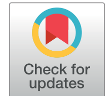

Infant Mesenchymal Stem Cell Insulin Action Is Associated With Maternal Plasma Free Fatty Acids, Independent of Obesity Status: The Healthy Start Study

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Preclinical rodent and nonhuman primate models investigating maternal obesity have highlighted the importance of the intrauterine environment in the development of insulin resistance in offspring; however, it remains unclear if these findings can be translated to humans. To investigate possible intrauterine effects in humans, we isolated mesenchymal stem cells (MSCs) from the umbilical cord tissue of infants born to mothers of normal weight or mothers with obesity. Insulin-stimulated glycogen storage was determined in MSCs undergoing myogenesis in vitro. There was no difference in insulin action based on maternal obesity. However, maternal free fatty acid (FFA) concentration, cord leptin, and intracellular triglyceride content were positively correlated with insulin action. Furthermore, MSCs from offspring born to mothers with elevated FFAs displayed elevated activation of the mTOR signaling pathway. Taken together, these data suggest that infants born to mothers with elevated lipid availability have greater insulin action in MSCs, which may indicate upregulation of growth and lipid storage pathways during periods of maternal overnutrition.

The increased incidence of obesity has led to a parallel rise in type 2 diabetes (1). Alarmingly, data from the SEARCH for Diabetes trial indicate the occurrence of type 2 diabetes in adolescents will quadruple by the year 2050 (2). Although the increased prevalence of Western-style diets and sedentary lifestyles clearly contributes to these trends, it is well accepted that the intrauterine environment has a lasting impact on offspring's risk of disease later in life. For example, children born to mothers with obesity have an increased risk of obesity (3), type 2 diabetes (4), metabolic syndrome (5), and premature death resulting from cardiovascular events (6). One underlying factor in the pathogenesis of these diseases is impaired insulin action in skeletal muscle and adipose tissues, which contributes to systemic insulin resistance (7).

Offspring in rodent (8,9) and nonhuman primate models (10) of maternal obesity exhibit skeletal muscle insulin resistance. Although much has been gained from studying animal models during pregnancy, discrepancies with humans exist, including a gestational period that is significantly shorter as well as differences in placental physiology, such as estrogen storage/release (11), expression of cell-surface markers (12), and accumulation of diet-specific metabolites (i.e., Neu5gc) (13). Therefore, it is unclear if these preclinical findings can be translated to humans. Our laboratories have used mesenchymal stem cells (MSCs) from the umbilical cord tissue of human offspring donors as an in vitro model of developmental programming in humans,

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because during fetal development, MSCs differentiate to develop mesodermal tissues, such as skeletal muscle and adipose, and are the primary progenitors for fetal myogenesis as well as postnatal skeletal muscle growth and repair (14–16). We previously reported that MSCs undergoing myogenesis from infants born to mothers with obesity have a greater capacity for adipogenesis and reduced lipid oxidation, both of which are correlated with adiposity of the neonate donor (17,18).

The primary aim of the current study was to determine the effects of maternal obesity on insulin action in human offspring MSCs when undergoing myogenesis in vitro. A secondary aim was to determine the relationships between indices of maternal metabolic health and offspring MSC insulin action. To explore potential mechanisms underlying the relationships between maternal obesity, maternal metabolic health, and offspring MSC insulin action, we also determined the expression of insulin-sensitive markers of cell growth and nutrient storage.

RESEARCH DESIGN AND METHODS

Ethics Statement

This study used umbilical cord MSCs collected from participants enrolled in the Healthy Start study. Approval for this study was obtained from the University of Colorado Hospital Institutional Review Board, and informed consent was obtained from each participant upon enrollment. All experimental procedures involving MSCs following initial collection and processing were approved and conducted at East Carolina University.

Participants

Maternal Measures

The Healthy Start longitudinal prebirth cohort study recruited women from obstetrics clinics at the University of Colorado Hospital during 2010 to 2014 and enrolled pregnant women age ≥16 years and at ≥23 weeks of gestation, as described in detail elsewhere (19). Briefly, women were excluded if they had prior diabetes, prior premature birth, serious psychiatric illness, or current multiple pregnancy. Pregnant women were evaluated at 27 weeks of gestation for demographics, tobacco use, height, and weight. Fasting blood samples taken at 27 weeks of gestation were drawn for measures of glucose, insulin, triglycerides, and free fatty acids (FFAs). HOMA for insulin resistance (HOMA-IR) was calculated as follows: (fasting serum insulin [mU/L] × fasting plasma glucose [mmol/L])/22.5 (20). Prepregnancy BMI was obtained through medical record abstraction (84%) or self-report (16%) at the first research visit. Umbilical cord–derived MSCs were cultured from a convenience sample of 165 infants collected under the mechanistic arm of Healthy Start: BabyBUMP (18). In this study, a subsample that included mothers with pregravid obesity (n = 10) and normal-weight mothers were frequency matched for maternal age, gestational age at delivery, infant sex, and MSC culture time to confluence (n = 9).

Infant Measures

At birth, umbilical cord blood was collected and used for measures of insulin, glucose, triglycerides, and leptin. Infant birth weight was obtained from medical records and measured weight and body composition (fat mass, fat-free mass, and whole-body air displacement plethysmography [PEA POD; COSMED, Inc.]) within 24 to 48 h after birth.

MSC Isolation, Culture, and Myogenesis

MSCs were cultured from fresh umbilical cord tissue explants of infants born to normal-weight mothers (NW-MSCs) (n = 9) and mothers with obesity (Ob-MSCs) (n = 10), as described previously (18). The MSCs used in the current study were shown to be >98% positive for MSC markers CD73, CD90, and CD105 and were negative for hematopoietic and lymphocyte markers CD34, CD45, and CD19 (18). All experiments were performed on cells in passages 4 to 6. Myogenesis was induced for 20 to 24 days based on visual inspection of myotube formation, which elicited elevated levels of myogenic markers, as described previously (18,21). Day of experimentation did not differ between study groups and did not affect the study results.

MSC Proliferation

Undifferentiated MSCs were incubated with BrdU for 24 h before measurement of BrdU incorporation into newly synthesized DNA using a kit (cat. no. 11647229001; Sigma-Millipore). BrdU incubation time was empirically determined in house for optimal sensitivity. For each cell line, BrdU was measured in triplicate with cell-only controls (no BrdU) and no-cell controls (with BrdU). Triplicate measures were averaged and normalized to cell-only controls.

Insulin Action

Following 3 h of serum starvation, myogenically differentiating MSCs were incubated for 2 h with media containing D-[1-^{14}C] glucose (Perkin-Elmer, Waltham, MA) (1.6 μCi/ml, 5.0 mmol/L glucose), with or without 100 nmol/L insulin, at 37°C (22). Cells were washed twice with ice-cold Dulbecco's PBS and then solubilized with 0.5% SDS. Lysates were spiked with carrier glycogen (1 mg) and hydrolyzed at 100°C for 1 h. Ice-cold 100% ethanol was added to the spiked lysate to a final concentration of 70% in order to precipitate glycogen. Lysates were centrifuged at 11,100g for 15 min at 4°C and washed with 70% ethanol followed by centrifugation. The glycogen pellets were resuspended with distilled water, and the incorporation of radioactive glucose into glycogen was determined with liquid scintillation. An aliquot of unspiked lysate was reserved to measure protein concentration using bicinchoninic acid assay (Pierce Biotechnology, Rockford, IL). Rates of glycogen synthesis were expressed as either absolute values (nmol glucose/mg protein/minute) to assess basal and maximal glycogen synthesis or log-transformed relative values (log [insulin stimulated/noninsulin stimulated and fold change]) to assess insulin-mediated

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glycogen synthesis. To clarify interpretation, fold change with insulin was used as the index of insulin action.

Protein Quantification

Myogenically differentiating offspring MSCs were washed with 1× PBS and harvested in lysis buffer (CelLytic MT; Sigma-Aldrich, St. Louis, MO) supplemented with protease and phosphatase inhibitor cocktails (Sigma-Aldrich). Total protein was determined by bicinchoninic acid assay, and equal total protein from each sample was loaded into Simple Western plates (Protein Simple, San Jose, CA) to measure proteins of interest, as described previously (17,18). Specific content of Akt, phospho-Akt^{S473}, mTOR, phospho-mTOR^{S2448}, phospho-p70S6K$^{T421/S424}$, phospho-p85S6k$^{T444/S447}$, liver x receptor α, sterol regulatory element-binding protein 1c (SREBP-1c), fatty acid synthase, diacylglycerol acyltransferase 1, stearoyl-CoA desaturase 1, glucose transporter 4, acetyl-CoA carboxylase (ACC), and phospho-ACC was measured. Results from Western plates were analyzed using ProteinSimple Compass software. All antibodies were optimized in house for this system; antibody specifics and assay conditions are listed in Supplementary Table 1, and complete chemiluminescent graphs are shown in Supplementary Fig. 5*A*–*N*. Each panel of the figure represents the chemiluminescent signals from each participant sample, displayed in an electropherogram format. The electropherogram shows the intensity of the chemiluminescent signatures (y-axis), detected along a spectrum of molecular weights (x-axis), displayed as peaks similar to a band on a Western blot. The area under the curve for the blue or yellow highlighted peak, indicating chemiluminescent intensity for the protein of interest, was used to quantify specific protein content.

Intracellular Triglyceride Measures

Cell pellets were collected from 21-day myogenically differentiating cells. Briefly, cells were rinsed twice with ice-cold PBS, collected, and pelleted by centrifugation. Pellets were flash frozen in liquid nitrogen and stored at −80°C until measurement. For measurement, cells were thawed on ice, resuspended in PBS, and then fortified with internal standards. Lipids were extracted and analyzed by the Colorado Nutrition Obesity Research Center lipidomics core. The core routinely runs triacylglycerol from human biologic samples, and the data are reported for the number of carbons and the number of double bonds. Samples were run on a Sciex 2000 triple quadrupole mass spectrometer (Framingham, MA). Saturated species included only those species in which all three acyl chains contained no double bonds. Concentration was determined by comparing ratios of unknowns to odd chain or deuterated internal standards with standard curves run with standards of each lipid species.

Statistical Analyses

Data were analyzed using GraphPad Prism 9.3 (GraphPad Software; San Jose, CA) or RStudio (version 1.4.1106). All data are presented as means ± SDs. Unpaired Student *t* tests and one-way repeated measures ANOVAs were used to determine statistical significance, where appropriate. Kolmogorov-Smirnov tests were used to assess normality of the data. If the data did not follow a Gaussian distribution, Mann-Whitney tests were used when indicated in the figure legends. Factors tested were group and/or insulin treatment. Pearson correlations were used to identify relationships between maternal, offspring, and MSC outcomes. Parallel linear regression models were run using the lm function in R, with model adjustment for infant sex, gestational age at delivery, and maternal prepregnancy BMI. Pearson correlations are reported, with adjusted linear model *P* values additionally reported. Addition of covariates did not appreciably affect results. Statistical significance was set at $P \leq 0.05$.

Data and Resource Availability

The data sets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request. No applicable resources were generated or analyzed during the current study.

RESULTS

Maternal and Infant Characteristics

By design, mothers with obesity had higher BMI but similar maternal age and gestational age at delivery (Table 1). Mothers with obesity had higher fasting plasma insulin concentrations and HOMA-IR values ($P \leq 0.05$). Infants from both cohorts were similar in sex distribution, birth weight, and cord blood insulin, glucose, and leptin at the time of delivery (Table 1).

Insulin Action Is Not Different Between NW-MSCs and Ob-MSCs

MSC proliferation was not different between groups (Supplementary Fig. 1). Protein expression of myogenic markers, myogenin, and myosin heavy chain was similar between NW-MSCs and Ob-MSCs at days 7 and 21 of differentiation, respectively (Supplementary Fig. 2). Insulin stimulation increased glycogen synthesis rates twofold over basal (Fig. 1*A*), demonstrating insulin responsiveness. However, neither basal nor maximal rates, nor insulin-mediated rates of glycogen synthesis (log fold change over basal), differed between groups (Fig. 1*A* and *B*) ($P > 0.05$). This log fold change value is hereafter referred to as insulin action. To assess whether this trend was affected by the sex of the offspring, we sex stratified the groups as well and found insulin-mediated glycogen synthesis rates were similar between male and female offspring, regardless of parent BMI classification (Supplementary Fig. 3).

MSC Insulin Action Is Positively Correlated With Maternal FFAs

Because we did not observe overt differences in MSC insulin action based on maternal BMI, and given the large

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Table 1—Maternal and infant characteristics

Characteristics	Normal weight (n = 9)	Obese (n = 10)	P
Maternal			
Age, years	30.0 ± 5.3	27.9 ± 8.2	0.54
Prepregnancy BMI, kg/m^2	21.1 ± 0.9	32.9 ± 2.1	≤0.05*
Glucose, mg/dL	75.0 ± 8.8	79.1 ± 8.2	0.31
Insulin, μU/mL	9.2 ± 3.7	16.4 ± 7.7	≤0.05*
HOMA-IR	1.7 ± 0.7	3.3 ± 2.0	≤0.05*
Triglycerides, mg/dL	150.6 ± 56.8	171.6 ± 47.9	0.39
FFAs, μEq/L	378.0 ± 126.4	462.9 ± 158.5	0.47
Gestational weight gain, kg	13.2 ± 3.7	11.9 ± 7.5	0.64
Gestational age at delivery, weeks	39.5 ± 0.8	39.7 ± 1.3	0.67
Neonate			
Sex, n (female/male)	9 (3/6)	10 (3/7)	0.27
Birth weight, g	3,258.6.2 ± 340.4	3,350.2 ± 394.5	0.60
Neonatal adiposity, %	7.6 ± 4.0	10.3 ± 3.5	0.16
Cord blood glucose, mg/dL	75.6 ± 25.1	73.0 ± 17.4	0.81
Cord blood insulin, μIU/mL	6.4 ± 3.0	9.8 ± 5.2	0.16
Cord blood leptin, ng/mL	11.5 ± 13.6	16.6 ± 9.6	0.50

Data are mean ± SD unless otherwise indicated. *Significant by independent Student t test, $P \leq 0.05$.

degree of variation between participants, we hypothesized that maternal metabolic exposures could influence MSC insulin action and assessed relationships between insulin action and blood indices of maternal metabolic health (glucose, insulin, triglycerides, and FFAs) at 27 weeks of gestation. MSC insulin action was not correlated with maternal glucose (Fig. 2*A*) ($P > 0.05$), insulin (Fig. 2*B*) ($P > 0.05$), or triglycerides (Fig. 2*C*) ($P > 0.05$). Only maternal FFAs displayed a positive correlation with MSC insulin action (Fig. 2*D*) ($r = 0.58$; $P \leq 0.05$).

MSC Insulin Action Is Positively Correlated With Cord Blood Leptin and Intracellular Triglycerides

Emerging evidence indicates excess lipid availability from the mother may accelerate fetal growth, increasing neonatal adiposity (23–25). Insulin is a key growth factor regulating these processes and is necessary for healthy fetal growth. Therefore, the positive correlation of MSC insulin action with maternal FFAs suggests that insulin action may be linked to growth and lipid storage in offspring. Therefore, we assessed this relationship using birth weight, fat mass at birth, and cord blood leptin as offspring indices of lipid storage. Insulin action in offspring MSCs did not correlate with offspring birth weight or fat mass at birth (Fig. 3*A* and *B*) ($P > 0.05$). However, insulin action showed a strong positive relationship with cord blood leptin (Fig. 3*C*) ($r = 0.69$; $P \leq 0.05$). To determine whether a similar phenomenon was evident within the cells, we assessed the relationship between MSC insulin action and intracellular triglyceride storage. We found that MSC insulin action was positively correlated with total (Fig. 3*D*) ($r = 0.49$; $P \leq 0.05$) and positively trending with saturated (Fig. 3*E*) ($r = 0.47$; $P = 0.07$) triglyceride content.

High Maternal FFAs Correspond With Elevated Insulin/IGF/mTOR Signaling in MSCs From Offspring

Because observed maternal FFAs were related to MSC insulin action (Fig. 2*D*), we examined MSC differences based on median stratification of maternal FFAs. Supplementary Table 2 shows maternal and infant characteristics based on this stratification. Mothers in the high FFA group were older at the time of conception and delivered their infants ∼6 days earlier, although this did not translate into differences in infant birth weight, adiposity, or other metabolic measures (Supplementary Table 2). MSCs from the offspring of mothers with higher FFAs displayed a trend toward higher insulin action ($P = 0.07$) (Supplementary Fig. 4*A*). Given the observed correlation of MSC insulin action with intracellular triglyceride stores, we also measured protein targets involved with lipid synthesis and storage. Although we observed a trend toward greater liver x receptor α protein content in the high maternal FFA group ($P = 0.10$) (Fig. 4*A*), there were no group differences in any downstream proteins (SREBP-1c, fatty acid synthase, or diacylglycerol acyltransferase 1 protein content) (Fig. 4*B–D*). There were also no differences in glucose transporter 4, ACC (total protein or phosphorylation at serine 79), or stearoyl-CoA desaturase 1, (Supplementary Fig. 4*B–D*).

Maternal overnutrition enhances placental insulin/IGF/mTOR signaling, which may help partition nutrients to the developing fetus, thereby promoting growth (26,27). Given these fetal growth properties of insulin, we next measured protein markers of the insulin/IGF/mTOR signaling pathway in MSCs from offspring. We observed that MSCs from offspring born to mothers with elevated FFAs during pregnancy also displayed elevated Akt^{S473} phosphorylation (Fig. 5*A*) ($P \leq 0.05$) and a trending elevation in mTOR protein content (Fig. 5*B*) ($P = 0.07$). Furthermore, protein content of the mTOR substrate, p70S6k$^{T421/S424}$ phosphorylation

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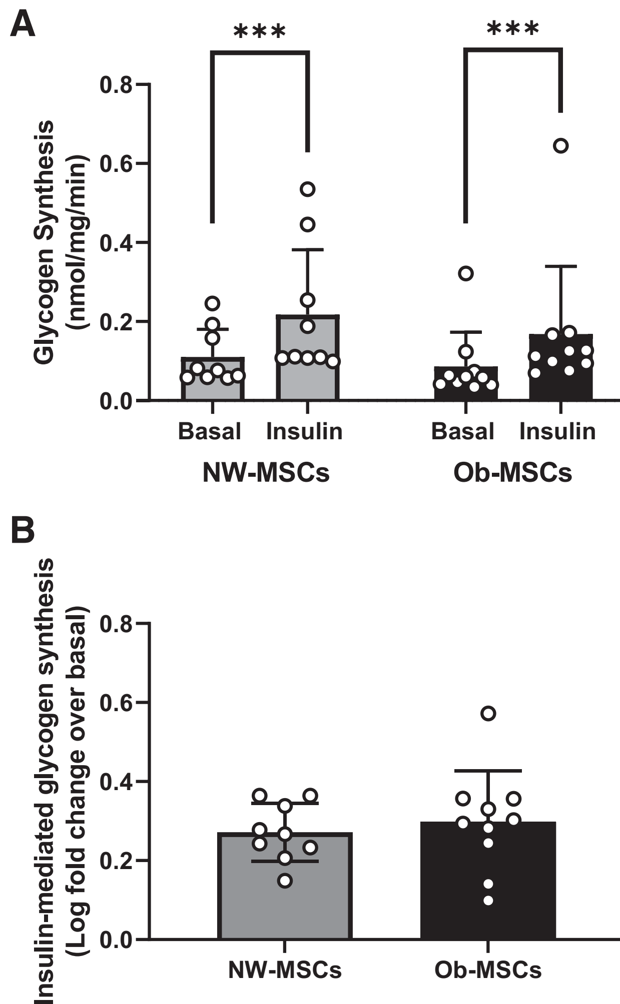

Figure 1—Maternal BMI does not affect insulin action in offspring MSCs. NW-MSCs and Ob-MSCs were myogenically differentiated for 20 to 24 days. Rates of glycogen storage were measured under basal and insulin-stimulated conditions and expressed as absolute (*A*) or insulin-mediated (*B*) rates of glycogen synthesis (log fold change over basal). Absolute rates of glycogen synthesis were analyzed via one-way ANOVA, and insulin-mediated rates were compared via Student *t* test and are expressed as mean ± SD (n = 9 and 10 for NW-MSCs and Ob-MSCs, respectively). ***$P \leq 0.001$ (significantly different from within-group basal rates.

(Fig. 5*C*) ($P \leq 0.05$) was elevated, with a trend toward higher p85S6k$^{T444/S447}$ phosphorylation (Fig. 5*D*) (P = 0.08). These data suggest that elevated maternal FFAs are linked to greater insulin action and insulin/mTOR pathway activation in offspring MSCs.

DISCUSSION

Animal models and human epidemiologic cohorts indicate that gestational obesity increases the risk of obesity as well as systemic and skeletal muscle insulin resistance in the offspring (3–5,10). However, the pathways by which maternal obesity exposure may induce poor metabolic health in offspring are not well defined, particularly in humans. Although we acknowledge that the MSC model is not a peripheral tissue contributing to infant metabolism at or after birth, recent evidence (17,18,28–30) supports that intrauterine exposures contribute to offspring MSC phenotypes, with relevance to long-term offspring metabolic health. The goal of this study was to determine insulin action in umbilical cord–derived MSCs from human infants exposed to obesity in utero.

Maternal obesity was not associated with differences in MSC insulin action (Fig. 1). Although screening for maternal obesity has provided a valuable prognostic tool for maternal health, metabolites from the mother that cross the placenta, such as glucose and FFAs, likely play a much larger role than maternal body size. For example, Gademan et al. (31) reported a positive relationship between maternal BMI and neonatal adiposity but also noted an independent relationship between maternal lipids and neonatal body composition. Samsuddin et al. (25) observed that gestational weight gain and maternal lipid levels were strongly associated with neonatal adiposity, independent of maternal insulin resistance, blood glucose, or pregravid BMI status. Furthermore, we previously reported that maternal metabolic health indicators, such as maternal glucose, insulin HOMA-IR, FFAs, and HDLs, may be more robust predictors of MSC metabolism than BMI (17,29). In line with these recent findings, we found a relationship between maternal FFAs and MSC insulin action (Fig. 2), which suggests that lipid availability in mid to late gestation, as opposed to maternal obesity status, may be a predictor of MSC insulin action. Importantly, the mothers in the obese group did not present with elevated FFAs, but infants born to mothers with higher FFA levels, regardless of body mass, had MSCs with trending elevations (P = 0.07) in insulin action (Supplementary Fig. 4*A*) and activation of insulin-mediated growth pathways (Fig. 5).

It may seem counterintuitive that MSCs from infants born to mothers with higher FFAs have elevated insulin action. However, when we consider that excess lipid availability may accelerate fetal growth (23–25,32,33), it is biologically plausible that increased FFA availability is concurrently occurring with enhanced insulin action. In our MSCs, insulin action was positively correlated with both triacylglycerol content in the MSCs and cord blood leptin (Fig. 3*C–E*), an index of fetal growth (34–37), but newborn body composition did not differ according to maternal FFA concentration (Supplementary Table 2) or MSC insulin action (Fig. 3*B*). Animal models of adverse intrauterine programming indicate that offspring metabolic consequences, such as insulin resistance, do not manifest until later in life (38–40). In humans, longitudinal analysis shows that positive associations between circulating FFAs and insulin resistance do not develop until adulthood (41). Therefore, it is plausible that the phenotype evident in the MSCs in the current study does not represent newborn body habitus but may reflect predisposition for

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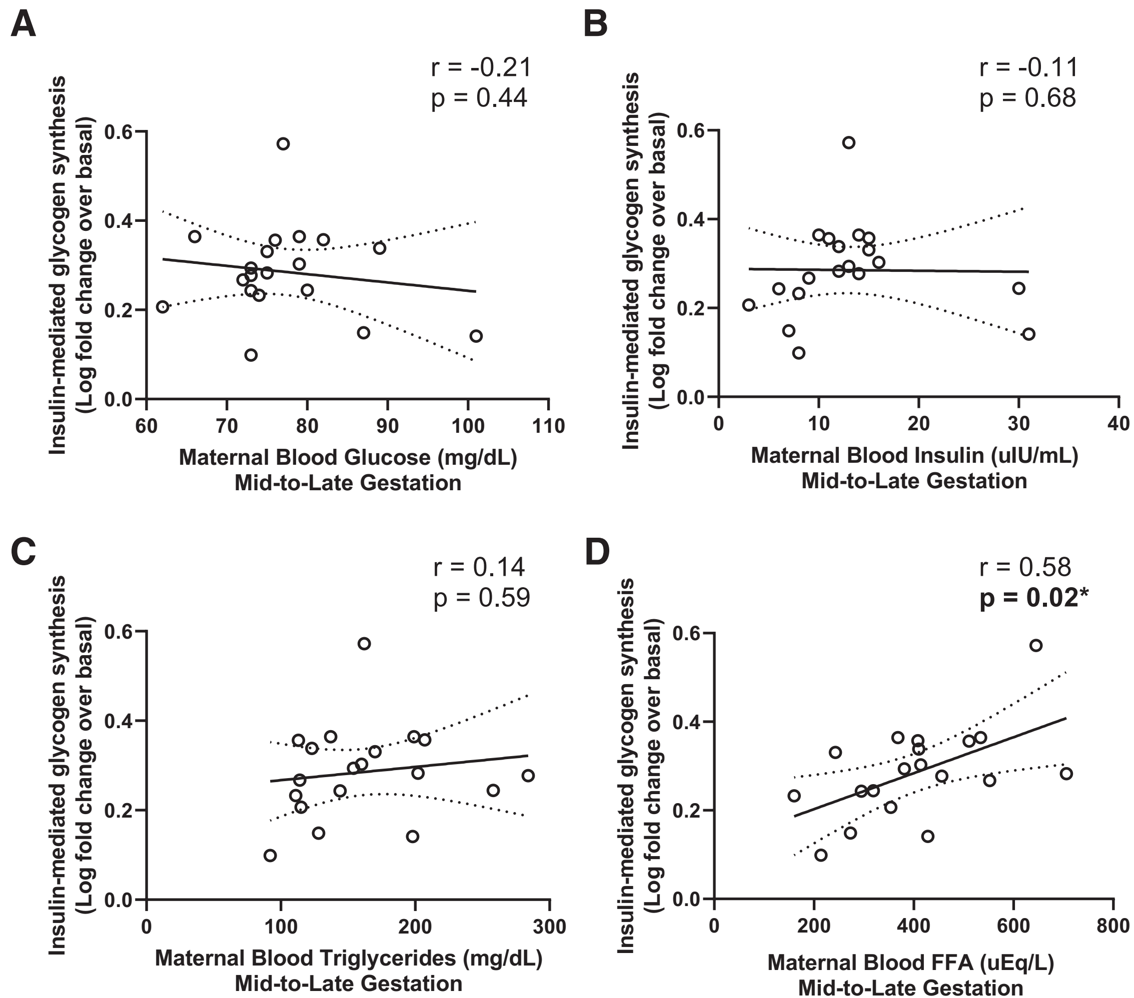

Figure 2—Maternal FFAs are positively associated with insulin action in offspring MSCs. Following myogenic induction, insulin-mediated glycogen synthesis rates were assessed in offspring MSCs. Pearson correlation analysis was used to determine the relationship between insulin-mediated glycogen synthesis rates (log fold change over basal) in offspring MSCs with maternal blood markers at 27 weeks of gestation. Insulin-mediated glycogen synthesis rate was not associated with maternal blood glucose (*A*), insulin (*B*), or triglycerides (*C*). Maternal FFAs were positively associated with insulin action in offspring MSCs (*D*) (n = 19). *$P \leq 0.05$.

altered metabolism. With respect to stored triglycerides, these lipids have been shown to be inert in the context of insulin sensitivity when compared with other reactive lipid species (e.g., ceramides, diacylglycerols, and acylcarnitines) (42–45). Therefore, it is possible that elevated triglyceride storage may serve as a means to minimize accumulation of other more reactive species, which would be detrimental to the cell. Taken together, the links between maternal obesity, maternal lipid availability, cord blood leptin, and offspring metabolic health outcomes are likely quite nuanced and require further study.

When mothers were divided into high and low FFA groups, both insulin and mTOR pathway induction in MSCs were elevated in the high FFA group (Fig. 5). Little is known about mTOR signaling in infants or infant MSCs in this context, but abundant research in placental tissue demonstrates that mTOR signaling is elevated with fetal overgrowth (26,27,46) and promotes nutrient transport, oxidative phosphorylation, protein synthesis, and hormone synthesis (47). Moreover, we recently reported that activation of the placental mTORC1 pathway is positively associated with offspring adiposity at birth (30), and placental activation of both mTORC1 and insulin signaling pathways is positively associated with adiposity in childhood (28,30). Recent work by Castillo-Castrejon et al. (48) revealed that insulin sensitivity in placental trophoblasts from mothers with obesity prior to pregnancy and/ or who developed gestational diabetes during pregnancy was similar compared with that from their lean counterparts without diabetes. The authors speculated the lack of an obesity effect, similar to that observed in the current study, may serve to ensure adequate nutrient delivery to the fetus (48). Although mTOR and insulin signaling are necessary for healthy fetal growth and tissue development,

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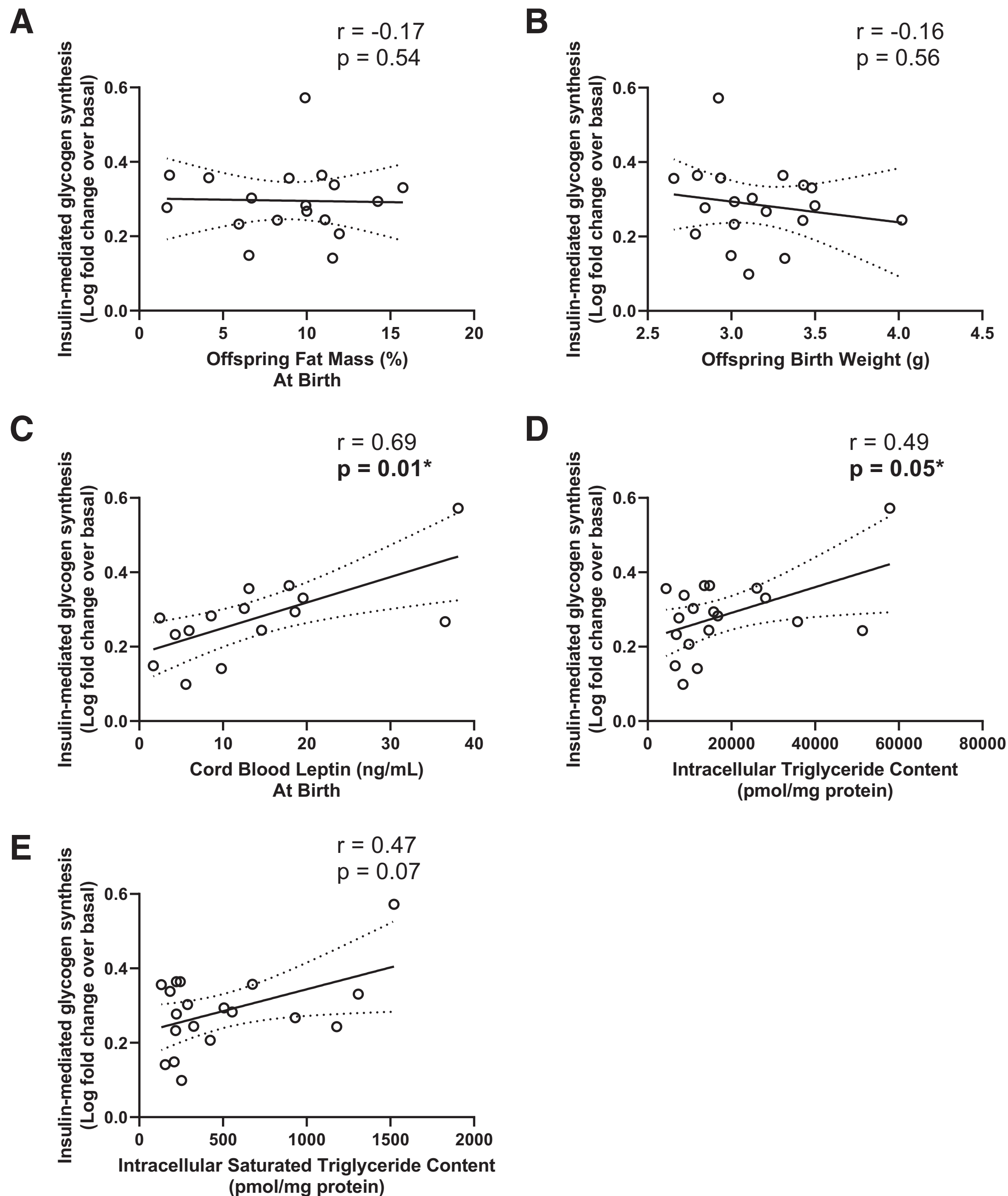

Figure 3—Insulin action in offspring MSCs is positively associated with intracellular triglyceride levels and cord leptin concentration at birth. Pearson correlation analysis was used to assess the relationship between MSC insulin-mediated glycogen synthesis with offspring fat mass at birth (*A*), offspring birth weight (*B*), cord blood leptin levels (*C*), and intracellular content of total (*D*) and saturated (*E*) triglycerides in offspring MSCs. Insulin-mediated glycogen synthesis (log fold change over basal) was not associated with offspring fat mass at birth or with offspring birth weight but was positively associated with offspring cord blood leptin levels. Furthermore, insulin action was positively associated with total and saturated levels of intracellular triglyceride content (n = 15–19). $^*P \leq 0.05$.

aberrant hyperactivity may contribute to metabolic dysfunction later in life, as indicated in reports of placental pathway activation being linked to greater child adiposity (28,30).

Despite observing a correlation between MSC insulin action and triacylglycerol stores in the myogenically differentiating MSCs, we did not observe differences in the content of proteins involved in insulin-mediated lipid storage when stratified by maternal FFAs (Fig. 4). mTOR activation can stimulate lipid storage through proteins, such as SREBP (49); however, in the current study, SREBP expression was not different. We recently reported that poor maternal metabolic health, independent of obesity, induces deficits in MSC fat oxidation following myogenic induction

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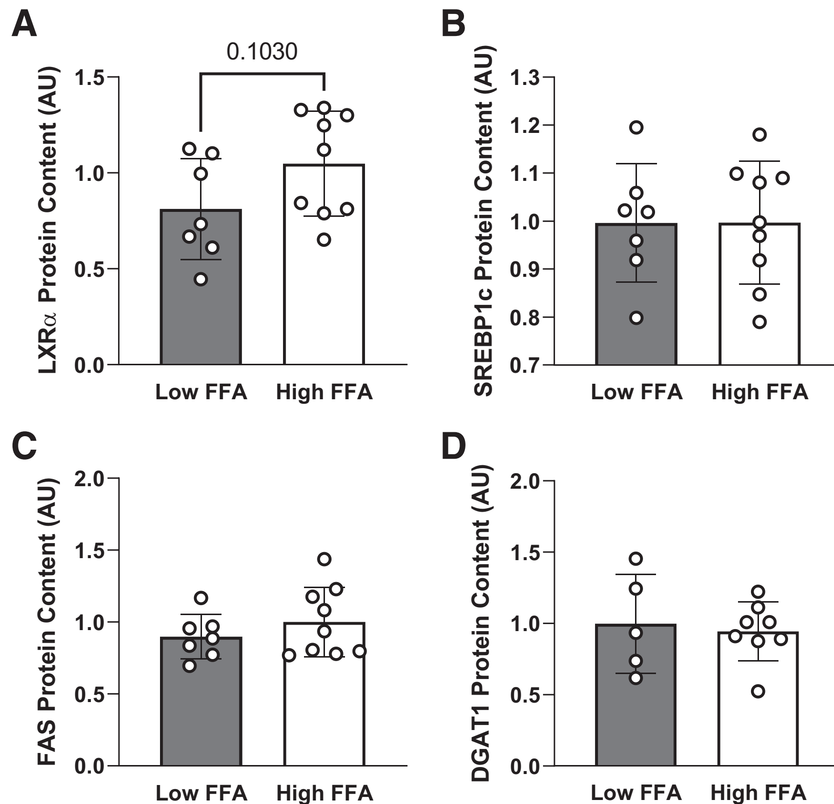

Figure 4—Maternal FFAs are not associated with lipogenic protein marker content in offspring MSCs. Following myogenic induction, protein quantification of targets involved in lipid synthesis and storage revealed trending elevations in offspring MSC protein content of liver x receptor α (LXRα) (*A*) but not SREBP-1c (*B*), fatty acid synthase (FAS) (*C*), or diacylglycerol acyltransferase (DGAT1) (*D*) when stratified by maternal FFAs at 27 weeks of gestation (low vs high FFAs) (n = 5–9 per group). Group comparisons were analyzed via Student t test. AU, arbitrary unit.

(17,29). Indeed, in the absence of upregulated lipid biosynthesis pathways or differences in cellular lipid uptake, we reported that MSCs from infants whose mothers had a less favorable metabolic milieu showed lower fat oxidation and greater triglyceride esterification than those from infants whose mothers had a more favorable metabolic milieu (17,29), suggesting a higher propensity for substrate storage.

Limitations and Future Directions

Although the current data uncover a potentially interesting role of maternal FFAs in the context of fetal programming, the study was not without limitations. First, the lack of a relationship between maternal FFAs and HOMA-IR and/or BMI status renders it difficult to explain the interparticipant variance in FFAs during pregnancy. Although HOMA-IR is useful in assessing whole-body insulin sensitivity, it is more reflective of hepatic insulin action (50,51). Therefore, future analysis should include dynamic measures of glucose or lipid tolerance examining peripheral tissues that may contribute to maternal FFA concentration. Because our original aim was to investigate the effect of maternal prepregnancy BMI, we selected MSC samples from a subset mothers who had obesity (BMI $\geq$30 kg/m^2) prior to pregnancy and compared them with samples from mothers with normal weight (BMI 18.5–24.9 kg/m^2). Therefore, our subsequent correlational and stratified analysis may not be generalizable to a BMI spectrum. Secondly, although our data provide evidence of storage phenotype being invoked by elevated maternal FFAs, we do not have sufficient follow-up data on the children to substantiate this hypothesis. Furthermore, the group with elevated FFAs was still within range of a healthy normal pregnancy. Therefore, future investigations should include a spectrum of maternal BMI, larger sample size, and more longitudinal data to help understand the utility of the MSC model in predicting aspects of offspring health (e.g., metabolic markers and body composition). Additionally, although we assessed the MSCs as they were undergoing myogenesis, we were limited in our ability to extrapolate these findings to the offspring's mature skeletal muscle. Future analyses should attempt to recapitulate aspects of the intrauterine environment in vitro to determine how this affects the metabolic phenotype of both undifferentiated and myogenically differentiating MSCs alike.

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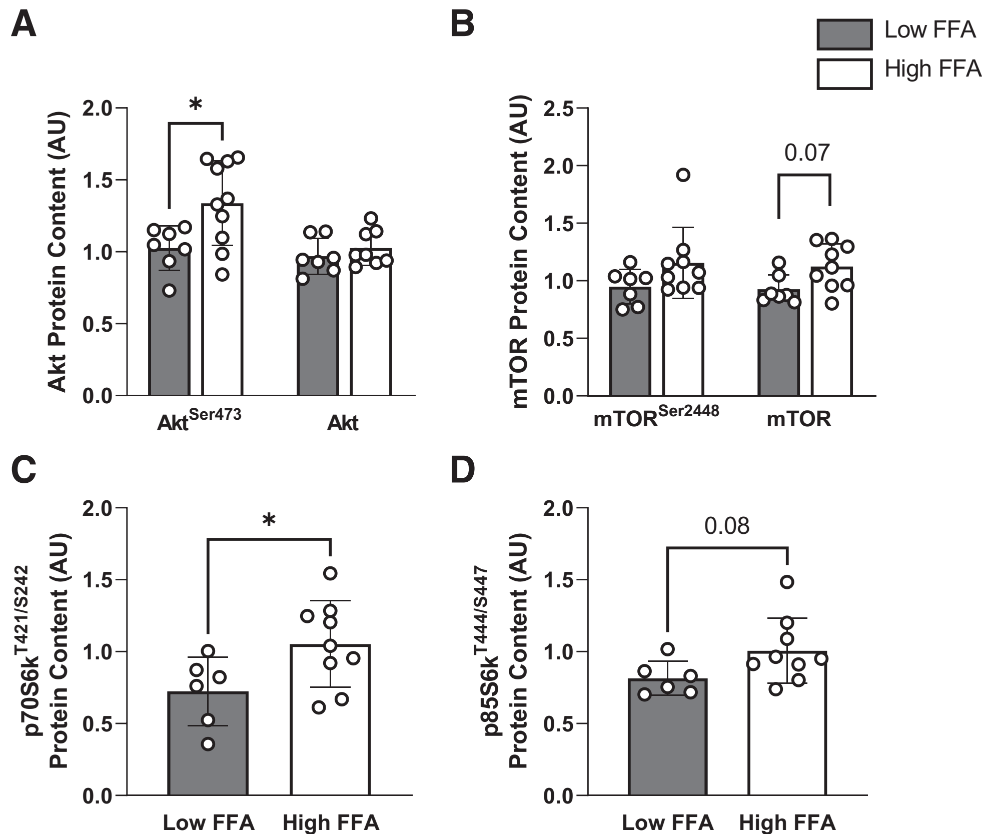

Figure 5—Elevated mTOR signaling in MSCs from offspring born to mothers with higher FFAs during pregnancy. Following myogenesis, protein quantification was conducted on members of the insulin/IGF/mTOR signaling pathway, including Akt (*C*), mTOR (*D*), p70S6k$^{T421/S424}$ (*E*), and p85S6k$^{T444/S447}$ (*F*), in offspring MSCs (n = 6–10 per group). Group comparisons for AktS473, mTOR^{S2448}, p70S6k$^{T421/S424}$, and p85S6k$^{T444/S447}$ were analyzed via Student t test. Kolmogorov-Smirnov test revealed data for mTOR did not follow a normal distribution, and therefore Mann-Whitney test was used for group comparisons. *$P \leq 0.05$. AU, arbitrary unit.

Conclusion

In the current study, maternal obesity was not related to insulin action in offspring-derived MSCs undergoing myogenesis. However, maternal FFA concentrations were positively correlated with MSC insulin action, which seems to be linked to cellular growth and lipid storage, as indicated by activation of mTOR signaling and a positive relationship with intracellular triglyceride content. These data support the importance of the intrauterine environment and extend findings to perturbations in insulin and mTOR signaling pathways in the tissue of offspring. Taken together, the data show MSCs from infants born to mothers with elevated FFAs seem to exhibit upregulation of growth and lipid storage, mediated by activation of insulin and mTOR pathways, which may serve to increase nutrient storage. Furthermore, these data highlight the importance of maternal health in dictating offspring outcomes, independent of BMI status.

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Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. A.B.C., J.A.H., and K.E.B. conceived this project. A.B.C., D.Z., J.A.J., B.C.B., Z.W.P., and V.Z. performed experiments and collected and analyzed the data. A.B.C., B.C.B., V.Z., E.M.B., P.K., N.T.B., L.E.M., and K.E.B. interpreted the results. A.B.C., J.A.H., and K.E.B. drafted the manuscript. D.D. conceptualized and implemented the parent Healthy Start study. All authors edited the manuscript and approved the final version. K.E.B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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References

1. Flegal KM, Kruszon-Moran D, Carroll MD, Fryar CD, Ogden CL. Trends in obesity among adults in the United States, 2005 to 2014. JAMA 2016;315:2284–2291
2. Imperatore G, Boyle JP, Thompson TJ, et al.; SEARCH for Diabetes in Youth Study Group. Projections of type 1 and type 2 diabetes burden in the U.S. population aged $<$20 years through 2050: dynamic modeling of incidence, mortality, and population growth. Diabetes Care 2012;35:2515–2520
3. Shankar K, Harrell A, Liu X, Gilchrist JM, Ronis MJ, Badger TM. Maternal obesity at conception programs obesity in the offspring. Am J Physiol Regul Integr Comp Physiol 2008;294:R528–R538
4. Dabelea D, Mayer-Davis EJ, Lamichhane AP, et al. Association of intrauterine exposure to maternal diabetes and obesity with type 2 diabetes in youth: the SEARCH Case-Control Study. Diabetes Care 2008;31:1422–1426
5. Boney CM, Verma A, Tucker R, Vohr BR. Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. Pediatrics 2005;115:e290–e296
6. Reynolds RM, Allan KM, Raja EA, et al. Maternal obesity during pregnancy and premature mortality from cardiovascular event in adult offspring: follow-up of 1 323 275 person years. BMJ 2013;347:f4539
7. DeFronzo RA, Tripathy D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. Diabetes Care 2009;32(Suppl. 2):S157–S163
8. Williamson DL, Li Z, Tuder RM, Feinstein E, Kimball SR, Dungan CM. Altered nutrient response of mTORC1 as a result of changes in REDD1 expression: effect of obesity vs. REDD1 deficiency. J Appl Physiol (1985) 2014;117:246–256
9. Mingrone G, Manco M, Mora MEV, et al. Influence of maternal obesity on insulin sensitivity and secretion in offspring. Diabetes Care 2008;31:1872–1876
10. Campodonico-Burnett W, Hetrick B, Wesolowski SR, et al. Maternal obesity and Western-style diet impair fetal and juvenile offspring skeletal muscle insulin-stimulated glucose transport in nonhuman primates. Diabetes 2020;69:1389–1400
11. Witorsch RJ. Low-dose in utero effects of xenoestrogens in mice and their relevance to humans: an analytical review of the literature. Food Chem Toxicol 2002;40:905–912
12. Soni C, Karande AA. Glycodelin A suppresses the cytolytic activity of CD8+ T lymphocytes. Mol Immunol 2010;47:2458–2466
13. Springer SA, Diaz SL, Gagneux P. Parallel evolution of a self-signal: humans and new world monkeys independently lost the cell surface sugar Neu5Gc. Immunogenetics 2014;66:671–674
14. Bailey P, Holowacz T, Lassar AB. The origin of skeletal muscle stem cells in the embryo and the adult. Curr Opin Cell Biol 2001;13:679–689
15. Du M, Zhao JX, Yan X, et al. Fetal muscle development, mesenchymal multipotent cell differentiation, and associated signaling pathways. J Anim Sci 2011;89:583–590
16. Amthor H, Christ B, Patel K. A molecular mechanism enabling continuous embryonic muscle growth - a balance between proliferation and differentiation. Development 1999;126:1041–1053
17. Boyle KE, Patinkin ZW, Shapiro ALB, et al. Maternal obesity alters fatty acid oxidation, AMPK activity, and associated DNA methylation in mesenchymal stem cells from human infants. Mol Metab 2017;6:1503–1516
18. Boyle KE, Patinkin ZW, Shapiro AL, Baker PR 2nd, Dabelea D, Friedman JE. Mesenchymal stem cells from infants born to obese mothers exhibit greater potential for adipogenesis: the Healthy Start BabyBUMP Project. Diabetes 2016; 65:647–659
19. Crume TL, Shapiro AL, Brinton JT, et al. Maternal fuels and metabolic measures during pregnancy and neonatal body composition: the healthy start study. J Clin Endocrinol Metab 2015;100:1672–1680
20. Gayoso-Diz P, Otero-González A, Rodriguez-Alvarez MX, et al. Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a general adult population: effect of gender and age: EPIRCE cross-sectional study. BMC Endocr Disord 2013;13:47
21. Gang EJ, Jeong JA, Hong SH, et al. Skeletal myogenic differentiation of mesenchymal stem cells isolated from human umbilical cord blood. Stem Cells 2004;22:617–624
22. Park S, Turner KD, Zheng D, et al. Electrical pulse stimulation induces differential responses in insulin action in myotubes from severely obese individuals. J Physiol 2019;597(2):449–466
23. Hakola L, Takkinen HM, Niinistö S, et al. Maternal fatty acid intake during pregnancy and the development of childhood overweight: a birth cohort study. Pediatr Obes 2017;12(Suppl. 1):26–37
24. Barbour LA, Hernandez TL. Maternal lipids and fetal overgrowth: making fat from fat. Clin Ther 2018;40:1638–1647
25. Samsuddin S, Arumugam PA, Md Amin MS, et al. Maternal lipids are associated with newborn adiposity, independent of GDM status, obesity and insulin resistance: a prospective observational cohort study. BJOG 2020;127:490–499
26. Rosario FJ, Powell TL, Jansson T. Activation of placental insulin and mTOR signaling in a mouse model of maternal obesity associated with fetal overgrowth. Am J Physiol Regul Integr Comp Physiol 2016;310:R87–R93
27. Jansson N, Rosario FJ, Gaccioli F, et al. Activation of placental mTOR signaling and amino acid transporters in obese women giving birth to large babies. J Clin Endocrinol Metab 2013;98:105–113
28. Keleher MR, Erickson K, Smith HA, et al. Placental insulin/IGF-1 signaling, PGC-1α, and inflammatory pathways are associated with metabolic outcomes at 4-6 years of age: the ECHO Healthy Start cohort. Diabetes 2021;70:745–751
29. Erickson ML, Patinkin ZW, Duensing AM, Dabelea D, Redman LM, Boyle KE. Maternal metabolic health drives mesenchymal stem cell metabolism and infant fat mass at birth. JCI Insight 2021;6:146606
30. Keleher MR, Erickson K, Kechris K, et al. Associations between the activity of placental nutrient-sensing pathways and neonatal and postnatal metabolic health: the ECHO Healthy Start cohort. Int J Obes 2020;44:2203–2212
31. Gademan MG, Vermeulen M, Oostvogels AJ, et al. Maternal prepregancy BMI and lipid profile during early pregnancy are independently associated with offspring's body composition at age 5-6 years: the ABCD study. PLoS One 2014;9:e94594
32. Shapiro ALB, Ringham BM, Glueck DH, et al. Infant adiposity is independently associated with a maternal high fat diet but not related to niacin intake: the Healthy Start study. Matern Child Health J 2017;21:1662–1668
33. Thaware PK, McKenna S, Patterson CC, Casey C, McCance DR. Maternal lipids at 28 weeks' gestation and offspring adiposity at age 5 to 7 years. J Clin Endocrinol Metab 2018;103:3767–3772
34. Euclydes VLV, Castro NP, Lima LR, et al. Cord blood concentrations of leptin, zinc-α2-glycoprotein, and adiponectin, and adiposity gain during the first 3 mo of life. Nutrition 2018;54:89–93
35. Tsai PJ, Yu CH, Hsu SP, et al. Cord plasma concentrations of adiponectin and leptin in healthy term neonates: positive correlation with birthweight and neonatal adiposity. Clin Endocrinol (Oxf) 2004;61:88–93
36. Boeke CE, Mantzoros CS, Hughes MD, et al. Differential associations of leptin with adiposity across early childhood. Obesity (Silver Spring) 2013;21:1430–1437
37. Shekhawat PS, Garland JS, Shivpuri C, et al. Neonatal cord blood leptin: its relationship to birth weight, body mass index, maternal diabetes, and steroids. Pediatr Res 1998;43:338–343
38. Stanford KI, Takahashi H, So K, et al. Maternal exercise improves glucose tolerance in female offspring. Diabetes 2017;66:2124–2136
39. Fernandez-Twinn DS, Alfaradhi MZ, Martin-Gronert MS, et al. Downregulation of IRS-1 in adipose tissue of offspring of obese mice is programmed cell-autonomously through post-transcriptional mechanisms. Mol Metab 2014;3:325–333
40. Borengasser SJ, Zhong Y, Kang P, et al. Maternal obesity enhances white adipose tissue differentiation and alters genome-scale DNA methylation in male rat offspring. Endocrinology 2013;154:4113–4125
41. Frohnert BI, Jacobs DR Jr, Steinberger J, Moran A, Steffen LM, Sinaiko AR. Relation between serum free fatty acids and adiposity, insulin resistance,

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and cardiovascular risk factors from adolescence to adulthood. Diabetes 2013;62:3163–3169
42. Haus JM, Kashyap SR, Kasumov T, et al. Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. Diabetes 2009;58:337–343
43. Bergman BC, Hunerdosse DM, Kerege A, Playdon MC, Perreault L. Localisation and composition of skeletal muscle diacylglycerol predicts insulin resistance in humans. Diabetologia 2012;55:1140–1150
44. Goodpaster BH, He J, Watkins S, Kelley DE. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. J Clin Endocrinol Metab 2001;86:5755–5761
45. Chen HC, Rao M, Sajan MP, et al. Role of adipocyte-derived factors in enhancing insulin signaling in skeletal muscle and white adipose tissue of mice lacking Acyl CoA:diacylglycerol acyltransferase 1. Diabetes 2004;53: 1445–1451
46. Gaccioli F, White V, Capobianco E, Powell TL, Jawerbaum A, Jansson T. Maternal overweight induced by a diet with high content of saturated fat activates placental mTOR and eIF2alpha signaling and increases fetal growth in rats. Biology Reprod. 2013;89:96.
47. Gupta MB, Jansson T. Novel roles of mechanistic target of rapamycin signaling in regulating fetal growth. Biol Reprod 2019;100:872–884
48. Castillo-Castrejon M, Jansson T, Powell TL. No evidence of attenuation of placental insulin-stimulated Akt phosphorylation and amino acid transport in maternal obesity and gestational diabetes mellitus. Am J Physiol Endocrinol Metab 2019;317:E1037–E1049
49. Porstmann T, Santos CR, Griffiths B, et al. SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. Cell Metab 2008;8:224–236
50. Hoffman RP. Indices of insulin action calculated from fasting glucose and insulin reflect hepatic, not peripheral, insulin sensitivity in African-American and Caucasian adolescents. Pediatr Diabetes 2008;9(3pt2):57–61
51. Tripathy D, Almgren P, Tuomi T, Groop L. Contribution of insulin-stimulated glucose uptake and basal hepatic insulin sensitivity to surrogate measures of insulin sensitivity. Diabetes Care 2004;27:2204–2210

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