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Maternal obesity is associated with a lipotoxic placental environment

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ABSTRACT

Maternal obesity is associated with placental lipotoxicity, oxidative stress, and inflammation, where MAPK activity may play a central role. Accordingly, we have previously shown that placenta from obese women have increased activation of MAPK-JNK. Here, we performed RNA-sequencing on term placenta from twenty-two subjects who were dichotomized based on pre-pregnancy BMI into lean (BMI 19–24 kg/m^2; $n = 12$) and obese groups (BMI, 32–43 kg/m^2; $n = 12$). RNA-seq revealed 288 genes to be significantly different in placenta from obese women by ≥1.4-fold. GO analysis identified genes related to lipid metabolism, angiogenesis, hormone activity, and cytokine activity to be altered in placenta from obese women. Indicative of a lipotoxic environment, increased placental lipid and CIDEA protein were associated with decreased AMPK and increased activation of NF-κB (p65) in placenta from obese women. Furthermore, we observed a 25% decrease in total antioxidant capacity and increased nuclear FOXO4 localization in placenta from obese women that was significantly associated with JNK activation, suggesting that maternal obesity may also be associated with increased oxidative stress in placenta. Maternal obesity was also associated with decreased HIF-1α protein expression, suggesting a potential link between increased inflammation/oxidative stress and decreased angiogenic factors. Together, these findings indicate that maternal obesity leads to a lipotoxic placental environment that is associated with decreased regulators of angiogenesis and increased markers of inflammation and oxidative stress.

1. Introduction

Currently over 60% of all pregnancies in the United States are in women who are overweight or obese at conception. Offspring of obese mothers are at an increased risk for being born large for gestational age (LGA) and for developing obesity, cardiovascular disease, and diabetes in adulthood [1–3]. As the interface between the maternal and fetal environment, the placenta plays a central role in how maternal obesity influences programming of offspring health. Studies have shown that maternal obesity is associated with augmented inflammation [4] and elevated proteins involved in nitrative [5] and oxidative stress [6] that may in turn contribute to impaired trophoblast invasion and differentiation (reviewed [7]), vascular development and function [8,9], and alterations in placental nutrient transport [10–12].

Using placental trophoblast cells (BeWo), we recently demonstrated that exposure to lipotoxic stimuli induces a pro-inflammatory response in placental cells that is regulated by JNK and early growth response protein-1 (EGR-1) [13]. Furthermore, our studies showed that JNK/EGR-1 signaling is activated in term placenta from obese women [13], suggesting that lipotoxicity may contribute to placental dysfunction associated with maternal obesity. Increased fatty acid uptake by trophoblasts has been demonstrated in animal and cell culture studies in response to obesogenic environments [14,15], suggesting that maternal obesity may induce a lipotoxic milieu within the placenta. Among other effects, elevated lipids in turn can adversely affect mitochondria, leading to increased ROS production, oxidative stress and cellular dysfunction.

In this study, we tested the hypothesis that maternal obesity promotes a lipotoxic placental environment characterized by increased placental lipid, inflammation and oxidative stress. Additionally, we examined the placental transcriptome using RNA-seq and employed gene ontology (GO) analysis to identify broad functional categories that are altered in placenta from obese women. Consistent with our hypothesis, we identified pathways affected by inflammation, lipotoxicity, and oxidative stress to be significantly increased in placenta from obese women. Furthermore, RNA-seq analysis identified genes related to angiogenesis

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and hormone activity to be significantly decreased in placenta from obese women, suggesting that an obese maternal environment may adversely affect placental development and function.

2. Methods

2.1. Collection of term placental samples

Placenta and umbilical cord blood (UCB) (mixed arterial and venous) were collected at the University of Arkansas for Medical Sciences (UAMS), after obtaining informed consent from mothers at term. The protocol was approved by the Institutional Review Board at UAMS (NCT01104454). Included in this study were non-smoking mothers without gestational diabetes or pre-eclampsia who had either vaginal or cesarean deliveries. Maternal clinical characteristics were obtained from medical records and presented in Table 1a [16]. Subjects were dichotomized based on self-reported pre-pregnancy BMI into lean (BMI 19–24 kg/m^2; $n = 12$) and obese groups (BMI, 32–43 kg/m^2; $n = 12$), $P < 0.05$.

2.2. Umbilical cord plasma analysis

Colorimetric assays were used to measure UC plasma glucose, triglycerides (TAGs) (Fisher Scientific) and non-esterified free fatty acids (NEFA) (Wako Chemicals). Concentrations of plasma insulin and leptin were measured by ELISA (Millipore). Cytokines interleukin 6 (IL-6) and tumor necrosis factor alpha (TNFα) were measured in plasma using the Milliplex Map Human Cytokine Panel.

2.3. RNA-seq libraries sequencing, alignment, and data analysis

Preparation of RNA-seq libraries, sequencing and data analyses were carried out as previously described [17]. Total RNA was isolated from placenta (9-lean, 11-obese) using a combination of TRI reagent (Molecular Research Center) and RNeasy-mini columns, including on-column deoxyribonuclease digestion (Qiagen). RNA integrity was assessed using Experion RNA StdSens analysis kit (BioRad). cDNA libraries for RNA-seq were prepared using polyA-mRNA from each individual RNA sample (Supplemental Methods for details) [17]. Single-read 36-bp sequencing of libraries was performed using Illumina GAII$_x$. Alignment to the human genome (hg19) was carried out using Bowtie [18]. All aligned reads were exported in SAM format, and subsequent data analysis was performed in Avadis-NGS and SeqMonk software packages (details in Supplemental Methods).

2.4. Placental lipid and total antioxidant capacity (TAC) analysis

Lipids were extracted from 300 to 500 mg of placental villi with chloroform-methanol (2:1, vol/vol) [19], allowed to dry to completion under nitrogen gas, and weighed. Data were expressed as total extractable lipids/g tissue. TAC was determined using the Antioxidant Assay Kit (#709001, Cayman, Ann Arbor, MI) as per manufacturer's instructions.

2.5. Immunoblotting

Total tissue lysates were prepared (12-lean, 12-obese) in RIPA buffer containing 1 mM PMSF and protease inhibitor cocktail. Nuclear and cytoplasmic proteins were isolated using NE-PER reagents (Thermo Fisher Scientific) on a subset of samples (9-lean, 9-obese). Immunoblotting was carried out following standard procedures [13]. See Table S1 for antibody details. Immunoblots were quantified using Quantity One software (BioRad).

2.6. Oil-red-O staining and immuno-histofluorochemistry

Oil-red-O in propylene glycol (Electron Microscopy Sources) was used to stain for neutral lipids in paraformaldehyde-fixed frozen sections from 6-lean and 6-obese placenta, following manufactures instructions. Vessel density was quantified in another set of sections following immunolabeling with primary antibodies against CD31 (#3528S, Cell Signaling). Immunoreactivity was visualized using secondary antibodies conjugated with Alexa-488 (Molecular Probes) and examined using an AxioVert 200 fluorescent microscope (Carl Zeiss).

2.7. Statistical analysis

Data are expressed as means ± SEM. Statistical differences between lean and obese groups were determined using two-tailed student's t test. Correlations between protein levels were determined using the Pearson's product–moment correlation coefficient (r). $P \leq 0.05$ was considered statistically significant. Statistical analyses were performed using SigmaStat 3.3 software (Systat Software Inc).

3. Results

3.1. Birth weight, placental weight, and UCB analysis

Birth weight and placental weight were not different in this cohort of women (Table 1b). There were no differences in UCB TG or NEFA content, however there was a 30% decrease ($P = 0.03$) in cholesterol in UCB from obese compared to the lean group (Table 1b). Maternal obesity was also associated with significantly increased glucose ($P = 0.01$) and leptin ($P = 0.03$) levels. Although there was not a significant difference in UCB IL-6 or TNFα levels, we observed a numerical increase of 50% and 15%, respectively, associated with maternal obesity (Table 1b).

3.2. RNA-seq analysis of term placenta from lean and obese women

RNA-seq revealed 288 genes to be significantly different in placenta from obese women (±1.4-fold, $P < 0.05$). Hierarchical clustering of differentially expressed genes is presented in Fig. S1A. Interactions of the gene ontology (GO) biological processes (development, stress response, immune response, differentiation, chromatin modification, and reproduction) significantly altered in placenta from obese women are depicted in Fig. S1B. Some of the most significantly altered processes are shown in Fig. 1A. Gene-set-enrichment-analysis (GSEA) of biological processes also identified genes involved in angiogenesis (*HIF1A*, *VEGFA*, *ANGPTL6*, *BTG1*, *HTATIP2*, *PROK2*, *FLIT1* ($P = 0.06$), and *PTGS2* ($P = 0.06$)) (Fig. 1B) and lipid metabolism (*DKK1*, *ANGPTL4*, *INSIG1*, and *NRIP1*) (Fig. 1C) to be significantly decreased ($P < 0.05$) in placenta from obese women. Maternal obesity was associated with a marked increase in lipid droplet-associated protein *CIDEA* ($P < 0.001$) mRNA.

GO analysis of molecular functions identified functions related to receptor signaling and hormone activity to be significantly altered in placenta from obese women (Fig. 1D). Likewise, GSEA of molecular functions confirmed that genes involved in cytokine activity were significantly altered (Fig. 1E), identifying a trending increase in pro-inflammatory interleukins (*IL-6* ($P = 0.06$) and *IL-23A* ($P = 0.08$)) and a significant increase in interleukin and chemokine receptors (*IL12RB2*, *IL21R*, *CX3CR1*, and *CCR3*) associated with maternal obesity ($P < 0.05$). In contrast, a significant decrease in pro-inflammatory interleukin-1 receptor-1 (*IL1R1*, $P < 0.01$) and receptor accessory proteins (*IL1RAP* and *IL1RAPL2*, $P < 0.02$) was found in placenta from obese compared to lean women. GSEA of genes associated with hormone activity revealed a significant decrease in a number of hormones (*CCK*, *Lep*, *CRH*, *INHA* and *INHBA*) and the adiponectin receptor (*ADIPOR1*) in placenta from obese compared to lean women ($P < 0.05$).

3.3. Maternal obesity is associated with a lipotoxic environment

Lipid accumulation was observed in placenta from both lean and obese women and was mainly localized to the villi stroma, however lipid droplets were also present in the syncytium (Fig. 2A). Placenta from obese women had 50% more lipid than placenta from lean women ($P < 0.03$, Fig. 2B). A significant increase in CIDE-A ($P < 0.001$ Fig. 2C,D) and a significant decreased in AMPK protein levels ($P < 0.001$ Fig. 2C,D) was associated with maternal obesity. Furthermore, CIDE-A and AMPK levels were inversely correlated ($r = -0.588$, $P < 0.01$, Fig. 2E). Finally, NF-κB activation (p-P65) was significantly increased in placenta from obese women compared to placenta from lean women ($P < 0.001$, Fig. 2C,D).

3.4. Decreased TAC and increased nuclear FOXO4 localization are associated with decreased angiogenic regulators in placenta from obese women

Maternal obesity was associated with a 25% decrease in placental TAC ($P < 0.02$, Fig. 3A). Additionally, placenta from obese women showed a 75% increase in nuclear FOXO4 ($P < 0.05$ Fig. 3B,D) when compared to placenta from lean women. Nuclear FOXO4 levels were also positively correlated with activated JNK (pJNK54/JNK54) in