Fetal Sex-Dependent Metabolic Influence on Placental Protein Synthesis and Signaling Pathways in Gestational Diabetes Mellitus

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Gestational diabetes (GDM) occurs in ~7-10% of pregnancies worldwide with obese women being up to 4 times more likely to develop GDM. GDM disposes the offspring to development of childhood obesity, and with the mother to cardiometabolic disorders and type 2 diabetes later in life. Women carrying a male fetus are at higher risk of GDM as well as associated co-morbidities. With obesity and GDM the placenta is exposed to, and adapts to, the maternal metabolic landscape of hyperglycemia and hyperinsulinemia to balance its own metabolic needs with maintenance of optimal fetal growth and development. The altered metabolic environment and insulin resistance of obesity and diabetes impact protein metabolism in non-pregnant individuals, but these effects have not been evaluated in placentas with GDM . Here, we determined if maternal obesity and GDM altered placental protein metabolism and if there is a sexual dimorphism in effect. Placental villous tissue was collected at C-section (no labor) from normoglycemic lean women (LN, BMI 18.5-24.9 kg/m2), normoglycemic obese women (OB, BMI>30) and obese women with type A2 gestational diabetes (GDM) with either a male or female fetus (n=6 per sex, per group). Proteins were extracted and expression of phosphorylated and total proteins were analyzed by Western blotting. The levels of phosphorylated/total AKT were higher (NS) in GDM compared to LN and OB placentas. When stratified by fetal sex, a notable increase ($p<0.05$) in the proportion of phosphorylated/total AKT was found in female tissue from the GDM group compared to LN and OB groups, while proportions in male tissues remained comparable suggesting that increase in AKT signaling in response to maternal obesity and GDM was specific to female placentas. We observed an increase in DEPTOR (inhibitor for mTOR that acts downstream of AKT) expression in GDM tissues compared to LN ($p<0.05$) and OB (NS) in the sex-combined analysis and these trends were maintained in the sex-stratified analysis, indicating downregulation of mTOR signaling in both male and female placentas with GDM. We also observed a decline in phosphorylated/total p70-S6 kinase in GDM tissue versus LN and OB ($p<0.05$) and phosphorylated/total 4E-BP1 in obese (NS) and GDM (NS) placentae compared to LN. p70-S6 kinase and 4E-BP1 act downstream of mTOR to regulate protein synthesis. Phosphorylation of p70-S6 kinase by mTOR results in its activation and downstream activation of protein S6 that regulates ribosome biogenesis and protein translation machinery. However, 4E-BP1 is a major translational repressor which inhibits cap-dependent translation by binding to eukaryotic translation initiation factor (eIF)4E. Phosphorylation of 4E-BP1 by mTOR is necessary for its dissociation from (eIF)4E and induction of translation. Decrease in phosphorylation of both p70-S6 kinase and 4E-BP1 in GDM placenta indicates an overall decrease in protein translation in these placentas. We did not observe any sex specific changes in p70-S6 kinase phosphorylation, but phosphorylation of 4E-BP1 was significantly higher in LN and OB ($p<0.05$) females compared to their respective males, with no differences in GDM males vs females. Taken together our results suggest that the maternal conditions of obesity and GDM

alter AKT signaling and protein synthesis in a fetal sex specific manner. Since proteins play crucial roles in all cellular functions and their synthesis requires substantial energy, the observed decrease in protein synthesis in our study suggests a potential energy-saving mechanism within the placenta.