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Maternal obesity programs mitochondrial and lipid metabolism gene expression in infant umbilical vein endothelial cells

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Abstract

Background/Objectives—Maternal obesity increases risk for childhood obesity, but molecular mechanisms are not well understood. We hypothesized that primary umbilical vein endothelial cells (HUVEC) from infants of overweight and obese mothers would harbor transcriptional patterns reflecting offspring obesity risk.

Subjects/Methods—In this observational cohort study, we recruited 13 lean (pre-pregnancy BMI <25.0 kg/m²) and 24 overweight-obese ('ov-ob', BMI ≥25.0 kg/m²) women. We isolated primary HUVEC, and analyzed both gene expression (Primeview, Affymetrix) and cord blood levels of hormones and adipokines.

Results—142 transcripts were differentially expressed in HUVEC from infants of overweight-obese mothers (false discovery rate, FDR <0.05). Pathway analysis revealed that genes involved in mitochondrial and lipid metabolism were negatively correlated with maternal BMI (FDR <0.05). To test whether these transcriptomic patterns were associated with distinct nutrient exposures in the setting of maternal obesity, we analyzed the cord blood lipidome and noted significant increases in levels of total free fatty acids (lean: 95.5 ± 37.1 ug/ml, ov-ob: 124.1 ± 46.0 ug/ml, P=0.049), palmitate (lean: 34.5 ± 12.7 ug/ml, ov-ob: 46.3 ± 18.4 ug/ml, P=0.03) and stearate (lean: 20.8 ± 8.2 ug/ml, ov-ob: 29.7 ± 17.2 ug/ml, P=0.04), in infants of overweight-obese mothers.

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There are no conflicts of interest to disclose.

Author Contributions

SMRC designed the study, collected/analyzed data, and reviewed the manuscript; SMRC is the guarantor of the clinical data. EI analyzed data and wrote the manuscript; EI is the guarantor of transcriptomics and lipidomics analyses. TM, KH, GD, and JMD assisted with data analysis. GAPD supervised clinical study design. MEP supervised data analysis and reviewed/edited the manuscript.

Conclusion—Prenatal exposure to maternal obesity alters HUVEC expression of genes involved in mitochondrial and lipid metabolism, potentially reflecting developmentally-programmed differences in oxidative and lipid metabolism.

Introduction

Evidence from human populations and animal models indicates that environmental exposures during early development are critical determinants of disease susceptibility throughout the lifespan, a phenomenon termed ‘developmental programming’ (1). A wide range of prenatal perturbations, including maternal undernutrition, obesity, diabetes, high-fat diet, and endocrine-disrupting chemicals, are now recognized as risk factors for chronic diseases including diabetes, obesity, and cardiovascular disease (2–4). Maternal obesity is of particular concern, as it is a potent risk factor for childhood obesity: offspring of mothers entering pregnancy with BMI >30 kg/m² have a 1.5 to 4-fold higher risk of childhood obesity (5). Studies of siblings born before vs. after a mother’s weight loss surgery - which minimize the contribution of shared genetics - suggest that *in utero* exposure to maternal obesity *per se* can increase risk of childhood obesity >2-fold (6, 7).

Unfortunately, the molecular mechanisms by which maternal obesity increases metabolic risk in offspring remain incompletely understood. Previous rodent and primate studies indicate that maternal insulin resistance, which is tightly correlated with maternal obesity (8), may be one contributor to obesity-associated developmental programming (9, 10). Other mediators may include shared environmental risk factors, epigenetics, and/or hormonal and metabolic adaptations to an ‘obese’ intrauterine environment. Fewer mechanistic studies have examined metabolic phenotypes in humans, largely due to the practical and ethical challenges of obtaining cells and tissues from infants. However, umbilical cords, which are usually discarded after delivery, provide an accessible source of infant cells for translational studies. Interestingly, analysis of umbilical cord segments from infants of women with type 1 diabetes identified differences in expression of genes related to vascular development and function (11).

Primary human umbilical vein endothelial cells (HUVEC) are readily isolated, remain viable and metabolically active in culture, and are insulin-responsive, features leading to their wide use in vascular biology for over 40 years (12), and more recently, in studies of fetal adaptations to maternal diabetes and placental insufficiency. For example, maternal gestational diabetes is associated with reduced vasodilation (13) and increased leukocyte adhesion in HUVEC (14), potentially mediated by specific miRNAs (15). Moreover, increased *eNos* promoter methylation has been reported in HUVEC from infants with intrauterine growth restriction (IUGR) (16).

We therefore hypothesized that maternal obesity would alter metabolism in HUVEC in a cell-autonomous fashion. We now demonstrate that maternal obesity is associated with a dramatic transcriptional response in infant HUVEC, particularly within pathways related to lipid metabolism and mitochondrial structure/function, and is accompanied by increases in cord blood insulin, palmitate, and stearate.

Methods

Human subjects - Recife Cohort

Pregnant women were recruited during prenatal visits at Instituto de Medicina Integral Prof. Fernando Figueira, Recife, Brazil. Eligibility criteria included: age ≥ 18 years and known gestational age (based on date of last menstrual period (LMP) or ultrasound before 16 weeks). Exclusion criteria included: diabetes diagnosed prior to or during the current pregnancy, multiple gestation, hypertension, artificial reproductive techniques, HIV, syphilis, maternal disease requiring medications known to affect fetal growth or glucose metabolism, fetal congenital malformations, premature delivery (<36 weeks), and deliveries occurring outside the study hospital. Participants were grouped by early pregnancy BMI, defined as BMI at the first prenatal visit (<12 weeks gestation): lean (<25.0 kg/m²) vs. overweight and obese ('ov-ob', ≥ 25.0 kg/m²). The study protocol was approved by the Hospital Institutional Review Board and the National Committee for Ethics in Research at National Health Council (CONEP-Statement # 387/2011), Brasilia, Brazil, and by the Committee for Human Subjects at Joslin Diabetes Center, Boston, Massachusetts. All participants provided written informed consent.

Assessments during pregnancy – Recife Cohort

At the first prenatal visit, maternal medical history, including systemic disease, smoking, obstetric history, and clinical data (height, weight, blood pressure, date of LMP, and fasting glucose values) were collected. Maternal glucose metabolism was assessed twice: a 1-hour 75 g oral glucose screen during the second trimester (24 weeks \pm 2 weeks), and a 2-hour 75 g oral glucose tolerance test (OGTT) during the third trimester (glucose and insulin at time 0 (fasting), 1, and 2 hours (30 weeks \pm 2 weeks). Homeostasis method assessment of insulin resistance (HOMA-IR) was calculated as $[(\text{Glucose} \times \text{Insulin}) / 405]$, and homeostatic method assessment of beta cell secretion (HOMA- β) as $[(360 \times \text{Insulin}) / (\text{Glucose} - 63)]$, as described (17). Fasting cholesterol, HDL, LDL, VLDL, and triglycerides were also assessed during the third trimester GTT visit. The final maternal BMI was calculated during the last prenatal visit (36 – 40 weeks). Cord blood was collected at delivery and plasma aliquots frozen. Newborn weight, length, head circumference, and abdominal girth were measured within the first 24 hours of life by trained nurses. Newborn weight for gestational age and sex percentiles were derived from the growth curves of Olsen et al.(18); small for gestational age was defined as birth weight $< 10^{\text{th}}$ percentile, while large for gestational age was defined as birth weight $>90^{\text{th}}$ percentile. Medical records were abstracted to obtain data regarding prenatal, labor and delivery, postpartum, and neonatal course.

Laboratory analysis

Plasma glucose, hemoglobin A1c, and lipids were analyzed using a colorimetric method (Architect c8000, Abbott); LDL cholesterol was calculated using the Friedwald formula. Insulin was measured using an electrochemiluminescence immunoassay (Siemens). Urinalysis, HIV, and syphilis tests were performed using Symex XT-1800 (Roche), and URiSCAN Pro II, YD, AxSYM System (Abbott) and ELISA immunoassay (Elisys Quattro) respectively. Cord blood IL-1 β , IL-6, IL-8, leptin, adiponectin, resistin, total PAI-1, MCP-1,

HGF and TNF- α levels were determined by multiplex ELISA (Milliplex) in the Joslin DRC Specialized Assay Core.

HUVEC isolation

Umbilical cord segments (10 cm) were transported to the lab within 1 hour of delivery, and kept on ice in buffer containing PBS and antibiotics until HUVEC isolation; all isolations were performed within 2h of delivery. Umbilical segments were rinsed with PBS, and the umbilical vein was cannulated using a 3-way stopcock (Poly Medicure Limited (India), MODEL #1021). The umbilical vein was rinsed twice with 50 ml PBS before infusion with 25 ml 0.1% collagenase (Gibco, Cat. No.# 17100-017) dissolved in 37°C PBS. The cord was clamped at both ends and incubated at room temperature for 30 minutes. The cord was then unclamped and the vein flushed with vascular cell complete growth media (ATCC, PCS-100-030, containing 100 mg/dl glucose), and the effluent collected. The cells were then centrifuged and resuspended in complete growth media. Vascular cell growth media contains VEGF (5 ng/mL), EGF (5 ng/mL), FGF basic (5 ng/mL), IGF-1 (15 ng/mL), L-glutamine (10 mM), heparin sulfate (0.75 Units/mL), hydrocortisone (1 μ g/mL), ascorbic acid (50 μ g/mL) and fetal bovine serum (2%) (ATCC Endothelial Cell Growth Kit, PCS-100-041).

RNA isolation and microarray analysis

For each cell line, an aliquot of freshly isolated HUVEC was stored in RNA Later reagent (Life Technologies) at -80°C until RNA extraction, using Trizol (Life Technologies) according to the manufacturer's protocol, with glycogen (AM9510, Life Technologies) added as a carrier. cRNA was prepared and hybridized to Affymetrix PrimeView microarrays. Data were normalized using Robust Multichip Average (RMA) (19) and log-2 transformed. Normalized microarray data were analyzed (p, FDR) using GenePattern Comparative Marker Selection module (Broad Institute). Heatmaps were created using GENE-E (Broad Institute). Pathway analyses were performed using Gene Set Enrichment Analysis (GSEA, Broad Institute) (20, 21) and DAVID (Bioinformatics Resources v6.7) (22).

Quantitative RT-PCR

Complementary DNA (cDNA) was generated using High-Capacity cDNA Reverse Transcription Kit (Life Technologies) with 500 ng input RNA. Expression of selected genes was assessed by qRT-PCR, with expression normalized to cyclophilin E (PPIE), using an ABI 7900 HT thermocycler. Primer sequences are available upon request.

Lipidomic analysis

Lipidomic analyses in cord plasma samples were performed at the Vanderbilt Lipid Core. Lipids were extracted (23), filtered, and recovered in the chloroform phase prior to separation by thin layer chromatography. Phospholipids, diacylglycerides, triglycerides and cholesteryl esters were scraped from the plates and methylated (24); methylated fatty acids were extracted and analyzed by gas chromatography (Agilent 7890A) with helium, and compared to known standards (C15:0, C17:0, C20:1, and cholesteryl eicosenoate (C20:1)). For total cholesterol, an internal standard (5- α -cholestane) was added to a portion of the

lipid extract and saponified at 80 C in 1 N KOH in 90% methanol for 1 hour. The non-saponifiable sterol was extracted into hexane, concentrated under nitrogen, and solubilized in carbon disulfide to inject onto the gas chromatograph. For unesterified cholesterol, an internal standard is added to the lipid extract, concentrated under nitrogen and solubilized in carbon disulfide to inject onto the gas chromatograph (25). We calculated the ratio of palmitoleate (16:1n7) to palmitate (16:0), and of oleic acid (18:1n9) to oleate (18:0) as a readout of delta-9 desaturase activity, as described (26).

Statistical analysis

Data are reported as mean \pm standard deviation. Between-group comparisons were performed by unpaired, unequal variance, two-sided T-tests for continuous data, and Fisher's exact test for categorical data (JMP 10, SAS Corporation, Cary, NC). To adjust for multiple comparisons, false discovery rates were calculated using R software.

Results

Clinical, metabolic, and hormonal characteristics of mothers and newborns

Maternal age tended to be higher in the overweight/obese ('ov-ob') group (lean: 24.6 ± 5.4 , ov-ob: 27.9 ± 5.9 years, $P=0.1$, Table 1). As expected, women in the ov-ob group had significantly higher first trimester BMI than lean women (lean: 21.9 ± 1.7 vs. ov-ob: 32.2 ± 5.7 kg/m², $P<0.0001$), and higher BMI at the last prenatal visit (lean: 27.6 ± 3.4 vs. ov-ob: 36.2 ± 6.6 kg/m², $P<0.0001$). Gestational weight gain was lower in the ov-ob group (lean: 11.5 ± 4.5 vs. ov-ob: 7.8 ± 4.5 kg, $P=0.03$), but the proportion of women with excessive gestational weight gain (2009 IOM criteria) (27) was numerically higher in ov-ob (lean: 1/13 or 7.7%, ov-ob: 6/24 or 25%, $P=0.38$). Diastolic blood pressure was higher in the ov-ob group (lean: 62 ± 7 mmHg, ov-ob: 69 ± 10 mmHg, $P=0.02$), but systolic blood pressure was unaltered. While fasting glucose did not differ at 24 or 30 weeks, blood glucose 1 hour after glucose challenge at 24 weeks tended to be higher in ov-ob (lean: 105.0 ± 26.8 vs. ov-ob: 126.4 ± 32.6 mg/dl, $P=0.09$), as was 2 hour glucose at 30 weeks (lean: 117.4 ± 14.2 , ov-ob: 134.9 ± 28.2 mg/dl, $P=0.02$). Insulin resistance (HOMA-IR), insulin secretion index (HOMA- β) and insulin levels during OGTT were similar, as were lipid levels.

Gestational age at delivery, birth weight, and newborn adiposity measures, including ponderal index and abdominal circumference, were similar between offspring of lean vs. ov-ob mothers (Table 2). Cord blood insulin was increased 2-fold in ov-ob (lean: 4.6 ± 3.9 , ov-ob: 9.0 ± 9.1 μ U/mL, $P=0.048$), as were leptin levels (lean: 23.9 ± 19.4 , ov-ob: 48.9 ± 43.6 μ g/mL, $P=0.02$). Other hormones and adipokines in cord blood approached statistical significance, notably hepatocyte growth factor (HGF), IL-6, and TNF- α , which tended to be higher, and PAI-1, which tended to be lower, in infants of ov-ob mothers.

Maternal obesity alters gene expression patterns in HUVEC

To test whether prenatal exposure to maternal obesity alters transcriptional patterns in infant cells, we analyzed global gene expression in a randomly selected subset of primary HUVEC (PrimeView microarray, Affymetrix). Characteristics of mothers and newborns included in the microarray analysis tended to be similar to the entire cohort, although cord blood levels

of HGF, resistin, and TNF- α were more significantly increased in the array subset (Supplemental Table 1). Principal components analysis showed only modest separation of lean vs. ov-ob infants based on gene expression, with substantial overlap between groups (Supplemental Figure 1). 4916 transcripts were altered by maternal obesity ($P < 0.05$), with 142 remaining different after adjustment for multiple comparisons ($FDR < 0.05$), all of which were upregulated in ov-ob (complete list in Supplemental Table 2 A). As seen in the volcano plot in Figure 1A, more transcripts were upregulated by maternal obesity than downregulated. Strikingly, many of the top-ranking differentially expressed genes were related to lipid metabolism (e.g. LPIN2, MGAT2, DGAT1, SREBP2), insulin signaling (e.g. MAP4K5, PIK3R1, PRKACB, MAP3K13) and mitochondrial metabolism (e.g. COX18, ATP5D) (Figure 1B). Similarly, several genes related to lipid metabolism (e.g. LDLR, ANGPTL4, LMNA) (28, 29) were top-ranking in terms of fold-change (increased in Ov-Ob, Supplemental Table 2C–D). We next examined correlations between maternal BMI and gene expression, and found that several mitochondrial genes (e.g. ATP5O, ATP5C1, COX7C, NDUFB6, TMEM14C) were inversely correlated with maternal BMI, whereas the lipid metabolism genes FADS2 and FADS3, insulin-like growth factor 1 receptor (IGF1R) and receptor tyrosine-protein kinase erbB-2 (ERBB2) were strongly positively correlated with maternal BMI (Figure 1C). qRT-PCR confirmed significant upregulation of LDL receptor (LDLR), UDP-glucose ceramide glucosyltransferase (UGCG), erb-b2 receptor tyrosine kinase 2 (ERBB2), G protein pathway suppressor 1 (GPS1), Kruppel-like factor 7, and TNFAIP3 interacting protein 2 (TNIP2) in HUVEC from infants exposed to maternal obesity, consistent with microarray patterns. We also confirmed a significant, positive correlation between maternal BMI and PRKAB1 (Figure 2A/D).

Mitochondrial and lipid metabolism transcriptional pathways are altered by maternal obesity

We next performed Gene Set Enrichment Analysis (GSEA) to analyze enrichment of Gene Ontology (GO) terms in genes correlating with maternal BMI. 98 GO terms were positively correlated with BMI ($q < 0.05$), including pathways related to ion channels, transmembrane transporters, and neurotransmitter activity. 73 GO terms were inversely correlated ($q < 0.05$). The top-ranking set inversely correlated with maternal BMI was “proton-transporting two sector ATPase complex”; many other inversely correlated pathways were related to mitochondria (e.g. mitochondrial small ribosomal subunit, matrix, aerobic respiration) and to lipid metabolism (e.g. fatty acid biosynthesis, beta-oxidation, lipid catabolism). Pathway analyses are shown in Supp Tables 3A/B.

Maternal obesity associated with reduced maternal LDL cholesterol and increased fatty acid and triglyceride species in cord blood

We next asked whether altered HUVEC gene expression, particularly within lipid and mitochondrial pathways, might reflect differences in nutrient exposure. Maternal LDL cholesterol tended to be lower in ov-ob mothers (lean: 146 ± 28 , ov-ob: 121 ± 40 mg/dl, $P = 0.06$), but levels of triglycerides and HDL were similar. In infant cord blood, there was a trend for increased levels of several triglyceride and fatty acid species from offspring of ov-ob mothers (Figure 4A). Notably, levels of total free fatty acids, and of two saturated fatty acids, palmitate (16:0) and stearate (18:0), were significantly increased (1.3, 1.3 and 1.4-

fold, respectively, $P < 0.05$, Figure 4B/C). Moreover, several triglyceride species containing long-chain and omega-6 fatty acids were increased by 1.5 to 2.3-fold in the ov-ob group (TAG18:3w6, TAG20:4, TAG22:4w6, and TAG22:5w6, $P < 0.05$) (Figure 4C). The ratio of TAG18:1n9 to TAG18:0, a surrogate for desaturase activity, was significantly higher in ov-ob, suggesting increased SCD-mediated lipogenesis (Figure 4E). Interestingly, cholesterol esters and phospholipids containing the rare essential fatty acid 20:3w6 (dihomo-gamma-linolenic acid, DGLA) were negatively correlated with pre-pregnancy BMI (CE20:3w6: Pearson $r = -0.45$, $P = 0.005$; PL20:3w6: $r = -0.37$, $P = 0.02$, Figure 4D). Since levels of DGLA may be influenced by activity of delta-5 and delta-6 desaturase enzymes, it is interesting that expression of FADS2 (delta-6 desaturase) and FADS3 (delta-5 desaturase) were positively correlated with maternal BMI (Supplemental Figure 2). Strikingly, we noted strong correlations between levels of cord blood CE20:3w6 and expression of several transcripts related to lipid metabolism, including monoglycerate lipase (*MGLL*) and *SREBP2*, and the mitochondrial gene *ATP5D* (Supplemental Figure 3A). Cord blood palmitate (16:0) and stearate (18:0) also correlated with expression of *PPARG2* (Supplemental Figure 3B) and several other transcripts dysregulated in ov-ob (e.g. correlation of 16:0 vs. *NDUFB6* $R = -0.62$, $P = 0.02$; *KLF7*, $R = 0.615$, $P = 0.02$; *ARID4B*, $R = 0.73$, $P = 0.003$). Together, these data raise the possibility that altered plasma lipid species might contribute to differential gene expression in HUVEC from infants of overweight-obese mothers.

Discussion

Given that HUVEC are exclusively fetal in origin, they provide a powerful model to test whether prenatal exposures such as maternal obesity result in cell autonomous transcriptional patterns in offspring. In this study, we perform the first analysis of transcriptional responses to maternal obesity in HUVEC. Strikingly, maternal obesity was associated with marked perturbations in gene expression in offspring HUVEC, with downregulation of numerous genes related to mitochondrial function and lipid metabolism. Transcriptional patterns in infant HUVEC were closely correlated with cord blood levels of the fatty acids palmitate and stearate, and with lipids containing 20:3w6, raising the possibility that plasma lipids may mediate altered gene expression patterns in HUVEC. Whether such transcriptional patterns might result from direct nutrient exposures, or indirect epigenetic mechanisms, will be a key question for future studies. Furthermore, it will be important to determine whether these expression patterns can serve as markers or mediators of metabolic disease risk in infants of overweight/obese mothers.

Many of the differentially expressed genes identified in our analysis have previously been linked to obesity, diabetes, or other metabolic traits. For example, decreased adipose expression of *SREBP2* and other genes involved in lipolysis and triglyceride synthesis, has been reported in adipose tissue from women with GDM and/or obesity (30). Expression of the cholesterol regulatory gene *LDLR* (31), was increased in HUVEC of ov-ob mothers. For several other top-ranking genes identified in our analysis (e.g. *LPIN2*, *KLF7*, *PPARG2*), SNPs within these loci have been linked to obesity and type 2 diabetes risk (32–34). Intriguingly, *ERBB2*, which was upregulated in infants of ov-ob mothers, has previously been linked to maternal obesity; differences in methylation of *ERBB2* in cord blood leukocytes have been linked to pre-pregnancy maternal BMI (35). It will thus be important

for future studies to examine whether differences in infant expression of ERBB2 and other genes identified in our analysis, may be associated with differences in DNA methylation or other forms of epigenetic regulation.

Another striking pattern in HUVEC from infants exposed to maternal obesity is altered expression of individual genes and transcriptional pathways related to mitochondrial metabolism. A large body of evidence has linked diabetes and obesity to reduced adipose, muscle, and liver expression of genes involved in mitochondrial oxidation (21, 36). Moreover, functional defects in mitochondrial oxidation have been reported in humans with diabetes and obesity, and in rodent models of adverse prenatal exposures (37). In mice, maternal high fat diet reduces oocyte mitochondrial number and function (38). However, evidence from human infants is much more limited. Maternal obesity has been associated with reduced mitochondrial function in human placenta (39, 40), but given that the placenta contains maternal and fetal cells in close proximity, dissecting the cause of such transcriptional patterns is challenging. Our data in HUVEC, cells of purely fetal origin, suggest that altered mitochondrial gene expression may be an early marker of infant metabolic disease risk resulting from maternal obesity. Transcriptional patterns in HUVEC from infants of Ov-Ob mothers, moreover, appear distinct from previous analyses of HUVEC from infants of mothers with GDM (41), which highlighted differences in gene pathways related to extracellular matrix remodeling and insulin binding proteins. Gene expression patterns in the Ov-Ob group were somewhat more similar to a published analysis of transcriptional patterns in HUVEC exposed to acute insulin treatment, which identified “Electron Transport Chain” as a top-ranking significantly altered pathway (42).

In our analysis of individual genes altered by maternal obesity, we found that several genes critical for insulin signaling, including PIK3R1, MAP4K5, and MAP3K13, ranked among the most significantly differentially regulated transcripts. These data are intriguing in light of the recent study by Thakali et al. reporting increased phosphorylation of AKT, an insulin signaling node downstream of PI3-kinase, in umbilical cord segments from infants of overweight/obese mothers (43), and several reports of impaired PI3 kinase activation in rodent models of developmental programming (44–46). Together, these data raise the possibility that the PI3-kinase pathway may be susceptible to modulation by prenatal perturbations.

To test whether nutrient exposures might mediate transcriptional responses to maternal obesity in infant HUVEC, we analyzed the cord blood lipidome. Total free fatty acids, as well as individual lipids including palmitate (16:0), stearate (18:0), and triglycerides containing 18:3w6, 22:4w6, 22:5w6 and 20:4, were significantly increased in cord blood from infants of ov-ob women. Moreover, the ratio of 18:1n9 to 18:0, a surrogate for SCD1 delta-9 desaturase activity, was increased in the ov-ob group, similar to patterns in infants of women with gestational diabetes (47). By contrast, lipid species containing 20:3w6 fatty acids (e.g. CE20:3w6, PL20:3w6) were negatively correlated with maternal BMI. Previous reports have documented increases in plasma 20:3w6 (di-homo gamma linolenic acid, DGLA), and in phospholipids containing DGLA, in obese humans (reviewed in (48)). Moreover, maternal plasma levels of (20:3w6, or DGLA) during pregnancy have been associated with childhood obesity risk at age 7 years (49). By contrast, dietary DGLA

supplementation in mice may protect against atherosclerosis (50). These data raise the intriguing possibility that reductions in plasma DGLA in infants of obese women might contribute to increased atherosclerosis risk. More broadly, the observation that transcriptional pathways involved in lipid metabolism, together with cord blood lipid levels, are altered by maternal obesity may have relevance to offspring vascular function given the recent discovery that fatty acid oxidation by endothelial cells is critical for proliferative capacity (51). Surprisingly, we did not observe any differences in plasma triglycerides in ov-ob vs. lean women, which stands in contrast to previous studies demonstrating associations between maternal BMI and serum triglycerides (52). It is difficult to assess whether the lack of association is due to the genetics, environment, or diet of this Brazilian group of women, as we unfortunately do not have information about these characteristics. This will be an important area for future studies, as maternal triglyceridemia may be an important contributor to fetal macrosomia, a key risk factor for future metabolic disease in offspring (53).

We acknowledge that our study has several limitations. First, our sample size is small, which limits our power to detect differences, and may limit the generalizability of our findings. Our limited sample size, moreover, prevented us from adjusting for offspring sex, mode of delivery, maternal age, and other covariates that might influence infant weight status or metabolism. Secondly, the infants were not observed beyond the newborn period, so we cannot assess whether the transcriptional patterns observed in HUVEC, or the hormonal/lipidomic differences in cord blood, were linked to the development of obesity or other metabolic diseases in offspring. Thirdly, we did not exclude infants with fetal growth restriction (SGA) or growth excess (LGA), so we cannot exclude the possibility that infant weight status, rather than maternal obesity, might drive the phenotypes we observed. Finally, we recognize that analyses of transcriptomic and metabolic variables in humans cannot prove causality. Nevertheless, as a readily isolated cell of purely fetal origin, HUVEC provide evidence for how prenatal perturbations such as maternal obesity may alter cellular responses in offspring. HUVEC provide a robust model for testing how maternal obesity may alter lipid metabolism, insulin signaling, nutrient sensing or other key cellular pathways in infants.

In summary, we tested whether prenatal exposure to maternal obesity influences transcriptional responses in infant cells. We found that maternal obesity alters expression of genes related to mitochondrial and lipid metabolism. Transcriptional patterns were associated with altered levels of cord blood lipids, notably palmitate, stearate, and lipids containing omega-6 fatty acids. These data suggest that altered lipid metabolism may be an early signature of mother-to-child transmission of obesity and metabolic disease risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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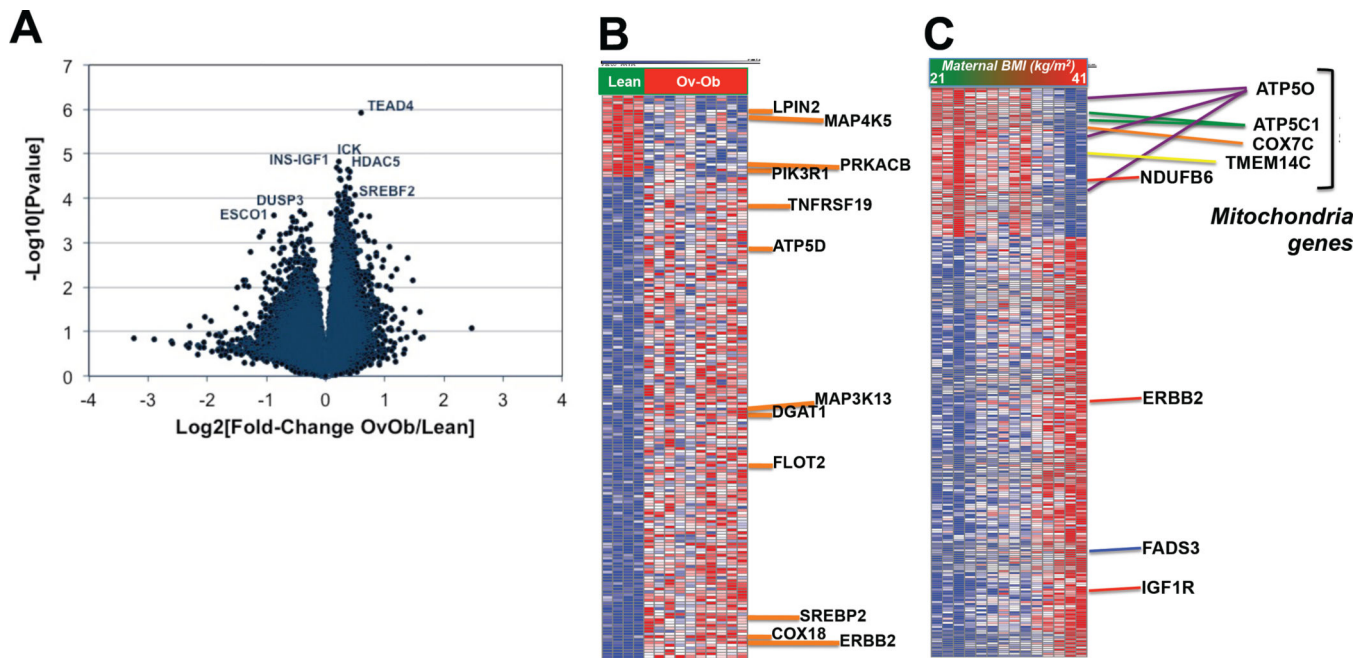


Figure 1. Global gene expression patterns in HUVEC isolated from infants of overweight-obese mothers vs. controls

A. Volcano plot.

B. Heatmap – top 200 differentially regulated probes, selected based on Comparative Marker Selection scores, GenePattern, Broad Institute.

C. Heatmap of top-ranking probesets correlating with maternal BMI, selected based on Pearson's $R = 0.75$ and $R = 0.75$. The full gene list is available in Supplemental Table 2B.

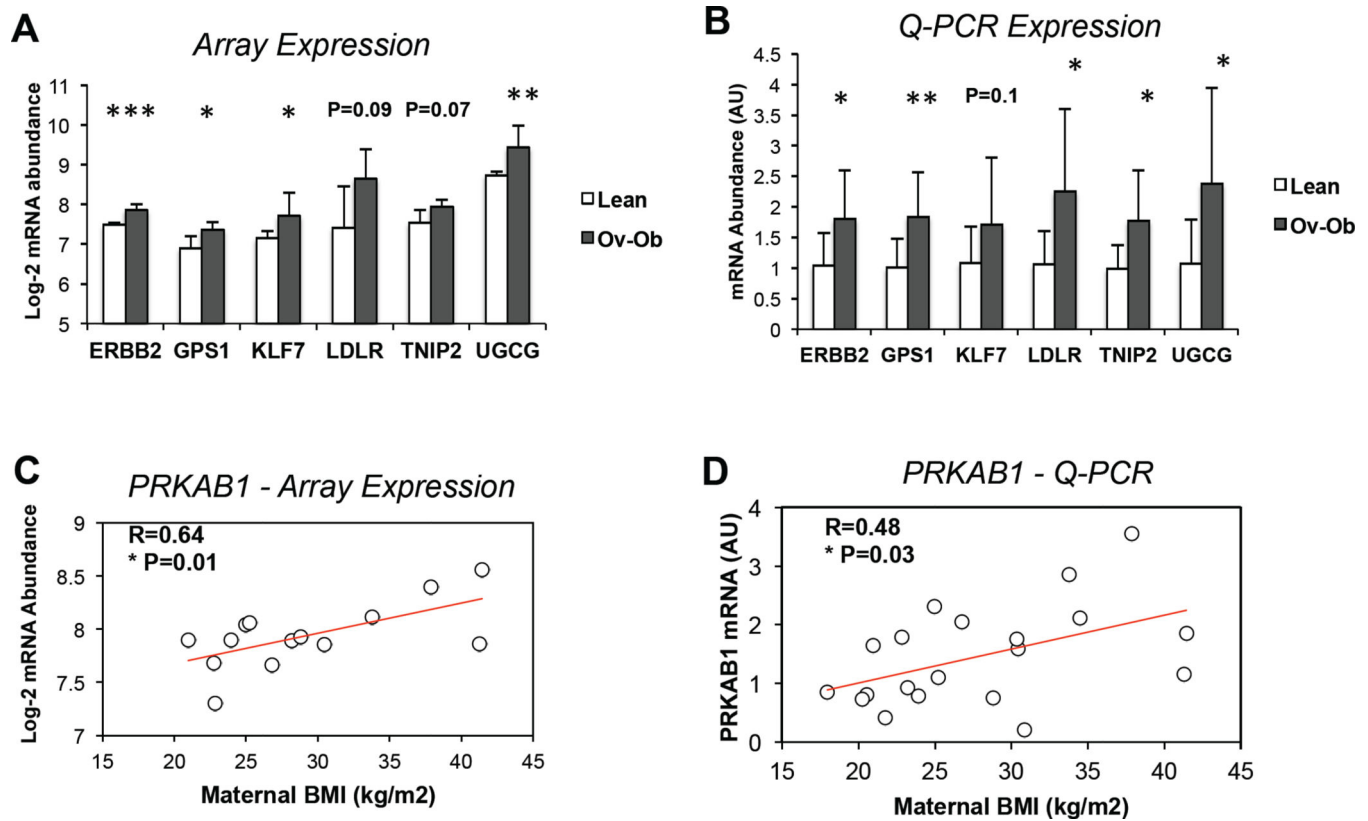


Figure 2. Validation of HUVEC gene expression patterns identified by array data using Q-PCR

A. Array expression of top-ranking differentially expressed genes in HUVEC from infants of Ov-Ob vs. lean mothers, including *erb-b2 receptor tyrosine kinase 2* (ERBB2), *G protein pathway suppressor 1* (GPS1), *Kruppel-like factor 7* (KLF7), *low density lipoprotein receptor* (LDLR), *TNFAIP3 interacting protein 2* (TNIP2), and *UDP-glucose ceramide glucosyltransferase* (UGCG). N=14.

B. qRT-PCR expression of ERBB2, GPS1, KLF7, LDLR, TNIP2, and UGCG, normalized to PPIE and to expression in lean controls. N=20.

C. Based on array analysis, HUVEC expression of *protein kinase, AMP-activated, beta 1 non-catalytic subunit* (PRKAB1) is positively correlated with maternal pre-pregnancy BMI. N=14.

D. qRT-PCR expression of PRKAB1, normalized to PPIE, is positively correlated with maternal pre-pregnancy BMI. N=20. * P<0.05, ** P<0.01, ***P<0.0001.

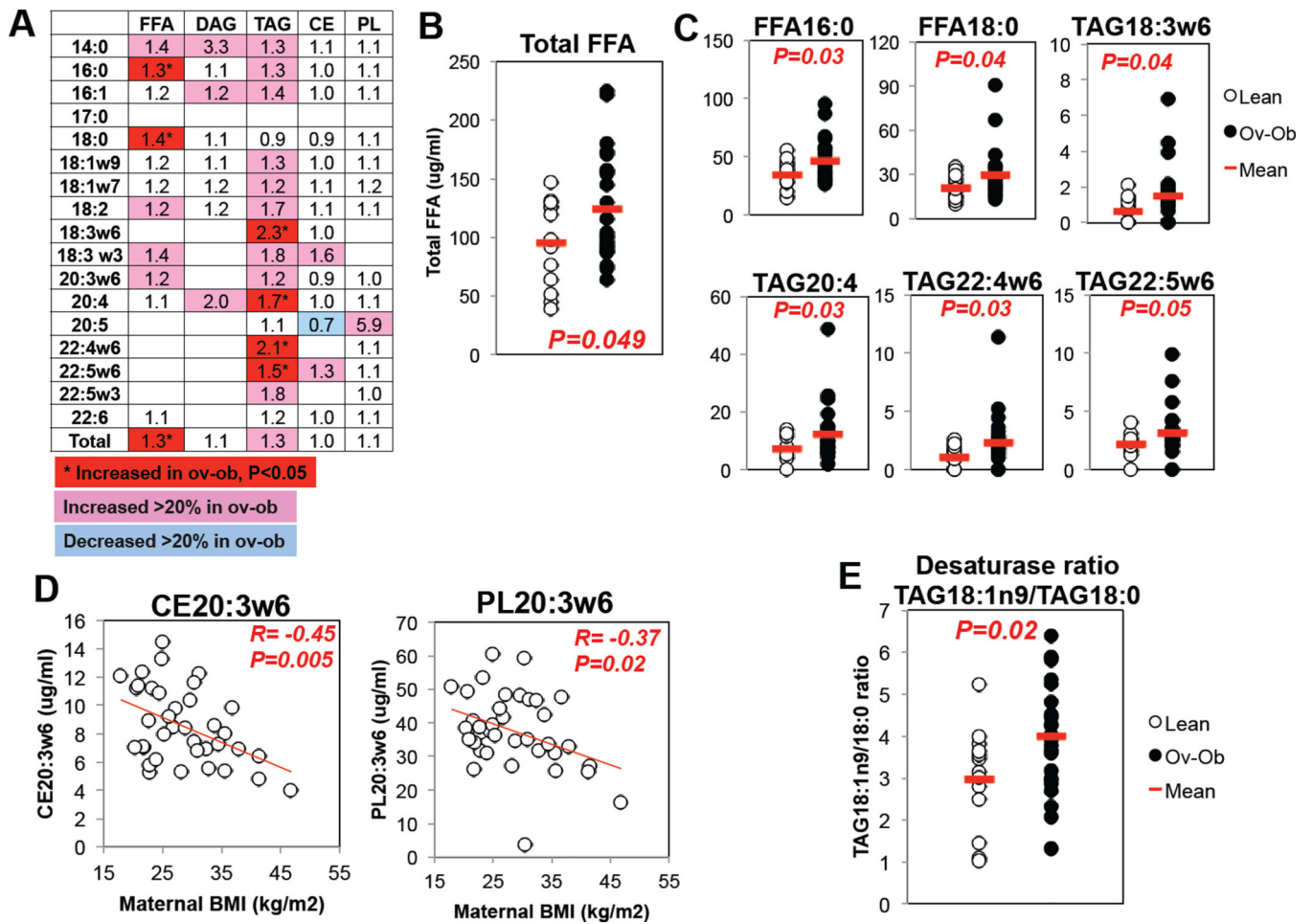


Figure 3. Maternal obesity alters infant cord blood lipidome

A. Overview of lipid composition of cord blood. Each column represents a class of lipids, and each row represents a fatty acid comprised within the lipid class. Each lipid subset was quantified by LC/MS; then normalized to the level in infants of lean mothers. Data are expressed as fold-change in ov-ob/lean. FFA: Free fatty acids; DAG: diacylglycerides; TAG: triacylglycerides; CE: cholesterol esters; PL: phospholipids.

B. Cord blood total fatty acids (ug/ml). N=37.

C. Cord blood FFA16:0, FFA18:0, omega-6 conjugated triglycerides and TAG20:4 are increased in infants of overweight/obese women. All lipid levels expressed as $\mu\text{g/ml}$. N=37.

D. 20:3w6-conjugated cholesterol esters and phospholipids are inversely correlated with maternal BMI. N=37.

E. The ratio of 18:1n9 to 18:0 triglycerides, a surrogate for *stearoyl-coA desaturase 1* (SCD1) activity, is increased in cord blood from infants of Ov-Ob women. N=37.

Table 1

Maternal pregnancy characteristics according to early pregnancy weight category.

Maternal Data	BMI < 25.0 kg/m ²	BMI ≥ 25.0 kg/m ²	P-value
Age (years)	24.6 ± 5.4 (13)	27.9 ± 5.9 (24)	0.1
Early BMI	21.9 ± 1.7 (13)	32.2 ± 5.7 (24)	<0.0001
Final BMI	27.6 ± 3.4 (13)	36.2 ± 6.6 (24)	<0.0001
Weight Gain (kg)	11.5 ± 4.5 (13)	7.8 ± 4.5(24)	0.03
Height (meters)	1.60 ± 0.06 (13)	1.59 ± 0.07 (24)	0.56
Systolic BP (mm Hg)	105 ± 15 (13)	109 ± 13 (24)	0.41
Diastolic BP (mm Hg)	62 ± 7 (13)	69 ± 10 (24)	0.02
Hematocrit (%)	36.3 ± 2.7 (13)	36.4 ± 3.5 (24)	0.98
Hemoglobin (g/dl)	11.8 ± 1.0 (13)	12.1 ± 1.3 (24)	0.49
Fasting Glucose 1st trimester (mg/dl)	78.6 ± 8.4 (11)	79.9 ± 7.2 (24)	0.64
Glucose 1h OGTT 24 weeks (mg/dl)	105.0 ± 26.8 (9)	126.4 ± 32.6 (22)	0.09
Fasting Glucose OGTT 30weeks (mg/dl)	75.7 ± 5.4 (12)	78.0 ± 10.2 (22)	0.40
Glucose 1h OGTT 30 weeks (mg/dl)	134.9 ± 18.9 (12)	139.0 ± 24.0 (22)	0.61
Glucose 2h OGTT 30 weeks (mg/dl)	117.4 ± 14.2 (12)	134.9 ± 28.2 (22)	0.02
Fasting Insulin OGTT 30 weeks (uU/ml)	9.9 ± 3.5 (11)	12.4 ± 7.0 (22)	0.27
Insulin 1h OGTT 30 weeks (uU/ml)	106.9 ± 52.3 (11)	112.3 ± 70.3 (21)	0.82
Insulin 2h OGTT 30 weeks (uU/ml)	106.4 ± 64.2 (11)	126.8 ± 83.4 (22)	0.48
HOMA-IR	1.9 ± 0.7 (11)	2.5 ± 1.6 (22)	0.24
HOMA-B (%)	478 ± 640 (11)	432 ± 347 (22)	0.79
Total Cholesterol (mg/dl)	252.7 ± 31.4 (12)	233.6 ± 41.5 (22)	0.18
HDL-Cholesterol (mg/dl)	66.0 ± 10.8 (12)	66.7 ± 16.6 (22)	0.90
LDL-Cholesterol (mg/dl)	146.2 ± 28.0(12)	121.4 ± 39.5 (22)	0.06
VLDL-Cholesterol (mg/dl)	40.6 ± 11.4 (12)	44.4 ± 19.6 (22)	0.55
Triglycerides (mg/dl)	202.9 ± 56.0 (12)	200.8 ± 49.6 (22)	0.91
C-section (%)	37.1 (13)	31.6 (12)	0.49*

Mean ± SD; sample size indicated in parentheses

P-value refers to T-test, except for categorical data, where Fisher's exact test was used (denoted by asterisk).

Table 2

Newborn characteristics and cord blood adipokines according to maternal weight category

Newborn Data	BMI < 25.0 kg/m ²	BMI ≥ 25.0 kg/m ²	P-value
Gestational Age (weeks)	39.1 ± 1.3 (13)	39.1 ± 1.3 (24)	0.85
Birth weight (grams)	3280 ± 662 (13)	3229 ± 508 (24)	0.81
Infant sex (M/F)	10M/3F	13M/11F	0.28
Weight for gestational age and sex (percentile)	41.5 ± 27.1 (13)	45.2 ± 26.5 (24)	0.69
Head circumference (cm)	33.9 ± 1.3 (13)	34.3 ± 2.1 (24)	0.56
Abdominal girth (cm)	31.6 ± 3.0 (13)	31.6 ± 2.0 (24)	0.96
Length (cm)	48.9 ± 2.8 (13)	48.8 ± 2.7 (24)	0.93
Ponderal Index (kg/m ³)	27.7 ± 2.4 (13)	27.6 ± 2.6 (24)	0.88
Cord blood hormones and adipokines			
Insulin (μU/ml)	4.6 ± 3.9 (12)	9.0 ± 9.1 (24)	0.048
HGF (ng/ml)	2.5 ± 1.0 (12)	3.1 ± 1.0 (24)	0.08
Leptin (ng/ml)	23.9 ± 19.4 (13)	49.0 ± 43.6 (24)	0.02
Adiponectin (ug/ml)	407 ± 377 (13)	337 ± 296 (24)	0.57
Resistin (pg/ml)	174 ± 97 (12)	196 ± 110 (24)	0.55
IL-1β (pg/ml)	0.12 ± 0.06 (12)	0.17 ± 0.29 (23)	0.50
IL-6 (pg/ml)	11 ± 10 (12)	41.9 ± 77.7 (23)	0.07
IL-8 (pg/ml)	61 ± 137 (12)	85 ± 210 (24)	0.68
MCP-1 (pg/ml)	386 ± 245 (13)	314 ± 205 (23)	0.38
TNF-α (pg/ml)	9.1 ± 2.5 (12)	10.8 ± 3.7 (24)	0.11
Total PAI-1 (pg/ml)	186 ± 62 (13)	152 ± 32 (23)	0.08

Mean ± SD; sample size is indicated in parentheses.

P-value refers to Student's T-test, except for Infant sex, which was analyzed by Fisher's exact test.