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Glucose and Fatty Acid Metabolism in Placental Explants From Pregnancies Complicated With Gestational Diabetes Mellitus

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Abstract

Placental metabolism is an important mechanism for the regulation of fetal growth and long-term health of the newborns. In this study, we investigated the effects of maternal metabolic environment on human placental fatty acid and glucose metabolism. We used placental explants from uncomplicated pregnancies or pregnancies complicated with gestational diabetes mellitus (GDM), undergoing vaginal delivery (VD) or cesarean section (CS). Fatty acid oxidation (FAO) and glucose uptake (2-DOG) were similar in both modes of delivery in normal and GDM pregnancies. However, placental explants from GDM exhibited 40% to 50% reduced FAO capacity compared to control placentas in women undergoing VD or CS. In contrast, 2-DOG uptake was 2- to 3-fold higher in placental explants from GDM compared to control placentas in women undergoing VD or CS, respectively. In conclusion, ex vivo placental fuel selection is influenced by maternal GDM, but placental metabolic characteristics are not altered by the mode of delivery.

Keywords

fatty acid oxidation, glucose uptake, gestational diabetes mellitus, placental villous explants

Introduction

Gestational diabetes mellitus (GDM) is a growing health concern in women of reproductive age because it is associated with a broad range of maternal and fetal complications.[1] Fetuses from women with GDM are exposed to excessive availability of nutrient supply at the maternal–fetal interface, leading to augmented transplacental nutrient transfer.[2] In this line of thinking, it has been proposed the placental metabolism as a regulatory step toward fetal nutrition.[2] However, metabolic characteristics of placental metabolism in women with pregnancies complicated with GDM remain incompletely understood.

On the other hand, the mode of delivery has been proposed to exert effects on placental energy metabolism.[3-5] Placentas undergoing vaginal delivery (VD) are exposed to physiological stress during labor, such as compression caused by uterine contractions and its associated intermittent hypoxia,[6,7] whereas placentas undergoing elective nonlabor cesarean section (CS) are not subjected to these conditions. However, no systematic investigation has been conducted on the effects of labor on placental metabolism in uncomplicated versus GDM pregnancies.

In this study, we have measured fatty acid oxidation (FAO) and glucose uptake (2-DOG) capacity in placental explants from pregnancies complicated with GDM or uncomplicated pregnancies, undergoing VD or nonlabor CS. Our data underscore ex vivo differences in placental fuel utilization in women with GDM.

Methods

Tissue Collection

The study was performed on placentas from pregnancies monitored at the Department of Obstetrics and Gynecology,

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Table 1. Anthropometrics and Metabolic Data of the Study Population.[a]

	Control Group (n = 10)		GDM Group (n = 10)	
Delivery Mode of Delivery	Labor	Cesarean Section	Labor	Cesarean Section
Maternal age, yr	29.5 ± 4.5	30.2 ± 4.2	32.9 ± 5.4	34.2 ± 6.0
Gestational age at delivery, wk	39.1 ± 1.5	38.8 ± 0.9	39.4 ± 1.7	38.2 ± 1.0
Maternal pregravid BMI, kg/m^2	23.8 ± 5.5	26.5 ± 6.0	25.8 ± 4.8	28.6 ± 6.1
Maternal plasma glucose, mg/dL	79.3 ± 15.4	77.5 ± 11.2	78.2 ± 16.2	84.5 ± 12.1
Maternal plasma insulin, pmol/L	19.3 ± 9.8	20.8 ± 8.0	26.1 ± 16.8	15.3 ± 10.0
Maternal plasma TG, mg/dL	172.0 ± 30.8	186.3 ± 31.4	205.0 ± 67.0	238.5 ± 71.4
Maternal plasma cholesterol, mg/dL	215.0 ± 63.1	233.0 ± 51.8	272.1 ± 52.1	257.5 ± 57.9
Maternal plasma HDL-C, mg/dL	65.0 ± 23.6	73.7 ± 18.2	63.6 ± 14.7	70.7 ± 19.1
Maternal plasma LDL-C, mg/dL	148.3 ± 42.0	163.0 ± 33.5	162.0 ± 49.2	141.5 ± 43.5
Maternal NEFA, mEq/L	0.4504 ± 0.14	0.4434 ± 0.03	0.6758 ± 0.36	0.6260 ± 0.09[b]
Placental weight, g	499 ± 106	510 ± 80	671 ± 60	639 ± 110
Birthweight, g	3315 ± 323	3122 ± 214	3270 ± 388	3145 ± 236

Abbreviations: BMI, body mass index; GDM, gestational diabetes mellitus; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; NEFA, nonesterified fatty acids; VA, vaginal delivery; CS, cesarean section; SEM, standard error of the mean.
[a]Data are given as mean ± SEM. No significant statistical differences were found among VD and CS in control or GDM group.
[b]$P < .05$ control CS versus GDM CS group.

University Hospital "Puerta del Mar" (HUPM). A total of 20 consecutive patients qualifying for the study were enrolled, and written informed consent was obtained in accordance with the HUPM Ethics Committee requirements and the Declaration of Helsinki. None of the 20 patients approved by the HUPM Ethics Committee was excluded from the study. Placentas were obtained from uncomplicated term deliveries (control group, n = 10) or pregnancies complicated with GDM (GDM group, n = 10), who had an elective CS (nonlabor group) for clinical reasons (breech presentation or prior CS) or spontaneous VD (labor group). Specific exclusion criteria included women younger than 18 years, smokers, or those with a history of long-chain 3-hydroxyacyl-CoA deficiency, hemolysis, elevated liver function, and low platelets syndrome or acute fatty liver of pregnancy, preeclampsia, chronic hypertension, or other comorbid disease. Maternal GDM was defined as previously described.[5] Randomly chosen subsets of 3 to 6 placentas were used for the experiments, as indicated in the figure legends. Table 1 shows demographics, baseline data, and perinatal variables. Biochemical parameters listed in Table 1 (maternal plasma glucose, insulin, triglycerides [TGs], total cholesterol [CHL], low-density lipoprotein cholesterol [LDL-c], high-density lipoprotein cholesterol [HDL-c], and nonesterified fatty acids [NEFA]) were analyzed as described previously.[5]

Placental Explant Culture

Placental villous explant culture was performed as described previously.[5,8] Term placenta obtained from elective CS or VD was placed on ice and arrived to the laboratory within 10 to 15 minutes of delivery. Afterward, placental villous explants (100 mg wet weight) were dissected and cultured in 6-well plate containing 2 mL of culture medium (RPMI-1640 supplemented with 5 mmol/L glucose, 10% fetal bovine serum [vol/vol], 100 units/mL penicillin G, and 100 μg/mL streptomycin).

Fatty Acid Oxidation Assay

Mitochondrial FAO was performed as previously described.[5,8] Stock of fatty acid solution was prepared by conjugating palmitate with essential fatty acid-free bovine serum albumin (BSA) to generate a stock solution of 25% (wt/vol) BSA, 4 mmol/L palmitate in culture medium. Freshly isolated explants were incubated in culture media in the presence of BSA and palmitate (1.25% BSA, 0.1 mmol/L [0.5 μCi/mL [^{3}H]-palmitate]) at 37°C for 18 hours. Afterward, tritiated water was determined by the vapor-phase equilibration method. The FAO was defined as nmol of palmitate per mg of tissue per hour.

Glucose Transport Assay

Uptake of 2-[1,2-^{3}H]-deoxy-D-glucose ([^{3}H]-2-DOG) was determined in freshly isolated explants as described[9] with the following modifications. Explants were washed in transport solution buffer (20 mmol/L HEPES-Na pH 7.4, 140 mmol/L NaCl, 5 mmol/L KCl, 2.5 mmol/L $MgSO_4$, and 1 mmol/L $CaCl_2$) at room temperature and immediately transferred to transport solution plus 10 μmol/L 2-DOG (0.5 μCi/mL [^{3}H]-2-DOG) and 39 mmol/L mannitol (0.32 μCi/mL [^{14}C]-mannitol) for 1 minute. Reactions were stopped and analyzed for ^{14}C and ^{3}H content.

Statistical Analysis

Statistical analysis of data was performed using the SPSS software (SPSS 20.0, Inc, Chicago, Illinois). Data were presented as mean ± standard error of the mean. Normality of FAO, 2-DOG, maternal age, gestational age, maternal body mass index, glucose, insulin, TG, CHL, HDL-c, LDL-c, NEFA, placental weight, and birthweight data was checked by the Kolmogorov-Smirnov test. Comparisons between more than 2 groups (control group VD or CS vs GDM group VD or

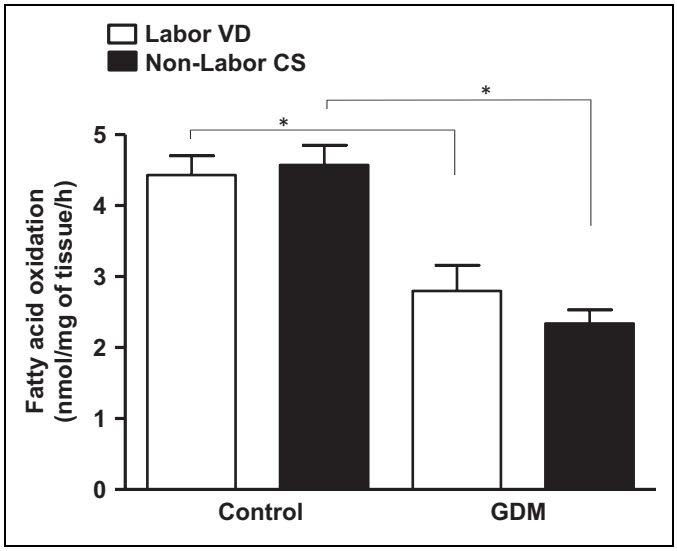

Figure 1. Effect of labor on fatty acid oxidation in placental explants form control and GDM women. Fatty acid oxidation was assessed, as described in the Methods section, using placental villous explants from women with no pregnancy complications undergoing vaginal delivery (labor VD, n = 6) or cesarean section (nonlabor CS, n = 4) or pregnancies complicated with GDM undergoing vaginal delivery (labor VD, n = 6) or cesarean section (nonlabor CS, n = 4). Values are represented as means ± standard error of the means (SEMs) for 3 to 6 independent experiments in triplicate. *$P < .05$ by analysis of variance (ANOVA). CS indicates cesarean section; GDM, gestational diabetes mellitus; VD, vaginal delivery.

CS) were performed by analysis of variance followed by a Tukey post hoc test. Differences were considered significant at $P < .05$.

Results and Discussion

To gain insights into the effects of labor on placental metabolism, we measured FAO capacity in control placentas after VD or CS. The FAO was similar in both conditions (Figure 1). Likewise, FAO in GDM placentas after labor or nonlabor delivery remained unchanged (Figure 1). These results suggest that physiological stress during labor did not alter ex vivo placental capacity for oxidation of exogenous fatty acids. However, we cannot rule out the possibility that mitochondrial FAO driven by an endogenous pool of fatty acids may be altered by the mode of delivery. Interestingly, the FAO rate was lower in placentas from GDM women compared to control women undergoing either VD or CS (Figure 1). These results support our previously published data on placental FAO in pregnancies complicated with GDM undergoing nonlabor CS[5] and indicate that in freshly isolated placental explants the FAO capacity was likely associated with the maternal metabolic environment rather than the mode of delivery.

Our results on FAO contrast with early studies performed by Mendez-Figueroa et al.[4] They showed that placentas undergoing labor, obtained from uncomplicated pregnancies, had lower FAO capacity than placentas undergoing nonlabor CS. The discrepant results between these 2 studies may be explained due to several technical factors that may contribute to variations in experimental measurement of placental energetics, such as speed of sample collection and processing of tissues, the ratio of tissue weight to culture medium, renewal of the medium, glucose concentration in the culture media, and addition of growth factors/hormones to the culture media to mimic the in vivo maternal environment.[3,10-12] On the other hand, in our experiments, explants were obtained from the maternal side and incubated in the presence of palmitate for 18 hours, whereas Mendez-Figueroa et al used explants from the fetal side, which were assayed for 1 hour.[4] Of note, it has been reported that syncytial degeneration in placental explants begins to occur after 6 to 7 hours in culture.[11,12]

Because in our experimental setting, we used placental explants from the maternal side, and Mendez-Figueroa et al used explants from the fetal side, it is difficult to draw conclusions about the relevance of FAO as placental energy source. Of note, Ramsay et al described that porcine maternal placental explants were more efficient in oxidizing palmitate than explants obtained from the fetal side, whereas fetal explants were more efficient in esterifying palmitate.[13] Therefore, it is plausible to propose that the metabolic fate of fatty acids in human placenta may be different according to anatomical locations. Further work is guaranteed to reveal the metabolic partitioning of fatty acids regarding placental human anatomy, as a critical step toward understanding regulation of fetal fatty acids supply.

Nonetheless, these studies led to an important question: What is the relevance of FAO metabolism during labor? According to the studies of Mendez-Figueroa et al, FAO is not an important placental energy source during labor.[4] However, analyses of placental metabolism were limited to placentas from normal pregnancies, and they acknowledge that GDM could theoretically cause alterations in FAO rate.

Maternal GDM could potentially alter placental FAO capacity as an adaptive or compensatory response to excessive intrauterine nutrient environment. To further investigate the metabolic characteristics of placentas from control or GDM women, we also measured 2-DOG uptake in placentas undergoing VD or CS. In normal and GDM pregnancies, 2-DOG uptake was similar between modes of delivery (Figure 2). Therefore, as shown for FAO, physiological stress during labor did not alter ex vivo placental glucose uptake capacity. However, the 2-DOG uptake rate was higher in placentas from GDM women compared to control women undergoing either VD or CS (Figure 2). These results are consistent with the notion that placental glucose transport is enhanced in maternal diabetes.[14] Since FAO was reduced in GDM placentas, it is plausible to suggest that augmented placental glucose uptake in GDM contributes to the decrease in FAO capacity. In this line of thinking, we have previously demonstrated in placental explants obtained from uncomplicated pregnancies that high glucose levels reduced mitochondrial FAO through inhibition of carnitine palmitoyltransferase I, shifting flux of fatty acids away from oxidation toward the esterification pathway.[5]

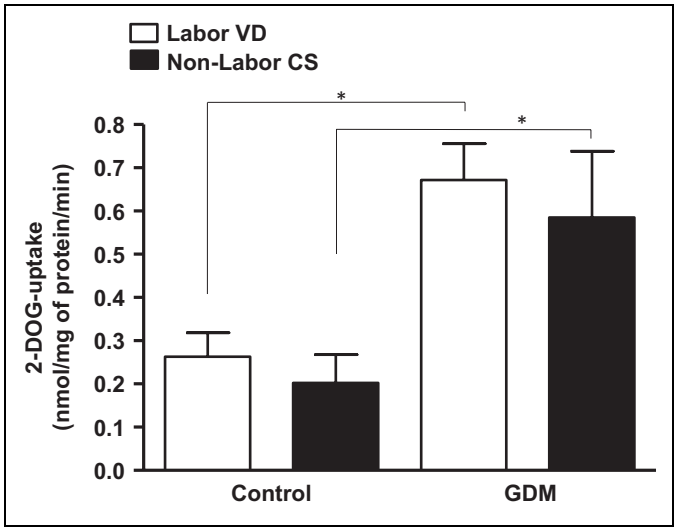

Figure 2. Effect of labor on glucose uptake in placental explants form control and GDM women. Glucose uptake was assessed, as described in the Methods section, using placental villous explants from women with no pregnancy complications undergoing vaginal delivery (labor VD, n = 5) or cesarean section (nonlabor CS, n = 5) or pregnancies complicated with GDM undergoing vaginal delivery (labor VD, n = 3) or cesarean section (nonlabor CS, n = 3). Values are represented as means ± standard error of the means (SEMs) for 3 to 6 independent experiments in triplicate. *$P < .05$ by analysis of variance (ANOVA). CS indicates cesarean section; GDM, gestational diabetes mellitus; VD, vaginal delivery.

Furthermore, in this study, we showed that NEFA levels were higher in GDM women than in control women undergoing CS. These data support the notion that in addition to the inhibition of FAO by high glucose levels in placentas from women with GDM, the esterification pathway may be fueled with higher maternal levels of NEFA. In support of this hypothesis, we have observed that esterification is 2-fold higher (12.3 ± 0.8 vs 5.8 ± 1 nmol/mg tissue/h; $P < .001$) in placental explants from GDM women than from control women (Visiedo et al unpublished data).

In conclusion, our data demonstrated that placental explants from pregnancies complicated with GDM preferentially used glucose as exogenous carbon source of energy, but placental metabolic characteristics are not altered by the mode of delivery. In addition, placental villous explants are useful tools for studying metabolic features of term placentas from pregnancies complicated with GDM.

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Declaration of Conflicting Interests

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