Increased Hepatic Glucose Production in Fetal Sheep With Intrauterine Growth Restriction Is Not Suppressed by Insulin

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Intrauterine growth restriction (IUGR) increases the risk for metabolic disease and diabetes, although the developmental origins of this remain unclear. We measured glucose metabolism during basal and insulin clamp periods in a fetal sheep model of placental insufficiency and IUGR. Compared with control fetuses (CON), fetuses with IUGR had increased basal glucose production rates and hepatic PEPCK and glucose-6-phosphatase expression, which were not suppressed by insulin. In contrast, insulin significantly increased peripheral glucose utilization rates in CON and IUGR fetuses. Insulin robustly activated AKT, GSK3β, and forkhead box class O (FOXO)1 in CON and IUGR fetal livers. IUGR livers, however, had increased basal FOXO1 phosphorylation, nuclear FOXO1 expression, and Jun NH2-terminal kinase activation during hyperinsulinemia. Expression of peroxisome proliferator–activated receptor γ coactivator 1α and hepatic nuclear factor-4a were increased in IUGR livers during basal and insulin periods. Cortisol and norepinephrine concentrations were positively correlated with glucose production rates. Isolated IUGR hepatocytes maintained increased glucose production in culture. In summary, fetal sheep with IUGR have increased hepatic glucose production, which is not suppressed by insulin despite insulin sensitivity for peripheral glucose utilization. These data are consistent with a novel mechanism involving persistent transcriptional activation in the liver that seems to be unique in the fetus with IUGR. Diabetes 62:65–73, 2013

Intrauterine growth restriction (IUGR) increases the risk of preterm birth, neonatal morbidity and mortality, and the development of diabetes and obesity during the life span (1–3). In animal models of IUGR, early developmental shifts in fetal metabolism include increased hepatic gluconeogenic gene expression, glucose production, and decreased pancreatic insulin secretion (2,4–8). Postnatal offspring from animal models of IUGR continue to have increased hepatic glucose production accompanied by insulin resistance and defects in insulin secretion (5,9–12). Activation of glucose production in the fetus with IUGR may be a beneficial adaptive response to maintain glucose supply to vital organs as placental glucose supply is diminished. If persistent after birth, however, this adaptation can have adverse consequences by promoting glucose production in excess of glucose utilization capacity, contributing to persistent hyperglycemia.

Gluconeogenesis is regulated such that expression of PEPCK (PCK1 gene), glucose-6-phosphatase (G6Pase, G6PC gene), and fructose-1,6-bisphosphatase is normally quiescent until just before birth, when increases in glucagon, cortisol, and catecholamines activate the glycolytic and gluconeogenic pathways (13–15). Experiments in fetal sheep indicate similar effects of these hormones on glucose metabolism (4,6,15–17). Expression of peroxisome proliferator–activated receptor γ coactivator (PGC) 1α and activation of cAMP-responsive element–binding protein (CREB) are increased in the IUGR fetal liver, supporting a role for increased cAMP in transcriptional activation of PEPCK and G6Pase (6,7,18,19). Previously, we found only modest increases in CCAAT enhancer binding proteins α and β and a lack of activation of several energy and stress sensors, indicating that IUGR produces a complex and robust set of mechanisms resulting in fetal glucose production (6,7).

Insulin is the dominant mechanism for suppressing gluconeogenic gene expression and glucose production; however, the mechanisms of insulin’s effects on postnatal hepatic glucose metabolism have become delineated only recently (20,21). Little is known about the regulation of glucose production in fetuses with premature activation of gluconeogenesis and no prior studies have examined how insulin regulates hepatic glucose metabolism in the IUGR fetus. In the IUGR fetus, basal insulin concentrations are reduced and those of counter-regulatory hormones are increased, favoring increased glucose production (4,6,16,17).

We hypothesized that IUGR produced by placental insufficiency results in an adaptive increase in hepatic glucose production, which is not suppressed by insulin, while peripheral insulin sensitivity for glucose utilization is maintained. Our aim was to test the ability of insulin to suppress hepatic glucose production and gluconeogenic gene activation and simultaneously increase peripheral glucose utilization in a fetal sheep model of IUGR. We also tested whether insulin could appropriately activate and regulate signaling pathways and nuclear proteins in the IUGR fetal liver that control glucose production and the persistence of these effects in isolated hepatocytes.

RESEARCH DESIGN AND METHODS

IUGR fetal sheep model. Pregnant Columbia-Rambouillet ewes were studied under regulatory compliance at the University of Colorado School of Medicine Perinatal Research Center, Aurora, Colorado. IUGR fetuses were created by exposing pregnant ewes to elevated humidity and temperature (40°C for 12 h, 35°C for 12 h) from 37 ± 1 days’ gestational age (dGA) (term = 147 dGA) to 116 ± 1 dGA in an environmentally controlled room (6,7,22). Control (CON) fetuses were from pregnant ewes exposed to normal humidity and temperatures daily (25°C) in an environmentally controlled room from 38 ± 1 to 117 ± 2 dGA. These ewes were pair-fed to the intake of the IUGR ewes. After treatment, ewes were exposed to normal humidity and temperatures until the time of the study. Indwelling catheters were surgically placed in the...
GLUCOSE PRODUCTION IN IUGR FETUS

Blood, Hormones, Nutrients, and Hematological status

66 DIABETES, VOL. 62, JANUARY 2013 diabetes.diabetesjournals.org

TABLE 1

Fetal arterial blood hematological values, plasma nutrient and hormone concentrations, and blood flow and oxygen uptake rates

|                          | Basal period | Insulin clamp period | Significance* |
|--------------------------|--------------|----------------------|---------------|
|                          | CON          | IUGR                 | CON           | IUGR         | IUGR         |
| **Hematological**        |              |                      |               |
| pH                       | 7.36 ± 0.02  | 7.36 ± 0.02          | 7.32 ± 0.03   | 7.22 ± 0.04  | 0.16         | <0.001       | <0.05       |
| Hematocrit (%)           | 35.1 ± 1.6   | 37.0 ± 1.5           | 34.7 ± 1.3    | 35.2 ± 1.1   | 0.52         | 0.12         | 0.30        |
| O2 content (mmol/L)      | 3.3 ± 0.3    | 2.0 ± 0.4            | 2.3 ± 0.3     | 1.1 ± 0.3    | <0.01        | <0.001       | 0.56        |
| SO2 (%)                  | 48.3 ± 2.9   | 26.5 ± 5.1           | 34.0 ± 5.2    | 16.1 ± 3.9   | <0.01        | <0.001       | 0.27        |
| PO2 (Torr)               | 19.4 ± 0.8   | 14.0 ± 1.4           | 17.0 ± 1.2    | 12.6 ± 1.1   | <0.01        | <0.005       | 0.36        |
| Pco2 (Torr)              | 48.7 ± 0.9   | 52.3 ± 1.7           | 52.8 ± 1.8    | 57.0 ± 2.4   | 0.16         | <0.001       | 0.77        |
| **Nutrients**            |              |                      |               |
| Glucose (mg/dL)          | 17.6 ± 1.1   | 15.9 ± 1.5           | 18.0 ± 1.5    | 15.6 ± 1.5   | 0.33         | 0.77         | 0.40        |
| Lactate (mmol/L)         | 2.0 ± 0.2    | 4.3 ± 0.9            | 3.3 ± 1.0     | 9.4 ± 2.0    | <0.05        | <0.01        | 0.06        |
| BCAA (ng/mL)†            | 0.60 ± 0.07  | 0.72 ± 0.05          | 0.56 ± 0.09   | 0.73 ± 0.09  | 0.18         | 0.81         | 0.50        |
| **Hormones**             |              |                      |               |
| Insulin (ng/mL)          | 0.35 ± 0.04  | 0.24 ± 0.03          | 18.94 ± 3.6   | 18.85 ± 4.5  | 0.97         | <0.001       | 0.99        |
| Cortisol (ng/mL)         | 3.1 ± 1.2    | 15.6 ± 4.9           | 7.4 ± 2.4     | 35.1 ± 8.8   | <0.05        | <0.005       | 0.16        |
| Glucagon (pg/mL)         | 44.1 ± 7.3   | 82.1 ± 31.9          | 83.6 ± 27.0   | 163 ± 70.0   | 0.37         | <0.05        | 0.46        |
| Norepinephrine (pg/mL)   | 679 ± 517    | 1201 ± 313           | 971 ± 282     | 1686 ± 238   | 0.13         | <0.001       | 0.33        |
| Blood flow and oxygen uptake |          |                      |               |
| Blood flow (mL/min/kg)   | 198.2 ± 19.0 | 128.0 ± 10.5         | 161.7 ± 10.8  | 121.8 ± 14.3 | <0.01        | <0.05        | 0.11        |
| VO2 (µmol/min/kg)        | 345.9 ± 20.6 | 300.1 ± 9.2          | 333.2 ± 17.4  | 308.0 ± 13.0 | 0.11         | 0.51         | 0.58        |

Means ± SE are shown. CON group, n = 6; IUGR group, n = 9. *Statistical significance is indicated by P value from 2 × 2 ANOVA for the main effect of IUGR, insulin, and interaction (I × I). †BCAA concentrations measured in all fetuses; BCAA concentrations in CON (n = 3) and IUGR (n = 7) fetuses receiving Tropihamine were 0.68 ± 1.15 and 0.71 ± 0.06, respectively, during the basal period and 0.63 ± 0.17 and 0.81 ± 0.09, respectively, during the clamp. ‡P < 0.05 compared with CON during the basal period by t test.

Western blotting. Whole-cell lysates and nuclear enriched protein lysates were prepared from liver tissue, and Western immunoblotting was performed (7). Antibodies were from Cell Signaling to detect phosphorylated (p-)FOXO1 (T24), total FOXO1, p-Jun NH2-terminal kinase (JNK) (T183/Y185), total JNK, and p-GSK3β (S9); from Calbiochem to detect total GSK3β; from Santa Cruz to detect G6Pase; and from Abcam to detect PEPCK; and as previously described (7). Samples were run on two blots (10 samples each plus a reference sample). Results were quantified on each blot and data for the reference sample on both blots were determined to be similar.

Primary fetal hepatocytes. Primary fetal sheep hepatocytes were isolated (32). A 15- to 30-g piece of the medial right lobe was flushed with perfusion buffer (PB) (10 mmol/L HEPES, 138 mmol/L NaCl, 5.3 mmol/L KCl, 0.7 mmol/L NaHPO4) containing 30 U/mL heparin, followed by perfusion with PB supplemented with 0.5 mmol/L EGTA, 2 mmol/L glucose, and 0.05% BSA, and then 500 mL PB supplemented with 2 mmol/L glucose, 3 mmol/L CaCl2, 1.2 mmol/L MgSO4, 0.5% BSA, 0.05% collagenase, and 0.001% DNase I. Digested tissue was filtered, cells were spun and washed three times with Dulbecco's modified Eagle's medium (DMEM) similar to fetal circulation (1.1 mmol/L glucose, 2 mmol/L lactate, 2 mmol/L glutamine, 1 mmol/L pyruvate, 1 × non-essential amino acids, 1 × penicillin-streptomycin) supplemented with 0.1 mmol/L insulin, 10 mmol/L dexamethasone, and 10% FBS. Cells were plated.
in complete DMEM (3 × 10⁵ cells/cm², six-well plates, 5 µg/cm² collagen). After a 4-h attachment period, cells were washed twice and media was replaced with fetal DMEM plus 0.2% BSA. After overnight incubation, hepatocytes were washed twice and incubated in phenol red and glucose-free DMEM with 10 mmol/L HEPES, 0.369% NaHCO₃, 2 mmol/L sodium pyruvate, 20 mmol/L sodium lactate containing 100 µmol/L cAMP, 500 nmol/L dexamethasone, or 100 nmol/L insulin, as indicated, for 24 h in duplicate wells. Glucose in the media (glucose oxidase assay) and protein content of cells (bicinchoninic acid protein assay) per well was measured.

**Statistical analysis.** Data were analyzed by mixed model ANOVA with fixed effects of group (CON, IUGR), period (basal, insulin), and the interaction and random effect for sheep using SAS software (PROC MIXED). Measurements in liver tissue were analyzed with fixed effects of group (CON, IUGR), treatment (saline, insulin), and the interaction. Hepatocyte data were analyzed similarly and included a random effect for animal of origin. When the interaction was significant, individual posttest comparisons were made. In CON fetuses, GPR was tested against a theoretical mean of zero by one-sample t test and Wilcoxon signed rank test. Statistical significance was declared at $P < 0.05$.

**FIG. 1.** Glucose metabolism flux rates measured in late gestation CON and growth-restricted (IUGR) fetal sheep during basal and hyperinsulinemic clamp (insulin) periods. Total fetal GER represents the sum of UGU (lower bars) and GIR (upper bars). GUR was measured with glucose tracer, and GPR represents the difference between GUR and GER. Means ± SE are shown for CON (n = 6; white bars) and IUGR (n = 8 or 9; black bars). Significant effects from two-way ANOVA are indicated.

**FIG. 2.** Fetal glucose metabolism rates and plasma hormones. A: The relationship between GUR (left) and GPR (right) and insulin infusion rate during the clamp in CON (n = 6) and growth-restricted (IUGR) (n = 8) fetuses. B: The relationship between GPR with plasma cortisol (log-transformed) (left) and norepinephrine (log-transformed) concentrations (right) in CON and IUGR fetuses during basal and hyperinsulinemic clamp (insulin) periods (n = 28).
RESULTS

Fetal characteristics. IUGR fetuses had smaller placenta (IUGR, 261 ± 52 g; CON, 444 ± 40 g; P < 0.05) and weighed ∼40% less than CON fetuses (IUGR, 2.1 ± 0.2 kg; CON, 3.4 ± 0.1 kg; P < 0.001). Under basal conditions, IUGR fetuses had 30% lower plasma insulin concentrations and similar glucose and BCAA concentrations compared with CON fetuses (Table 1). Insulin infusions during the clamp increased plasma insulin concentrations similarly by more than 50-fold, and, by design, glucose and BCAA concentrations in CON and IUGR fetuses were not different compared with the basal period (Table 1). This allowed us to test the effect of hyperinsulinemia independently of glucose.

IUGR fetuses had lower arterial blood oxygenation compared with CON fetuses but had similar oxygen consumption rates (Table 1). Hyperinsulinemia further reduced fetal arterial oxygenation in both CON and IUGR groups, but oxygen consumption rates remained similar (Table 1). Fetal arterial blood pH and the partial pressure of CO2 were similar in CON and IUGR fetuses during the clamp periods. Hyperinsulinemia further increased arterial pH in CON and IUGR fetuses compared with CON fetuses but had similar oxygen consumption rates (Table 1). Concentrations of cortisol, glucose, and BCAA were higher in IUGR fetuses under basal conditions and increased twofold in both groups during hyperinsulinemia (Table 1). Weight-specific umbilical blood flow rates were 35% lower in IUGR fetuses during both periods and insulin reduced flow by 5–15% in CON and IUGR fetuses (Table 1).

Glucose metabolism rates. Insulin infusion rates based on necropsy-determined fetal weight were similar between groups (CON, 2.5 ± 0.3 mU/min/kg; IUGR, 2.9 ± 0.2 mU/min/kg; P = 0.3). UGU was not different between CON or IUGR fetuses during the basal or insulin periods (Fig. 1). GIR and total GER (GER = GIR + UGU) during the clamp were similar between CON and IUGR fetuses, but GER was higher in both groups during hyperinsulinemia due to the GIR (Fig. 1). IUGR fetuses had a higher GPR under basal conditions compared with CON fetuses, which is consistent with prior data (6). In response to hyperinsulinemia, IUGR fetuses maintained this increased GPR. GIR was not significantly different from zero in CON animals during basal or insulin periods (P > 0.05).

In response to insulin, the GUR increased nearly twofold in both CON and IUGR fetuses. GUR was positively correlated with the insulin infusion rate across all fetuses and study periods. When compared separately, however, the relationship was significant only in IUGR fetuses and not in CON fetuses (Fig. 2A). GPR did not correlate with insulin infusion rate in CON or IUGR fetuses (Fig. 2A).

Hormone levels. IUGR fetuses had fivefold increases in arterial plasma cortisol concentrations during basal and insulin periods (Table 1). Concentrations of cortisol, glucagon, and norepinephrine increased by approximately twofold in both CON and IUGR fetuses during hyperinsulinemia (Table 1). Glucagon was positively correlated with fetal cortisol and norepinephrine concentrations (Fig. 2B) but not glucagon (data not shown).

Hepatic gluconeogenic gene expression. The IUGR fetal liver had a more than 30-fold increase in mRNA abundance of PCK1, the cytosolic form of PEPCK, and a 20-fold increase in G6PC under basal conditions (Fig. 3A). In response to hyperinsulinemia, PCK1 mRNA was not suppressed in the IUGR liver and remained elevated by 30-fold. Expression of G6PC was reduced 75% by insulin in the IUGR livers, yet was still more than fourfold higher compared with CON livers under insulin conditions (Fig. 3A). Given the short half-life (30 min) of PCK1 mRNA, we examined the levels of these proteins. Protein expression of PEPCK was increased by 50% in IUGR livers and expression of G6Pase increased more than twofold during both basal and hyperinsulinemic periods (Fig. 3B).

Molecular effects of insulin. The lack of suppression of PGR and PEPCK by insulin prompted us to evaluate the effect of hyperinsulinemia on the insulin signaling pathway. Expression of components in the proximal pathway (IR-β, IRS-2, p85, AKT1, and AKT2) were relatively similar between CON and IUGR fetal livers during the basal and insulin periods (Supplementary Fig. 1). Phosphorylation of (p-)AKT at S473 was modestly increased under basal and insulin conditions in IUGR livers (Fig. 4). In response to insulin, p-AKT (S473 and T308) increased nearly 10-fold in both the CON and IUGR groups (Fig. 4 and Supplementary Fig. 1). We next measured activation of two AKT target proteins, GSK3β and FOXO1 (33,34). Insulin increased
p-GSK3β twofold in CON and IUGR livers (Fig. 4). Under basal conditions, p-FOXO1 (T24) was more than twofold higher in IUGR livers. Furthermore, insulin increased p-FOXO1 by nearly threefold in CON livers but failed to do so in the IUGR livers (Fig. 4).

Given the importance of cellular localization of AKT and FOXO1 in mediating insulin’s effects, we measured expression in nuclear and cytosol-enriched protein lysates. Nuclear p-AKT (S473) was increased 10-fold in both the CON and IUGR groups (Fig. 5A). Basal nuclear FOXO1 protein localization was similar between the CON and IUGR groups. Insulin reduced nuclear FOXO1 expression in CON livers by 50%, yet in the IUGR livers nuclear FOXO1 expression was unchanged by insulin and was twofold higher compared with CON livers (Fig. 5A). Cytosolic expression of FOXO1 was similar under basal and insulin conditions (Fig. 5B). The ratio of nuclear to cytosolic FOXO1 protein expression during the insulin period was 25% higher in IUGR livers compared with CON livers (Fig. 5B). Nuclear JNK phosphorylation was increased more than fivefold in the IUGR fetal livers (Fig. 5A) in response to insulin.

We next examined the mRNA expression of IGFBP1, a target of insulin and a measure of FOXO1 activity (34,35). IGFBP1 was increased fourfold in the IUGR livers under basal conditions and remained increased during hyperinsulinemia, consistent with increased FOXO1 activity (Fig. 6A). Likewise, expression of PGC1A mRNA was increased nearly threefold in the IUGR livers during basal and insulin conditions (Fig. 6A). Nuclear expression of p-CREB was higher in the IUGR livers by 30% during basal conditions and nearly 50% during hyperinsulinemia, although neither increase was statistically different (Fig. 6B). Nuclear expression of hepatocyte nuclear factor 4α (HNF4α) was increased by 20% in IUGR livers (Fig. 6B).

**Isolated primary fetal hepatocytes.** Isolated primary hepatocytes from the IUGR fetal livers had higher glucose output in response to treatment with dexamethasone and cAMP compared with untreated IUGR hepatocytes and compared with CON hepatocytes similarly treated (Fig. 7). IUGR hepatocytes also tended to have higher glucose output ($P = 0.18$) compared with CON hepatocytes under all treatment conditions.

**DISCUSSION**

This study is the first to show that increased glucose production and hepatic gluconeogenic gene expression ($PCK1$, $G6PC$, $PGC1A$) is not suppressible by insulin in late gestation IUGR fetal sheep. During hyperinsulinemia, GPR remained increased in IUGR fetuses. As expected, CON fetuses had a low basal GPR (not significantly greater than zero) because the gluconeogenic pathway is not activated until just before birth (15), leaving limited GPR for insulin to suppress. In other studies, acute fetal hypoglycemia alone increases GPR and gluconeogenic gene activation, similar to the IUGR fetus (26,36–38). In the hypoglycemic fetus, GPR is suppressed by hyperinsulinemia (26,36), indicating that factors besides hypoglycemia may be contributing to the development of hepatic insulin resistance in the IUGR fetus. Furthermore, these studies demonstrate that fetal GPR can be suppressed using an insulin clamp technique similar to the one performed here. Hyperinsulinemia also robustly increased glucose utilization in both CON and IUGR fetuses, supporting maintained peripheral sensitivity to insulin.

Mechanisms responsible for increased hepatic glucose production and the inability of insulin to suppress this in the IUGR fetus may include the combined effects of hormonal cues and persistently increased nuclear factors. Components in the proximal insulin pathway are similar...
between CON and IUGR fetal livers and, even in IUGR fetuses, insulin robustly activates nuclear AKT. Furthermore, insulin-stimulated phosphorylation of GSK3β, a target of AKT, is similar between CON and IUGR fetal livers. Thus, there is discordance between normal proximal insulin signaling to AKT and the failure of insulin to suppress gluconeogenic gene expression and GPR in the IUGR fetus. This is consistent with a nuclear mechanism and alteration in transcriptional regulation that seems to be unique in the IUGR fetus compared with adults and rodents with hepatic insulin resistance (39).

Improper FOXO1 activation and regulation may contribute to increased GPR and gluconeogenic gene expression. The phosphorylation and nuclear exclusion of FOXO1 is one well-characterized mechanism for insulin-mediated suppression of gluconeogenic gene expression, and recent studies indicate the importance of proper FOXO1 regulation for hepatic metabolism (40–43). Basal phosphorylation of FOXO1 is higher in the IUGR liver and is not increased further with insulin, nor is nuclear localization reduced, despite normal AKT activation. This is in contrast to the nearly threefold increase in phosphorylation and 50% reduction in nuclear FOXO1 in response to insulin in the CON liver. Expression of PCK1 and IGFBP1 mRNA, both transcriptional targets of FOXO1 (34,35), remain increased during hyperinsulinemia in the IUGR liver, supporting increased nuclear FOXO1 activity. Nuclear phosphorylation of JNK is increased in the IUGR liver with insulin. JNK-mediated phosphorylation of FOXO1 has been shown to prevent 14-3-3 protein binding and subsequent nuclear export and degradation (44). We speculate that increased JNK activation in IUGR may antagonize AKT-induced phosphorylation, which has classically been viewed as the inactivation signal for FOXO1 nuclear exclusion. In addition, decreased phosphatase activity may contribute to increased p-FOXO1 in the IUGR liver. Mechanistic studies in isolated hepatocytes are needed to test these hypotheses.

Expression of PGC1α mRNA, nuclear HNF4α, and p-CREB are increased in the IUGR liver during basal and hyperinsulinemic conditions, supporting the potential role of cAMP-dependent signaling in mediating glucose production (6,7,18,45). IUGR fetuses have increased cortisol concentrations, and cortisol and norepinephrine concentrations positively correlate with GPR, suggesting that these hormones may potentiate glucose production. Indeed, in normal fetal sheep, experimentally induced changes in

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FIG. 5. The effect of growth restriction (IUGR) and hyperinsulinemia (insulin) on nuclear AKT, FOXO1, and JNK in the fetal liver. A: Nuclear protein expression of p-AKT (S473), total AKT, total FOXO1, p-JNK (p54 and p46 at T183/Y185), total JNK (p54 and p46), and lamin A/C measured by Western blotting. B: Cytosolic protein expression of FOXO1 and β-actin. Representative images and quantification of results and ratio of nuclear to cytosolic FOXO1 protein expression are shown. Means ± SE are shown for saline (CON, n = 4; IUGR, n = 4) and insulin (CON, n = 6; IUGR, n = 6). Significant effects from two-way ANOVA are indicated. Bars with different letters are significant differences when interaction (I × I) is significant.
cortisol, glucagon, and catecholamines have been implicated in regulating glucose production (15–17). Recent studies and our data also support an inverse relationship between fetal oxygenation and catecholamine concentrations (results not shown), which may drive glucose production in the IUGR fetus (6).

The increase in GPR is likely due to gluconeogenesis rather than glycogenolysis because liver glycogen content is similar between CON and IUGR fetuses (data not shown), and PCK1 and G6PC gene expression were both higher in IUGR fetuses during hyperinsulinemia (6,7).

Given that G6PC expression was reduced with insulin in IUGR liver, it is possible that there is a change in glycogen flux, as observed in humans and canines during hyperinsulinemia (20,21,46,47). Additional studies are needed to address this in fetal sheep. It is interesting that acute hyperglycemia (fourfold) accompanied by a twofold increase in insulin for 3 h was able to suppress glucose production rates in our fetal sheep model of IUGR (6). This suggests that glucose and insulin regulate glucose production via different mechanisms in the fetus, similar to the differential regulation observed in other species and physiological states (46,48).

Offspring that were IUGR are at an increased risk for the development of insulin resistance and uncontrolled hepatic glucose production (1–3). Recent data suggest that IUGR produces early tissue differences in insulin sensitivity that eventually evolve into insulin resistance as the offspring mature (2). Our data support this: along with the inability of insulin to suppress GPR, IUGR fetal sheep maintained and potentially increased insulin sensitivity for glucose uptake and utilization by peripheral tissues. This finding is consistent with previous data in our model (6) and two other fetal sheep models of placental insufficiency and IUGR (9,25), suggesting maintained or increased peripheral insulin sensitivity for glucose utilization. Despite a nearly 50% reduction in plasma insulin concentrations during basal conditions, IUGR fetuses had a GUR similar to that of CON fetuses (4,6). During the insulin clamp, CON fetuses increased their GUR to a potentially maximal rate because there was little variation in GUR and no relationship with insulin infusion rate. Previous studies in normal fetal sheep have shown that insulin infusions producing more than fivefold increases in insulin concentration (>5 ng/mL) during euglycemia result in a maximal GUR of ~47 μmol/min/kg (24). In contrast, IUGR fetuses in this study demonstrated dose-response sensitivity to insulin infusion rate, with some having a GUR nearly twofold higher than that observed in CON fetuses.
Isolated primary IUGR fetal hepatocytes maintain increased glucose production. The persistence of this phenotype and the lack of induction in CON hepatocytes implicate developmental programming that is independent of in vivo factors such as circulating hormones, hypoxia, and nutrient supply. Future studies are needed to test whether persistent increases in expression of nuclear regulatory factors (PGC1α, FOXO1, or JNK) or alterations in PCK1 promoter are responsible for glucose production in the IUGR liver.

Conclusions. Glucose diffuses across the placenta to the fetus by concentration-dependent, facilitated transport (49). It is advantageous for the IUGR fetus to have a lower glucose concentration to maximize UGU because it has reduced placental glucose transport capacity (22,50). Maintained or increased peripheral tissue glucose utilization and insulin sensitivity in the IUGR fetus also helps maintain glucose metabolism. The development of GPR adds an additional adaptive capacity of the fetus to maintain plasma glucose concentrations to support glucose uptake and energy metabolism. Together, the increased GPR and GUR maintain the survival of vital organs despite reduced restricted nutrient supply. These changes in fetal glucose metabolism have important implications for managing glucose homeostasis in UGR neonates. Furthermore, the persistence of these phenotypes across the life span may underlie the increased susceptibility of IUGR offspring to develop hyperglycemia and type 2 diabetes.

ACKNOWLEDGMENTS

This research was supported by National Institutes of Health Grants F32-DK-08220, K01-DK-090199 (S.R.T.), K08-HD-060688, R01-DK-088139 (P.J.R.), K12-HD-04332 (L.D.B.), and T32-HD-07186 (W.W.H.) and a pilot award to S.R.T. (P50-DK-048520).

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

S.R.T. designed and performed the experiments, analyzed data, and wrote the manuscript. L.D.B., P.J.R., W.W.H., and J.E.F. designed the experiments and reviewed and edited the manuscript. S.R.T. is the guarantor of this work and, as such, had full access to all the data and takes full responsibility for the integrity of the data and the accuracy of the data analysis.

The authors thank David Caprio, Karen Trembler, Alex Cheung, Gates Roe, Meghan O’Meara, and Dan LoTurco, from the University of Colorado, for technical support and Dr. Giacomo Meschia, also from the University of Colorado, for critically evaluating the data.

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