High glucose levels reduce fatty acid oxidation and increase triglyceride accumulation in human placenta

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Abstract—Placentas of women with gestational diabetes mellitus (GDM) exhibit an altered lipid metabolism. The mechanism by which GDM is linked to alterations in placental lipid metabolism remains obscure. We hypothesized that high glucose levels reduce mitochondrial fatty acid oxidation (FAO) and increase triglyceride accumulation in human placenta. To test this hypothesis, we measured FAO, fatty acid esterification, de novo fatty acid synthesis, triglyceride levels, and carnitine palmitoyltransferase I activities (CPT) in placental explants of women with GDM or no pregnancy complication. In women with GDM, FAO was reduced by ~30% without change in mitochondrial content, and triglyceride content was threefold higher than in the control group. Likewise, in placental explants of women with no complications, high glucose levels reduced FAO by ~20%, and esterification increased linearly with increasing fatty acid concentrations. However, de novo fatty acid synthesis remained unchanged between high and low glucose levels. In addition, high glucose levels increased triglyceride content approximately twofold compared with low glucose levels. Furthermore, etomoxir-mediated inhibition of FAO enhanced esterification capacity by ~40% and elevated triglyceride content 1.5-fold in placental explants of women, with no complications. Finally, high glucose levels reduced CPT I activity by ~70% and phosphorylation levels of acetyl-CoA carboxylase by ~25% in placental explants of women, with no complications. We reveal an unrecognized regulatory mechanism on placental fatty acid metabolism by which high glucose levels reduce mitochondrial FAO through inhibition of CPT I, shifting flux of fatty acids away from oxidation toward the esterification pathway, leading to accumulation of placental triglycerides.

carnitine palmitoyltransferase I; de novo fatty acid synthesis; esterification of fatty acids; fatty acid oxidation; gestational diabetes mellitus; hyperglycemia; placenta; triglycerides

Pregnancies affected by gestational diabetes mellitus (GDM) are characterized by various degrees of maternal glucose intolerance, hyperglycemia, and hyperinsulinemia (6). Several epidemiological studies have shown that GDM is independently associated with adverse perinatal outcomes (9, 36, 42). The main adverse outcome of maternal diabetes is fetal macrosomia, which is characterized by fetal fat accretion and overgrowth (27, 42). The HAPO (Hyperglycemia and Adverse Pregnancy Outcome) Study Cooperative Research Group has demonstrated an association between maternal hyperglycemia and fetal macrosomia (18, 26), suggesting that maternal hyperglycemia is a contributing factor to fetal macrosomia by enhancing substrate availability to the fetus, stimulating excessive growth and formation of adipose tissue (13, 34).

The underlying mechanisms by which maternal hyperglycemia translates into fetal adiposity are incompletely understood. In 1954, Pedersen (31) proposed that maternal hyperglycemia results in augmented transplacental glucose transfer leading to hyperglycemia in the fetus, which stimulates the production and secretion of insulin by the fetal pancreatic β-cells. Hence, glucose surplus and hyperinsulinemia would play a direct role in the accumulation of fat in fetal adipose tissue (30, 31). However, Szabo and Szabo (41) proposed a different hypothesis to explain fetal macrosomia in diabetic women. The hypothesis postulates that high maternal plasma nonesterified fatty acid levels (NEFA), secondary to maternal insulin resistance, lead to increased transplacental transfer of NEFAs to the fetus, which are subsequently transported to fetal adipocytes and esterified into triglycerides. In this scenario, maternal hyperglycemia does not contribute directly to fetal fat accretion in the form of energy oversupply, but rather, maternal glucose is used as a source of the glycerol necessary for NEFA esterification. Several clinical studies have reinforced the idea that elevated maternal plasma triglyceride levels may account for fetal fat accretion (15, 22, 23, 28, 38).

Recently, it has been shown that placental lipid metabolism is altered in placentas from diabetic women (16, 25, 34, 35). These findings have prompted the notion that placental lipid metabolism may represent a regulatory step toward fetal macrosomia (14, 34, 39, 41). In this study, we aimed to further understand the role of maternal hyperglycemia on the regulation of placental lipid metabolism. To this end, we tested the hypothesis that high-glucose levels inhibit placental fatty acid oxidation, leading to enhanced NEFA esterification and accumulation of placental triglycerides.

Methods

Study subjects. The study was performed on placentas from pregnancies monitored at the Department of Obstetrics and Gynecology, University Hospital “Puerta del Mar” (HUPM). Patient samples were obtained after written informed consent was obtained in accordance with the HUPM Ethics Committee requirements and the Declaration of Helsinki. The HUPM Ethics Committee approved our study. Patients were eligible among consecutive pregnant women attending
our antenatal clinic who planned to deliver by an elective Caesarean section because of clinical reasons other than diabetes and potentially not affecting placental metabolism (breech presentation or prior Caesarean section). This was to rule out potential effects of labor on placental energy metabolism. Specific exclusion criteria included women under the age of 18, smokers, or those with a history of long-chain 3-hydroxyacyl-CoA deficiency, hemolysis elevated liver function syndrome or acute fatty liver of pregnancy, preeclampsia, chronic hypertension, or other comorbid disease. The diabetic group was composed of eight gestational diabetic women. Only cases needing insulin therapy for metabolic control were eligible and offered to participate in the study to include only cases with clear metabolic impairment. Maternal diabetes mellitus was defined as an abnormal glucose tolerance according the criteria defined by the National Diabetes Data Group (1) that have been accepted by the Spanish Group of Diabetes in Pregnancy (11). Screening was performed using a two-step approach in pregnant women between 24 and 28 wk of gestation. The initial screening procedure consisted of a 50-g glucose challenge test, with a 1-h blood glucose cutoff set at \( \geq 7.76 \) mmol/l. Women with a positive screening test underwent a confirmatory 3-h, 100-g oral glucose tolerance test (fasting glucose: \( \geq 5.82 \) mmol/l; 1 h, \( \geq 10.54 \) mmol/l; 2 h, \( \geq 9.15 \) mmol/l; and 3 h, \( \geq 8.04 \) mmol/l). Gestational diabetes mellitus was defined when two or more plasma glucose measurements were equal or higher than the cutoff points. Insulin therapy was indicated if more than one-third of capillary peripheral glucose measurements were higher than the targets (\( >5.27 \) mmol/l fasting, \( >5.82 \) mmol/l preprandial and \( >7.76 \) mmol/l 1 h postprandial). In total, 14 women with no pregnancy complication participated in the control group. Randomly chosen subsets of either six or eight controls were used for the experiments, as indicated in the figure legends. Demographics and baseline data, as well as perinatal variables, are shown in Table 1. All Caesarean sections were performed at term. Placental samples and fasting maternal blood samples from the control and GDM groups were obtained at the time of the elective Caesarean section. At this time, no significant differences were found in lipids, glycemia, or insulinemia levels. Neonatal anthropometric measurements were performed immediately after delivery as usual. Fetuses of women with GDM showed a slight tendency to have higher birth weight, and placental weight was significantly higher in this group.

**Biochemical parameters.** All biochemical parameters were analyzed at the Clinical laboratory, HUPM, using reagents and modular systems from Roche Diagnostics. Plasma insulin was measured by electrochemiluminescence immunoassay by E-170, using 20 \( \mu l \) of sample. Plasma glucose, triglycerides, total cholesterol, and HDL cholesterol were measured by standard enzymatic methods by C-711, using between 2 and 3 \( \mu l \) of sample. LDL cholesterol was calculated using the Friedewald-Fredrickson formula.

**Placental explants culture.** Term placenta obtained from elective Caesarean section was placed on ice and arrived to the laboratory within 10–15 min of delivery. Then, decidual tissue and large vessels were removed from villous placenta by blunt dissection on aseptic culture conditions. Afterward, small fragments of villous tissues (\( \leq 100 \) mg wet wt) were rinsed twice in cold PBS, and six explants were transferred to each well of a six-well plate containing 2 ml of culture medium [RPMI-1640 supplemented with 5 mmol/l glucose, 10% FBS (vol/vol), 100 U/ml penicillin G, and 100 \( \mu l \) of streptomycin] and maintained at 37°C in a humidified atmosphere of 5% CO\(_2\)-95% O\(_2\) for 1 h prior to the experiments. Villous explant viability and morphological integrity were assessed by XTT (XTT kit; Roche) and hematoxylin and eosin staining, respectively.

Materials. Cell culture reagents (RPMI-1640 medium without glucose and fetal bovine serum) were from Invitrogen-Gibco. The [9,10-\(^{3}H\)palmitic acid, \( \mathrm{[^{3}H]H}_{2} \mathrm{O}, \mathrm{n-[^{14}C]} \mathrm{glucose}, \) and \( 1-\mathrm{N}-\mathrm{methyl-}^{[14]} \mathrm{carnitine-HCl} \) were from PerkinElmer. Etomoxir and essentially fatty acid-free bovine serum albumin were from Sigma (St. Louis, MO).

**Fatty acid solution preparation.** Stock of fatty acid solution was prepared by conjugating palmitate with essentially fatty acid-free bovine serum albumin (BSA) to generate a stock solution of 25% (wt/vol) BSA and 4 mmol/l palmitate in glucose-free culture medium. Stock solution was filtered/sterilized and diluted into the final culture medium to give concentrations of 1.25% BSA and 0.1 or 0.2 mmol/l palmitate.

**Fatty acid oxidation assay in placental explants.** Mitochondrial fatty acid oxidation (FAO) assays were performed ex vivo in placental explants, as described previously (2, 32), with the following modifications. Freshly isolated villous explants were incubated in culture medium supplemented with low (5 mmol/l) or high (11 mmol/l) glucose concentrations and in the presence of 1.25% BSA, 0.1 mmol/l cold palmitate, and 18,500 Bq/ml \( \mathrm{[^{3}H]} \) palmitate at 37°C for 18 h. The glucose concentration in culture medium for the experiments in which glucose was not an experimental factor was 5 mmol/l. Glucose was added to medium from a sterile stock solution of 1 mol/l glucose. At the end of the incubation period, the medium was collected and tritiated water determined by the vapor phase equilibration method of Hughes et al. (21). FAO was defined as nanomoles of palmitate per milligram of tissue per hour.

**Esterification into total lipids in placental explants.** The esterification rate in placental explants was determined as described previously, with some modifications (5). Briefly, after incubation conditions similar to those used for measurements of \( \beta \)-oxidation, with low or high glucose levels in the presence of 1.25% BSA, 0.1 mmol/l cold palmitate, and 18,500 Bq/ml \( \mathrm{[^{3}H]} \) palmitate for 18 h, explants were washed three times with 2 ml of ice-cold PBS and homogenized in 500 \( \mu l \) of PBS. An aliquot of 100 \( \mu l \) was used to extract the lipid content from samples according to Bligh and Dyer (3). Afterward, the radioactive content was determined by liquid scintillation counting. Esterification was defined as nanomole of palmitate per milligram of tissue per hour.

**De novo lipid synthesis in placental explants.** De novo lipid synthesis was determined using \([^{14}C] \) glucose according to the procedure described by Brown et al. (5), with some modifications. Villous placental explants from the control group were incubated in RPMI-1640 culture media with low and high glucose levels (5 and 11 mmol/l, respectively) and 37,000 Bq/ml \( \mathrm{[^{14}C]} \) glucose at 37°C for 18 h. At the end of the incubation period, culture media were discarded and explants collected and rinsed three times with 2 ml of ice-cold PBS, followed by homogenization in 500 \( \mu l \) of PBS. After a total lipid extraction (as described for measurements of placental esterification.
rate), the radioactive content was determined. De novo lipid synthesis is expressed as picomoles per milligrams of tissue per hour.

Placental triglyceride determination. Placental triglyceride determination was as described previously (33). Frozen placental explants from control and GDM group (~20 mg) were used for experiments shown in Fig. 1. For the rest of the experiments, placental explants were preincubated in low or high glucose in the presence of 0.1 mmol/l palmitate for 18 h, as described above. Tissues were homogenized in 400 μl of HPLC-grade acetone. After incubation with agitation at room temperature overnight, aliquots of 5 μl of acetyl-extracted lipid suspension were used to determine triglyceride concentrations, using a triglyceride reagent kit (Biosystems, Barcelona, Spain). Proteins were quantified using the bicinchoninic acid method (Thermo Scientific, Madrid, Spain). Placental lipid content was defined as milligrams of triglyceride per milligrams of total placental proteins.

Western blot analysis. Placental explants from the control group were preincubated in RPMI-1640 culture media containing low or high glucose levels for 18 h. At the end of the incubation period, culture media were discarded and explants collected and washed with ice-cold PBS, followed by homogenization in lysis buffer [20 mmol/l Tris-HCl, pH 7.5, 150 mmol/l NaCl, 1 mmol/l EDTA, 1 mmol/l EGTA, 1% (vol/vol) Triton X-100, 2.5 mmol/l sodium pyrophosphate, 1 mmol/l β-glycerophosphate, 1 mmol/l Na3VO4, 1 μg/ml leupeptin, and 1 mmol/l phenylmethylsulfonyl fluoride] plus protease inhibitors (Protease Inhibitor Cocktail; Sigma). After 10 min on ice, extracts were sonicated and centrifuged at 18,000 g for 10 min at 4°C. Pellets were discarded, and solubilized proteins (40–60 μg/sample) were resolved by 5% SDS-PAGE for phospho-acetyl-CoA carboxylase (p-ACC) and 10% SDS-PAGE for actin and electrotransferred onto polyvinylidene difluoride filters for immunoblotting by conventional means. After probing with specific p-ACC antibody (1:1,000; Cell Signaling Technology, Barcelona, Spain), the membranes were stripped and reprobed with antibody against actin (1: 3,000; Sigma). Signals were detected by chemiluminescence (Immuno-Start Western Chemiluminescence Kit; Bio-Rad, Madrid, Spain), and band densitometry was quantified with Image J software (National Institutes of Health).

Mitochondrial citrate synthase assay. As an index of mitochondrial content, citrate synthase activity was measured using the Citrate Synthase Assay Kit (Sigma) according to the manufacturer’s instructions in placenta from the control and GDM groups. Protein content was determined as above. Citrate synthase activity was defined as nanomoles per milligram per minute.

Carnitine palmitoyltransferase assay. Activities of carnitine palmitoyltransferase I (CPT I) and carnitine palmitoyltransferase II (CPT II) were determined in the direction of acyl-carnitine formation, with [14C]carnitine used as substrate (4). Briefly, placental explants were preincubated in RPMI-1640 culture medium containing low or high glucose levels at 37°C for 18 h. At the end of the incubation period, culture media were discarded and explants collected and washed with ice-cold PBS prior to homogenization in lysis buffer (5 mmol/l Tris-HCl, pH 7.2, and 150 mmol/l KCl) with a glass homogenizer. For assay of CPT I, 100 μl of cell homogenate, in which the mitochondria remain largely intact, was incubated in the presence of 50 μmol/l palmitoyl-CoA, 500 μmol/l carnitine, and 9,250 Bq/ml [14C]carnitine in a 30°C shaking water bath for 10 min. For assay of CPT II, a portion of the homogeneate was adjusted to 1% (wt/vol) of the detergent octylglucoside, which solubilizes the mitochondrial membranes, inactivating CPT I and releasing CPT II from the mitochondrial matrix in active form. Afterward, reactions were stopped by adding 500 μl of 1.2 N HCl, and palmitoyl-[14C]carnitine was extracted by adding 500 μl of l-butanol. Radioactive content was determined by liquid scintillation counting.

Statistical analysis. Statistical analysis of data was performed using the SPSS software (SPSS, Chicago, IL). Distributions were checked with a histogram and the Kolmogorov-Smirnov test. When a variable was distributed normally, data were presented as means ± SD. In cases of nonnormal distribution, data were shown as median and interquartile range. Comparisons were done by using the Mann-Whitney U-test or ANOVA. Differences were considered significant at *P < 0.05.

RESULTS

Reduced fatty acid oxidation and elevated triglyceride levels in placentas from women with gestational diabetes. To reveal the metabolic characteristics of placentas from women with GDM, we determined the FAO capacity in placental explants from control and diabetic women. As shown in Fig. 1A, FAO was reduced by ~30% in placentas of women with gestational diabetes compared with the control group. A reduction in FAO capacity could be explained by a lower mitochondrial number in the GDM group. However, as assessed by citrate synthase activity, mitochondrial content was similar between placental explants from control and diabetic women, suggesting that the molecular mechanism underlying reduced FAO capacity in the
diabetic group may be related to other factors rather than to mitochondrial number (Fig. 1B). Coinciding with reduced FAO, triglyceride levels in the GDM group were threefold higher compared with control group (Fig. 1C). Taken together, these results indicate an association between reduced FAO capacity and accumulation of triglycerides in placentas from diabetic women.

**Effect of high glucose levels on fatty acid oxidation and triglyceride levels in explants of human placenta.** Maternal hyperglycemia is a hallmark of women with gestational diabetes. Therefore, it is reasonable to hypothesize that the impaired ability of placentas from women with GDM to oxidize fatty acids is a direct consequence of placental glucose surplus environment, leading to accumulation of placental triglycerides. To test this hypothesis, we measured the effect of low or high glucose levels on FAO in placental explants from the control group. As shown in Fig. 2A, high glucose levels reduced the FAO rate significantly in placental explants. In parallel, high glucose levels enhanced fatty acid esterification in the presence of 0.1 and 0.2 mmol/l palmitate (Fig. 2B). Likewise, esterification was augmented at increasing concentrations of palmitate from 0.1 to 0.2 mmol/l (Fig. 2B). However, de novo fatty acid synthesis using [14C]glucose as a carbon source remained unchanged (Fig. 2C). Similar findings for de novo fatty acid synthesis were found, using [14C]acetate as carbon source (data not shown). High glucose levels significantly increased the placental triglyceride content, approximately twofold (Fig. 2D), consistent with the expectation that fatty acids are preferentially directed toward esterification under that condition. Taken together, these data indicate that high glucose levels alter the placental triglyceride content through inhibition of FAO.

**Etomoxir-mediated inhibition of fatty acid oxidation increases triglyceride accumulation in placental explants.** To gain further insight into the molecular mechanism by which high glucose levels alter placental fatty acid partitioning, we used etomoxir, a specific and irreversible inhibitor of CPT I, to evaluate the impact of inhibition of mitochondrial fatty acid entry on FAO, fatty acid esterification, and the storage pool of triglycerides in placenta from healthy women. Etomoxir treatment significantly inhibited FAO capacity in placental explants (Fig. 3A), resulting in augmented esteri-

![Placental TG content](image)

**Fig. 2.** High glucose levels inhibit fatty acid oxidation in placentas from healthy women. A: effect of high glucose levels on fatty acid oxidation. A subset of 6 placentas from women with no pregnancy complication (as described in Table 1) was used to obtain villous explants. Placental explants from control group were incubated at 5 or 11 mmol/l glucose (Gl) in the presence of 0.1 mmol/l palmitate (Pa) for 18 h. Afterward, [3H]water was determined as described in METHODS. Means ± SD for 6 independent experiments in triplicate. Significance is indicated (*P < 0.05) relative to 5 mmol/l Gl. B: effect of high glucose levels on de novo lipid synthesis. A subset of 4 placentas from women with no pregnancy complication (as described in Table 1) was used to measure the esterification capacity. Placental explants from control group were incubated at 5 or 11 mmol/l Gl in the presence of 0.1 or 0.2 mmol/l Pa for 18 h. Afterward, [3H]palmitate incorporation into total lipids was determined as described in METHODS. Mean ± SD for 6 independent experiments in triplicate. *P < 0.05 relative to 5 mmol/l Gl/0.1 Pa. †P < 0.05 relative to 0.1 mmol/l Pa. C: effect of high glucose levels on de novo lipid synthesis. A subset of 6 placentas from women with no pregnancy complication (as described in Table 1) was used to measure the esterification capacity. Placental explants from control group were incubated at 5 or 11 mmol/l Gl in the presence of 0.1 or 0.2 mmol/l Pa for 18 h. Afterward, [3H]palmitate incorporation into total lipids was determined as described in METHODS. Mean ± SD for 4 independent experiments in triplicate. D: effect of high glucose levels on placental TG content. The same subset of placentas used for fatty acid oxidation and esterification experiments described above was used to measure TG content. Placental explants were incubated as described above, and the TG content was determined as described in METHODS. Mean ± SD for 5 independent experiments in triplicate. *P < 0.05 relative to 5 mmol/l Gl.
High glucose levels decrease CPT I activity in placental explants. We further investigated the mechanisms by which high glucose reduced FAO capacity in human placental explants. To this end, we measured the activity of CPT I and CPT II in placental explants from the control group preincubated in low or high glucose levels for 18 h. As shown in Fig. 4, A and B, high glucose levels reduced the activity of CPT I by ~70%, whereas CPT II activity remained unchanged as expected. Because malonyl-CoA is a physiological regulator of CPT I activity, we quantified the phosphorylation levels of ACC, the enzyme that catalyzes the ATP-dependent carboxylation of acetyl-CoA to form malonyl-CoA. Interestingly, phosphorylation levels of ACC were reduced by ~25% in the presence of high glucose levels (Fig. 4C), suggesting an increased production of malonyl-CoA in placental explants.

### DISCUSSION

The availability of maternal nutrients to the fetus is regulated by the placenta involving three main mechanisms: direct transfer of nutrients, placental consumption of nutrients, and placental conversion of nutrients into alternative fuel sources (19). Direct transfer has been considered the main mechanism by which placenta regulates the nutrient exchange between the mother and the fetus (19). However, the placenta exhibits a high metabolic activity that is severely affected by the intrauterine milieu of diabetic and/or obese women. Specifically, studies performed on placentas from diabetic women have shown that major changes in expression levels of genes involved upregulation of pathways of lipid synthesis and transplacental lipid fluxes (16, 25, 34, 35). These findings have spurred the notion that alterations in placental lipid pathways perhaps contribute to fetal fat accumulation and adiposity in diabetic women (8, 34).

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**Fig. 3.** Etomoxir (ETX)-dependent inhibition of fatty acid oxidation increases TG accumulation in placentas from healthy women. **A:** fatty acid oxidation in placental explants treated with various concentrations of ETX. A subset of 6 placentas from women with no pregnancy complication (as described in Table 1) was used to assess FAO capacity. Placental explants were incubated in the absence or presence of 50, 100, or 200 µmol/l ETX with 0.1 mmol/l (18,500 Bq/ml) palmitate for 18 h, and the production of [3H]water was determined as described in METHODS. Means ± SD for 6 independent experiments in triplicate is shown. **B:** fatty acid esterification in placental explants treated with various concentrations of ETX. The same subset of placental explants described in **A** was used to assess esterification into total lipids, as described in METHODS. Means ± SD for 6 independent experiments in triplicate. **C:** the same subset of placental explants described in **A** was used to assess TG content, as described in METHODS. Means ± SD for 6 independent experiments in triplicate. *P < 0.05 relative to untreated placental explants; †P < 0.05 relative to 200 µmol/l ETX-treated placental explants.

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**Fig. 4.** High glucose levels inhibit carnitine palmitoyltransferase I (CPT I) activity and reduce phosphorylation levels of acetyl-CoA carboxylase. A subset of 6 placentas from women with no pregnancy complication (as described in Table 1) was used to obtain villous explants and perform the following experiments. CPT I activity (A) and CPT II activity (B) were determined as described in METHODS in placental explants incubated at low (5 mmol/l) or high glucose (11 mmol/l) concentrations for 18 h. Means ± SD for 6 independent experiments in duplicate is shown. **C:** Western blot analysis of phospho-acetyl-CoA carboxylase (p-ACC) in protein extracts from placental explants incubated at low (5 mmol/l) or high glucose (11 mmol/l) concentrations for 18 h. Top: a representative picture of the Western blot is shown. **Bottom:** the y-axis represents the ratio of p-ACC vs. β-actin in arbitrary units (AU). Means ± SD for 4 independent experiments in triplicate. *P < 0.05 relative to 5 mmol/l glucose.
The FAO pathway has not been evaluated in placenta from GDM women. In this study, we demonstrated that these women exhibited lower FAO oxidation capacity without change in mitochondrial content. To explain these observations, we hypothesized that lower FAO capacity may be related to maternal hyperglycemia, a hallmark of GDM women. However, the metabolic environment of women with GDM is characterized also by the presence of excessive NEFA levels and proinflammatory cytokines (6, 35), which makes it difficult to tease apart the causing factor involved in reduced placental FAO observed in these women. Thus, we attempted to mimic maternal milieu of women with GDM in our ex vivo studies, using low and high glucose levels and low and high NEFA levels. Therefore, a limitation of this study is that although our ex vivo culture conditions for placental explants clearly allowed mechanistic studies, they may not accurately reflect a GDM milieu and replicate in vivo pathology. Thus, our findings in placenta from women with GDM may be explained by other factors related to obesity, such as elevated NEFA and/or proinflammatory cytokines, rather than maternal hyperglycemia. However, obesity is not a confounding factor in the phenotype of the GDM women group in our study population (BMI was similar between both groups), which supports the notion that only GDM-related factors, such as higher glucose levels, may trigger the observed modifications. Although glycosuria and insulinemia levels were determined only in the fasting state, it may be highlighted that the absence of differences between the two groups may also be attributed to the prescription of a strict metabolic control in patients with GDM. Along this line, there were no differences in the levels of glycosylated hemoglobin between the two groups. Nevertheless, further studies are warranted to investigate the regulation of FAO pathways using placental explants from women with type 1 and type 2 diabetes and obese nondiabetic women.

Using placental explants from women with no pregnancy complication, we demonstrated that high glucose levels inhibited FAO and increased triglyceride accumulation. These results are in agreement with our findings in placenta from GDM women. Because de novo fatty acid synthesis remained unchanged, and because etomoxir-mediated inhibition of CPT I recapitulated the effects of high glucose on FAO and esterification pathways, we thought that the mechanistic link between high glucose levels and lower FAO was inhibition of CPT I activity by its physiological inhibitor malonyl-CoA, which is synthesized from glucose-derived acetyl-CoA by ACC. Following this rationale, we demonstrated that CPT I activity and phosphorylation of ACC was decreased significantly by high glucose levels. Because phosphorylation of ACC inhibits its enzymatic activity, our results support the notion that FAO is diminished by high glucose levels through decreased ACC phosphorylation and enhanced production of malonyl-CoA levels in placental explants, which resulted in lower CPT I activity. Interestingly, this mechanism results in a shift of fatty acid partitioning away from the β-oxidation pathway toward esterification, allowing the accumulation of triglycerides in human placenta.

These alterations in lipid metabolism mediated by high glucose levels lead to two important questions. 1) What are the consequences of triglyceride accumulation in placenta; and 2) is placental storage of triglycerides a contributing factor to fetal macrosomia? Several studies have demonstrated that maternal serum triglyceride levels are associated with abnormal fetal growth in women with GDM and type 1 and type 2 diabetes (17, 38), spurring the notion that increased maternal lipid availability results in fetal fat accretion. In a hypothetical scenario of maternal triglycerides, oversupply, and elevated lipolysis rate at the maternal-placental side, esterification of NEFA into triglycerides in placental cells may indicate a regulatory system to limit maternal fatty acid transfer to the fetus and serve as a protective mechanism against fetal macrosomia. However, there are no data about the lipolysis rate of very low-density lipoproteins and chylomicron remnants in placenta from women with GDM. Thus, although placental lipid metabolism has been proposed as a regulatory step toward fetal macrosomia (14, 34, 39, 41), it is still missing direct evidence demonstrating that unbalanced triglyceride storage in placental cells results in augmented transplacental delivery of adipogenic substrates to the fetus. On the other hand, accumulation of triglycerides or its harmful intermediaries, such as ceramide and diacylglycerol, in trophoblast cells may exacerbate the basal proinflammatory state of pregnancy. In this hypothetical scenario, accumulation of triglycerides in placental cells would trigger inflammatory pathways in trophoblast cells and deleterious effects on placental and fetal metabolism. Several studies support the idea that GDM and/or obesity induces inflammatory pathways in placenta (7, 12, 24, 35).

Our results on fatty acid partitioning contrast with early studies performed by Pathmapeura et al. (29) in trophoblast isolated from normal-term human placentas. They showed that low or high glucose (0.5–18 mmol/l) levels had no significant effects on FAO or esterification processes in cultured trophoblast exposed to short (2 h) or longer periods (24 h) of time. The differences between both studies may be explained by the experimental models employed. First, Pathmapeura et al. (29) used cultured trophoblast isolated from human placentas, whereas we used placental explants. The latter technique allows the possibility to investigate trophoblast function in a context that contains other cell types (fibroblasts, macrophages, and endothelial cells, etc.) and retains the cellular architecture of the tissue in vivo. Second, trophoblast cells were maintained in culture media for 16 h prior to initiation of experimental procedures, whereas placental explants were maintained in culture media for only 1 h. Finally, they investigated the effects of glucose levels on fatty acid partitioning for 24 h in the presence of 0.25 mmol/l nonesterified fatty acids (palmitate/oleate ratio 1:1), whereas we used 0.2 mmol/l palmitate as a source of nonesterified fatty acids.

We showed that placentas from healthy women can incorporate [14C]glucose into lipids, corroborating previous studies concerning the de novo fatty acid synthesis capacity of human placenta (10, 20, 40). Whereas high glucose did not result in a significant increase in de novo lipid synthesis in placental explants, FAO was decreased, suggesting an increase in glucose-derived malonyl-CoA. Under these experimental conditions, ACC activity appeared to function primarily as a regulator of the FAO pathway rather than as a regulator of the de novo fatty acid synthesis pathway. A similar role for ACC has been described in tissues with low de novo fatty acid synthesis capacity, such as skeletal and cardiac muscle (37). Earlier studies have suggested that the de novo fatty acid synthesis pathway plays a minor role in triglyceride accumulation in diabetic placenta, which is consistent with our observation on
ex vivo metabolism (10, 20, 39, 40). Finally, we acknowledge that a limitation of our study is that placental explants were preincubated in the absence of insulin, which is present in the in vivo milieu and is required for de novo lipid synthesis. Therefore, taking into consideration our experimental conditions without insulin, and given the nonsignificant trend toward increased [14C]glucose incorporation into lipid in the presence of high glucose, we cannot conclude that elevations in glucose do not increase placental de novo lipid synthesis in vivo.

In conclusion, we demonstrate that high glucose levels alter the metabolic partitioning of fatty acids in human placenta, shifting flux of fatty acids away from oxidation toward the esterification pathway, leading to accumulation of placental triglycerides. The mechanistic link between high glucose levels and lower FAO capacity is through reduced activity of the enzyme CPT I, which regulates the first step of the entry of long-chain acyl-CoA into the mitochondrial matrix for β-oxidation. These findings shed light on the biochemical mechanisms by which maternal hyperglycemia may regulate placental lipid pathways in diabetic mothers.

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DISCLOSURES

The authors declare no conflicts of interest, financial or otherwise, in the research.

AUTHOR CONTRIBUTIONS

F.V., V.S., and I.C.-C. performed the experiments; F.V., I.C.-C., and G.P. analyzed the data; F.V., F.B., J.L.B., and G.P. interpreted the results of the experiments; F.V., I.C.-C., and G.P. prepared the figures; F.V., F.B., V.S., I.C.-C., J.L.B., and G.P. approved the final version of the manuscript; F.B., V.S., I.C.-C., and G.P. edited and revised the manuscript; J.L.B. and G.P. contributed to the conception and design of the research; G.P. drafted the manuscript.

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