



# Placental Insulin/IGF-1 Signaling, PGC-1 $\alpha$ , and Inflammatory Pathways Are Associated With Metabolic Outcomes at 4–6 Years of Age: The ECHO Healthy Start Cohort

Madeline Rose Keleher,<sup>1,2</sup> Kathryn Erickson,<sup>3</sup> Harry A. Smith,<sup>4</sup> Katerina J. Kechris,<sup>2,4</sup> Ivana V. Yang,<sup>2,5</sup> Dana Dabelea,<sup>2,6</sup> Jacob E. Friedman,<sup>7,8</sup> Kristen E. Boyle,<sup>1,2</sup> and Thomas Jansson<sup>3</sup>

*Diabetes* 2021;70:745–751 | <https://doi.org/10.2337/db20-0902>

**An adverse intrauterine environment is associated with the future risk of obesity and type 2 diabetes. Changes in placental function may underpin the intrauterine origins of adult disease, but longitudinal studies linking placental function with childhood outcomes are rare. Here, we determined the abundance and phosphorylation of protein intermediates involved in insulin signaling, inflammation, cortisol metabolism, protein glycosylation, and mitochondrial biogenesis in placental villus samples from healthy mothers from the Healthy Start cohort. Using MANOVA, we tested the association between placental proteins and offspring adiposity (fat mass percentage) at birth ( $n = 109$ ) and infancy (4–6 months,  $n = 104$ ), and adiposity, skinfold thickness, triglycerides, and insulin in children (4–6 years,  $n = 66$ ). Placental IGF-1 receptor protein was positively associated with serum triglycerides in children. GSK3 $\beta$  phosphorylation at serine 9, a readout of insulin and growth factor signaling, and the ratio of phosphorylated to total JNK2 were both positively associated with mid-thigh skinfold thickness in children. Moreover, peroxisome proliferator-activated receptor  $\gamma$  coactivator (PGC)-1 $\alpha$  abundance was positively associated with insulin in children. In conclusion, placental insulin/IGF-1 signaling, PGC-1 $\alpha$ , and inflammation pathways were positively associated with metabolic outcomes in 4- to 6-year-old children,**

**identifying a novel link between placental function and long-term metabolic outcomes.**

A range of noncommunicable diseases, including obesity and type 2 diabetes, have intrauterine origins (1–3). The intrauterine environment is largely determined by the function of the placenta, the maternal-fetal interface performing an array of functions critical for normal fetal growth and development, including nutrient transport and hormone synthesis. Emerging evidence suggests that placental structure and function impact life-long health (4–7). We recently demonstrated that the activity of placental mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) is associated with cardiometabolic risk factors at 4–6 years (8). Thus, a better understanding of the mechanisms linking placental function to childhood and adult metabolic disease risk may offer innovative avenues to preventing them in future generations.

Here, we tested the hypothesis that altered abundance and phosphorylation of key proteins involved in placental insulin/IGF-1 and inflammation signaling, cortisol metabolism, protein glycosylation, and mitochondrial biogenesis (Fig. 1) are associated with infant and child markers of cardiometabolic health. We obtained placentas from

<sup>1</sup>Section of Nutrition, Department of Pediatrics, University of Colorado Anschutz Medical Campus, Aurora, CO

<sup>2</sup>The Lifecourse Epidemiology of Adiposity and Diabetes (LEAD) Center, Aurora, CO

<sup>3</sup>Department of Obstetrics and Gynecology, University of Colorado Anschutz Medical Campus, Aurora, CO

<sup>4</sup>Department of Biostatistics and Informatics, Colorado School of Public Health, Aurora, CO

<sup>5</sup>Department of Medicine, University of Colorado School of Medicine, Aurora, CO

<sup>6</sup>Department of Epidemiology, Colorado School of Public Health, Department of Pediatrics, University of Colorado Anschutz Medical Campus, Aurora, CO

<sup>7</sup>Section of Neonatology, Department of Pediatrics, University of Colorado School of Medicine, Aurora, CO

<sup>8</sup>Harold Hamm Diabetes Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK

Corresponding author: Madeline Rose Keleher, [madeline.keleher@cuanschutz.edu](mailto:madeline.keleher@cuanschutz.edu)

Received 4 September 2020 and accepted 24 December 2020

This article contains supplementary material online at <https://doi.org/10.2337/figshare.13491417>.

© 2021 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. More information is available at <https://www.diabetesjournals.org/content/license>.

109 mother/infant pairs from the larger Environmental Influences on Child Health Outcomes (ECHO) Healthy Start prebirth cohort and determined protein levels, testing their association with metabolic outcomes measured in the children at birth, infancy (4–6 months), and childhood (4–6 years).

## RESEARCH DESIGN AND METHODS

### Ethics Statement

We used placental samples, body composition data, and serum biomarker data collected by the Healthy Start Study (ClinicalTrials.gov, NCT02273297). This study was approved by the Colorado Multiple Institutional Review Board at the University of Colorado Hospital (Aurora, CO). All participants gave written, informed consent at enrollment.

### Participant Measures and Sample Collection

#### Pregnant Women

The Healthy Start longitudinal prebirth cohort study comprises 1,410 women aged  $\geq 16$  years who enrolled in the study between 2010 and 2014 at  $\leq 23$  weeks' gestation. We recruited women from the University of Colorado Hospital's obstetrics clinics and excluded participants if they currently had a multiple pregnancy or had a prior history of premature

birth, diabetes, or serious psychiatric illness. Data collection included maternal height, weight, demographics, and a fasting blood sample between 24 and 32 weeks' gestation (median 27 weeks), and is reported in detail elsewhere (9,10).

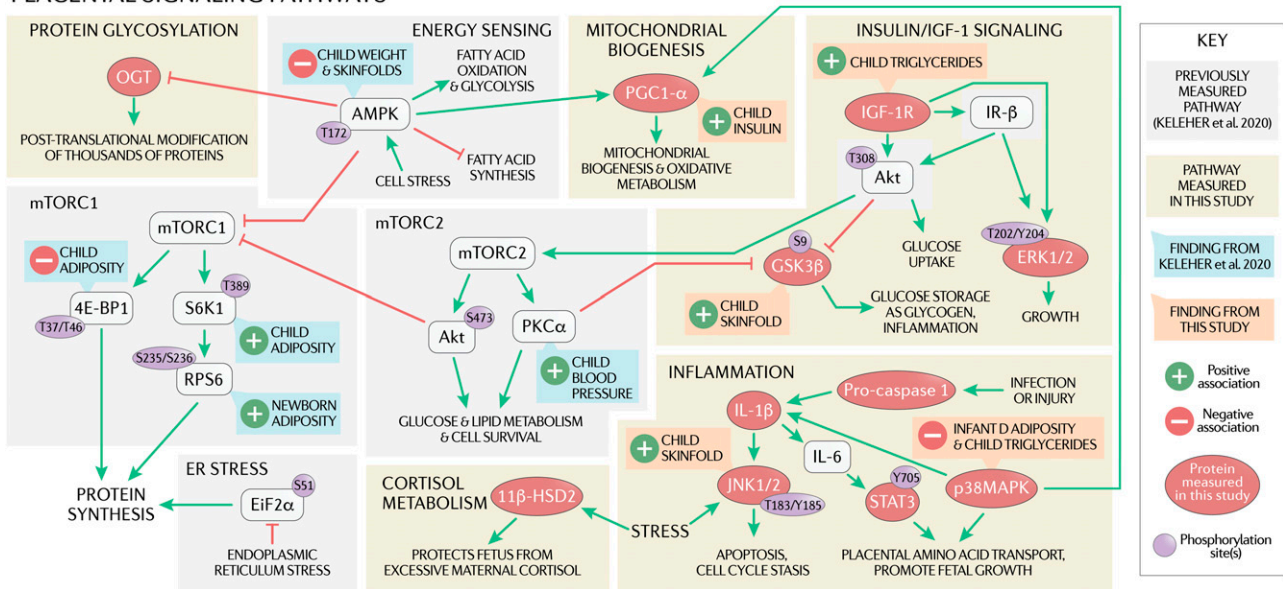
### Placental Samples

In a convenience subsample of the Healthy Start cohort, as part of the ancillary Healthy Start BabyBUMP (biology of intrauterine metabolic programming) Project, we collected trophoblast villi samples from placentas after delivery ( $n = 111$ ), snap froze them in liquid nitrogen, and stored them at  $-80^{\circ}\text{C}$ . We excluded samples from two subjects because they were from preterm births ( $< 37$  weeks), resulting in a sample size of 109 for analysis.

### Placental Signaling

We homogenized  $\sim 20$  mg frozen placental villus tissue in  $75 \mu\text{L}$  ice-cold buffer D (250 mmol/L sucrose, 10 mmol/L HEPES, pH 7.4) with a 1:100 dilution of protease and phosphatase inhibitors. Subsequently, we used Wes (ProteinSimple, Santa Clara, CA) to perform Simple Western assays to measure the phosphorylated and total abundance of key proteins (Table 1). We ran the Wes plates according to the manufacturer's instructions, with minor modification (200 V, 55 m separation time) using

## PLACENTAL SIGNALING PATHWAYS



**Figure 1**—The placenta may play a role in the intrauterine origin of adult metabolic disease through several mechanisms, such as by determining the flux of oxygen, nutrients, and methyl donors to the fetus, thereby regulating fetal growth and development. The activity of placental insulin/IGF-1 signaling is positively correlated with fetal growth and regulates multiple downstream signaling pathways, including mTOR and critical cellular processes such as mitochondrial biogenesis. Placental inflammation, which is activated in maternal obesity, may also impact short- and long-term fetal outcomes, as increased p38MAPK and STAT3 signaling promote placental amino acid transport, which is associated with increased fetal growth. Placental 11 $\beta$ -HSD2 protects the fetus from excessive maternal cortisol, which causes fetal growth restriction and predisposes the offspring to metabolic disease later in life. OGT, a broad marker of nutrient status and downstream target of AMPK, is elevated in human skeletal muscle in type 2 diabetes, and placental OGT has been linked to poor neurodevelopment in mice. We previously demonstrated that placental proteins involved in the mTOR and energy-sensing pathways (shown in gray) are associated with child outcomes at 4–6 years of age (shown in green). Here, we investigated additional pathways (shown in yellow) and found that child outcomes (shown in orange) are also linked to placental proteins involved in inflammation, mitochondrial biogenesis, and insulin signaling. ER, endoplasmic reticulum; IL, interleukin.

a 0.1 mg/mL total protein concentration (Supplementary Table 1 and Supplementary Figs. 1 and 2). To control for batch variation, we included an equalizer sample on each plate that had clean median values for each protein. We also multiplexed a loading control (vinculin or  $\beta$ -actin) in each capillary to normalize the protein levels to.

### Offspring Outcomes

At birth, we obtained birth weight and length from medical records ( $n = 105$ ) and saved a cord blood sample to measure insulin, glucose, free fatty acids, cholesterol, and triglycerides. We measured body composition (fat mass [FM] and fat-free mass [FFM]) with whole-body air displacement plethysmography (PEA POD; COSMED)

**Table 1—Participant characteristics**

Characteristics	
<b>Maternal characteristics</b>	<i>n</i> = 109
Prepregnancy BMI (kg/m <sup>2</sup> )	25.0 (23.8–26.0)
Gestational weight gain (kg)	13.5 (12.2–15.1)
Cesarean delivery, <i>n</i> (%)	23 (21.1)
Maternal insulin at 24–32 weeks' gestation ( $\mu$ IU/mL)	12.9 (11.6–14.2)
Cord blood insulin ( $\mu$ IU/mL)	7.27 (6.20–8.34)
Cord blood glucose (mg/dL)	80.6 (77.3–84.0)
<b>Newborn characteristics</b>	<i>n</i> = 109
Gestational age at delivery (weeks)	39.5 (39.3–39.8)
Sex, <i>n</i>	
Female	58
Male	51
Adiposity (FM %)	9.0 (8.4–9.9)
Body weight (kg)	3.1 (3.0–3.2)
BMI z-score	−0.49 (−2.39 to 1.42)
<b>Infant characteristics (4–6 months)</b>	<i>n</i> = 104
Age (months)	4.50 (4.36–4.63)
Adiposity (%)	24.38 (23.4–25.6)
Body weight (kg)	6.6 (6.5–6.8)
BMI z-score	−0.35 (−2.32 to 1.61)
<b>Child characteristics (4–6 years)</b>	<i>n</i> = 66
Age (years)	4.62 (4.56–4.68)
Adiposity (%)	18.2 (16.7–19.8)
Body weight (kg)	17.2 (16.6–17.8)
BMI z-score	−0.06 (−1.72 to 1.61)
Midhigh skinfold (mm)	14.4 (13.3–15.5)
Triceps skinfold (mm)	10.3 (9.5–11.1)
Subscapular skinfold (mm)	5.1 (4.7–5.5)
Triglycerides (mg/dL)	59.8 (51.4–68.2)
Insulin ( $\mu$ IU/mL)	5.5 (4.8–6.2)
<b>Protein pathway</b>	<b>Protein measured</b>
Insulin/IGF-1 signaling	IGF-1r GSK3 $\beta$ <sup>Ser9</sup> GSK3 $\beta$ <sup>Ser9</sup> /GSK3 ERK(1/2) <sup>Thr202/Tyr204</sup> ERK(1/2) <sup>Thr202/Tyr204</sup> -to-ERK(1/2)
Inflammation	Procaspase 1 STAT3 <sup>Tyr705</sup> STAT3 <sup>Tyr705</sup> -to-STAT3 JNK1 <sup>Thr183/Tyr185</sup> JNK1 <sup>Thr183/Tyr185</sup> -to-JNK1 JNK2 <sup>Thr183/Tyr185</sup> JNK2 <sup>Thr183/Tyr185</sup> -to-JNK2
Cortisol metabolism	IL-1 $\beta$
Protein glycosylation	p38MAPK
Mitochondrial biogenesis	11 $\beta$ -HSD2 OGT PGC-1 $\alpha$

Data are presented as mean with 95% CI in parentheses, unless otherwise stated. 11 $\beta$ -HSD2, 11 $\beta$ -hydroxysteroid dehydrogenase type 2; ERK, extracellular signal-regulated kinase; GSK3 $\beta$ , glycogen synthase kinase-3 $\beta$ ; IGF-1r, IGF-1 receptor; IL-1 $\beta$ , interleukin-1 $\beta$ ; JNK, c-Jun N-terminal kinase; OGT, O-linked *N*-acetylglucosamine transferase.

and calculated adiposity as  $FM/(FM + FFM) \times 100$ . We measured body composition again in infancy, between 4 and 6 months (PEA POD,  $n = 101$ ). When the children reached 4–6 years ( $n = 66$ , median age 4.54 years), we assessed body composition (BOD POD; COSMED,  $n = 62$ ), as previously described (10), and skinfold thickness twice at the triceps, subscapular, and midhigh points using skinfold calipers, with a third measurement if the first two were off by  $>1$  cm. We collected a fasted venous blood sample from consenting children to measure circulating levels of insulin, triglycerides, cholesterol, adiponectin, and glucose.

### Statistical Analysis

We analyzed the protein data as the phosphorylated form or as the ratio of phosphorylated to total. We analyzed only total protein in cases where the phosphorylated form was not measured or not relevant. We performed all statistical analyses in R (version 4.0.2). We tested the normality of the data (Shapiro-Wilk test) and used Box-Cox transformations to normalize variables that were not normal. In the one case where the transformed variable still did not pass the Shapiro-Wilk test, we used an inverse normal transformation. We performed the statistical tests on the transformed values (Supplementary Table 2) but presented the untransformed values in the graphs for interpretation. We identified outliers with the Grubbs test and removed one outlier for one subject (for serum insulin).

We performed MANOVA to test for a relationship between the protein pathways and offspring body composition and cardiometabolic traits (Supplementary Table 3). When MANOVA showed a significant overall association, we performed multivariate multiple regression (MMR) to evaluate each dependent variable. Sex and age affected the offspring cardiometabolic outcomes, so they were included as covariates. Where indicated, linear regression and correlation were used post hoc to further explore complex relationships. All associations remained

unchanged after reanalyzing data using BMI  $z$ -scores instead of the actual BMI (data not shown).

### 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

### Subject Characteristics

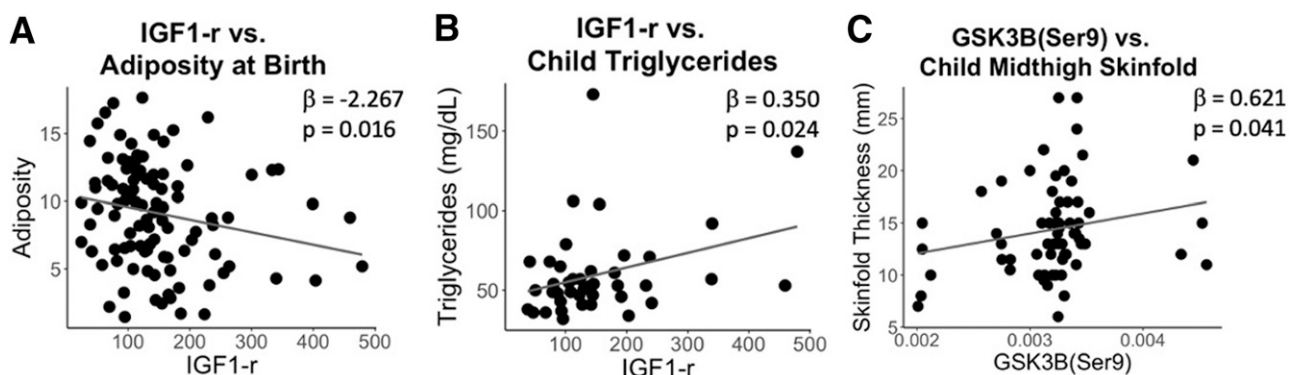
Our analysis included 109 mother/infant pairs at birth, 104 infants at 4–6 months, and 66 children at 4–6 years (Table 1). Mothers had prepregnancy BMIs ranging from 19.9 to 45.8  $kg/m^2$ . Infants had an even sex distribution (53% female), their gestational age at delivery ranged from 37 to 42 weeks, and two infants were born with macrosomia ( $>4$  kg).

### No Effect of Maternal Prepregnancy BMI, 11 $\beta$ -HSD2, or OGT

Maternal BMI did not significantly affect any of the signaling pathways (Supplementary Table 4). MANOVA did not detect any association between placental proteins involved in cortisol metabolism (Supplementary Table 5) or protein glycosylation (Supplementary Table 6) on child outcomes.

### Proteins in the Insulin/IGF-1 Signaling Pathway Are Associated With Child Outcomes at Birth and 4–6 Years

MANOVA of the insulin/IGF-1 signaling pathway proteins (Supplementary Table 7) revealed a significant association between placental IGF-1 receptor (IGF-1r) abundance and body composition at birth ( $P = 0.045$ ), with MMR showing a negative association with adiposity ( $\beta = -2.267$ ,  $P = 0.016$ ) (Fig. 2A). By childhood, that association was no longer significant; however, placental IGF-1r expression was positively associated with child triglycerides ( $\beta = 0.350$ ,  $P = 0.024$ ) (Fig. 2B). At that same age, placental



**Figure 2**—Placental insulin signaling proteins and child outcomes. MANOVA and MMR were performed for placental proteins in the growth factor/insulin signaling pathway vs. child outcomes, adjusting for child sex and age. A–C: Scatter plots are shown for significant MMR results that first passed significance with MANOVA.

GSK3 $\beta$ <sup>Ser9</sup> was associated with skinfolds ( $P = 0.009$ ), with a positive association with midhigh skinfold thickness ( $\beta = 0.621$ ,  $P = 0.041$ ) (Fig. 2C). Previous research has shown a positive association between maternal insulin levels and placental GSK3 $\beta$ <sup>Ser9</sup> (11), prompting our post hoc linear regression showing a nonsignificant positive trend between maternal insulin and placental GSK3 $\beta$ <sup>Ser9</sup> ( $\beta = 0.162$ ,  $P = 0.112$ ) and a significant positive association between cord blood insulin and GSK3 $\beta$ <sup>Ser9</sup> ( $\beta = 0.245$ ,  $P = 0.036$ ). ERK(1/2)<sup>Thr202/Tyr204</sup>-to-ERK(1/2) had a significant relationship with skinfolds overall; however, it did not have a clear relationship with any individual skinfold measurements.

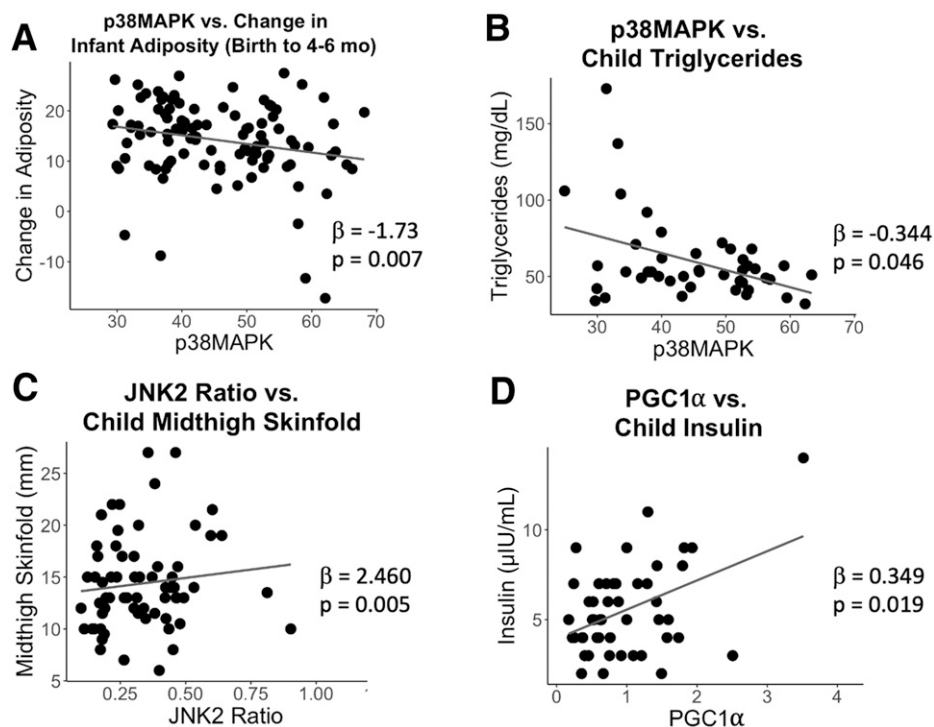
### Placental JNK2, p38 Mitogen-Activated Protein Kinase, and PGC-1 $\alpha$ Are Associated With Child Outcomes at 4–6 Years

MANOVA on the inflammatory pathway (Supplementary Table 8) revealed a significant relationship between placental p38 mitogen-activated protein kinase (MAPK) abundance and growth trajectory from birth to 4–6 months ( $P = 0.008$ ), driven by a negative association with change in adiposity from birth to 4–6 months ( $\beta = -1.73$ ,  $P = 0.007$ ) (Fig. 3A). Similarly, p38MAPK had a negative association with child triglycerides at 4–6 years of age ( $\beta = -0.344$ ,  $P = 0.046$ ) (Fig. 3B). JNK2<sup>Thr183/Tyr185</sup>-to-JNK2 was also associated with child skinfolds ( $P = 0.037$ ), with a positive association with midhigh skinfold thickness ( $\beta = 2.460$ ,  $P = 0.005$ ) (Fig. 3C). At

4–6 months, there was a significant effect of both JNK2<sup>Thr183/Tyr185</sup>-to-JNK2 ( $P = 0.006$ ) and p38MAPK ( $P = 0.0124$ ) on infant body composition. Although the univariate tests were not significant for either protein, there was a positive correlation between JNK2<sup>Thr183/Tyr185</sup>-to-JNK2 and 4–6 month adiposity ( $r = 0.253$ ,  $P = 0.0115$ ) (Supplementary Fig. 3A). Similarly, MANOVA revealed a significant effect of STAT3<sup>Tyr705</sup> on child body composition at 4–6 years ( $P = 0.047$ ), without significant associations with adiposity or body weight, although there was a positive correlation between STAT3<sup>Tyr705</sup> and child adiposity ( $r = 0.324$ ,  $P = 0.010$ ) (Supplementary Fig. 3B). The mitochondrial biogenesis pathway analysis (Supplementary Table 9) revealed a significant positive association between placental peroxisome proliferator-activated receptor  $\gamma$  coactivator (PGC)-1 $\alpha$  and child insulin at 4–6 years ( $\beta = 0.349$ ,  $P = 0.019$ ) (Fig. 3D). In a post hoc analysis, PGC-1 $\alpha$  was positively correlated with IGF-1r ( $r = 0.39$ ,  $P < 0.001$ ) and OGT ( $r = 0.27$ ,  $P = 0.005$ ).

### DISCUSSION

A better understanding of the mechanisms linking placental function to childhood and adult disease will allow us to develop innovative avenues to prevent obesity and diabetes in future generations. Here, we report that the activity of placental pathways involving inflammation and growth factor/insulin signaling were positively associated with midhigh skinfold thickness and serum triglycerides



**Figure 3**—Placental inflammation and mitochondrial biogenesis proteins and child outcomes. MANOVA and MMR were performed for placental proteins in the growth factor/insulin signaling pathway vs. child outcomes, adjusting for child sex and age. A–D: Scatter plots are shown for significant MMR results that first passed significance with MANOVA.

in healthy 4- to 6-year-old children. Additionally, PGC-1 $\alpha$ , which regulates mitochondrial biogenesis, was positively associated with child insulin. Collectively, these findings suggest that activation of placental signaling pathways promoting nutrient transport, protein synthesis, and oxidative phosphorylation are related to growth and development of offspring born to healthy pregnant mothers.

Placental IGF-1r expression was positively correlated with childhood serum triglycerides. Increased GSK3 $\beta$  phosphorylation—indicating activation of placental insulin signaling—was linked to child skinfold thickness. These findings are in general agreement with our previous observations that the activity of placental mTORC1, downstream of insulin signaling, is positively associated with child adiposity (8). The activation of placental insulin/IGF-1 and mTOR signaling may be mechanistically involved in the well-established link between maternal obesity/gestational diabetes mellitus (GDM) and metabolic disease in the offspring (12,13). Given that these signaling pathways promote placental nutrient transport, it is possible that increased fetal nutrient delivery contributes to this association by increasing fat deposition in utero and/or altering fetal nutrient sensing systems.

In addition to being linked to infant adiposity, JNK2 was positively associated with midhigh skinfold thickness in childhood. These findings are in general agreement with studies reporting activation of placental JNK2 in maternal obesity (14) and suggest that placental inflammation in maternal obesity may predispose the infant to future obesity. The inverse correlation between placental p38MAPK and child triglycerides is contradictory. However, we speculate that this may reflect a positive correlation between the ratio of phosphorylated to total p38MAPK (which we were unable to measure) and adiposity in childhood.

Placental PGC-1 $\alpha$  has been reported to be decreased in GDM (15,16), possibly reflecting a homeostatic downregulation of mitochondrial respiration by glucose abundance. In our cohort consisting largely of healthy pregnant women and their children, placental PGC-1 $\alpha$  was positively associated with child serum insulin. At first glance, this may be counterintuitive as PGC-1 $\alpha$  is phosphorylated by AMPK, which we previously reported was negatively associated with child weight and skinfold thickness at 4–6 years (8). However, we found that PGC-1 $\alpha$  was not correlated with the AMPK values measured in our previous study in the same individuals. Instead, PGC-1 $\alpha$  was positively correlated with IGF-1r, a known stimulator of mitochondrial biogenesis. PGC-1 $\alpha$  was also positively correlated with OGT. These two proteins form a complex that mediates inappropriate gluconeogenesis in the diabetic liver via PGC-1 $\alpha$  recruiting OGT to activate FoxO via GlcNAcylation (17). Thus, greater IGF-1/insulin signaling in the placenta may underlay the positive association between placental PGC-1 $\alpha$  and child insulin. Increased placental mitochondrial respiration in response to

enhanced PGC-1 $\alpha$  signaling may provide additional ATP for placental protein synthesis and active nutrient transport promoting fetal growth and fat deposition, predisposing the infant for future metabolic disease.

The strengths of the study include having a cohort of women across the BMI spectrum, a large sample size, and follow-up with the children from birth to 4–6 years of age. Because recruited pregnant women were largely healthy by design, limitations of the study include the lack of women with pregnancy complications, such as GDM and preterm birth, known to be associated with programming of offspring metabolic disease.

Our findings suggest that activation of signaling pathways promoting placental nutrient transport, protein synthesis, and oxidative phosphorylation could be involved in metabolic pathways that contribute to metabolic risk in young children. Our data provide additional evidence that changes in placental function are linked to long-term health in children and may serve as a foundation for mechanistic studies in animal models and the development of placental biomarkers and novel intervention strategies to prevent future cardiometabolic disease.

---

**Acknowledgments.** The authors thank M. Martinez (the Healthy Start Study project coordinator, Colorado School of Public Health, University of Colorado), the Healthy Start team, and the Healthy Start participants for their hard work and dedication to this study. The authors thank KIMEN Design4Research for the graphic design of Fig. 1.

**Funding.** This work was supported by Colorado Clinical and Translational Sciences Institute UG30D023248-03 (to D.D.). The Healthy Start BabyBUMP Project is supported by grants from the American Heart Association (predoctoral fellowship 14PRE18230008) and by the parent Healthy Start Study (to D.D.). The Healthy Start Study was supported by the National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases (R01 DK076648 to D.D.) and the Colorado Clinical and Translational Sciences Institute via National Institutes of Health, National Center for Advancing Translational Sciences (UL1 TR001082) for maternal visits and collection of birth measures.

The funders had no influence on the results of the study.

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

**Author Contributions.** M.R.K., K.E., H.A.S., and K.J.K. contributed to the statistical analysis. M.R.K., I.V.Y., D.D., J.E.F., K.E.B., and T.J. contributed to the interpretation of the results. M.R.K., K.E.B., and T.J. drafted the manuscript. K.E., K.E.B., D.D., and T.J. contributed to data collection. T.J. contributed to the conception of the manuscript. All authors approved the final version of the manuscript. T.J. 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.

## References

1. Gluckman PD, Hanson MA. The developmental origins of the metabolic syndrome. *Trends Endocrinol Metab* 2004;15:183–187
2. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. *N Engl J Med* 2008;359:61–73
3. Barker DJ. The developmental origins of insulin resistance. *Horm Res* 2005;64(Suppl. 3):2–7
4. Burton GJ, Fowden AL, Thornburg KL. Placental origins of chronic disease. *Physiol Rev* 2016;96:1509–1565

5. Barker DJ, Gelow J, Thornburg K, Osmond C, Kajantie E, Eriksson JG. The early origins of chronic heart failure: impaired placental growth and initiation of insulin resistance in childhood. *Eur J Heart Fail* 2010;12:819–825
6. Barker DJ, Larsen G, Osmond C, Thornburg KL, Kajantie E, Eriksson JG. The placental origins of sudden cardiac death. *Int J Epidemiol* 2012;41:1394–1399
7. Chen L, Guilmette J, Luo Z-C, et al. Placental 11 $\beta$ -HSD2 and cardiometabolic health indicators in infancy. *Diabetes Care* 2019;42:964–971
8. 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 (Lond)* 2020;44:2203–2212
9. Starling AP, Brinton JT, Glueck DH, et al. Associations of maternal BMI and gestational weight gain with neonatal adiposity in the Healthy Start Study. *Am J Clin Nutr* 2015;101:302–309
10. Sauder KA, Stamatiou AV, Leshchinskaya E, Ringham BM, Glueck DH, Dabelea D. Cord blood vitamin D levels and early childhood blood pressure: the Healthy Start Study. *J Am Heart Assoc* 2019;8:e011485
11. Lassance L, Miedl H, Absenger M, et al. Hyperinsulinemia stimulates angiogenesis of human fetoplacental endothelial cells: a possible role of insulin in placental hypervascularization in diabetes mellitus. *J Clin Endocrinol Metab* 2013;98:E1438–E1447
12. Lindell N, Carlsson A, Josefsson A, Samuelsson U. Maternal obesity as a risk factor for early childhood type 1 diabetes: a nationwide, prospective, population-based case-control study. *Diabetologia* 2018;61:130–137
13. Santangeli L, Sattar N, Huda SS. Impact of maternal obesity on perinatal and childhood outcomes. *Best Pract Res Clin Obstet Gynaecol* 2015;29:438–448
14. Saben J, Zhong Y, Gomez-Acevedo H, et al. Early growth response protein-1 mediates lipotoxicity-associated placental inflammation: role in maternal obesity. *Am J Physiol Endocrinol Metab* 2013;305:E1–E14
15. Wang L, Fan H, Zhou L, Wu Y, Lu H, Luo J. Altered expression of PGC-1 $\alpha$  and PDX1 and their methylation status are associated with fetal glucose metabolism in gestational diabetes mellitus. *Biochem Biophys Res Commun* 2018;501:300–306
16. Jiang S, Teague AM, Tryggstad JB, Aston CE, Lyons T, Chernausk SD. Effects of maternal diabetes and fetal sex on human placenta mitochondrial biogenesis. *Placenta* 2017;57:26–32
17. Housley MP, Udeshi ND, Rodgers JT, et al. A PGC-1 $\alpha$ -O-GlcNAc transferase complex regulates FoxO transcription factor activity in response to glucose. *J Biol Chem* 2009;284:5148–5157