cholesterol (B), and phospholipids (C) were made in all extracts. Each point represents the data from an individual placenta. Maternal diabetes had a significant effect on the placental TG content ($P < 0.05$), which increased with increasing hemoglobin A1c (HbA1c) (P trend < 0.02). Comparisons between groups were performed after ANOVA with Dunnett's multiple comparisons test (* $P < 0.05$).
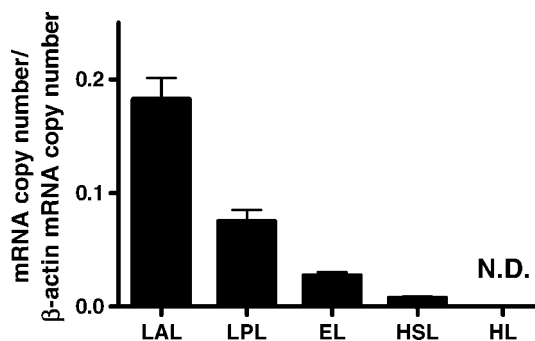

Fig. 2. mRNA copy numbers of lipases with TG lipase activity in human term placentas. Copy numbers were determined in transplacental biopsies (one from the center of the placenta close to insertion of the umbilical cord, four from the periphery, and four from the region in between) from three normal placentas. HL mRNA was not detectable (N.D.) in placenta. EL, endothelial lipase; HSL, hormone-sensitive lipase; LAL, lysosomal acid lipase.
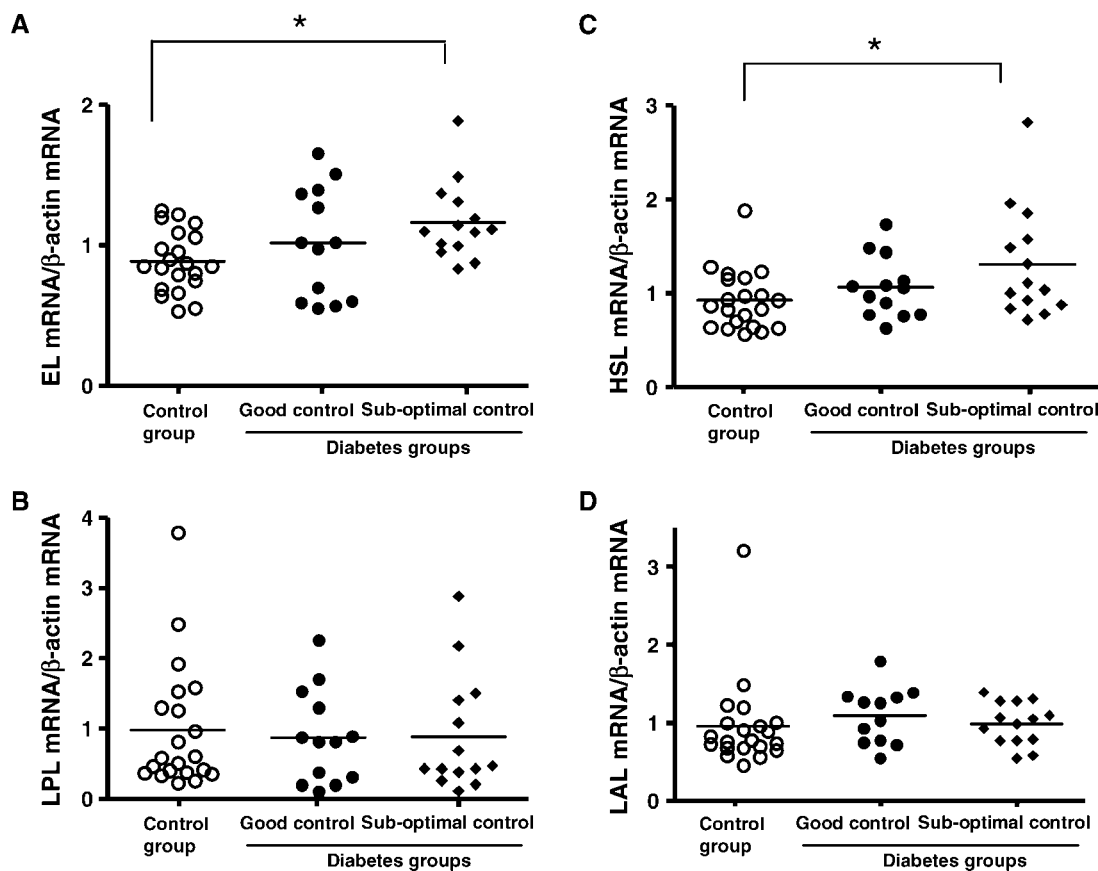
Fig. 3. Impact of maternal type 1 diabetes on mRNA expression of lipase genes in placenta. Gene expression of EL (A), LPL (B), HSL (C), and LAL (D) in placentas from normal women (n = 21) and women with type 1 diabetes with good (n = 13) or suboptimal (n = 14) metabolic control. mRNA was measured with real-time PCR in samples with equal amounts of tissue from the center of the placenta, the periphery, and the region between. In each sample, mRNA expression was normalized with β-actin mRNA content. Gene expression for an individual sample is expressed as the fraction of the average gene expression in all samples. Each point represents the data from an individual placenta. Maternal diabetes had a significant effect on the expression of EL ($P < 0.03$ by ANOVA) and HSL ($P < 0.04$ by ANOVA). Both EL and HSL mRNA expression increased with increasing HbA1c (P trend ≤ 0.01 for both EL and HSL). Comparisons between groups were performed after ANOVA with Dunnett's multiple comparisons test (* $P < 0.05$). For the LAL results, exclusion of the outlier in the control group did not change the overall results of the statistical test.

Protein localization of placental extracellular lipases

The cellular pattern of EL expression in placenta was examined with immunohistochemistry and compared with that of LPL. Both proteins were associated with syncytiotrophoblasts facing the maternal blood and endothelial cells facing the fetal blood. LPL was also seen in macrophage-like cells. The staining patterns of EL and LPL were similar in placentas from two normal women and two women with diabetes (**Fig. 5**).

DISCUSSION

Previous studies suggested that pregnancies of women with diabetes are associated with dyslipidemia not only in the mother but also in the fetus, at least when the maternal diabetes is poorly controlled (2). Thus, it has been suggested that fetal lipid accumulation is caused by an increased maternal-to-fetal lipid concentration gradient (32). During the last decade, it has become known that strict glycemic control before and during pregnancy is important to minimize the risk of fetal complications, including macrosomia (3). In this study, we examined mothers with type 1 diabetes that was fairly well controlled, as reflected by the relatively low HbA1c concentrations (5.2–7.0%) at the time of birth. Interestingly, although the mothers with diabetes had plasma lipid concentrations and placental weights that did not differ from those in the control group, the placental TG concentration and fetal-to-maternal ratio of plasma TGs were increased in pregnancies of women with diabetes. These results may reflect increased lipid uptake by the placenta and perhaps delivery to the fetus in mothers with diabetes, even though they are good glycemic control and have plasma lipid levels that are similar to those of controls. Of note, relatively high plasma LDL and TG concentrations, similar to those in the present study, have been discovered in pregnant women during late gestation (33). The finding of increased expression of EL and HSL mRNAs in placentas from the women with diabetes further supports the idea that maternal diabetes has direct effects on placental lipid metabolism.
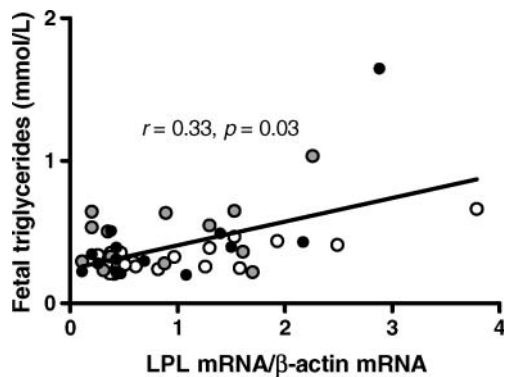
Fig. 4. Correlation of fetal TGs and LPL mRNA expression in placentas of normal and diabetic mothers (n = 48). r = 0.33, correlation coefficient of the Spearman rank test (P = 0.03). White circles, controls; gray circles, good metabolic control; black circles, suboptimal metabolic control.

EL is a member of the TG lipase family. Like the other members of this family, it works extracellularly and is associated with heparan sulfate proteoglycans in the lumen of the vessel, where it exerts phospholipase and TG lipase activities on plasma lipoproteins (34, 35). Its lipase activity results in the release of free fatty acids, which subsequently can be transported into cells and stored as TGs. EL may also "bridge" lipoproteins to the cell surface and mediate the uptake of lipoprotein lipids in a lipase-independent manner (34, 36). Thus, we speculate that increased placental EL expression in pregnancies of women with diabetes might enhance the supply of free fatty acids and/or TGs to the syncytiotrophoblasts and subsequently be responsible for the increased deposits of TGs, as detected in the placentas from women with diabetes. It should be noted that EL was present on both the maternal and fetal sides of the placenta in women with diabetes. There was no association on linear regression analysis between placental EL mRNA expression and fetal plasma HDL concentration. As such, our results cannot definitively establish whether increased EL expression will increase the uptake of fatty acids from the maternal or fetal circulation or both.

HSL is an intracellular lipase that hydrolyzes triacylglycerols, diacylglycerols, monoacylglycerols, and cholesteryl esters. Thus, if increased HSL mRNA expression results in increased HSL activity, the present finding of increased HSL expression in placentas from women with diabetes implies that maternal diabetes may result in an increased release of free fatty acids from intracellular TGs and a subsequent increased supply to the fetal circulation. Hence, increased HSL expression could be at least partly accountable for the increased ratio of fetal to maternal plasma TG concentrations we found in pregnancies of women with diabetes. We must emphasize, however, that our conclusions based on mRNA expression levels must be subject to reservations because we do not have matching protein levels to support the data.

Although previous studies have found that the HSL mRNA level in adipose tissue is increased in response to streptozotocin-induced diabetes in rats (23), we are not aware of reports on the regulation of EL gene expression attributable to metabolic disturbances. Because diabetes
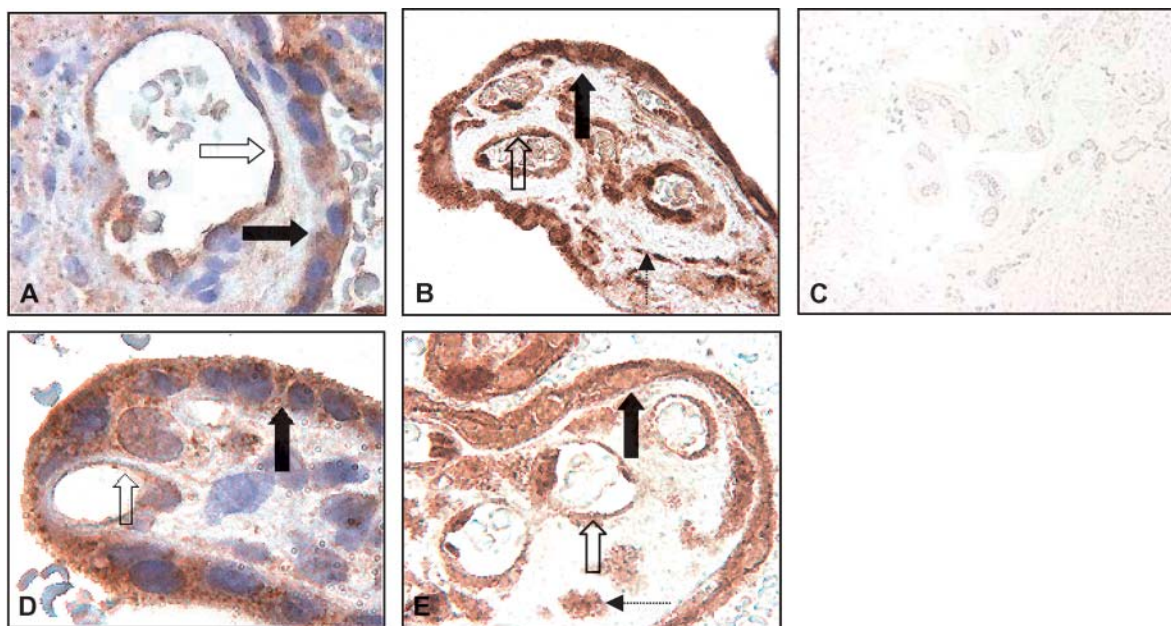

Fig. 5. Immunohistochemistry of EL and LPL protein in human normal placentas and placentas from women with diabetes. Sections from normal placentas (A, B) and placentas from women with diabetes (C–E) were incubated with an anti-EL antiserum (A, D; original magnification, ×63), anti-LPL antibodies (B, E; original magnification, ×40), or porcine serum as a control (C; original magnification, ×20). Black arrows point at syncytiotrophoblasts, white arrows point at endothelial cells, and dotted arrows point at macrophage-like cells. A, D: EL staining in endothelial cells and in syncytiotrophoblasts. B, E: LPL staining in syncytiotrophoblasts, endothelial cells, and macrophage-like cells.

has been associated with increased LPL expression in various tissues (19–22), we would not have been surprised to discover an upregulation of placental LPL mRNA expression, but this was not the case. Also, we did not find any difference between the groups in the expression of LAL mRNA or in secretory phospholipase A2 group IIa mRNA (data not shown). We cannot rule out an up-regulation of placental LPL gene expression in the setting of more severe diabetes. Indeed, a recent study reported increased LPL activity in placentas from women with type 1 diabetes (17). We recently found that the major part (~95%) of LPL protein in placenta is inactive and that only a small fraction appears to be active (10). In rats, feeding results in the conversion of inactive monomeric LPL to active dimeric LPL (37). Therefore, it is possible that maternal diabetes could result in the conversion of inactive to active LPL. Human postheparin plasma LPL activity and LPL activity in rat adipose tissue decrease late in gestation (38–40). Thus, LPL mRNA expression at parturition might not reflect LPL mRNA expression earlier in pregnancy. Of note, however, there was no correlation between placental LPL mRNA expression and gestational age in this study. Interestingly, there was a positive association between placental LPL mRNA expression and fetal plasma TG concentration, which might imply interactions between placental and fetal TG metabolism. Thus, it is conceivable that the dissociation of FFAs from TGs by placental LPL is important for delivering fat from the maternal to the fetal circulation.

Previous studies of placental lipases have focused mainly on placental lipase activities rather than on the expression of specific individual lipase genes or their products, and the relative contributions of the individual lipase gene products to total placental lipase activity are unknown. In this study, we compared gene copy numbers of five major TG lipases, of which four were expressed in placenta, albeit at different levels. Because the activities of the different lipases are difficult to separate and because the lipases work at different cellular locations in the placenta, it will be a challenge in the future to dissect the functional roles of the lipases in normal and pathological conditions. For this purpose, genetically modified animals may be helpful. For instance, we recently found an upregulation in EL expression in placentas of LPL-deficient mice, suggesting that the functions of LPL and EL may be at least partly redundant (10).

In conclusion, this study suggests that despite efforts to normalize blood glucose homeostasis during pregnancy, maternal type 1 diabetes changes the gene expression of placental TG lipases that work both extracellularly and intracellularly and increases the placental accumulation of TGs. These results indicate that maternal diabetes affects placental lipid metabolism and thus the transport of lipids from mother to fetus.■

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