Early exercise regimen improves insulin sensitivity in the intrauterine growth-restricted adult female rat offspring

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Garg M, Thamotharan M, Oak SA, Pan G, MacLaren DC, Lee PW, Devaskar SU. Early exercise regimen improves insulin sensitivity in the intrauterine growth-restricted adult female rat offspring. Am J Physiol Endocrinol Metab 296: E272–E281, 2009. First published November 11, 2008; doi:10.1152/ajpendo.90473.2008.—We examined the effect of early exercise training (Ex) on glucose kinetics, basal, and insulin-stimulated skeletal muscle (SKM) plasma membrane (PM) GLUT4 in pre- and/or postnatal nutrient-restricted adult rat offspring compared with sedentary (Sed) state. Gestational control female (Ex CON vs. Sed CON) and offspring exposed to prenatal (Ex IUGR vs. Sed IUGR), postnatal (Ex PNGR vs. Sed PNGR), or pre- and postnatal (Ex IUGR + PNGR vs. Sed IUGR + PNGR) nutrient restriction were studied. The combined effect of exercise and pre/postnatal nutrition in the Ex IUGR demonstrated positive effects on basal and glucose-stimulated insulin production (HGP) with suppression of endogenous hepatic glucose production (HGP) compared with sedentary state. Ex PNGR was hyperglycemic after glucose challenge with no change in glucose-stimulated insulin production or HGP compared with sedentary state. Basal SKM PM-associated GLUT4 was unchanged by exercise in all four groups. Whereas Ex PNGR and Ex IUGR + PNGR insulin responsiveness was similar to that of Ex CON, Ex IUGR remained nonresponsive to insulin. Early introduction of regular Ex in the gestational female offspring had a positive effect on hepatic adaptation to GSIR and HGP in IUGR and IUGR + PNGR, with no effect in PNGR. Change in insulin responsiveness of SKM GLUT4 translocation was observed in exercised IUGR + PNGR and PNGR but not in exercised IUGR.

glucose tolerance test; metabolic programming; glucose transporter 4; insulin-responsive glucose transporter 4 translocation

RESTRICTION OF EARLY GROWTH prenatally or postnatally is linked to adult-onset insulin resistance, visceral adiposity, and type 2 diabetes mellitus (T2DM) (1–4). Animal models consisting of fetal and/or postnatal malnutrition as either global (19, 36, 41) or selective nutrient restriction (17) causing intrauterine growth restriction (IUGR) and/or postnatal growth restriction (PNGR) predispose the adult offspring to selective tissue-specific insulin resistance (17, 19, 36). Prenatal nutrient restriction with IUGR enhances glucose-induced insulin response (19) and causes hyperinsulinemia (17, 19), hepatic insulin resistance per hyperglycemic euglycemic clamp experiments (22), and insulin resistance of skeletal muscle (SKM) (32, 41) and adipose tissue (17) GLUT4 translocation. In contrast, postnatal nutrient restriction (PNGR) programs adult females toward unsuppressed hepatic glucose production, hypoinsulinemia with a lean body habitus, and relative hepatic insulin resistance (19) but partially retained insulin sensitivity of SKM GLUT4 translocation (41). The glucose metabolic adaptations and this insulin-resistant phenotype in the IUGR (19, 22) are transgenerationally inherited and amplified in the next generation (7, 9, 39).

Acute exercise or endurance training lowers circulating insulin concentrations and increases counterregulatory hormones. Exercise overcomes glucose intolerance by attenuating hepatic glucose production via suppression of glycogenolysis (13) and enhancing SKM glucose utilization (5, 11). Exercise improves insulin sensitivity by augmenting insulin signaling and non-insulin-mediated alternative signaling mechanisms (37). Exercise and muscle contraction overcome insulin resistance by increasing adenosine 5’-monophosphate kinase (AMPK) enzyme activity that in turn increases cellular glucose uptake. This occurs by AMPK-mediated SKM GLUT4 translocation to the plasma membrane (PM), thereby circumventing the insulin resistance of GLUT4 translocation (20).

On the basis of this information, we hypothesized that early introduction of submaximal exercise training (ET) will ameliorate certain forerunning features of selective insulin resistance in the adult IUGR and/or PNGR postgestational female offspring. To test this hypothesis, we subjected postweaned IUGR or PNGR female rats to a moderate exercise regimen for ~6 wk and determined its effect on hepatic glucose kinetics and SKM subcellular GLUT4 protein distribution.

RESEARCH 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 carbohydrate 63.9%, fat 4.5%, and protein 14.5%). 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 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 (CON) counter-
parts that received ad libitum access to rat chow (22 g/day). Both groups had ad libitum access to drinking water. At birth, the litter size was culled to six to ensure no interlitter nutritional variability. Postnatally, the cross-fostering of animals generated four experimental groups, as described previously by us (19). Briefly, the newborn pups born to ad libitum feeding CON mothers were reared either by mothers on seminutrient restriction from PN21–PN21 (PNGR) or by CON mothers (Fig. 1A). The intrauterine semi-nutrient-restricted progeny was fed either by CON mothers with ad libitum access to nutrients representing intrauterine nutrient restriction (IUGR) alone or by semi-nutrient-restricted mothers representing a combination of intrauterine and postnatal nutrient restriction (IUGR + PNGR) (Fig. 1A). After being weaned from the mother, all animals had ad libitum access to food and water.

**Moderate ET**

Postweaned animals in all four groups underwent daily supervised ET, whereas another set of postweaned animals in each group were maintained under sedentary conditions from PN21 to PN60 (~2 mo). The ET comprised of running on a motorized treadmill at a speed of 11 m/min for 15 min/day, spanning 5 days/wk and lasting for a total of 6 wk (Fig. 1B). This moderate exercise regimen was devised to accommodate the perinatally energy-restricted animal groups in our study.

**Surgical Catheter Placement**

The adult females were anesthetized using an anesthetic cocktail of ketamine HCl (50 mg/kg) and xylazine (4.5 mg/kg) by intraperitoneal injection (19). Jugular vein catheters were inserted aseptically and maintained patent with heparinized saline, as described previously (19). All animals were allowed full recovery from the surgical procedure prior to glucose tolerance tests (GTTs) being conducted.

**Intravenous GTT**

All tests were performed in the resting state 48–72 h after ET was completed following an overnight fast. The 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 glucose and insulin concentrations and isotopomer enrichment (19).

**Insulin Tolerance Test**

Awake adult females received 0.75 U/kg of human insulin via the jugular venous catheter, and blood was obtained at 0, 15, 30, and 60 min subsequently to measure glucose concentration (41).

**Plasma Assays**

Plasma was separated and aliquots stored for measurement of glucose by the glucose oxidase method (sensitivity = 0.1 mM; Sigma Diagnostics, St. Louis, MO). Insulin was quantified by enzyme-linked immunoabsorbent assays using rat standards and anti-rat insulin antibody (sensitivity: insulin = 0.2 ng/ml; Linco Research, St. Charles, MO). Homeostasis model of insulin resistance (HOMA-IR) was calculated on the basis of these measured values.

**Gas Chromatography-Mass Spectrometry Analysis**

Glucose was analyzed by modified gas chromatography-mass spectrometry method, as described previously (19, 43, 44). All isotopomeric determinations were performed using a Hewlett-Packard gas chromatograph (model 5890) connected to a mass selective detector (model 5973A) (Hewlett-Packard, Palo Alto, CA). Electron impact ionization was used to characterize glucose positional isotopomers of [6,6-2H2]glucose at mass-to-charge ratio (m/z) of 187 for C3–C6 of [2-2H]glucose at m/z of 242 for C1–C4 fragments (19, 44).

**Analysis and Interpretation of Glucose Tolerance Test**

Mass isotopomer distribution was determined using the method of Lee et al. (27). Briefly, disappearance of the two isotopes [2-2H]- and [6,6-2H2]glucose was determined for the M1 label that represented [2-2H]glucose and the M2 label that represented the [6,6-2H2]glucose (19, 43, 44). The difference between disappearance rates of M1 and M2 was used as a measurement of futile cycling (i.e., glucose to glucose 6-phosphate and back) (19, 27).

**SKM AMPK Studies**

To validate our exercise regimen, SKM AMPK enzyme activity was initially measured after an acute 15-min bout of similar treadmill training in control animals and compared with their respective sedentary controls. To guard against detecting remnant effects from the last 15-min bout of acute exercise that can potentially contaminate the chronic effects of exercise lasting over 6 wk duration, we measured SKM total AMPK, phosphorylated AMPK (pAMPK) (32), and AMPK enzyme activity (28, 42) in all exercise (Ex) groups 48–72 h after their last 15-min bout of exercise and compared them with their respective Sed groups.
Western Blot Analysis

The animals were deeply anesthetized with inhalational isoflurane to maintain organ blood flow, and SKM was rapidly separated from surrounding tissues, quickly snap-frozen in liquid nitrogen, and stored at −70°C. Fifty micrograms of prepared SKM homogenates were separated on SDS-PAGE and subjected to Western blot analysis, as described previously (32). The primary antibodies consisted of one raised against total AMPK (containing α1 and α2 isoforms, H-300 AMPK IgG; Santa Cruz Biotechnology) or specifically against either total AMPK (containing H-300 AMPK IgG), 0.2 mM AMP, 80 mM NaCl, 8% glycerol, 0.8 mM EDTA, 0.8 mM dithiothreitol, 5 mM MgCl2, and 0.2 mM ATP (+2 μCi [32P]ATP), pH 7.0, in a final volume of 40 μl for 10 min at 37°C. At the end of incubation, an aliquot was spotted on Whatman P81 filter paper. The unbound [32P]ATP was removed with six washes of 1% phosphoric acid and one wash of acetone. The filter papers were air-dried, and radioactivity was quantified using a scintillation counter (42).

Skeletal Muscle GLUT4 Studies

To investigate the effect of exercise, skeletal muscle homogenates from all four Ex and Sed groups were employed. To examine insulin responsiveness of SKM GLUT4 translocation, 2-mo-old female animals from all four Ex groups received either vehicle or insulin (8 U/kg by intraperitoneal injection). After 20 min, the predetermined optimal time point hindlimb SKM was harvested, and subcellular fractions were prepared. PM and low-density microsomal (LDM) subfractions were isolated as described previously, and the relative purity was determined by marker enzyme enrichment (40, 41). The homogenate, fractionated sacrolemmal PM, and LDM samples were subjected to Western blot analysis (40, 41). The affinity-purified rabbit anti-rat GLUT4 antibody (1:2,500 dilution) was used as the primary antibody (40, 41).

Table 1. NT length and organ weights in all Ex and Sed groups: CON, IUGR, PNGR, and IUGR + PNGR

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body Weight, g</th>
<th>NT Length, 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>Ex CON (5)</td>
<td>282.5 ± 7.8</td>
<td>41.8 ± 0.49*</td>
<td>1.09 ± 0.02b</td>
<td>1.94 ± 0.02*</td>
<td>9.54 ± 0.35</td>
<td>1.95 ± 0.40b</td>
<td>0.35 ± 0.04</td>
<td>10.42 ± 1.4b</td>
</tr>
<tr>
<td>Sed CON (8)</td>
<td>271.6 ± 9.7</td>
<td>37.75 ± 0.25</td>
<td>0.87 ± 0.02</td>
<td>2.13 ± 0.07</td>
<td>9.87 ± 0.31</td>
<td>1.73 ± 0.06</td>
<td>0.29 ± 0.03</td>
<td>5.86 ± 0.81</td>
</tr>
<tr>
<td>Ex PNGR (6)</td>
<td>272.3 ± 4.6</td>
<td>43.3 ± 0.42</td>
<td>1.08 ± 0.05</td>
<td>1.79 ± 0.02</td>
<td>8.73 ± 0.3a</td>
<td>1.9 ± 0.03b</td>
<td>0.29 ± 0.04</td>
<td>6.26 ± 1.16a</td>
</tr>
<tr>
<td>Sed PNGR (7)</td>
<td>277.3 ± 4.8</td>
<td>42.57 ± 0.29</td>
<td>0.97 ± 0.08</td>
<td>1.95 ± 0.02*</td>
<td>10.61 ± 0.67</td>
<td>1.74 ± 0.02</td>
<td>0.21 ± 0.06</td>
<td>6.98 ± 0.92</td>
</tr>
<tr>
<td>Ex IUGR (6)</td>
<td>290.5 ± 7.0</td>
<td>42.5 ± 0.34a</td>
<td>1.08 ± 0.04</td>
<td>1.92 ± 0.07</td>
<td>9.63 ± 0.31</td>
<td>1.94 ± 0.04a</td>
<td>0.33 ± 0.02</td>
<td>9.42 ± 1.01a</td>
</tr>
<tr>
<td>Sed IUGR (8)</td>
<td>293.7 ± 12.7</td>
<td>36.88 ± 0.35</td>
<td>0.896 ± 0.05</td>
<td>1.88 ± 0.06</td>
<td>8.51 ± 0.49</td>
<td>1.67 ± 0.04</td>
<td>0.28 ± 0.03</td>
<td>4.33 ± 0.53</td>
</tr>
<tr>
<td>Ex IUGR + PNGR (6)</td>
<td>256 ± 11.6</td>
<td>41.84 ± 0.4a</td>
<td>0.986 ± 0.51b</td>
<td>1.59 ± 0.08</td>
<td>8.21 ± 0.3</td>
<td>1.87 ± 0.04a</td>
<td>0.21 ± 0.04</td>
<td>3.63 ± 0.53#</td>
</tr>
<tr>
<td>Sed IUGR + PNGR (8)</td>
<td>246.8 ± 10.5</td>
<td>36.1 ± 0.44</td>
<td>0.79 ± 0.08</td>
<td>1.54 ± 0.04*</td>
<td>7.88 ± 0.37*</td>
<td>1.52 ± 0.04*</td>
<td>0.3 ± 0.04</td>
<td>1.68 ± 0.15*</td>
</tr>
</tbody>
</table>

Data are shown as means ± SE; n is shown in parentheses. NT, nose-tail; Ex, exercise; Sed, sedentary; CON, control; IUGR, intrauterine growth restricted; PNGR, postnatal growth restricted; BAT, brown adipose tissue; WAT, white adipose tissue. *b, P < 0.001, P < 0.001, and P < 0.03, respectively, for Ex vs. Sed states in the pre/postnatal nutrition groups (Holm-Sidak test). *P < 0.0001 and **P < 0.02, Sed pre/postnatal nutrition groups vs. Sed CON (Holm-Sidak test). #P < 0.0001, Ex pre/postnatal nutrition groups vs. Ex CON (Holm-Sidak test).

RESULTS

Anthropometric Changes, Basal Glucose and Insulin Concentrations, and HOMA-IR

ET did not affect body weight but significantly increased nose-to-tail length in Ex CON, Ex IUGR, and Ex IUGR + PNGR groups (P < 0.0001). Body length in sedentary (Sed) PNGR was significantly greater than all other sedentary groups and did not change significantly after exercise. There was a significant exercise × pre/postnatal nutritional effect on nose-to-tail length (F = 18.66, P < 0.001), a pre/postnatal nutrition effect alone (F = 43.58, P < 0.001), and an exercise effect alone (F = 226.7, P < 0.001). Similarly, heart weights revealed a significant effect of exercise (F = 32.13, P < 0.001) and pre/post nutrition (F = 3.55, P < 0.02) but no effect of Ex × pre/postnatal nutrition (F = 0.556, P < 0.64). Heart weights increased after ET in Ex CON, Ex IUGR, and Ex PNGR groups (P < 0.0001). Body length in sedentary (Sed) PNGR was significantly greater than all other sedentary groups and did not change significantly after exercise. There was a significant exercise × pre/postnatal nutritional effect on nose-to-tail length (F = 18.66, P < 0.001), a pre/postnatal nutrition effect alone (F = 43.58, P < 0.001), and an exercise effect alone (F = 226.7, P < 0.001). Similarly, heart weights revealed a significant effect of exercise (F = 32.13, P < 0.001) and pre/post nutrition (F = 3.55, P < 0.02) but no effect of Ex × pre/postnatal nutrition (F = 0.556, P < 0.64). Heart weights increased after ET in Ex CON, Ex IUGR, and Ex PNGR groups (P < 0.0001). Body length in sedentary (Sed) PNGR was significantly greater than all other sedentary groups and did not change significantly after exercise. There was a significant exercise × pre/postnatal nutritional effect on nose-to-tail length (F = 18.66, P < 0.001), a pre/postnatal nutrition effect alone (F = 43.58, P < 0.001), and an exercise effect alone (F = 226.7, P < 0.001). Similarly, heart weights revealed a significant effect of exercise (F = 32.13, P < 0.001) and pre/post nutrition (F = 3.55, P < 0.02) but no effect of Ex × pre/postnatal nutrition (F = 0.556, P < 0.64). Heart weights increased after ET in Ex CON, Ex IUGR, and Ex PNGR groups (P < 0.0001). Body length in sedentary (Sed) PNGR was significantly greater than all other sedentary groups and did not change significantly after exercise. There was a significant exercise × pre/postnatal nutritional effect on nose-to-tail length (F = 18.66, P < 0.001), a pre/postnatal nutrition effect alone (F = 43.58, P < 0.001), and an exercise effect alone (F = 226.7, P < 0.001). Similarly, heart weights revealed a significant effect of exercise (F = 32.13, P < 0.001) and pre/post nutrition (F = 3.55, P < 0.02) but no effect of Ex × pre/postnatal nutrition (F = 0.556, P < 0.64). Heart weights increased after ET in Ex CON, Ex IUGR, and Ex PNGR groups (P < 0.0001). Body length in sedentary (Sed) PNGR was significantly greater than all other sedentary groups and did not change significantly after exercise. There was a significant exercise × pre/postnatal nutritional effect on nose-to-tail length (F = 18.66, P < 0.001), a pre/postnatal nutrition effect alone (F = 43.58, P < 0.001), and an exercise effect alone (F = 226.7, P < 0.001). Similarly, heart weights revealed a significant effect of exercise (F = 32.13, P < 0.001) and pre/post nutrition (F = 3.55, P < 0.02) but no effect of Ex × pre/postnatal nutrition (F = 0.556, P < 0.64). Heart weights increased after ET in Ex CON, Ex IUGR, and Ex PNGR groups (P < 0.0001).
IUGR + PNGR groups ($P < 0.001$, Holm-Sidak test), whereas brain weights increased in Ex CON, Ex IUGR, Ex PNGR, and Ex IUGR + PNGR groups vs. their Sed counterparts ($P < 0.001$, Holm-Sidak test). Brown adipose tissue weight was no different, but abdominal white adipose tissue increased in Ex CON and Ex IUGR compared with corresponding Sed counterparts ($P < 0.001$ and $P < 0.001$, Holm-Sidak test; Table 1). ET did not alter fasting plasma glucose concentration (Table 2); a significant decrease in fasting plasma insulin concentration was observed by exercise alone ($F = 11.16$, $P < 0.002$) and by pre/postnatal nutrition alone ($F = 3.69$, $P < 0.001$, Holm-Sidak test; Table 2). The Ex
PNGR and Ex IUGR + PNGR groups remained unchanged from the sedentary insulin-sensitive state.

**GTT and Glucose-Stimulated Insulin Release**

An intravenous glucose challenge led to a lower peak plasma glucose concentration at 5 min in Ex CON (Holm-Sidak test, \( P < 0.007; \) Fig. 2A, graph 1) compared with that of Sed CON. The plasma glucose concentration was higher at 5 and 15 min in Ex PNGR (Holm-Sidak test, \( P < 0.02 \) and \( P < 0.05 \) respectively; Fig. 2A, graph 2) vs. Sed PNGR, but Ex IUGR (Fig. 2A, graph 3) was similar to Sed IUGR. The interactive effect of exercise \( \times \) pre/postnatal nutrition resulted in a reduced glucose area under the curve (AUC) in Ex CON (\( P < 0.04 \) by Holm-Sidak test; Fig. 2B) but significantly increased AUC in Ex PNGR (\( P < 0.03 \) by Holm-Sidak test). Euglycemia during GTT was maintained by a significant lowering of the glucose-stimulated insulin release (GSIR) at 5, 15, and 30 min (Fig. 2C, graphs 1 and 3) and a lower insulin AUC (\( P < 0.001 \) by Holm-Sidak test; Fig. 2D) in Ex Con and Ex IUGR groups. These findings support improved insulin sensitivity. The GSIR in Sed PNGR and Sed IUGR + PNGR was decreased vs. Sed CON but did not change further after ET (Fig. 2C, graphs 2 and 4).

**Insulin Tolerance Test Reflecting Insulin-Stimulated Glucose Uptake**

The percent decrease in plasma glucose concentration from the zero time value was greater at 15 and 30 min in Ex PNGR, Ex IUGR, and Ex IUGR + PNGR groups and at 60 min in Ex IUGR and Ex IUGR + PNGR (Fig. 3, graphs 1–4) vs. their Sed counterparts. This resulted in decreased percent glucose AUC in Ex IUGR and Ex IUGR + PNGR after ET (\( P < 0.00001 \) by Holm-Sidak test; Fig. 3B).

**Glucose Metabolic Adaptations After a Glucose Challenge**

**Hepatic glucose production.** ET resulted in greater suppression of endogenous hepatic glucose production (HGP) during the GTT in Ex CON and Ex IUGR at various time points (5, 15, 30, and 60 min) compared with that of Sed CON and Sed IUGR, respectively. Sixty minutes after the glucose challenge, HGP in Ex IUGR was suppressed to a greater extent than that observed in Ex CON, Ex PNGR, and Ex IUGR + PNGR (\( P < 0.04 \) by Holm-Sidak test). This translated into a greater suppression of HGP AUC in Ex CON and Ex IUGR (\( P < 0.05 \) by Holm-Sidak test; Fig. 4A). The HGP AUC in Ex PNGR did not change, whereas it increased by 11% in Ex IUGR + PNGR.

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**Fig. 3. A:** %change in plasma glucose concentration from the “0” time point value during insulin tolerance test is shown in Ex and Sed states in individual graphs (graph 1, CON; graph 2, PNGR; graph 3, IUGR; graph 4, IUGR + PNGR). Holm-Sidak test shows significant response in Ex PNGR, Ex IUGR, and Ex IUGR + PNGR groups (†\( P < 0.01 \), ††\( P < 0.03 \), *\( P < 0.0001 \), **\( P < 0.0004 \)) compared with corresponding Sed group at the same time point. B: AUC for %change in plasma glucose concentration during the insulin tolerance test for all Ex and Sed groups is shown. Two-way ANOVA revealed a significant effect of exercise alone (\( F = 34.793, P < 0.001 \)) and exercise \( \times \) pre/postnatal nutrition (\( F = 3.16, P < 0.03 \)) but not of pre/postnatal nutrition alone (\( F = 2.763, P = 0.057 \)). Holm-Sidak test demonstrated a difference at *\( P < 0.0001 \) between Ex IUGR and Ex IUGR + PNGR vs. their corresponding Sed groups; \( n = 6 \) for all study groups, except \( n = 5 \) each for Sed and Ex PNGR groups alone.

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**Fig. 4. A:** Blood glucose response - insulin tolerance test.
from their sedentary counterparts (Fig. 4A). These findings support improved insulin sensitivity only in the Ex IUGR vs. the Sed IUGR being similar in range to that achieved by the Ex CON vs. Sed CON.

Glucose clearance and hepatic glucose recycling. The resting glucose clearance rate was unchanged after ET (Fig. 4B). Hepatic glucose futile cycling (GFC) increased in Ex IUGR + PNGR compared with the nonexercised state, an adaptation geared toward meeting the glucose requirements. Hepatic glucose recycling in Ex CON, Ex PNGR, and Ex IUGR groups was similar to that of their respective sedentary group.

SKM Studies

AMPK protein concentrations and enzyme activity. The AMPKα1 and AMPKα2 activity was increased in Ex CON 15 min after an acute bout of exercise compared with Sed CON (P < 0.04 and P < 0.01, respectively; Fig. 5A). In contrast, when pAMPK and total AMPK protein concentrations were examined 48–72 h after cessation of the chronic exercise regimen, there was no statistical difference in all experimental groups between Sed and exercised rat SKM (Fig. 5B). Separate and combined AMPKα1 and -α2 enzyme activity in all exercised groups was also not significantly different from that of the sedentary counterparts (Fig. 5C).

Total and subcellular GLUT4 protein distribution. Examination of total GLUT4 protein revealed no differences between Ex and Sed states in all four experimental groups (Fig. 5D). We have previously reported increased SKM basal PM-associated GLUT4 concentrations in Sed IUGR and Sed IUGR + PNGR groups, which was unresponsive to exogenous insulin administration (41). After ET, the basal SKM PM- and LDM-associated GLUT4 protein concentrations in Ex IUGR, Ex PNGR, and Ex IUGR + PNGR groups were no different from each other, being similar to that of Ex CON (Fig. 5E). Insulin-responsive SKM GLUT4 translocation to PM from LDM was observed in Ex IUGR + PNGR (P < 0.05) and Ex PNGR (P < 0.05) groups, the former being more and the latter being less to that seen in Ex CON, but was absent in Ex IUGR (Fig. 5E). Similar to Ex CON, the attaining of insulin-responsive SKM GLUT4 protein translocation from LDM to PM over that not present in the previously reported sedentary counterpart (41) was observed only in the Ex IUGR + PNGR group that overshot the Ex CON group (P < 0.05).

DISCUSSION

Effect of Exercise

The positive effect of exercise on glucose kinetics, GSIR, and skeletal muscle PM GLUT4 association was observed in the CON group as anticipated. Most animal investigations link IUGR to adult-onset glucose intolerance and tissue-specific insulin resistance, lending credence to the Barker hypothesis (1, 2). The IUGR adult female develops gestational diabetes (7) and poses additional risk of transgenerational inheritance of the insulin-resistant phenotype (9, 39). Therefore, we focused the present study on the critical pregestational phase by initiating submaximal exercise training prior to the appearance of gestational diabetes with the onset of pregnancy (7). Exercise training in IUGR offspring improved insulin sensitivity by suppressing glucose-stimulated insulin production and hepatic glucose production. It also altered baseline skeletal muscle PM GLUT4 concentrations similar to that seen in Ex CON but did not further affect the insulin-responsive PM association of GLUT4.

Epidemiological human studies indicate that premature birth and catchup growth are associated with insulin resistance and higher susceptibility to adult chronic diseases (4, 33, 34). Further poor growth during infancy also predisposes toward glucose intolerance and type 2 diabetes mellitus (6). Contrary to our expectation in the pregestational PNGR group, early exercise training resulted in hyperglycemia with a 20% increase in glucose AUC without perturbations in basal glucose.
EXERCISE IN THE IUGR OFFSPRING

A

AMPK enzyme activity

B

pAMPK/AMPK

C

AMPK enzyme activity

D

GLUT4

E

Insulin Responsive Skeletal Muscle GLUT4 Distribution

- Insulin
- + Insulin
or basal and GSIR compared with the sedentary counterpart and Ex CON. This was despite partial insulin responsiveness of skeletal muscle PM GLUT4 association. Inadequacy of basal energy stores (fat and glycogen) and the superimposed increased metabolic demands of early exercise training may cause a shortfall in replenishing depleted stores. This in turn may cause a dependency on unsuppressed hepatic glucose production to meet the exercise-induced metabolic demands. The resultant hyperglycemia may have decreased hepatic glucose recycling.

Early implementation of exercise training in the Ex IUGR + PNGR group improved insulin sensitivity and maintained eu-glycemia due to the adaptive decrease in GSIR and increase in HGP, GFC, and insulin responsiveness of skeletal muscle PM GLUT4 association (41). Thus early exercise training in the pregestational state may prove protective when exposed to additional metabolic stress as encountered in a pregnant state.

These results indicate that the exercise-induced glucose metabolic adaptations are specifically tailored to the preexisting metabolic phenotype. Although these adaptations ameliorate hepatic insulin resistance in IUGR, they have no effect in PNGR. Thus, when considering exercise regimen in the prevention of T2DM, the lack of an effect in a preexisting PNGR phenotype is a distinct possibility.

Insulin Sensitivity

Benefits of acute exercise and endurance training in human and rat consist of lowered insulin concentrations, improved glucose tolerance, and insulin sensitivity. The latter is achieved by decreasing hepatic glucose production and enhancing skeletal muscle glucose utilization (8, 11, 14, 20, 21, 30). In the adult female IUGR offspring, we have previously observed increased GSIR and diminished HGP during GTT (19). Several studies demonstrate that modest weight loss and physical activity can reduce the risk of diabetes (16, 26, 31). Human studies show that moderate levels of endurance and resistance training protect adult males and females with impaired glucose tolerance from developing type 2 diabetes (26). Furthermore, regular exercise protects adult human males and females born with a low birth weight (16). Similar to these observations, our present results demonstrate beneficial effects of exercise training in the presence of preexisting conditions such as IUGR that are high risk for adult-onset diabetes mellitus. T2DM is a progressive disease where the β-islets fail in the face of increasing insulin resistance, which in turn is linked to obesity and sedentary lifestyle (29). The acquisition of these phento-
typic features can be prevented by regular exercise and physical activity (10, 15, 18, 23, 24, 45). Moderate exercise prior to overt symptoms may preserve pancreatic β-islet cell function and prevent the onset of chronic insulin resistance. Our present results demonstrated enhanced insulin-responsive glucose uptake during insulin tolerance test in all exercised nutrient-restricted groups (Ex IUGR, Ex PNGR, and Ex IUGR + PNGR) compared with their corresponding sedentary counterparts. These responses further support that moderate exercise training prior to developing glucose intolerance or obesity improved insulin sensitivity in the IUGR female offspring.

Anthropometric Effects

Unlike the various adult studies reported, our studies are novel in that they consist of early postweaning exercise training in growing animals that have not yet developed insulin resistance, diabetes, or obesity. This early intervention compared with sedentary counterparts did not affect total body weight but had a positive anabolic effect on nose-tail length and certain organ/tissue weights in all four groups, improved glucose tolerance, and decreased GSIR without affecting glucose clearance or recycling. Thus, although an increase in brain and heart weights was observed, more surprising was the increase in abdominal white adipose tissue weight despite improved insulin sensitivity in the Ex CON, Ex IUGR, and Ex IUGR + PNGR vs. the respective sedentary counterparts. These changes may collectively reflect a positive growth-promoting effect of exercise when introduced early in life at a time when cellular growth in different organs/tissues translates into exponential growth and development. Our present observations confirm the positive effects of early exercise training in pre- and postnatal nutrient-restricted rat phenotypes, similar to previous reports in healthy human adults (11, 30).

Skeletal Muscle

Skeletal muscle is the predominant site of insulin- and exercise-dependent glucose disposal. Insulin and exercise have additive effects on skeletal muscle glucose uptake mediated by GLUT4 proteins via differing signaling cascades (25, 35). In this study we implemented a submaximal treadmill exercise regimen that was validated by an increase in skeletal muscle AMPK activity (28, 42). Additionally, exercise training-induced increase in skeletal muscle GLUT4 expression is well described in human and animal studies (8). However, no information exists in the sedentary IUGR and IUGR + PNGR...
regarding the effect of exercise training on reversing preexisting perturbed skeletal muscle subcellular GLUT4 distribution (41). Six weeks of exercise training led to partial reversal in Ex IUGR by bringing basal PM GLUT4 concentration closer to that of Ex CON. Therefore, although this submaximal exercise training improved basal insulin sensitivity of skeletal muscle PM GLUT4 association in Ex IUGR, no further response to exogenous insulin administration was observed. Such disparity in the exercise-induced response of glucose homeostasis was previously described to depend on the severity of the preexisting metabolic perturbation. This includes an example of improved glucose tolerance in a mild state of diabetes (20), with no effect in the severe insulin-deficient state (21). Along similar lines, perhaps exogenous insulin in IUGR has no further effect on chronically exercised skeletal muscle PM GLUT4 concentrations while affecting that of IUGR + PNGR. An alternate explanation may be that, in the IUGR, exercise had a maximal impact with no further room for an added insulin effect. In contrast, the IUGR + PNGR group while exercised still retained some insulin sensitivity of the skeletal muscle GLUT4. At the same time, intertissue differences between the effects of exercise on liver vs. skeletal muscle within the same experimental group (IUGR or IUGR + PNGR) may relate to end-organ specificity and sensitivity. Whether a more intense exercise regimen than the one we employed would demonstrate insulin responsiveness of skeletal muscle PM GLUT4 association in Ex IUGR remains unknown. Since skeletal muscle GLUT4 expression in insulin-resistant obese Zucker rat parallels the intensity of the exercise regimen instituted (12), this is a possibility in the Ex IUGR as well. Total glucose clearance in Ex CON was similar to Ex IUGR, Ex PNGR, and in IUGR + PNGR. These observations support a previous report of unaltered skeletal muscle glucose uptake due to moderate exercise of endurance training in female rats (38).

Summary

Early exercise training in the nutrient-restricted offspring that positively regulates the growth potential impacts tissue-specific adaptations involved in nutritional programming of glucose kinetics. This occurs in a phenotype-specific manner, proving advantageous to the IUGR and IUGR + PNGR, but without affecting the PNGR adult female offspring.

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