Glucose metabolic adaptations in the intrauterine growth-restricted adult female rat offspring

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Garg, Meena, Manikkavasagar Thamotharan, Lisa Rogers, Sara Bassilian, W. N. Paul Lee, and Sherin U. Devaskar. Glucose metabolic adaptations in the intrauterine growth-restricted adult female rat offspring. Am J Physiol Endocrinol Metab 290: E1218–E1226, 2006. First published January 31, 2006; doi:10.1152/ajpendo.00474.2005—We studied glucose metabolic adaptations in the intrauterine growth-restricted (IUGR) rat offspring to decipher glucose homeostasis in female rat offspring. The IUGR rats exposed to either prenatal (CM/SP, n = 5 per age), postnatal (SM/CP, n = 6), or pre- and postnatal (SM/SP, n = 6) nutrient restriction were compared with age-matched controls (CM/CP, n = 5). At 2 days, IUGR pups (SP) were smaller and glucose intolerant and had increased hepatic glucose production and increased glucose disposal (P < 0.01) compared with controls (CP). At 2 mo, the GTT, glucose clearance, and GFC did not change. However, a decline in hepatic glucose-6-phosphatase (P < 0.05) and fructose-1,6-biphosphatase (P < 0.05) enzyme activities in the IUGR offspring was detected. At 15 mo, prenatal nutrient restriction (CM/SP) resulted in greater weight gain (P < 0.01) and hyperinsulinemia (P < 0.001) compared with postnatal nutrient restriction (SM/CP). A decline in GFC in the face of a normal GTT occurred in both the prenatal (CM/SP, P < 0.01) and postnatal calorie (SM/CP, P < 0.03) and growth-restricted offspring. The IUGR offspring with pre- and postnatal nutrient restriction (SM/SP) were smaller, hypoinsulinemic (P < 0.03), and hypoalimentic (P < 0.03), with no change in GTT, hepatic glucose production, GFC, or glucose clearance. We conclude that there is pre- and postnatal programming that affects the postnatal compensatory adaptation of GFC and disposal initiated by changes in circulating insulin concentrations, thereby determining hepatic insulin sensitivity in a phenotype-specific manner.

Glucose tolerance test; glucose homeostasis; glucose recycling; hepatic futile cycling; metabolic programming

Epidemiological investigations have linked prenatal nutrient restriction presenting with low birth weight to the metabolic syndrome in the adult, consisting of insulin resistance, a forerunner of type 2 diabetes mellitus, obesity, hypertension, and coronary artery disease (1, 2, 14, 20, 21). Mimicking this paradigm, hyperinsulinemic euglycemic clamp studies in intrauterine growth-restricted (IUGR) animal models encountered the development of insulin resistance and glucose intolerance (13, 32, 25, 26). Glucose intolerance has been observed as early as 2 or 7 days of age that persists into adulthood when IUGR was induced in rat models either by nutrient restriction (28) or ischemia (25), respectively. In transition to postnatal life, the neonate balances its glucose production and utilization by the availability of gluconeogenic precursors, such as lactate, alanine, and glycerol, in addition to hormonal regulation of substrate utilization (16). This is in contrast to adults, in whom plasma insulin concentration plays the primary role (14). Observations in IUGR human infants show increased insulin sensitivity that serves as a precursor to the subsequent emergence of insulin resistance during childhood (3, 12).

Although many investigations in animals have built this link between IUGR and insulin resistance on the basis of tissue-specific analyses of insulin-regulated processes, there are limited studies addressing whole body responses in the calorie- and growth-restricted offspring. Furthermore, although most animal models have focused on changes in the male (22, 25, 32), female rat offspring have been reported to develop gestational diabetes (4) and become insulin resistant later in life when muscle- and fat-specific insulin-signaling mechanisms were examined (7). In addition, we have previously observed (28) that, at an early age, the female IUGR offspring demonstrate insulin resistance of skeletal muscle GLUT-4 translocation to the plasma membrane along with glucose intolerance. Although 8-wk-old male adult IUGR offspring have been noted to develop glucose intolerance due to enhanced hepatic glucose production (32), there is limited information regarding the in vivo glucose kinetics in female IUGR offspring. Furthermore, whether hepatic glucose production, cycling, and/or disposal are altered in the IUGR female offspring remains unknown. The preservation of glucose homeostasis and the maintenance of a balance between hepatic glucose production and utilization require glycogenolysis, gluconeogenesis, and glucose recycling. Normal glucose recycling plays a significant role in providing this necessary balance to glucose homeostasis. The process by which glucose enters the hepatocytes and is partially metabolized to glucose 6-phosphate (G-6-P) by phosphorylation and then recycled back into dephosphorylated glucose constitutes “hepatic futile cycling” (33, 34). Glucose futile cycling (GFC) contributes to the resultant hyperglycemia observed in streptozotocin-induced insulin-dependent diabetes.
Hepatic glucose production, GFC between glucose and G-6-phosphate access to nutrients and examined the ontogeny of diabetic rat model with postnatal seminutrient restriction or ad libitum access to milk intake during the suckling phase would cause perturbations in glucose production, GFC, and disposal as age-dependent adaptations toward maintaining normalcy in the unstressed basal state. To test this hypothesis, we employed the age-dependent adaptations toward maintaining normalcy in diabetes mellitus (6, 23). On the basis of available information, we hypothesized that prenatal calorie restriction modified postnatally by ad libitum access to milk intake during the suckling phase would cause perturbations in glucose production, GFC, and disposal as age-dependent adaptations toward maintaining normalcy in the unstressed basal state. To test this hypothesis, we employed the mid-to late-gestation maternal semi-nutrient-restricted pregnant rat model with postnatal seminutrient restriction or ad libitum access to nutrients and examined the ontogeny of hepatic glucose production, GFC between glucose and G-6-P, and glucose disposal at 2-day newborn, 2-mo peak fertility, and 15-mo postfertility stages. This was accomplished by cross-fostering of animals, which generated four experimental groups. The control mother with ad libitum access to nutrients fed the intrauterine semi-nutrient-restricted progeny (CM/SP) and represented intrauterine nutrient restriction; the semi-nutrient-restricted mother fed the IUGR progeny (SM/SP) and represented intrauterine and postnatal nutrient restriction. These groups were compared with the control mother that fed control progeny (CM/CP) and with one another.

MATERIALS AND METHODS

Animals

Sprague-Dawley rats (Charles River Laboratories, Hollister, CA) were housed in individual cages, exposed to 12:12-h light-dark cycles at 21–23°C, and allowed ad libitum access to standard rat chow (composition: carbohydrate 63.9%, fat 4%, protein 14.5%, ash 4.7%, fiber 4.5%, and the rest is water). The National Institutes of Health guidelines were followed, and the study was approved by the Animal Research Committee of the University of California, Los Angeles.

Animal Model

We provided 50% of daily food intake (11 g/day) of a pregnant rat with ad libitum access to water from embryonic day (e)11 to e21 as previously described by us (28). This prenatal seminutrient restriction led to relative brain-sparing IUGR (28). At birth, the litter size was culled to six to ensure no interlitter nutritional variability. Postnataally, the cross-fostering of animals generated four experimental groups, as previously described by us (28). The newborn pups born to ad libitum-fed control mothers (CP) were reared either by mothers continued on seminutrient restriction from postnatal day (PN)1–PN21 (SM/CP) or by control mothers (CM/CP, Fig. 1). During the suckling phase, the intrauterine semi-nutrient-restricted progeny were fed either by control mothers with ad libitum access to nutrients (CM/SP), representing intrauterine nutrient restriction, or by semi-nutrient-restricted mothers that continued to receive 50% of daily food intake (SM/SP), representing intrauterine and postnatal nutrient restriction.

Glucose Tolerance Tests: 2-Day-Old Newborn Pups

An intraperitoneal glucose tolerance test (IPGTT) was performed in 2-day-old control pups (CP) and IUGR pups (SP). Glucose at the dose of 1 g/kg body wt was administered intraperitoneally as a 1:1 mixture of [2-2H]- and [6,6-2H2]glucose (34). Timed blood samples were collected from the jugular vein and pooled from six randomly chosen pups at 0, 15, 30, 60, 120, and 180 min (Fig. 2) for a single IPGTT.

Adult Females

Surgical catheter placement. Two month- and 15-mo-old adult female rats were anesthetized using an anesthetic cocktail of ketamine HCl (50 mg/kg), acepromazine (1 mg/ml), and xylazine (4.8 mg/kg) intraperitoneally. The surgical site was closely shaved and using sterile precautions, a skin incision was made, and the jugular vein was exposed. Catheters were inserted into the jugular vein, tunneled subcutaneously, exteriorized, and maintained patent with heparinized saline. All animals were allowed full recovery from the surgical procedure prior to conducting the glucose tolerance tests (28).

Intravenous glucose tolerance test (IVGTT). Two-month- and 15-mo-old adult female awake animals received 1 g/kg body wt of the 1:1 mixture of [2-2H]- and [6,6-2H2]glucose via the surgically placed jugular vein catheters. Blood (500 μl) was obtained at 0, 5, 15, 30, 60, and 120 min for assessment of hormone and glucose concentrations, and isotopomer enrichment (Fig. 2).

Plasma Assays. The plasma was separated, and aliquots were made for measurement of glucose by the glucose oxidase method (Sigma Diagnostics, St. Louis, MO; sensitivity 0.1 mM). Insulin and leptin were quantified by enzyme-linked immunosorbent assays using rat standards and anti-rat insulin or leptin antibodies (Linco Research, St. Louis, Mo; sensitivity insulin = 0.2 ng/ml, leptin = 0.04 ng/ml, using 10-μl sample). Corticosterone was quantified using a radioimmunoassay and an anti-rat corticosterone antibody (Coat-A-Count; Diagnostic Products, Los Angeles, CA; sensitivity ~5.7 ng/ml with apparent concentration at 95% bound fraction of sample/bound fraction of the zero standard).
Gas Chromatography-Mass Spectrometry Analysis

Glucose was separated in 50 to 100 μl of blood plasma by deproteinization and deionization and subsequently converted to its aldonitrile pentaacetate derivative by dissolving in ethyl acetate (150 μl) in preparation for gas chromatography-mass spectrometry (GC-MS) analysis per a modified method described by Szafranek et al. (27). All isotopometric determinations were performed using a Hewlett-Packard gas chromatograph (model 6890; Hewlett-Packard, Palo Alto, CA) connected to a Hewlett-Packard Mass Selective Detector (model 5973A). The glucose derivative was separated on an HP5 capillary column with 30 m × 250 μm internal diameter. GC conditions were as follows: helium as carrier gas at a flow rate of 1.0 ml/min, sample injector temperature of 250°C, oven temperature at 220°C, and retention time of 2.9 min. Electron impact ionization of the glucose derivative was used to characterize glucose positional isotopomers of [6,6-2H2]glucose at mass-to-charge ratio (m/z) 187 for C3–C6 and of [2-2H]glucose at m/z 242 for C1–C4 fragments. Selected ion monitoring was used to follow specific ions. Isotope enrichment in these fragments was used to determine the disappearance of [2-2H]- and [6,6-2H2]glucose (33, 34).

Mass isotopomer distribution was determined using the method of Lee et al. (17), which corrects the contribution of the derivatizing agent and natural abundance to the mass isotopomer distribution of the compound of interest. Results of the mass isotopomers in glucose (enrichment of glucose isotopomers) are reported as molar fractions of M0, M1, and M2, according to the number of labeled hydrogen in the molecule (17) (15). The sum of all isotopomers of the glucose molecules, M1 for 1 out of n (n = 6 for glucose), is equal to 1, or 100%. The disappearance of the two isotopes [2-2H]- and [6,6-2H2]glucose was determined by mass fragmentography by assessing the M1 label in the C1–C4 fragment (for [2-2H]glucose) and the M2 label in the C3–C6 fragment (for [6,6-2H2]glucose) (33, 34).

Data Analysis and Interpretation of GTT

A typical time course of total plasma glucose and M0, M1, and M2 glucose isotopomer concentrations after administering intravenous glucose demonstrates the increase in total glucose concentration to greater than twice the baseline within 5 min (Fig. 3) (34). The labeled glucose fractions M1 and M2 concentrations leveled off between 60 and 120 min. This was accompanied by a concomitant increase in the concentration of unlabeled glucose “M0” derived from glycogenolysis and/or gluconeogenesis between 60 and 120 min (Fig. 3).

**Plasma M0 glucose.** M0 glucose is unlabeled glucose. It can be generated by the liver via glycogenolysis from unlabeled glycogen or gluconeogenesis from unlabeled substrates.

**Plasma M1 and M2 glucose.** Timed plasma M1 or M2 ([2-2H]- or [6,6-2H2]glucose) enrichment was plotted on semilog plots. The rate of tracer disappearance was analyzed by a one-exponential model to determine the fractional clearance of glucose (KM1 and KM2). Hepatic and peripheral tissue uptake of glucose and subsequent glycolysis leads to loss of the deuterium-labeled glucose. However, when the [6H]glucose is taken up by the liver, the deuterium in the carbon-2 position is lost in the equilibrium reaction of G-6-P to fructose 6-phosphate (F-6-P). Therefore, the fractional clearance of [2-2H]glucose is greater than that of [6,6-2H2]glucose when hepatic GFC occurs. The difference between the disappearance rates of M1 and M2 is then used as a measure of GFC (i.e., glucose to glucose fractional clearance, or glucose disposal).

**Liver Enzyme Activity**

G-6-Pase (8) and fructose-1,6-biphosphatase (FBPase) enzyme (29) activities were assessed by previously described methods (8, 29).

**Statistical Analysis**

All data are expressed as means ± SE. The ANOVA models were used to compare the various treatment groups at different ages, and F values were determined. The intergroup differences were determined by Fisher’s paired least significance difference test when ANOVA revealed significance. Significance levels were computed on the basis of exact methods accounting for small sample size, and P values at <0.05 were considered significant.

**RESULTS**

2-Day-Old Newborns: CP vs. SP Group

**Phenotypic changes:** body weight and baseline hormonal profile. Two-day-old pups born to mothers with semi-restricted nutrient intake (SM) were significantly smaller in weight (SP) compared with control pups (CP) born to control mothers (CM) on the ad libitum chow diet (P < 0.02; Table 1). The plasma glucose at baseline was not significantly different (90.29 ± 5.3 in CP vs. 102.50 ± 15.5 mg/dl in SP).

Baseline hormonal assessments were made after a 4-h separation from mother in 2-day-old pups. The plasma insulin concentration at baseline in the 2-day-old SP was lower but not significantly different from that of CP (P 1.35 ± 0.19 vs. SP 1.21 ± 0.18 ng/ml; Table 1). The baseline plasma leptin concentration in SP was 0.16 ± 0.09 ng/ml, which was significantly lower than in CP (0.71 ± 0.13 ng/ml, P < 0.001), corresponding to the low body weight. The baseline plasma corticosterone concentrations in 2-day-old control pups were not significantly different from those of the small pups (CP 190.42 ± 0.55 vs. SP 203.22 ± 6.08 ng/ml; Table 1).

**Glucose metabolic adaptations: IPGTT.** The 2-day-old SP group was significantly hyperglycemic after an intraperitoneal glucose load (1 g/kg body wt) compared with CP [area under the curve (AUC) 966.5 ± 20.2 vs. 679.5 ± 52.8, P < 0.0004]. The time course of plasma glucose isotopomers from their baseline level in response to the [2-2H]- and [6,6-2H2]glucose challenge (1 g/kg body wt) in the 2-day-old CP and SP (Fig. 4,
A and B) demonstrates unsuppressed hepatic unlabeled glucose (M0) production. The AUC for unlabeled glucose produced by the liver during the 180 min of monitoring after IPGTT was greater for SP at 401.1 ± 44.3 compared with 208.4 ± 19.4 in CP (P < 0.001). M0 represents glucose molecules without the deuterium label. Because deuterium in [2-2H]- and [6,6-2H2]glucose is not recycled during gluconeogenesis, the M0 molecule represents hepatic glucose production. Therefore, increased hepatic glucose production after a glucose challenge contributed to the hyperglycemia in pups that experienced in utero nutrient restriction.

In addition, glucose clearance determined from the rate of disappearance of M2 label as the C3–C6 fragment (GTT using stable isotope [6,6-2H2]glucose) was significantly increased in the 2-day-old SP at 17.2 ± 0.11 mg·kg⁻¹·min⁻¹ compared with 14.76 ± 0.26 mg·kg⁻¹·min⁻¹ in CP (P < 0.01; Fig. 5A). However, the rate of hepatic GFC measured from the difference in clearance of M1 label in the C1–C4 fragment (for [2-2H]glucose) and the M2 label in the C3–C6 fragment (for [6,6-2H2]glucose) was not significantly different between the two groups (Fig. 5B).

Developmental Study: Control Animals (2-Day-Old CP and 2-Mo-Old and 15-Mo-Old CM/CP)

Phenotype changes: body weight and baseline hormonal profile. Table 1 shows the age-dependent increase in body weight with corresponding increase in serum leptin concentration. The fasting insulin and baseline corticosterone increased from 2 days to 2 mo of age without any further change at 15 mo of age.

Glucose metabolic adaptations: glucose clearance and hepatic GFC. Glucose clearance rate declined in an age-dependent manner. The glucose clearance in 2-day-old CP was 14.76 ± 0.26 mg·kg⁻¹·min⁻¹, which decreased to 8.56 ± 0.31 at 2 mo and to 4.1 ± 0.34 mg·kg⁻¹·min⁻¹ at 15 mo of age (P < 0.01; Fig. 6A). The 2-day-old CP showed GFC at a much lower rate compared with the 2-mo- and 15-mo-old control animals (P < 0.001), whereas there was no difference in GFC between 2 mo and 15 mo of age (Fig. 6B).

The decrease in glucose turnover from 2 mo to 15 mo of age (P < 0.001) without a net change in the amount of glucose recycled (2.1 ± 0.28 vs. 1.92 ± 0.21 mg·kg⁻¹·min⁻¹; Table 2) is ascribed to a nonsignificant change in the contribution of hepatic glucose production by recycling from 24.7 ± 3.4% at 2 mo of age to 31.7 ± 1.8% at 15 mo of age (Table 2).

Adult IUGR Study: CM/SP, SM/SP, and SM/SP vs. CM/CP Group

Phenotype changes: body weight and baseline hormonal profile. The 2-mo-old offspring of prenatal nutrient restriction (CM/SP) gained weight on an ad libitum postnatal diet and weighed the same as control animals (CM/CP), but at 15 mo of age they weighed more than SM/CP and SM/SP (P < 0.01) groups. In contrast, the animals subjected to semi-

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### Table 1. Phenotype changes in body weights and plasma insulin, corticosterone, and leptin concentrations in all groups at 2 days, 2 mo, and 15 mo of age

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Body Weight, g</th>
<th>Insulin</th>
<th>Corticosterone</th>
<th>Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-day CP (4)</td>
<td>6.9 ± 0.1*</td>
<td>1.35 ± 0.19</td>
<td>190.42 ± 0.55</td>
<td>0.71 ± 0.13*</td>
</tr>
<tr>
<td>2-day SP (6)</td>
<td>5.3 ± 0.07</td>
<td>1.21 ± 0.18</td>
<td>203.22 ± 6.08</td>
<td>0.16 ± 0.09</td>
</tr>
<tr>
<td>2-mo CM/CP (5)</td>
<td>271.6 ± 9.7</td>
<td>4.51 ± 0.72</td>
<td>674.82 ± 34.73</td>
<td>4.34 ± 0.52</td>
</tr>
<tr>
<td>15-mo CM/CP (6)</td>
<td>565.2 ± 38.4</td>
<td>5.7 ± 0.73</td>
<td>540.04 ± 40.84</td>
<td>37.25 ± 2.84</td>
</tr>
<tr>
<td>2-mo CM/SP (5)</td>
<td>293.7 ± 12.7</td>
<td>4.26 ± 0.88</td>
<td>464.0 ± 65.6</td>
<td>9.43 ± 1.74</td>
</tr>
<tr>
<td>15-mo CM/SP (5)</td>
<td>586.0 ± 41.5</td>
<td>5.07 ± 1.02</td>
<td>601.84 ± 20.17</td>
<td>38.21 ± 6.14</td>
</tr>
<tr>
<td>2-mo SM/CP (5)</td>
<td>273.3 ± 4.8</td>
<td>2.29 ± 0.22</td>
<td>553.36 ± 11.9</td>
<td>3.73 ± 0.54</td>
</tr>
<tr>
<td>15-mo SM/CP (5)</td>
<td>486.6 ± 34.2a</td>
<td>2.7 ± 0.7</td>
<td>618.47 ± 34.18</td>
<td>28.48 ± 6.4b</td>
</tr>
<tr>
<td>2-mo SM/SP (5)</td>
<td>246.8 ± 10.5</td>
<td>2.72 ± 0.22</td>
<td>625.64 ± 16.77</td>
<td>3.61 ± 1.38</td>
</tr>
<tr>
<td>15-mo SM/SP (6)</td>
<td>456.2 ± 20.3</td>
<td>3.9 ± 0.5a</td>
<td>616.23 ± 23.57</td>
<td>29.18 ± 2.64a</td>
</tr>
</tbody>
</table>

All data are shown as means ± SE, concentrations in ng/ml. CP, control pups; SP, semi-nutrient-restricted pups; CM, control mothers; SM, semi-nutrient-restricted mothers. *P < 0.02, **P < 0.001: CP vs. SP; †P < 0.002, ‡P < 0.03, ¶P < 0.05: vs. age-matched controls (CM/CP). *P < 0.01: 15-mo CM/SP vs. 15-mo SM/SP and 15-mo CM/CP. **P < 0.001: 15-mo CM/SP vs. 15-mo SM/CP and 15-mo SM/SP. ¶P < 0.001: 15-mo SM/CP vs. 15-mo CM/SP. *P < 0.001: 15-mo SM/SP vs. 15-mo CM/SP. *P < 0.0001: 2-mo CM/SP vs. 2-mo CM/CP, SM/CP, and SM/SP.

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![Fig. 4](http://ajpendo.physiology.org/ Downloaded from by AJP-Endocrinol Metab • VOL 290 • JUNE 2006 • www.ajpendo.org)
restricted nutrient intake postnatally (SM/CP, P < 0.03) and nutrient restriction, both in utero and postnatally (SM/SP, P < 0.002), demonstrated ongoing growth restriction from 2 mo to 15 mo of age. Thus body weight patterns at 15 mo of age showed opposite effects of prenatal (CM/SP) vs. postnatal (SM/CP) nutrient restriction, with the former being heavier than the latter, whereas the lightest SM/SP group demonstrated an additive effect of pre- and postnatal nutrient restriction (Table 1).

The baseline hormonal assessments were made after a 12-h overnight fast in 2-mo- and 15-mo-old female offspring. The fasting plasma insulin concentrations in the 2-mo-old CM/SP, SM/CP, and SM/SP groups were not significantly different from controls (CM/CP; Table 1). The fasting plasma leptin in the 2-mo-old CM/SP tended to be high but was not statistically different between groups (Table 1). At 15 mo, the prenatal nutrient restriction group (CM/SP) had fasting plasma insulin and leptin levels similar to those of controls (CM/CP) but higher than those of the 15-mo-old SM/CP and SM/SP (P < 0.001). The SM/CP animals in the absence of IUGR with postnatal nutrient restriction alone and SM/SP representing the IUGR offspring with pre- and postnatal nutrient restriction had lower fasting insulin (P < 0.05 and P < 0.03, respectively; Table 1). The plasma leptin concentrations in the 15-mo-old SM/CP were lower than in CM/SP (P < 0.001), whereas the SM/SP group was lower than controls (CM/CP, P < 0.03) and CM/SP (P < 0.001). The plasma corticosterone concentrations in the 2-mo-old CM/SP were significantly lower than in the age-matched CM/CP, SM/CP, and SM/SP groups (P < 0.0001). There was no significant difference in baseline plasma corticosterone concentrations at 15 mo among all four groups (Table 1).

Hormonal adaptations during IVGTT: glucose/insulin. The glucose AUC during IVGTT in 2-mo-old offspring of all three groups with nutritional restriction (CM/SP, SM/CP, and SM/SP) was less than that of age-matched controls (CM/CP; Table 3). The insulin AUC during IVGTT in 2-mo-old IUGR offspring with ad libitum postnatal intake (CM/SP) was similar to that of controls, whereas the insulin AUC was decreased in the 2-mo-old SM/CP and SM/SP groups compared with the age-matched controls (CM/CP, P < 0.001; Table 3).

At 15 mo of age, the glucose AUC during IVGTT in the IUGR offspring with ad libitum postnatal intake (CM/SP) was significantly lower (P < 0.006), whereas the insulin AUC during GTT was significantly higher (P < 0.05) compared with controls (CM/CP; Table 3). The glucose and insulin AUC in the 15-mo-old SM/CP offspring were similar to those of controls.

The 15-mo-old offspring with pre- and postnatal nutrient restriction (SM/SP) had a lower glucose AUC (P < 0.004) without a change in the insulin AUC compared with the age-matched controls (Table 3).

Glucose metabolic adaptation: hepatic glucose production (M0). At 2 mo of age, the fraction of unlabeled glucose produced by the liver (M0) during GTT in all three groups with nutrient restriction was no different from controls. At 15 mo of age, the unlabeled glucose fraction was maximally suppressed in the IUGR offspring with ad libitum postnatal nutrient intake (CM/SP) compared with age-matched controls (CM/CP, P < 0.01; Table 3) and compared with the group with postnatal nutrient restriction without IUGR (SM/SP, P < 0.04), reflecting the IVGTT-induced insulin response. There was no change in the M0 glucose fraction of the SM/SP group with pre- and postnatal nutrient restriction.

Glucose metabolic adaptation: glucose clearance during IVGTT. The glucose clearance rate (in mg·kg⁻¹·min⁻¹) measured by fractional disappearance of the stable isotope M2 fragment at 2 mo of age was not significantly different among all four experimental groups. At 15 mo of age, all three groups with nutrient restriction (CM/SP, SM/CP, and SM/SP) had lower glucose clearance rates compared with the corresponding group at 2 mo (P < 0.001); however, they were not significantly different from age-matched controls (CM/CP; Table 2). The glucose clearance at 15 mo of age after nutrient restriction (CM/SP, SM/CP, and SM/SP), ranged from 4.1 to 5.7 vs. 7.2 to 8.6 mg·kg⁻¹·min⁻¹ at 2 mo of age (P < 0.001; Table 2).
G-6-Pase were decreased in livers from the three nutrient-restricted groups (CM/SP, SM/SP, and SM/CP) compared with age-matched controls (CM/CP; Table 4). At 15 mo of age, these intergroup differences were no longer apparent. The adaptive changes in hepatic enzymes regulate glucose production after perturbations in pre- and postnatal nutrition.

**DISCUSSION**

We have shown differential hepatic adaptive responses in glucose production, futile cycling, and disposal in the female IUGR offspring depending on the timing of nutrient restriction that caused intrauterine and/or postnatal growth restriction. These adaptive responses were associated with alterations in hepatic enzyme activities and circulating insulin concentrations geared towards maintaining normal glucose homeostasis up to 15 mo of age in the unstressed state. This is the first report examining the in vivo glucose kinetics at different ages (2 days, 2 mo, and 15 mo) in response to nutrient restriction during the mid to late gestation alone, the postnatal suckling phase alone, or a combination of both developmental periods. Although phenotypic presentations secondary to nutrient restriction during these different developmental periods have been examined previously by imposing severe and prolonged prenatal nutrient restriction followed by a high-caloric diet after weaning in male progeny (30) and in female progeny with a similar extent of prenatal nutrient restriction as in the present investigation (28), no reports determining in vivo glucose kinetics through postnatal development exist.
Most animal investigations linking IUGR to adult onset glucose intolerance and insulin resistance were performed mainly in male offspring, lending credence to the Barker hypothesis (22, 25, 26, 30). In contrast, female offspring subjected to pre- and postnatal protein restriction were reported to demonstrate insulin sensitivity at 3 mo of age (9), but at 21 mo of age, tissue analysis for insulin signaling revealed insulin resistance in skeletal muscle and adipose tissue (7). In contrast, by employment of nutrient restriction similar to that in the CM/SP and SM/SP groups in the present study, minimal hepatic insulin resistance was reported in 3-mo-old females (13). Further studies in the uterine artery ligation model of IUGR demonstrated the development of gestational diabetes in female offspring (4). In light of these contradictory age-dependent observations in female IUGR offspring, it became important to confirm the in vivo presence of insulin resistance. Hence, investigation of the status of glucose tolerance, glucose kinetics, and insulin sensitivity in female IUGR offspring at differing ages, namely the newborn and adult at peak and postpeak fertility stages, was necessary.

As reported previously (28), we observed glucose intolerance soon after birth, at 2 days of age, that was associated with a hypoinsulinemic response. This glucose intolerance was a culmination of increased hepatic glucose production with increased glucose turnover in the IUGR offspring (SP), with minimum GFC. These changes in glucose kinetics appear to reflect the diminished insulin response to a glucose load, causing a blunting of insulin action that manifested as an uncontrolled increase in hepatic glucose production and a concomitant increase in clearance to meet the demand of increased glucose production. In contrast, at 2 mo, despite the persistent presence of a hypoinsulinemic response in the SM/SP and SM/CP groups, the offspring in all four groups became glucose tolerant (28). This state of glucose tolerance was associated with no change in hepatic glucose production, futile cycling, or clearance despite an adaptive decrease in hepatic FBPase and G-6-Pase enzyme activities in all three groups, namely the CM/SP, SM/CP, and SM/SP vs. CM/CP. Glucose recycling depends on both the G-6-Pase and glucokinase enzymes. However, accurate assessment of glucokinase enzyme activity proved to be difficult given the inseparable contamination with hexokinase enzyme activity. When these groups were previously subjected to glucose tolerance tests at 6 mo of age, glucose intolerance was observed particularly in the CM/SP and SM/SP groups compared with the CM/CP and the SM/CP groups (28). Contrary to our expectation that the glucose intolerance would worsen with increasing age, we observed preservation of glucose tolerance at 15 mo of age in the present study. This difference is perhaps related to GTT’s being performed either via the tail vein (28) or in the unstressed state via previously placed jugular vein catheters. Thus blood collection via the tail vein could potentially result in stress-induced glucose intolerance, similar to the state of pregnancy causing glucose intolerance in glucose-tolerant female IUGR offspring (4). These observations collectively suggest that, although adaptive mechanisms are geared toward normalizing glucose homeostasis, any unexpected stressor can serve to push this finely balanced homeostatic state over the edge, culminating in overt aberrations of glucose control.

At 15 mo of age, the female IUGR offspring is glucose tolerant, with a decrease in glucose production and GFC and no change in glucose clearance, particularly in the CM/SP group. These changes were associated with hyperinsulinemia, which may be suggestive of declining peripheral insulin sensitivity. In contrast, the SM/SP group demonstrated no change in hepatic glucose production, futile cycling, or clearance despite the persistent presence of hypoinsulinemia. This resulted in the highest fraction of recycled glucose contributing to the hepatic glucose production. Compared with these two variants of the IUGR offspring, the SM/CP group, which was exposed only to postnatal nutrient restriction, demonstrated a phenotype that was between these two groups, namely no change in glucose production or clearance but a decrease in GFC in the presence of hypoinsulinemia. Thus it appears that the changes in glucose kinetics are phenotype specific, with the CM/SP group demonstrating adaptive changes consistent with heightened hepatic insulin sensitivity. However, this was achieved at the expense of an escalating insulin response perhaps secondary to impaired peripheral insulin action in other tissues.

During GTT, CM/SP hepatic glucose production was suppressed. However, previous work by another group of investigators in CM/SP and SM/SP groups reported a dampened suppression of endogenous glucose production by utilizing a hyperinsulinemic euglycemic clamp such that plasma insulin concentrations were perhaps sustained at levels relatively higher than the endogenous concentrations observed during GTT in the present investigation (13). These findings are dissimilar to those reported by Hales et al. (9) and Ozanne et al. (22) at 3 mo, which demonstrated an increased insulin sensitivity. Our previous investigations focusing on skeletal muscle GLUT4 translocation to the plasma membrane revealed insulin resistance as early as 2 days old through 2 mo of age (28), thereby setting the stage for subsequent development of impaired peripheral insulin action. Similar to the CM/SP group, the SM/CP female offspring demonstrated a phenotype consistent with preservation of hepatic insulin sensitivity reflective of concomitant hypoinsulinemia. In contrast, the SM/SP group, despite the presence of hypoinsulinemia, failed to demonstrate features of hepatic insulin sensitivity, supporting maladaptation to an adverse in utero/postnatal nutrient environment consistent with insulin resistance. Thus the CM/SP group demonstrates adaptive changes in GFC in response to the increased insulin concentrations at 15 mo of age. The development of insulin resistance may be organ specific, with skeletal muscle leading the way while liver continues to be insulin sensitive in the 15-mo-old female IUGR offspring of the SM/SP group. Whether the presence of enhanced hepatic insulin sensitivity with hyperinsulinemia serves as a forerunner for the subsequent development of total body insulin resistance in this group remains unknown. In contrast, the SM/SP group with pre- and postnatal nutrient restriction may be prone earlier to hepatic insulin insensitivity despite hypoinsulinemia, perhaps due to added islet β-cell derangement (19).

Our present observations in the context of previous investigations support the presence of adaptive mechanisms that develop postnatally in the IUGR female offspring in response to in utero dysregulation and are targeted at maintaining normal glucose homeostasis. As tissue-specific studies have revealed the presence of insulin resistance in skeletal muscle (7, 28) in the absence of a change in whole body glucose utilization (13) or clearance, it suggests that hepatic insulin sensitivity and glucose kinetics play a major role in counter-
balancing and normalizing whole body glucose homeostasis and insulin sensitivity (28). We speculate that this normalcy is achieved in the CM/SP and SM/CP groups by heightened hepatic insulin sensitivity and in the SM/SP group by relative hepatic insulin resistance. These adaptive changes allow the female IUGR offspring to maintain normal whole body insulin sensitivity during the peak stages of fertility.

The female CM/SP offspring were less obese or insulin resistant compared with the previously reported male counterparts (26, 30, 32). The cause for this sex-related difference remains elusive. Infusion of testosterone during fetal life causes insulin resistance in the offspring (24). These investigations set the stage for a role of fetal sex steroids in modifying subsequent insulin sensitivity, with the male sex steroids predisposing toward insulin resistance and female sex steroids perhaps offering relative protection against the earlier development of insulin resistance. The hyperinsulinemia may perhaps serve as a compensatory adaptation to the tissue-specific development of insulin resistance. The hyperinsulinemia may potentially cause glucose intolerance and insulin resistance in the offspring (10, 18). In our present study, using the calorie-restricting IUGR model, we failed to demonstrate any increase in circulating corticosterone concentrations in the newborn or adult offspring. Hence, changes in the hormonal milieu and glucose kinetics in this model are due to nutrient restriction and IUGR rather than being due to changes in the corticosteroid concentration and thus may be responsible for the ultimate phenotype.

In addition to assessing the impact of nutrient-restricted IUGR on glucose kinetics in an age-dependent manner, our present study allowed us to make age-related comparisons in the control group. This revealed an increase in GFC in the adult compared with the newborn, with the values remaining the same between 2 and 15 mo of age. In contrast, there was a progressive decline in glucose clearance with age. The age-dependent decrease in hepatic glucose production is associated with an increase in the fraction of GFC, suggesting that the adult liver is more insulin resistant than the newborn’s. The higher glucose turnover in the newborn perhaps promotes anabolism and growth, lending itself to decreased recycling, whereas the increased GFC in the adult lends itself to decreasing metabolic requirements by various tissues.

In summary, nutritional programming of glucose kinetics occurs with nutrient/calorie restriction during prenatal and early suckling periods, with postnatal adaptations predetermining the ultimate adult phenotype in a sex-specific manner. Although prenatal or postnatal nutrient restriction alone is associated with changes in GFC consistent with heightened hepatic insulin sensitivity, the culmination of pre- and postnatal nutrient restriction lends itself to hepatic insulin insensitivity. We speculate that the insulin response to a glucose load in all of these groups may reflect an aggregation of the state of the islet β-cells and peripheral insulin sensitivity in skeletal muscle and adipose tissue.

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REFERENCES


