Early exposure of the pregestational intrauterine and postnatal growth-restricted female offspring to a peroxisome proliferator-activated receptor-γ agonist

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Submitted 3 June 2009; accepted in final form 8 December 2009

Garg M, Thamotharan M, Pan G, Lee PW, Devaskar SU. Early exposure of the pregestational intrauterine and postnatal growth-restricted female offspring to a peroxisome proliferator-activated receptor-γ agonist. Am J Physiol Endocrinol Metab 298: E489–E498, 2010. First published December 15, 2009; doi:10.1152/ajpendo.00361.2009.—Prenatal nutrient restriction with intrauterine growth restriction (IUGR) alters basal and glucose-stimulated insulin response and hepatic metabolic adaptation. The effect of early intervention with insulin-sensitizing peroxisome proliferator-activated receptor γ agonists was examined in the metabolically maladapted F1 pregestational IUGR offspring with a propensity toward pregnancy-induced gestational diabetes. The effect of rosiglitazone maleate [RG; 11 μmol/day from postnatal day (PN) 21 to PN60] vs. placebo (PL) on metabolic adaptations in 2-mo-old F1 female rats subjected to pregestational (IUGR), postnatal (PNGR), or pre- and postnatal (IUGR + PNGR) nutrient restriction was investigated compared with control (CON). RG vs. PL had no effect on body weight or plasma glucose concentrations but increased subcutaneous white and brown adipose tissue and plasma cholesterol concentrations in all three experimental groups. Glucose tolerance tests with a 1:1 mixture of [2-13C]- and [6,6-2H2]glucose in RG IUGR vs. PL IUGR revealed glucose tolerance with a lower glucose-stimulated insulin release (GSIR) and suppressed endogenous hepatic glucose production (HGP) with no difference in glucose clearance (GC) and recycling (GR). RG PNGR, although similar to PL CON, was hyperglycemic vs. PL PNGR with reduced GR but no difference in the existing low GSIR, HGP, and GC. RG IUGR + PNGR overall was no different from the PL counterpart. Insulin tolerance tests revealed perturbed recovery to baseline from the exaggerated hypoglycemia in RG vs. the PL groups with the only exception being RG PNGR where further worsening of hypoglycemia over PL PNGR was minimal with full recovery to baseline. These observations support that early intervention with RG suppressed HGP in IUGR vs. PL IUGR, without increasing GSIR similar to that seen in CON. Although RG reversed PNGR to the PL CON metabolic state, no such insulin-sensitizing effect was realized in IUGR + PNGR.

A glucose tolerance test; hepatic glucose production; stable isotopes

LOW BIRTH WEIGHT ALONE and in association with slow growth during early childhood has been epidemiologically associated with the acquisition of type 2 diabetes mellitus among various other adult-onset chronic diseases (2–4, 12, 24). Animal investigations employing differing nutrient restriction models of intrauterine growth restriction (IUGR) and postnatal growth restriction suggest that diabetes mellitus stems from an imbalance between insulin production because of decreased pancreatic β-cell mass (5, 22, 40) and reduced insulin sensitivity in select organs (15, 21, 43). Regardless of the exact pathophysiology, pregnancy in the adult IUGR rat offspring results in gestational diabetes (6). This gestational diabetic state in the adult IUGR rat offspring permits transgenerational propagation of dysregulated insulin sensitivity (30, 52). To understand this phenomenon, we and others have previously characterized the metabolic adaptations encountered in the female adult IUGR rat offspring and compared them with that seen in the postnatal growth-restricted rat offspring (PNGR) (15, 21, 33, 38, 43). Although the IUGR rat offspring demonstrated increased glucose-induced insulin release with emerging hepatic insulin resistance, the PNGR demonstrated glucose intolerance and diminished glucose-induced insulin release with relative hepatic insulin sensitivity (21).

In the IUGR rat offspring, various early interventions have been introduced toward reversing some of the metabolic adaptations that predetermine subsequent development of diabetes mellitus (1, 31, 39). This subsequent development of diabetes mellitus is akin to that seen in human studies (7, 16, 26), the idea being that these interventions will carry over to humans and prevent the subsequent onset of diabetes mellitus (type 2 diabetes mellitus), before the expression of disabling symptoms and complications that require long-term limitations on life style and pharmacotherapy. Additionally, women with pregestational and gestational diabetes have an increased risk of malformations and adverse outcome in the offspring (18, 35). To this end, glucagon-like peptide-1 agonists have been administered in rats to improve β-cell proliferation toward overcoming the reduced mass instrumental in causing gestational diabetes mellitus (1, 31, 39). More recently, we have demonstrated early introduction of chronic exercise to have a beneficial effect on hepatic insulin sensitivity of the pregestational female adult IUGR rat offspring (20). However, precription of such life style changes in humans encounters considerable noncompliance, defeating the achievement of a disease prevention goal. Hence the search continues in a preclinical setting for an ideal pharmacological agent that will have a beneficial effect on hepatic insulin resistance of the IUGR female offspring.

Thiazolidinediones (TZDs) are peroxisome proliferator-activated receptor-γ (PPARγ) agonists with insulin-sensitizing ability (37) that are FDA-approved and being routinely used in the clinical setting, for treatment of type 2 diabetes mellitus (25, 46, 51). Animal experiments show that PPARγ is predom-
inantly expressed in white adipose tissue (WAT), necessary for the development of WAT, and mediates inhibition of lipolysis responsible for redistribution of WAT (17, 27). In animals, TZDs improved insulin sensitivity in high-fat-diet-fed rats (44), obese Zucker rats, and Zucker diabetic fatty rats (45, 47). However, in these situations, the rats demonstrated a prior accumulation of WAT providing a platform for TZD action (27, 28). Whether a similar beneficial effect will be realized in the metabolically maladaptive pregestational female adult IUGR and/or PNGR rat offspring that lack a comparable accumulation of WAT is not known. We therefore hypothesized that early intervention with PPARγ agonists will have a beneficial effect on the perturbed metabolic homeostasis of the IUGR but not the PNGR offspring. To test this hypothesis, we treated postweaned pregestational female rats exposed to pre-natal, postnatal, and pre- and postnatal nutrient restriction with rosiglitazone maleate (RG), a PPARγ agonist and investigated the effect on metabolic adaptations.

STUDY DESIGN 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: 63.9% carbohydrate, 4% fat, 14.5% protein, 10% ash, and 7–8% fiber and crude fiber). The National Institutes of Health guidelines were followed as approved by the Animal Research Committee of the University of California, Los Angeles.

Maternal Nutrient Restriction Model

Pregnant rats (F₀) received 50% of their daily food intake (11 g/day) beginning from day 11 through day 21 of gestation, which constitutes mid- to late gestation, compared with their control counterparts fed ad libitum that consumed 22 g/day of rat chow. Both groups had free access to drinking water. At birth (F₁), the litter size was culled to six to ensure no interlitter postnatal nutritional variability. Cross fostering of pups generated four experimental groups as previously described by us (21). Pups born to ad libitum-feeding control mothers were reared by either mothers on seminunrient restriction from postnatal day (PN) 1 to PN21 (PNGR) or by control mothers (CON) (Fig. 1A). During the suckling phase, the progeny born to seminunrient-restricted mothers was reared by either control mothers (IUGR) or by seminunrient restricted mothers (IUGR + PNGR). After being weaned from mothers, all groups received ad libitum access to rat chow and water (21).

Gestational Studies

A subgroup of 2-mo-old untreated female offspring (F₁) in all four experimental groups (CON, IUGR, PNGR, IUGR + PNGR) was mated with control males, resulting in a pregnant state. Glucose tolerance tests (GTTs) were performed as described previously on pregnant F₁ females at day 18 of gestation (n = 4 each) to detect the presence of gestational diabetes mellitus (43). Restrained awake F₁ 18-days-gestation pregnant rats received 0.5 g of d-glucose via the tail vein, and blood samples were collected at 0, 15, 30, 60, and 120 min from the tail vein for assessment of glucose concentrations by the glucose oxidase method as previously described (43). This avoided the use of an anesthetic during pregnancy in these animals. For similar reasons, to avoid hypoglycemic stress during pregnancy, these animals were not subjected to insulin tolerance tests.

Pregestational Studies

RG administration. RG tablets were powdered and mixed in pre-measured amounts of rat chow so that daily food intake from PN21 to PN60 would provide orally 11 μmol/day of RG per pregestational F₁ animal. Another set of comparable F₁ animals received a placebo in their rat chow. Five to eight animals per experimental group (CON, n = 14; PNGR, n = 13; IUGR, n = 14; IUGR + PNGR, n = 13) and per treatment (RG, n = 23; placebo (PL), n = 31) were included in the study (Fig. 1B).

Vascular catheter placement. Adult pregestational 2-mo-old F₁ female rats were anesthetized using an anesthetic cocktail of ketamine hydrochloride (2.8 mmol/kg) and xylazine (4.8 mg/kg) by the intraperitoneal route. The surgical site was closely shaved, and, with the use of sterile precautions, a skin incision was made, and the jugular vein was exposed. Catheters were inserted in the jugular vein, tunnelled subcutaneously, exteriorized, and maintained patent with heparinized saline. All animals were allowed full recovery from the surgical procedure before GTTs were conducted.

Intravenous glucose tolerance tests. Intravenous glucose tolerance tests (IVGTT) were performed in 2-mo-old pregestational F₁ rats after an overnight fast in the awake state within 48–72 h of completing RG or PL administration (4 + 4 = 8 groups). PL- and RG-treated pregestational animals received 1 g/kg body wt of a 1:1 mixture of [2-^2H]_2- and [6,6-^2H]_2-glucose via the surgically placed jugular vein catheters (21, 42). Blood (500 μl) was obtained at 0, 5, 15, 30, 60, and 120 min from the jugular vein catheters for assessment of glucose with hormone concentrations and isotopomer enrichment (21, 42).

Plasma assays. Plasma was separated and stored as aliquots. Glucose was measured by the glucose oxidase method (sensitivity = 0.1 mM; Sigma Diagnostics, St. Louis, MO). Insulin and leptin were quantified by enzyme-linked immunosorbent assays using rat standards and anti-rat insulin or leptin antibodies (sensitivity: insulin = 0.2 ng/ml leptin = 0.04 ng/ml using 10 μl samples; Linco Research, St. Charles, MO). All hormone assays were performed in triplicates using 96-well plates. The intra-assay and interassay coefficient of variation was 6.48 ± 0.84%. Serum triglyceride, cholesterol, high-density lipoprotein (HDL), unesterified cholesterol, and free fatty acids were measured by colorimetric assays as previously described (46a). HDL cholesterol was measured from the measurement of the supernatant following the precipitation of apolipoprotein B (apolipoprotein B-containing lipoproteins) with heparin and MnCl₂ (34). Each lipid determination was measured in triplicate. An external control sample with known analyte concentration was run in each plate to ensure accuracy. All lipids were analyzed by the University of California Los Angeles Lipid and Lipoprotein Laboratory, which is certified by the Centers for Disease Control and Prevention and the National Heart, Lung, and Blood Institute Lipid Standardization Program.

Gas chromatography/mass spectrometry analysis. Glucose was analyzed by gas chromatography/mass spectrometry using a modified method described by Szafranek et al. (41). All isotopomer determinations were performed using a Hewlett-Packard gas chromatograph (model 6890) connected to a Mass Selective Detector (model 5973A; Hewlett-Packard, Palo Alto, CA). Electron-impact ionization was used to characterize glucose positional isotopomers of [6,6-^2H]_2-glucose at mass-to-charge ratio (m/z) 187 for C-3 to C-6 and [2-^2H]_2-glucose at m/z 242 for C-1 to C-4 fragments (21, 29).

Analysis and interpretation of GTT. Mass isotoper distribution was determined using the method of Lee et al. (29). The disappearance of the two isotopes, [2-^2H]_2- and [6,6-^2H]_2-glucose, was determined with the M₁ label for [2-^2H]_2-glucose and the M₂ label for [6,6-^2H]_2-glucose (21, 49, 50). Results of the mass isotopomers in glucose (enrichment of glucose isotopomers) are reported as molar fractions namely M₀, M₁, and M₂, etc., according to the number of labeled hydrogen in the molecule (50). The sum of all isotopomers of the glucose molecules, n Mᵢ for i = 0 to n (n = 6 for glucose), is equal to 1 or 100%. Timed plasma M₁ or M₂ ([2-^2H]_2- or [6,6-^2H]_2-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).

$M_2$ represents the rate of disappearance of total glucose clearance or glucose disposal. The difference between the disappearance rates of $M_1$ and $M_2$ was used as a measure of futile cycling or recycling (i.e., glucose to glucose 6-phosphate and back) (21, 49, 50).

$M_0$ glucose is unlabeled glucose that is generated by the liver via glycogenolysis from unlabeled glycogen or gluconeogenesis from unlabeled substrates. During IVGTT, unsuppressed endogenous hepatic glucose production (HGP) was derived from the increase in unlabeled glucose concentration $M_0$, which was assessed by subtracting the labeled glucose fraction from the total glucose concentration.

Insulin tolerance tests. Awake pregestational animals received 0.75 U/kg of human insulin via a jugular venous catheter, and blood was subsequently collected at 0, 15, 30, and 60 min to measure glucose concentrations. Insulin tolerance tests were performed 48 h after completing IVGTT.

Body and organ weights. Body weight and nose-tail length were measured in 2-mo-old animals from all RG- and PL-treated groups. The animals were then deeply anesthetized by inhalation of isoflurane, and organs/tissues [brain, heart, liver, kidneys, WAT, and brown adipose tissue (BAT)] were mechanically isolated and weighed individually.

Data Analysis

All data are expressed as means ± SE. The effect of two interventions (RG and early nutrition) was compared simultaneously using the two-way ANOVA, and the $F$-values were determined. Independent effects of RG and early nutrition along with the effect of their interaction were also determined. Intergroup differences were established by the post hoc Holm-Sidak test. When the data were not
normally distributed, the nonparametric Kruskal-Wallis ANOVA followed by the post hoc Dunn’s tests was employed. Significance was assigned when $P$ values were $<0.05$. When multiple comparisons were undertaken by the one-way ANOVA followed by the post hoc Dunn’s test, the Bonferroni correction was implemented.

**RESULTS**

**Gestational Glucose Tolerance in the Adult F1 Offspring During Late Pregnancy**

A glucose challenge during late pregnancy in the F1 IUGR female offspring led to glucose intolerance, most notable at 5 min, whereas PNGR and IUGR + PNGR pregnant offspring were glucose tolerant similar to CON (Fig. 1C). These baseline observations during pregnancy in the four untreated F1 groups prompted our early RG vs. PL intervention study during the F1 pregestational state.

**RG vs. PL F1 Pregestational Study at 2 mo of Age**

**Anthropometric measurements.** RG treatment made no difference to the total body weights in all three experimental groups compared with their PL counterparts. In both RG and PL treatments, CON and IUGR exhibited a similar body weight, with the IUGR + PNGR group weighing significantly less ($P < 0.01$) (Table 1). Hence, compared with the PL CON (gold standard), the RG IUGR + PNGR was lighter. The nose to tail length was longer in RG IUGR vs. the PL IUGR with no change in RG PNGR and IUGR + PNGR vs. their PL counterparts. Compared with the PL CON, the RG IUGR, RG PNGR, and RG IUGR + PNGR were no different in length.

RG revealed a higher liver weight in the IUGR group compared with PL IUGR, but the liver weight in RG IUGR + PNGR was similar to the gold standard PL-CON (Table 1). In contrast, RG was associated with substantially heavier BAT in three experimental groups compared with corresponding PL groups as well as the PL CON. The WAT was increased in IUGR and IUGR + PNGR groups over that seen in the PL counterparts and PL CON (Table 1). RG also increased WAT in the PNGR and IUGR + PNGR groups achieving values closer to that of PL CON. There was a striking RG-associated visible increase in subcutaneous WAT that acquired a firm and lobular texture in all four experimental groups.

**Plasma glucose and hormone concentrations.** RG did not alter the basal plasma glucose and insulin concentrations in all three treatment (IUGR, PNGR, and IUGR + PNGR) groups compared with the corresponding PL group (Table 2). However, basal plasma insulin concentration in all RG-treated groups was significantly lower than that of the PL CON (Table 2). RG vs. PL significantly decreased the glucose-stimulated insulin release (GSIR) in CON and IUGR 5 min after the intravenous glucose challenge (Table 2). There was no effect of RG vs. the PL counterpart on GSIR at 5 min in PNGR and IUGR + PNGR. However, compared with the gold standard PL CON, RG decreased GSIR at 5 min in all RG-treated groups (Table 2).

Plasma leptin concentration in RG IUGR was significantly lower compared with PL IUGR, since PL IUGR alone was hyperleptinemic compared with PL CON. No other experimental group demonstrated a change in leptin concentration after RG treatment.

Calculated HOMA-IR, a measure of insulin resistance, was significantly lower in the RG CON compared with PL CON, with a trend toward lower values in RG IUGR vs. PL IUGR that did not achieve statistical significance. HOMA-IR in RG PNGR and RG IUGR + PNGR was no different from their corresponding PL counterparts or the RG CON, but was lower than PL CON (Table 2).

**Lipid profile.** RG did not alter serum triacylglycerol concentration in all four experimental groups, although in RG IUGR + PNGR the concentration was lower than that of RG CON. In contrast, RG treatment was associated with higher plasma cholesterol, HDL, and unesterified cholesterol concentrations in RG PNGR and RG IUGR compared with the corresponding PL counterparts and PL CON. Higher cholesterol and unesterified cholesterol concentrations without a change in HDL were observed in RG CON compared with the PL CON. In contrast, no significant effect of RG over that of PL was observed on serum-free fatty acid concentrations in all four experimental groups (Table 3).

**GTT and GSIR.** No effect of RG was observed on glucose tolerance in the PL CON, PL IUGR, and PL IUGR + PNGR groups (Fig. 2A). However, in the RG PNGR vs. the PL PNGR, glucose intolerance at 5 and 15 min translated into increased glucose area under the curve (AUC) ($P < 0.0001$ RG PNGR vs. PL PNGR, Holm-Sidak test). RG PNGR, however, was no different from PL CON.

GSIR was lowered by RG in IUGR similar to CON without such an effect observed in RG PNGR and RG IUGR + PNGR (Fig. 2B). The calculated insulin AUC reflected this change as an ~4.5-fold reduction in RG CON vs. PL CON and a 2-fold

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Table 1. Body weight, nose-tail length, and organ weights in RG- and PL-treated experimental groups measured at 2 mo of age

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body Wt. a g</th>
<th>N-T Length. b cm</th>
<th>Heart. g</th>
<th>Kidney. g</th>
<th>Liver. g</th>
<th>Brain. g</th>
<th>BAT. g</th>
<th>WAT. g</th>
</tr>
</thead>
<tbody>
<tr>
<td>RG CON (n = 6)</td>
<td>309.5 ± 9d</td>
<td>41 ± 0.4c</td>
<td>1.4 ± 0.1</td>
<td>1.8 ± 0.1i</td>
<td>10.4 ± 0.4</td>
<td>1.8 ± 0.1</td>
<td>2.7 ± 0.3</td>
<td>20.1 ± 1.3c</td>
</tr>
<tr>
<td>PL CON (n = 8)</td>
<td>271.6 ± 9.8</td>
<td>37.8 ± 0.3</td>
<td>0.9 ± 0.1</td>
<td>2.1 ± 0.1i</td>
<td>10.0 ± 0.3</td>
<td>1.7 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>5.3 ± 0.1</td>
</tr>
<tr>
<td>RG PNGR (n = 6)</td>
<td>297.8 ± 9.6</td>
<td>41.8 ± 1.7</td>
<td>1.5 ± 0.1i</td>
<td>1.8 ± 0.1i</td>
<td>10.3 ± 0.7</td>
<td>1.8 ± 0.1</td>
<td>3.6 ± 0.4d</td>
<td>10.6 ± 1.1</td>
</tr>
<tr>
<td>PL PNGR (n = 7)</td>
<td>277.3 ± 9.1</td>
<td>42.6 ± 0.3d</td>
<td>1.0 ± 0.1</td>
<td>2.0 ± 0.1i</td>
<td>10.6 ± 0.7</td>
<td>1.7 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>7.0 ± 1.4</td>
</tr>
<tr>
<td>RG IUGR (n = 6)</td>
<td>300.7 ± 9.4</td>
<td>41.7 ± 0.2c</td>
<td>1.6 ± 0.1f</td>
<td>1.6 ± 0.1f</td>
<td>10.4 ± 0.5e</td>
<td>2.2 ± 0.1i</td>
<td>3.8 ± 0.4d</td>
<td>15.1 ± 1.7</td>
</tr>
<tr>
<td>PL IUGR (n = 8)</td>
<td>293.7 ± 9.1</td>
<td>36.9 ± 0.4</td>
<td>0.9 ± 0.1</td>
<td>1.9 ± 0.1i</td>
<td>8.5 ± 0.7</td>
<td>1.7 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>4.3 ± 1.3</td>
</tr>
<tr>
<td>RG IUGR + PNGR (n = 5)</td>
<td>259.2 ± 5.8c</td>
<td>39.8 ± 0.2</td>
<td>1.3 ± 0.1</td>
<td>1.4 ± 0.1i</td>
<td>9.0 ± 0.1</td>
<td>1.8 ± 0.1c</td>
<td>4.2 ± 0.3d</td>
<td>8.7 ± 1.1c</td>
</tr>
<tr>
<td>PL IUGR + PNGR (n = 8)</td>
<td>246.8 ± 9.1</td>
<td>36.1 ± 0.5</td>
<td>0.8 ± 0.1</td>
<td>1.5 ± 0.1f</td>
<td>7.9 ± 0.4f</td>
<td>1.5 ± 0.1c</td>
<td>0.3 ± 0.1</td>
<td>1.7 ± 0.2c</td>
</tr>
</tbody>
</table>

Data are shown as means ± SE; n, no. of rats. RG, rosiglitazone maleate; PL, placebo; N-T, nose-tail; BAT, brown adipose tissue; WAT, white adipose tissue; CON, control; PNGR, postnatal growth restriction; IUGR, intrauterine growth restriction. aTwo-way ANOVA with Holm-Sidak test; bKruskal-Wallis ANOVA with Dunn’s test. cP < 0.0001, dP < 0.001, and eP < 0.004, RG vs. the respective PL-treated group within each early nutrition group. fP < 0.0001, gP < 0.003, hP < 0.01 represent comparison of the PL and RG early nutrition group with the PL CON. iP < 0.001, RG early nutrition group vs. RG CON.
diminution in RG IUGR vs. PL IUGR. Compared with PL CON (gold standard), GSIR decreased 2.5-fold in RG IUGR and RG PNGR and 3.2-fold in RG IUGR + PNGR. This consists of an approximately twofold lower RG effect on glucose-induced insulin release in IUGR vs. CON. This difference between IUGR and CON may indirectly support reduced hepatic RG or insulin sensitivity in the former vs. the latter. In contrast, PL PNGR and PL IUGR + PNGR insulin AUC was threefold lower than that observed in PL CON, with no further change in RG PNGR or RG IUGR + PNGR (Fig. 2C).

Insulin tolerance tests. RG administration resulted in lower insulin-induced plasma glucose concentrations compared with the corresponding PL counterparts in all four experimental groups (Fig. 3A). This change is reflected in the lower calculated glucose AUC by ~30% in RG CON, RG IUGR, and RG IUGR + PNGR vs. the PL counterpart. In contrast, only an ~10% lower glucose AUC was observed in RG PNGR vs. PL PNGR (P < 0.0001, in RG CON, RG IUGR, and RG IUGR + PNGR vs. the corresponding PL groups, P < 0.035 RG PNGR vs. PL PNGR, and P < 0.006 for RG PNGR vs. RG CON and RG IUGR). This intergroup difference is exaggerated when expressed as a percent of the baseline plasma glucose concentration. PL-treated groups demonstrate a slightly higher glucose concentration over that observed in the corresponding PL group (Fig. 3B). The failure of glucose release 60 min after insulin administration in all RG-treated groups may reflect a decrease in endogenous hepatic glucose release or failure of the counterregulatory hormonal effect.

Glucose metabolic adaptations during IUGTT. After the glucose challenge, there was a significant suppression of endogenous HGP in response to RG administration only in the IUGR group vs. the PL IUGR. Suppression of endogenous HGP in RG PNGR and RG IUGR + PNGR was unchanged compared with corresponding PL groups, but significantly greater suppression was noted in RG IUGR + PNGR compared with PL CON (Fig. 4A). In contrast, glucose clearance was relatively higher in the PL IUGR group compared with PL CON. Early administration of RG caused a trend toward lowering glucose clearance, which did not achieve statistical significance in RG IUGR, RG PNGR, and RG IUGR + PNGR vs. their corresponding PL-treated counterparts and PL CON (Fig. 4B). Glucose recycling was significantly lower in PNGR alone following RG vs. PL treatment (Fig. 4C). However, compared with PL CON, glucose recycling was higher in RG IUGR (P < 0.0001) but similar in RG PNGR and RG IUGR + PNGR.

DISCUSSION

In this investigation, we examined the effect of a PPARγ agonist, an insulin sensitizer, on the young pregestational female IUGR, PNGR, and IUGR + PNGR offspring. The idea behind this present study was to regulate metabolic adaptations initiated by either the intrauterine or postnatal adverse nutritional and growth environment. These metabolic adaptations serve as biomarkers of an imbalance between the pancreatic

<table>
<thead>
<tr>
<th>Groups</th>
<th>Basal Glucose, mmol/l</th>
<th>Basal Insulin, nmol/l</th>
<th>Stimulated Insulin, nmol/l</th>
<th>Basal Leptin, ng/ml</th>
<th>HOMA-IR</th>
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<tbody>
<tr>
<td>RG CON (n = 6)</td>
<td>6.4 ± 0.5</td>
<td>5.0 ± 1.4</td>
<td>19.4 ± 3.2</td>
<td>4.7 ± 1.1</td>
<td>11.2 ± 1.8</td>
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<td>PL CON (n = 5)</td>
<td>6.0 ± 0.5</td>
<td>13.7 ± 1.5</td>
<td>83.5 ± 9.9</td>
<td>4.4 ± 1.2</td>
<td>28.6 ± 4.7</td>
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<td>RG PNGR (n = 6)</td>
<td>5.7 ± 0.5</td>
<td>6.1 ± 1.4</td>
<td>19.7 ± 2.4</td>
<td>NA</td>
<td>12.2 ± 0.9</td>
</tr>
<tr>
<td>PL PNGR (n = 6)</td>
<td>6.1 ± 0.5</td>
<td>6.9 ± 1.4</td>
<td>23.9 ± 3.3</td>
<td>3.7 ± 0.5</td>
<td>14.7 ± 1</td>
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<tr>
<td>RG IUGR (n = 6)</td>
<td>5.4 ± 0.5</td>
<td>6.4 ± 1.4</td>
<td>23.9 ± 12.4</td>
<td>4.9 ± 1.1</td>
<td>12.2 ± 0.7</td>
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<tr>
<td>PL IUGR (n = 6)</td>
<td>6.4 ± 0.5</td>
<td>12.9 ± 1.4</td>
<td>47.2 ± 7.3</td>
<td>9.4 ± 1.1</td>
<td>29.9 ± 7.3</td>
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<tr>
<td>RG IUGR + PNGR (n = 5)</td>
<td>5.9 ± 0.5</td>
<td>5.8 ± 1.5</td>
<td>18.2 ± 2.1</td>
<td>3.5 ± 1.2</td>
<td>12.1 ± 1.8</td>
</tr>
<tr>
<td>PL IUGR + PNGR (n = 5)</td>
<td>5.6 ± 0.5</td>
<td>8.2 ± 1.4</td>
<td>18.5 ± 3</td>
<td>3.6 ± 1.1</td>
<td>15.9 ± 2.8</td>
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</table>

Data are shown as means ± SE; n, no. of rats. HSL, high density lipoprotein; UC, unesterified cholesterol; FFA, free fatty acids. *P < 0.05, aP < 0.001, bP < 0.001, and cP < 0.005, RG vs. the respective PL treatment in each early nutrition group and the PL CON. dP < 0.007, RG early nutrition group vs. the RG CON.
Fig. 2. A: plasma glucose concentrations at all time points during GTT in PL- and RG-treated groups. 1, CON; 2, postnatal growth restriction (PNGR); 3, intrauterine growth restriction (IUGR); and 4, IUGR + PNGR. *P < 0.0001 and **P < 0.006, RG vs. PL is significantly higher. †P < 0.02 ad ††P < 0.0001, RG vs. PL is significantly lower. B: plasma insulin concentrations at all time points during GTT in PL- and RG-treated groups. 1, CON; 2, PNGR; 3, IUGR; and 4, IUGR + PNGR. The scale of the ordinate was adjusted to show the lower values in PNGR and IUGR + PNGR groups. Holm-Sidak test shows a significant decrease in RG CON and RG IUGR vs. corresponding PL groups: *P < 0.0001 **P < 0.004, and †P < 0.001. C: area under the curve (AUC) for plasma insulin concentration during GTT. Two-way ANOVA revealed a significant effect of RG treatment (F = 34.512, P < 0.001), early nutrition (F = 12.729, P < 0.001), and RG treatment × early nutrition (F = 12.139, P < 0.001). Holm-Sidak test revealed significant decrease in insulin AUC for RG CON and RG IUGR vs. the corresponding PL group (*P < 0.0001) and †P < 0.0001 for PL PNGR and PL IUGR + PNGR vs. the PL CON.
The initiation of RG at an early age before any substantive WAT deposition led to an abnormal accumulation of subcutaneous rather than visceral WAT along with an increase in BAT. Previous investigations in pregnant rats demonstrated that accretion of visceral fat was associated with hepatic insulin resistance (11). Furthermore, surgical removal of visceral adipose tissue or exercise training that led to redistributing visceral to subcutaneous fat in nonpregnant rats improved insulin action (19, 23). Thus it is possible that RG may partially exert its insulin-sensitizing effect by redistributing fat depots from visceral to a subcutaneous location (27, 28). This WAT...

![Diagram](image-url)
distribution was associated with lowering of the circulating leptin concentration, mainly in the IUGR. Furthermore, by increasing BAT, RG may increase lipolysis and energy expenditure. This may offer an explanation for the lack of increase in body weight despite the visible addition of subcutaneous fat deposits in all four groups. Alternatively, a diminution in skeletal muscle mass may also provide an explanation for the lack of change in body weight, although not substantiating the observed insulin-sensitizing effect of RG in the IUGR. Some of the RG-induced insulin-sensitizing metabolic effects in the gestational IUGR consisted of the lower GSIR and suppression of endogenous HGP without affecting glucose clearance or recycling. When a suppressed glucose-induced insulin response existed previously as in the PNGR when compared with CON, RG had no metabolic effect. These findings are significant in the context of pregnancy, since the IUGR offspring alone demonstrated gestational diabetes in our study. One may speculate that the early introduction of RG primarily affected the response of pancreatic β-islet cell insulin release, hepatic insulin action, redistribution of WAT, and an increase in BAT stores. PPARγ agonists target small adipocytes, promote lipogenesis, and increase subcutaneous fat depots (17). This tissue-specific action of PPARγ agonists in WAT may partially spare pancreatic β-islets and liver and skeletal muscle from the potential hazards of gluco- and lipotoxicity (44).

We targeted our studies at the pregestational female, since previous investigations demonstrated a need to control maternal diabetes, before embryonic organogenesis, to successfully reverse adversity to the developing embryo (9, 13). Hence considerable emphasis has been placed on proper metabolic control at a stage before conception in pregestational females. In a similar vein, as seen in our present study, the IUGR adult rat offspring develops gestational diabetes and, as reported previously, transmits insulin resistance to its offspring (38). Intervention on day 1 of gestation by embryo-transfer experiments failed to ameliorate the transmission of insulin resistance to the offspring (42). This led to our present study design, where we introduced an insulin sensitizer before pregnancy to examine its effect on metabolic adaptations. In contrast to the insulin-sensitizing metabolic effects observed in the IUGR, the PNGR became hyperglycemic with decreased glucose recycling compared with its nutritionally matched PL counterpart. However, compared with the nutritionally sufficient PL-treated controls, RG PNGR were no different. Although this observation may support the concept that RG treatment reverts the PNGR pregestational metabolic state to that of the PL CON, it remains to be seen if the glucose intolerance observed compared with PL PNGR is of clinical significance. In this latter context, despite redistribution of WAT stores and an increase in BAT accumulation, liver size and nose-tail length, PPARγ agonists may not reverse the reduced pancreatic β-islets in the PNGR pregestational female rats. In contrast, the IUGR + PNGR exhibited metabolic features and RG sensitivity that were in between that seen in the IUGR and PNGR. Hence, postnatal control of caloric intake superimposed on IUGR partially ameliorated the subsequent propensity toward an insulin-resistant state, as described previously (21).

Although multiple studies in humans have demonstrated the beneficial effects of insulin sensitizers in delaying the development of type 2 diabetes mellitus and its complications, including starving type 2 diabetes mellitus in gestational diabetic women (7, 48), no such studies in children or adolescents exist to date. There is no safety or efficacy data for drug therapy, and only diet and exercise are recommended (32). When extrapolating our present preclinical animal studies to the clinical setting, it is important to remember that side effects during this phase of life involving active growth pose significantly different problems. Accumulation of subcutaneous fat deposits may not be a welcome aspect of this treatment, particularly during preteenage, teenage, and young adult stages of development. Furthermore, the possibility of developing cognitive impairments because of insulin-induced hypoglycemia warrants attention. In addition, while there is ongoing development of better PPARγ agonists with fewer side effects than the one used in this study, RG provided a proof of principle in this specific subpopulation. However, before considering clinical trials, it is important to conduct additional preclinical trials fine tuning the dosage schedule of later generations of PPARγ agonists toward maximizing the sensitizing and minimizing the side effects. Furthermore, it is essential in these future preclinical trials to characterize tissue-specific changes that involve the β-islets, liver, and adipose tissue, similar to recent investigations in skeletal muscle (33).

We conclude that daily administration of PPARγ agonists to the pregestational female has insulin-sensitizing metabolic effects in the IUGR offspring akin to that of CON, although vigilance against subcutaneous WAT accumulation and exaggerated exogenous insulin-induced hypoglycemia is necessary. In contrast, while no such metabolic effects are evident in the IUGR + PNGR, PPARγ agonists in the PNGR revert the growth and metabolic status back to that of PL CON. Whether this effect sets the PNGR for subsequently developing clinically significant glucose intolerance requires further study. Although there may be merit in early introduction of insulin sensitizers toward prevention of adult-onset insulin resistance, gestational diabetes mellitus, type 2 diabetes mellitus, visceral adiposity, and other cardiovascular complications in the IUGR offspring, future preclinical investigations consisting of fine tuning the use of these agents by maximally harnessing the benefits while avoiding the side effects are warranted.

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AJP-Endocrinol Metab • VOL 298 • MARCH 2010 • www.ajpendo.org


