OBSTETRICS

Identification of early transcriptome signatures in placenta exposed to insulin and obesity

Luciana Lassance, PhD; Maricela Haghiac, PhD; Patrick Leahy, PhD; Subhabrata Basu; Judi Minium; Joanna Zhou, BS; Mitchell Reider, MD; Patrick M. Catalano, MD; Sylvie Hauguel-de Mouzon, PhD

OBJECTIVE: The purpose of this study was to investigate the effects of insulin on human placental transcriptome and biological processes in first-trimester pregnancy.

STUDY DESIGN: Maternal plasma and placenta villous tissue were obtained at the time of voluntary termination of pregnancy (7-12 weeks) from 17 lean (body mass index, 20.9 ± 1.5 kg/m^2) and 18 obese (body mass index, 33.5 ± 2.6 kg/m^2) women. Trophoblast cells were immediately isolated for in vitro treatment with insulin or vehicle. Patterns of global gene expression were analyzed using genome microarray profiling after hybridization to Human Gene 1.1 ST and real time reverse transcription—polymerase chain reaction.

RESULTS: The global trophoblast transcriptome was qualitatively separated in insulin-treated vs untreated trophoblasts of lean women. The number of insulin-sensitive genes detected in the trophoblasts of lean women was 2875 ($P < .001$). Maternal obesity reduced the number of insulin-sensitive genes recovered by 30-fold. Insulin significantly impaired several gene networks regulating cell cycle and cholesterol homeostasis but did not modify pathways related to glucose transport. Obesity associated with high insulin and insulin resistance, but not maternal hyperinsulinemia alone, impaired the global gene profiling of early gestation placenta, highlighting mitochondrial dysfunction and decreased energy metabolism.

CONCLUSION: We report for the first time that human trophoblast cells are highly sensitive to insulin regulation in early gestation. Maternal obesity associated with insulin resistance programs the placental transcriptome toward refractoriness to insulin with potential adverse consequences for placental structure and function.

Key words: early pregnancy, gene profiling, insulin, obesity, placenta

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Extensive changes in maternal metabolic homeostasis take place over the course of human pregnancy.[1] The development of a physiological state of insulin resistance is a required adaptation to pregnancy. There is a transformation from an insulin sensitive-anabolic phase at early stages to an insulin resistant-catabolic phase in late gestation.[2,3]

The metabolic switch is facilitated by sequential changes in the action of insulin. Toward the end of normal pregnancy, all women develop physiological hyperinsulinemia in the basal state, about twice higher compared with pregravid levels.[4-6] The increase in basal plasma insulin concentrations results from a 30% increase in hepatic glucose production to compensate for the decrease in insulin sensitivity in maternal peripheral tissues.[5,6]

Most of the maternal adaptations are intensified by maternal obesity, which exaggerates the metabolic abnormalities in response to higher level of insulin resistance.[7] Pregnancy-specific changes in insulin action are influenced by various hormones and adipokines that are produced by maternal adipose tissue and the placenta.[8-10]

The placenta is also a significant source of a variety of cytokines, which link the low-grade inflammation to the insulin resistance of pregnancy.[11] As in nonpregnant individuals,[12] tumor necrosis factor alpha impairs the post-receptor insulin signaling cascade in skeletal muscle of pregnant women.[13] Human placental lactogen has long been cited as a contributor to the decreased insulin sensitivity of pregnancy because of its massive production by the placenta and its increasing concentrations through advancing gestation.[14,15]

Although in late gestation a high number of insulin receptors are expressed in placental syncytial membranes, sensitivity of the placenta to maternal insulin has remained controversial.[16-18] The placenta is not considered a classical insulin target tissue with regard to the

From the Center for Reproductive Health, MetroHealth Medical Center (Drs Lassance, Haghiac, Catalano, and Hauguel-de Mouzon, Ms Basu, Ms Minium, and Ms Zhou), and Case Comprehensive Cancer Center (Dr Leahy), Case Western Reserve University, and Department of Obstetrics and Gynecology, Cleveland Clinic (Dr Reider), Cleveland, OH.

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Corresponding author: Sylvie Hauguel-de Mouzon, PhD. shdemouzon@metrohealth.org

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regulation of glucose transport and utilization.[19,20] Whereas glucose transporter type 4 insulin-sensitive glucose transporters are expressed in the placenta, they are not being translocated upon insulin stimulation.[21]

Of note, most studies in humans have been performed late in gestation when maternal insulin levels are highest. In contrast to late stages, early pregnancy may be a time of increased placental sensitivity to maternal insulin. Placental size estimated by volume in early pregnancy and by weight at term delivery was strongly related to maternal insulin secretory response in early pregnancy.[22] Interestingly, insulin receptors are more abundant on the syncytiotrophoblast in the first trimester placenta compared with the third trimester.[23]

We hypothesize that early pregnancy is an optimal period for maternal insulin to regulate insulin sensitive pathways in the placenta. The objective of this study was to examine the response of human placental cells to insulin action in early pregnancy of normal-weight women. Secondarily, obesity was designed as a model to investigate the effects of a high maternal insulin environment in early pregnancy, before pregnancy-induced insulin resistance develops.

Materials and Methods

Study subjects

In this study we define lean as subjects whose pregravid weight for height (body mass index [BMI] kg/m^2) was less than 25 kg/m^2 and obese as subjects whose BMI was greater than 30 kg/m^2.

This study was approved by the Institutional Review Boards of MetroHealth Medical Center. Volunteers provided written informed consent in accordance with institution guidelines for the protection of human subjects prior to sample collection. Women without medical complications or laboratory signs of infection or history of autoimmune disorders were recruited at the time of voluntary pregnancy termination during the first trimester of pregnancy (weeks 7–12). Placental tissue and maternal blood samples were collected at the time of termination. Anthropometric and metabolic parameters obtained from 33 women are presented in Table 1.

Biological specimen collection

Blood and tissue samples were obtained from 33 women following an 8–10 hour fast. Maternal venous blood samples (10 mL) were collected prior to placement of intravenous lines before the pregnancy termination procedure. The placenta was obtained immediately after the termination procedure. Fresh minced placental villous tissue ($\sim$0.5 $\times$ 0.5 cm) was washed and digested with trypsin and deoxyribonuclease. Trophoblast cells were purified by density gradient centrifugation as previously described.[24] The average yield was 5–8 $\times$ 10^6 cells per gram of tissue, with the cell viability greater than 80%.

Cell culture

Freshly isolated first-trimester trophoblast cells were plated into 12 well plates at a density of 2 $\times$ 10^6 cells/well and cultured overnight in Iscoves's modified Dulbecco's modified Eagle's medium culture medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C under 5% CO_2. Cells were washed and subsequently serum deprived for 18 hours prior to insulin treatment at a final concentration of 300nM or vehicle for control (saline) for 24 hours. In vitro insulin treatment was performed in trophoblast cells derived from the placenta of obese (n = 6) and lean women (n = 4). Untreated trophoblast cells isolated from first-trimester placenta of obese (n = 5) vs lean women (n = 4) were used as controls and for in vivo gene expression.

RNA extraction and microarray processing

Total ribonucleic acid (RNA) was extracted from untreated and treated placental trophoblasts using an RNeasy kit (QIAGEN Inc, Valencia, CA) and electrophoresed to verify integrity. Purified RNA samples were quantified using a NanoDrop spectrophotometer (Thermo Fisher, Wilmington, DE), and integrity was also assessed by spectrometry (Agilent, Santa Clara, CA). Samples with 28S/18S ratio greater than 1.8 were selected. RNA samples were reversed transcribed into complementary deoxyribonucleic acid (cDNA) using SuperScript first-strand synthesis (Invitrogen, Valencia, CA). Complementary RNA was prepared using 100 ng of each RNA using the SMART cDNA synthesis kit (CLONTECH, Palo Alto, CA).

Gene expression was analyzed via whole-genome microarray profiling after hybridization to Human Gene 1.1 ST Affymetrix arrays (around 36,000

TABLE 1
Characteristics of the study cohort

Characteristic	Maternal BMI	Maternal age	GA	Insulin, μU/mL	Glucose, mg/dL	HOMA-IR index	Leptin, ng/mL
Lean (BMI <25 kg/m^2)	21.2 ± 1.9	25.52 ± 7.0	9.5 ± 2.2	7.0 ± 5.2	75 ± 8	1.3 ± 1.1	16.5 ± 12.8
Obese (BMI >30 kg/m^2)	35.2 ± 6.5	24.3 ± 4.9	9.9 ± 2.2	13.2 ± 4.9	77 ± 6	2.1 ± 1.3	40.8 ± 20.8
P value	< .0001	.30	.40	.002	.40	.03	< .001

Data are expressed as means ± SD with 17 lean and 16 obese subjects. Student *t* test was used for statistical analysis.

BMI, body mass index; *GA*, gestational age; *HOMA-IR*, homeostatic assessment model for insulin resistance.

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