Superimposition of Postnatal Calorie Restriction Protects the Aging Male Intrauterine Growth-Restricted Offspring from Metabolic Maladaptations

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Intrauterine growth restriction (IUGR) results in dysregulated glucose homeostasis and adiposity in the adult. We hypothesized that with aging, these perturbations will wane, and superimposition of postnatal growth restriction (PNGR) on IUGR [intrauterine and postnatal growth restriction (IPGR)] will reverse the residual IUGR phenotype. We therefore undertook hyperinsulinemic-euglycemic clamp, energy balance, and physical activity studies during fed, fasted, and refed states, in light and dark cycles, on postweaned chow diet-fed more than 17-month aging male IUGR, PNGR, and IPGR vs. control (CON) rat offspring. Hyperinsulinemic-euglycemic clamp revealed similar whole-body insulin sensitivity and physical activity in the nonobese IUGR vs. CON, despite reduced heat production and energy expenditure. Compared with CON and IUGR, IPGR mimicking PNGR was lean and growth restricted with increased physical activity, O₂ consumption (VO₂), energy intake, and expenditure. Although insulin sensitivity was no different in IPGR and PNGR, skeletal muscle insulin-induced glucose uptake was enhanced. This presentation proved protective against the chronologically earlier (5.5 months) development of obesity and dysregulated energy homeostasis after 19 wk on a postweaned high-fat diet. This protective role of PNGR on the metabolic IUGR phenotype needs future fine tuning aimed at minimizing unintended consequences. (Endocrinology 153: 4216–4226, 2012)

Developmental origins of health and adult disease hypothesis (DoHAD) states that adverse prenatal and early postnatal nutritional environments increase the risk for adult onset obesity, diabetes, and associated metabolic syndrome (1).

Consistent with DoHAD, animal models of prenatal nutritional restriction, especially global calorie restriction (2–6), low protein maternal diet (7, 8), and uterine artery ligation (9–13), have detected glucose and lipid metabolic abnormalities in low birth weight offspring that experience subsequent accelerated catch-up growth.

A recent “predictive adaptive hypothesis” along with the “match-mismatch theory” extended DoHAD by stating that the structure and function of fetal organs are programmed in response to prenatal malnutrition to confer an advantage to handle the future postnatal environment (14, 15). When the postnatal environment fails to match the experienced prenatal environment, maladaptation occurs, resulting in adult onset chronic diseases. In contrast, if the postnatal environment matches the fetal prediction, the offspring is protected from metabolic syndrome (14, 15).

The “lean” phenotype with glucose tolerance has been reported in mice on chow (16, 17) or hypercaloric diets (18), when postnatal calorie or protein restriction (19) reversed the development of insulin resistance. Male mice on chow or cafeteria diets that were exposed to postnatal
protein restriction demonstrated prolonged longevity compared with mice with fetal growth restriction and postnatal catch-up growth (20, 21). However, in these studies, the mechanisms responsible for the lean phenotype were not investigated (20, 21). Furthermore, the studies were pursued in younger adult offspring ranging in age from 5 to 12 months (16–18). Previous investigations in rats employing early protein restriction demonstrated that insulin resistance is encountered during aging at 21 months of life (7), whereas others have contended that aging in the intrauterine growth restriction (IUGR) offspring continues to demonstrate an insulin-sensitive state (22).

Hence, we undertook the present study to test the hypotheses that 1) aging partially ameliorates the overt metabolic perturbations and obesity of the IUGR adult offspring, and 2) a match between pre- and postnatal nutrition will further protect the aging IUGR male offspring from developing metabolic abnormalities and obesity.

Materials and Methods

Sprague Dawley rats (Charles River Laboratories, Hollister, CA) were housed in individual cages, exposed to 12-h light, 12-h dark cycles at 21–23 C, and allowed ad libitum access to standard rat chow (Laboratory Rodent Diet 5001: protein 23%, fat 4.5%, fiber 6%, ash 8.0%, and minerals 2.5%; LabDiet, Rancho Cucamonga, CA). The National Institutes of Health guidelines for care and maintenance of animals were followed as approved by the Animal Research Committee of the University of California, Los Angeles.

Maternal calorie restriction model

Pregnant Sprague Dawley rats received 50% of their daily food intake beginning from d 11 through d 21 of gestation, causing caloric restriction during mid to late gestation, compared with their control (CON) counterparts, who received ad libitum rat chow. Both groups had ad libitum access to drinking water. At birth, the litter size was culled to six. Four groups were created by cross-fostering all pups on d 2 of life, with control mothers rearing control pups or prenatally calorie-restricted pups (IUGR) and pre- and postnatally calorie-restricted mothers rearing prenatally calorie-restricted pups [intrauterine and postnatal growth restriction (IPGR) or control postnatal growth restriction (PNGR)] pups (Fig. 1) (23, 24). At d 21, the male pups from all four groups were weaned from the respective mothers and maintained on the same rat chow ad libitum. At 17 months of age, their phenotype was examined.

Energy intake, body weight, and white adipose tissue (WAT)

Food intake and body weight of male rats at 17 months of age were measured weekly over a 24-h period, and four readings were averaged. Energy intake was assessed based on the conversion factor of 4.07 kcal/g 5001 rat chow food intake (values calculated are based on ingredient analysis by manufacturer; Harlan Laboratories, Madison, WI). Visceral WAT was mechanically isolated and weighed. Energy intake and WAT were expressed as a percent of body weight.

Plasma assays

Plasma glucose concentration was measured by a HemoCue glucose 201 analyzer. Plasma insulin was measured using a Rat Insulin ELISA kit (Linco Research, Billerica, MA).

Hyperinsulinemic euglycemic clamp

One week before the study, under isoflurane (2–3.5% vapor concentration in O2), two internal jugular vein catheters extending to the right atrium, and a right carotid artery catheter extending to the level of the aortic arch, were implanted. Analgesics (ketoprofen) and antibiotics (ampicillin) were given daily for the first 48 h after surgery. All rats were fasted for 16 h before the study. The steady state in rats was achieved by a bolus injection of (3-3H) glucose (5–9262 Ci bolus) followed by the infusion of (3-3H) glucose (0.05–9262 Ci/min) for 90 min. This time point is referred to as 0. At time 0, hyperinsulinemic-euglycemic clamp study was started, and rats received an infusion of insulin (Novolin R, regular human insulin; Novo Nordisk, Bagsværd, Denmark) at 4 μU/kg/min and (3-3H) glucose at 0.2 μCi/min simultaneously. A variable infusion of 50% glucose solution was started.
and adjusted to clamp the plasma glucose concentration between 100 and 120 mg/dl. To assess the insulin-stimulated glucose uptake [2-deoxyglucose (2-DG)] in individual tissues, 10 μCi of 2-deoxy-D-[1-14C] glucose (PerkinElmer, San Jose, CA) were infused as a bolus 45 min before the end of the clamp study. At the end of the clamp study, rats were euthanized with pentobarbital sodium (50 mg/ml; 100 mg/kg) injection.

**Analytical procedure**

Plasma (3-1H) glucose-specific radioactivity was measured using Somogyi method as described previously (12). The glucose disposal rate (Rg) at steady state was determined by dividing the infusion rate of labeled glucose by the specific activity at the same time point. Endogenous glucose production was determined by subtracting the unlabeled glucose infusion rate (GIR) from Rg.

**Accumulation of phosphorylated derivatives of radioactive 2-DG by tissue**

Total and free 2-DG radioactivity in tissue samples that included liver, WAT, soleus, extensor digitorum longus, and gastrocnemius muscles were assessed, and 2-DG-phosphate was calculated as described previously (25).

**Indirect calorimetry**

Energy expenditure in rats was measured using the Oxymax Lab Animal Monitoring System (Columbus Instruments, Columbus, OH). Seventeen-month-old male rats were individually housed and allowed to acclimate for at least 24 h in experimental cages with free access to food and water (26–28). O2 consumption (VO2), heat production, respiratory exchange ratio (RER), and physical activity were recorded during light and dark cycles over a 24-h period during the fed state. Energy expenditure in rats under the fasting condition constituted monitoring during the last 2 h of a 16-h overnight fast. The refed condition was monitored over a 24-h period after ad libitum access to food after the overnight fast. Energy expenditure in rats under resting conditions (resting energy expenditure) was based on a 24-h period measurement by indirect calorimetry and was computed in the overnight fast. Energy expenditure in rats under resting conditions (fed or refed) being two factors as within metabolic settings (refed and fed) were compared using three-way ANOVA analysis (split-plot factorial), with light (dark) cycle and metabolic settings (fed or refed) being two factors as within subjects factor and group as between the subjects factor. Tukey-Kramer pairwise comparison was employed for post hoc analysis. For energy and water intake, three factors of group, light (dark) cycle, and metabolic settings (fed or refed) were compared using three-way ANOVA analysis (split-plot factorial), with light (dark) cycle and metabolic settings (fed or refed) being two factors as within subjects factor and group as between the subjects factor. Tukey-Kramer pairwise comparison was employed for post hoc analysis. For the HFD study, Student’s t-test was employed to compare body weight and WAT between CON-HFD and IPGR-HFD groups.

**Results**

**Postweaning chow diet studies**

**Body weights, WAT weights, and plasma glucose and insulin concentrations**

Birth weight of male pups born to calorie-restricted mothers (IUGR, 6.5 ± 0.1 g) was 82% of pups born to CON mothers (CON, 7.9 ± 0.1 g; P < 0.05). By d 50 of age, IUGR “caught up” to CON (92%; P > 0.05), whereas IPGR and PNGR remained lighter than CON, at approximately 70% of age matched CON (P < 0.05). By 17 months, PNGR caught up to CON, but IPGR remained growth restricted at 77% of CON (P < 0.05). The body weights of IUGR (P > 0.05) and PNGR (P > 0.05) were similar to that of CON. Reflecting the body weight, WAT depicted as a percent of body weight in IUGR was no different from CON. In contrast, IPGR exhibited a significant reduction in percent of WAT/body weight vs. both CON and IUGR (see figure 3 below). Plasma glucose and insulin were not different between the four groups (Table 1).
Glucose kinetics

IUGR was insulin sensitive and both PNGR and IPGR had no effect on glucose $R_d$, GIR and hepatic glucose production (HGP) at 17 months of age. Despite IPGR’s lighter body weight, no significant differences existed in $R_d$ among the four groups in the basal state. Insulin-stimulated $R_d$, HGP, and GIR were also similar among the four groups. However, significant differences were detected in $R_d$ between basal and hyperinsulinemic-euglycemic clamped states within the same group, that is, insulin-stimulated $R_d$ was significantly higher compared with basal $R_d$ in all four groups ($P < 0.05$ each) (Fig. 2A). Basal and insulin-stimulated HGP was similar among the four groups. In response to an exogenous insulin infusion, CON, IUGR, IPGR, and PNGR demonstrated 30% reduction in HGP (Fig. 2B). Steady state GIR required to maintain euglycemia during hyperinsulinemic-euglycemic clamp were similar in four groups (Fig. 2C). These results suggest that whole-body insulin sensitivity of IUGR, IPGR, and PNGR was similar to CON.

Tissue glucose uptake

IUGR remained insulin sensitive, whereas IPGR revealed increased skeletal muscle 2-DG uptake. As shown in Fig. 2D, insulin-stimulated glucose uptake by skeletal muscle was significantly increased in IPGR compared with CON, IUGR, and PNGR. Specifically the 2-DG uptake in the soleus (type 1 fibers; slow twitch muscle) was approximately 2-fold over CON ($P < 0.05$) and PNGR ($P < 0.05$). Similarly, in IPGR, insulin-stimulated 2-DG uptake was increased in extensor digitorum longus (mainly fast twitch fibers) and gastrocnemius (mixture of slow and fast twitch muscle fibers) by approximately 2-fold over CON ($P < 0.05$ each and IUGR ($P < 0.05$ each). Although in IPGR insulin-stimulated 2-DG uptake by extensor digitorum longus was 2-fold over PNGR ($P < 0.05$), in PNGR, insulin-stimulated gastrocnemius 2-DG uptake was 1.3-fold over CON ($P < 0.05$). No difference between IUGR and CON was observed in the case of soleus, extensor digitorum longus, and gastrocnemius. There were no intergroup differences noted in either liver or WAT (Fig. 2D).

Skeletal muscle protein expression studies

Skeletal muscle (soleus, extensor digitorum longus, and gastrocnemius combined) Sirt1, total AMPK, pAMPK, and total PKCζ protein concentrations were no different in the four experimental groups (Table 2), although pAMPK in IUGR trended toward an increase vs. that of CON.

Energy balance

IUGR demonstrated reduced whole-body energy expenditure, whereas PNGR and IPGR enhanced whole-body energy expenditure in aging rats.

Energy intake

Energy intake during light and dark cycles was similar among the four groups during fed and refed states (data not shown). When normalized to body weight, regardless of the fed or refed state, IPGR consumed more energy vs. CON and IUGR ($P < 0.05$ each, three-way ANOVA), whereas PNGR consumed more energy than IUGR ($P < 0.05$, three-way ANOVA) (Fig. 3A). Significantly more energy was consumed during dark cycle compared with light cycle in each group in both fed and refed states. Water intake was similar among four groups with or without normalization to body weight (data not shown).

Energy expenditure ($VO_2$)

IUGR demonstrates decreased basal metabolic rate (BMR) in the absence of obesity, whereas PNGR and IPGR remain unchanged. During the resting state, in the IUGR, $VO_2$ (~50–60% of CON, IPGR, and PNGR; $P < 0.05$) (Fig. 3C) and heat production decreased (~60% of CON, $P < 0.05$; IPGR and PNGR, $P > 0.05$ each) (Fig. 3D), whereas intergroup RER was unchanged ($P > 0.05$,

<table>
<thead>
<tr>
<th>TABLE 1. Body weights and serum metabolites</th>
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<td><strong>Con</strong></td>
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<td>CON</td>
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<tr>
<td>IUGR</td>
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<td>IPGR</td>
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<td>PNGR</td>
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Body weight (BW) of four experimental groups at 50 d and 17 months. At 50 d, IUGR was lighter than CON (*), IPGR was lighter than CON and IUGR (†), and PNGR was even lighter than CON, IUGR, and IPGR (‡), n = 6 in each group. By 17 months, IPGR was significantly smaller than CON ($P < 0.05$), whereas the BW of IUGR ($P > 0.05$) and PNGR ($P > 0.05$) were similar to that of CON. Serum metabolites of four experimental groups at 17 months are shown. Serum glucose and insulin were not significantly different between the four groups ($P > 0.05$): CON (n = 8), IUGR (n = 7), IPGR (n = 6), and PNGR (n = 8).

* $P < 0.05$ vs. CON.

$P < 0.05$ vs. CON and IUGR.

$P < 0.05$ vs. CON, IUGR, and IPGR.
uptake increased in IPGR (IPGR 2-DG uptake increased in IPGR groups. SOL: 2-DG uptake increased in IPGR (IPGR longus; GAS, gastrocnemius), liver (LIV), and WAT is shown during a HI-EG clamp for all ANOVA analysis). D, 2-DG uptake in skeletal muscle (SOL, Soleus; EDL, extensor digitorum longus; GAS, gastrocnemius), liver (LIV), and WAT is shown during a HI-EG clamp did not show significant changes in CON, IUGR, and PNGR (Fig. 3C), RER (data not shown), and heat production (Fig. 3D) in IPGR and PNGR were similar to CON.

IUGR exhibited decreased energy expenditure (VO2) in fed, fasting, and refed states. VO2 was reduced during the fed state in light cycle in IUGR vs. the other three groups (~60–70% of CON, IPGR, and PNGR; P < 0.05) (Fig. 4Ai). When compared with IPGR and PNGR, a decrease in VO2 also occurred in IUGR in light cycle during fasting (IUGR vs. CON, P > 0.05; but 67% of IPGR and PNGR, P < 0.05 each) and refed states (IUGR is 66% of IPGR, 73% of PNGR; P < 0.05 each) (Fig. 4A, ii and iii). During dark cycle, VO2 of IUGR decreased in fed (IUGR is 68% of IPGR and PNGR; P < 0.05 each) and refed states (IUGR is 63% of PNGR; P < 0.05 each) vs. IPGR and PNGR (Fig. 4A, i and iii).

IPGR expressed increased energy expenditure (VO2) in fed, fasting, and refed states. VO2 in fed state during dark cycle of IPGR increased vs. that of CON and IUGR (P < 0.05 each). During fasting state, VO2 of IPGR increased vs. that of IUGR (P < 0.05). In refed state during light cycle, IPGR demonstrated an increase in VO2 vs. that of CON (P < 0.05). During dark cycle as well, VO2 of IPGR increased over that of CON and IUGR (P < 0.05 each) (Fig. 4A, i–iii).

PNGR demonstrated an increase in energy expenditure (VO2) in fed, fasting, and refed states. VO2 of PNGR in fed state during dark cycle increased over that of CON (P < 0.05) and IUGR (P < 0.05). During refed state as well in dark cycle, VO2 of PNGR increased over that of IUGR (P < 0.05) (Fig. 4A, i–iii).

**Respiratory exchange ratio**

No intergroup differences were detected in RER during fed, fasting, or refed states. However, in all four experimental groups, fasting decreased RER from 0.9 of fed and refed states to 0.7. This change supported a switch in the use of fuel from carbohydrates in fed and refed states to fat in the fasting state (Supplemental Fig. 1A, published on The Endocrine Society’s Journals Online web site at http://endo.endojournals.org).

**Heat production**

IUGR reduced heat production. Heat production by IUGR during fed state in light cycle was reduced to that of CON (P < 0.05). During the refed state in dark cycle, heat

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**TABLE 2.** Skeletal muscle protein expression profile

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>IUGR</th>
<th>IPGR</th>
<th>PNGR</th>
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<tr>
<td>Sirt1</td>
<td>100.0±7.8</td>
<td>95.0±8.7</td>
<td>88.8±7.3</td>
<td>90.2±9.6</td>
</tr>
<tr>
<td>PKCζ</td>
<td>100.0±1.8</td>
<td>92.1±2.3</td>
<td>98.2±2.6</td>
<td>104.3±1.8</td>
</tr>
<tr>
<td>AMPK</td>
<td>100.0±5.2</td>
<td>98.3±9.4</td>
<td>110.8±9.7</td>
<td>118.9±11.2</td>
</tr>
<tr>
<td>PAMPK</td>
<td>100.0±19.5</td>
<td>122.7±12.5</td>
<td>110.8±19.0</td>
<td>111.7±25.5</td>
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</table>

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**FIG. 2.** Hyperinsulinemic-euglycemic (HI-EG) clamp. HI-EG clamp results are shown in the four experimental groups: CON (n = 8), IUGR (n = 7), IPGR (n = 6), and PNGR (n = 8). A, During HI-EG clamp, glucose use (Rg) (shown in black bars) increased significantly compared with basal Rg (shown in light bars) in CON, IUGR, IPGR, and PNGR [two-way repeated measures ANOVA, F(3,1) = 1.54, a, P < 0.001; Tukey’s post hoc analysis: a, P < 0.05]. Basal Rg and Rg during HI-EG clamp were not significantly different between the four experimental groups (P > 0.05). Statistical methods: two-way repeated measures ANOVA analysis, compares the groups as between subject factor and treatment (basal vs. clamp) as within subject factor. B, HGP during HI-EG clamp (shown in light bars) increased significantly compared with basal HGP (shown in light bars) in CON, IUGR, IPGR, and PNGR [two-way repeated measures ANOVA, F(3,1) = 31.993, P < 0.001; Tukey’s post hoc analysis: a, P < 0.05]. Basal HGP and HGP during HI-EG clamp were not significantly different between the four experimental groups (P > 0.05). C, GIR required to maintain euglycemia during a HI-EG clamp (shown in light bars) increased over that of CON (P < 0.05 each) and refed states (IUGR is 66% of IPGR, 73% of PNGR; P < 0.05 each) (Fig. 4A, ii and iii). During dark cycle, VO2 of IUGR decreased in fed (IUGR is 68% of IPGR and PNGR; P < 0.05 each) and refed states (IUGR is 63% of PNGR; P < 0.05 each) vs. IPGR and PNGR (Fig. 4A, i and iii).
production by IUGR was also diminished vs. that of CON and IPGR ($P < 0.05$ each) (Fig. 4B, i–iii).

**Physical activity**

IUGR was not inactive, whereas IPGR and PNGR expressed enhanced physical activity.

**Total activity**

Total activity of IPGR during fed state in dark cycle increased over that of CON ($P < 0.05$). During fasting state, total activity of IPGR increased 2.4-fold of CON and only 1.6-fold of IUGR and PNGR ($P < 0.05$ each). During refed state in dark cycle as well, IPGR demonstrated increased total activity vs. CON and IUGR ($P < 0.05$ each) (Fig. 4C, i–iii).

**Ambulation (horizontal)**

During fed and refed states in dark cycle, IPGR demonstrated increased ambulation compared with CON ($P < 0.05$) (Supplemental Fig. 1B, i–iii). Similar to IPGR, during fed state in dark cycle, PNGR exhibited more ambulation compared with CON and IUGR ($P < 0.05$ each), respectively. During fasting, IPGR demonstrated a 2.6-fold increase in ambulation vs. CON, IUGR, and PNGR ($P < 0.05$ each) (Supplemental Fig. 1B).

**Rearing (vertical)**

The rearing of IPGR during fed state in dark cycle increased over that of CON ($P < 0.05$). Similar increases in rearing of IPGR during refed state in light cycle were also observed, reflected as 2.5-fold of IUGR ($P < 0.05$). During the refed state in dark cycle, rearing of IPGR increased over that of CON, IUGR, and PNGR ($P < 0.05$ each) (Supplemental Fig. 1C).

**Postweaning HFD studies**

**Postweaning HFD intake in IPGR and CON**

IPGR raised on HFD was relatively protected from obesity seen as lower body weight and lower WAT expressed as a percent of body weight vs. the CON counterpart raised on a HFD ($P < 0.05$) (Fig. 5, A and B). IPGR and CON raised on HFD were no different in energy (Fig. 5C) and water intake (data not shown), although VO$_2$ in light and dark cycles ($P < 0.05$) (Fig. 5D), heat production (dark cycle, $P < 0.05$) (Fig. 5E), and physical activity (total, $P < 0.05$) in dark cycle were increased in IPGR vs. CON (Fig. 5F). Although no change in RER was evident, an increase in ambulation and rearing was also observed in dark cycle in IPGR vs. CON (Supplemental Fig. 2).

Summary of our results is schematically depicted in Table 3.

**Discussion**

**General**

The IUGR male rat offspring has previously been examined at various stages of the lifespan and described to be obese with perturbed glucose and insulin concentrations, glucose intolerance, and insulin resistance with associated hyperphagia and physical inactivity (4–8, 12, 13,
Regardless of the cause of IUGR, the phenotype of the adult offspring is similar. This is because nutrient restriction in utero was followed by postnatal access to ad libitum milk intake resulting in catch-up growth. The superimposition of catch-up growth resulted in a mismatch between the intrauterine and postnatal nutritional environments, ultimately leading to a chronic disease state (34). The implications of when this phenotype emerges and how it affects the next generation sired by these male offspring are topics of ongoing investigation (35).

In our investigation, the most notable observation was the lack of hyperglycemia, hyperinsulinemia, and insulin resistance in the aging male IUGR offspring. Investigations in younger 10-month-old male IUGR offspring demonstrated a development of sc and visceral adiposity but retention of glucose tolerance and insulin sensitivity (36). The question was whether normal insulin sensitivity encountered in the robust adult male IUGR offspring despite adiposity was: 1) a matter of age, so that aging will display accumulating whole-body insulin resistance and glucose intolerance; or 2) a result of in utero programming from calorie restriction that would be permanently seen throughout life into aging. Hence, it was important to examine the male IUGR adult offspring later in life during aging to resolve this question.
Insulin sensitivity

Intrauterine growth restriction

Previous investigations reporting insulin resistance in the adult male IUGR rat offspring were only based on insulin, glucose, and glucose to insulin ratios (6, 8, 33). Some studies included calculations of the insulin sensitivity index based on glucose and insulin concentrations and surmised the presence of insulin resistance (37, 38).

To overcome the limitations of these previous studies, we conducted hyperinsulinemic-euglycemic clamp experiments. In the presence of exogenous insulin, hyperinsulinemia with normal circulating glucose concentrations, no difference in HGP and glucose $R_d$ was observed in the IUGR vs. CON. Thus, our present observation of retained insulin sensitivity in the aging male IUGR offspring supports a permanently programmed effect due to in utero caloric restriction. This normalcy is achieved by key adaptations, examples being the changes observed in tissue-specific insulin signaling (23, 24) and glucose transporter 4 biology (23).

Previously, other investigators have shown hepatic insulin resistance at 8 wk (12) in the uterine artery-ligated male IUGR offspring. However, the absence of insulin resistance in anesthetized 8-month-old prenatally calorie-restricted male IUGR offspring (22), on the backdrop of insulin resistance seen in 100-d-old anesthetized female

TABLE 3. Schematic representation of the summary of the results

<table>
<thead>
<tr>
<th>BW (17 months)</th>
<th>EI/BW</th>
<th>Insulin sensitivity</th>
<th>Skeletal muscle 2-DG uptake</th>
<th>BMR</th>
<th>EE</th>
<th>PA</th>
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<tbody>
<tr>
<td>IUGR</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>↓</td>
<td>NC</td>
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<td>IPGR</td>
<td>↓</td>
<td>↑</td>
<td>NC</td>
<td>↑</td>
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<tr>
<td>PNGR</td>
<td>NC</td>
<td>↑</td>
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Increase (↑) or decrease (↓) compared with CON. NC, No change; BW, body weight; EI, energy intake; EE, energy expenditure; PA, physical activity.
IUGR offspring, created some controversy (39). The common approach was hyperinsulinemic-euglycemic clamp but in anesthetized rats. Our present study along with our previous investigation performed at 10 months of age (36) confirms the unequivocal absence of insulin resistance in awake IUGR male offspring in a milieu of adequate or high circulating insulin concentrations.

**PNGR and IPGR**

Examination of the PNGR and IPGR groups also revealed no changes in basal and insulin-stimulated HGP and $R_g$. Thus, on the surface, it appeared that superimposition of postnatal caloric restriction on the IUGR offspring failed to have a positive impact particularly during aging. However, when tissue-specific insulin-stimulated glucose uptake was examined, the PNGR demonstrated an increase in the gastrocnemius, whereas the IPGR demonstrated an increase in soleus, extensor digitorum longus, and gastrocnemius muscles vs. that seen in CON or IUGR. This supports a positive impact of IPGR on the IUGR offspring seen as enhanced insulin sensitivity in skeletal muscle. In contrast, no intergroup changes in insulin-stimulated glucose uptake were observed in WAT or liver, the other two insulin-responsive tissues.

**Energy metabolism and physical activity**

**Intrauterine growth restriction**

The lack of effect on insulin-induced WAT glucose uptake complements the lack of overt obesity in the aging male IUGR offspring. This may be related to WAT experiencing hypoxic injury and apoptosis during aging (40). However, the energy metabolism particularly in the IUGR reflects a diminution in the basal VO$_2$ and heat production unlike that seen in the PNGR and IPGR groups. Although fasting with refeeding revealed a similar pattern between the four groups overall, a diurnal variation was evident. VO$_2$ and heat production were much higher during the dark vs. the light cycle in each group, with the IUGR exhibiting a diminution greater than that of CON. More importantly, the IUGR lacked flexibility in adjusting VO$_2$ between the fed, fasted, and refed states. In addition, the increase in VO$_2$ during the dark vs. light cycles was minimal if any in this group.

These perturbations in the energy metabolism are seen in the absence of obesity in the IUGR. However, in the aging IUGR offspring, in the face of no change in energy intake, a reduction in VO$_2$ and heat production particularly during the light cycle in fed, fasting, and refed states do not reflect physical activity, because no change consistent with inactivity was evident in any cycle. Thus, these changes in the IUGR are reflective of a reduction in the BMR. Significant change in insulin-induced skeletal muscle glucose uptake was associated with no change in skeletal muscle Sirt1, total PKC$_z$, total AMPK, and pAMPK protein expression. The trend toward an increase of pAMPK in the IUGR vs. CON was suggestive of an energy deficit. The reduction in VO$_2$, heat production, and BMR are the classical hallmarks of obesity. Hence, it appears that with aging, although overt obesity is not present, features consistent with previous existence of obesity during an earlier age exist.

Other investigations have demonstrated a reduction in physical activity at an earlier adult age in the male IUGR offspring (32), unlike our present observations during aging. It appears that aging results in waning of some of the maladaptive features, such as overt obesity and inactive state, encountered during an earlier adult stage (9–10 months).

**PNGR and IPGR**

The PNGR and IPGR demonstrated increased energy intake during the two light and dark cycles. The PNGR and IPGR also displayed enhanced VO$_2$ and physical activity particularly in the dark when compared with CON and IUGR. This change was more acute in the IPGR during fasting and fed rather than the refed states. Further, the increased activity was due to enhanced ambulation and rearing activities. Thus, an argument can be made that in the PNGR and IPGR, particularly during the fed state and in the dark, energy consumption and insulin-stimulated glucose uptake reflect physical activity, because resting energy expenditure was unchanged.

These important observations, particularly in the IPGR, proved to be relatively protective compared with CON when both were placed on a HFD postweaning. Although the CON-HFD were heavier than the IPGR-HFD with more white fat, despite no difference in food (energy) and water intake, enhanced VO$_2$ and heat production in the IPGR-HFD paralleled the heightened ambulation and rearing activity in this group. Thus, during aging, the impact of postnatal dietary changes modifies the in utero programming effect, influencing the aging process. Early caloric restriction imposed postnatally to match the intrauterine caloric restriction positively affects physical activity and energy balance. All these parameters play a critical role in preventing the adult-type obesity unrelated to in utero programming but encountered when exposed to a postweaning hypercaloric diet. Although augmented insulin-induced skeletal muscle glucose uptake was evident in these two experimental groups, no effect was noted in skeletal muscle Sirt1, total PKC$_z$, total AMPK, or pAMPK protein concentrations.
Although IPGR had an ameliorating effect on the aging IUGR offspring particularly with respect to glucose and energy metabolism and physical activity further protecting the offspring when exposed to a HFD, it is imperative that future studies ensure the safety of such an early life intervention targeted at preventing the chronic phenotype of the robust and aging adult; for example, postnatal caloric restriction, although retarding growth may result in osteoporosis with aging (41). A significant and beneficial impact of such an early life postnatal intervention on the subsequent metabolic phenotype with aging may also result in unintended consequences on cognition and neuropsychological development.

Summary

In summary, the aging male IUGR offspring was non-obese, not insulin resistant, not hyperphagic but physically active, displaying reduced VO$_2$, and heat production that were inflexible in response to fasting vs. fed states while still demonstrating minimal diurnal variation. These findings suggest a waning of the overt obesity, inactivity, and dysregulated metabolic phenotype encountered earlier at 10 months of age (36). Specifically, we noted a diminution of energy expenditure reflected by BMR rather than changes in physical activity. In contrast, IPGR similar to PNGR caused a lean, hyperphagic phenotype with enhanced physical activity, which was reflected in increased VO$_2$ flexible in response to fasting vs. fed states with retained diurnal variation and an enhanced skeletal muscle insulin-stimulated glucose uptake. The enhanced energy expenditure with augmented skeletal muscle insulin sensitivity particularly reflected the state of physical activity in the IPGR unlike that encountered in the IUGR. In addition, IPGR was protective against postweaned consumption of a HFD and its metabolic consequences of obesity (overt and visceral adiposity), energy imbalance, and physical inactivity. Thus, although introduction of postnatal caloric restriction had long-term positive implications reaching into the aging adult, caution must be exercised in balancing the long-term benefits against metabolic dysregulation with the unintended consequences of negatively impacting cognition and neuropsychological state that develops during the early postnatal period. Future investigations should focus on fine tuning postnatal interventions to achieve optimal metabolic and cognitive functions.

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