Voluntary exercise improves metabolic profile in high-fat fed glucocorticoid-treated rats

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Beaudry JL, Dunford EC, Leclair E, Mandel ER, Peckett AJ, Haas TL, Riddell MC. Voluntary exercise improves metabolic profile in high-fat fed glucocorticoid-treated rats. J Appl Physiol 118: 1331–1343, 2015. First published March 19, 2015; doi:10.1152/japplphysiol.00467.2014.—Diabetes is rapidly induced in young male Sprague-Dawley rats following treatment with exogenous corticosterone (CORT) and a high-fat diet (HFD). Regular exercise alleviates insulin insensitivity and improves pancreatic β-cell function in insulin-resistant/diabetic rodents, but its effect in an animal model of elevated glucocorticoids is unknown. We examined the effect of voluntary exercise (EX) on diabetes development in CORT-HFD-treated male Sprague-Dawley rats (~6 wk old). Animals were acclimatized to running wheels for 2 wk, then given a HFD, either wax (placebo) or CORT pellets, and split into 4 groups: placebo-sedentary (SED) or -EX and CORT-SED or -EX. After 2 wk of running combined with treatment, CORT-EX animals had reduced visceral adiposity, and increased skeletal muscle type Iib/x fiber area, oxidative capacity, capillary-to-fiber ratio and insulin sensitivity compared with CORT-SED animals (all *P < 0.05). Although CORT-EX animals still had fasting hyperglycemia, these values were significantly improved compared with CORT-SED animals (14.3 ± 1.6 vs. 18.8 ± 0.9 mM). In addition, acute in vivo insulin response to an oral glucose challenge was enhanced ~2-fold in CORT-EX vs. CORT-SED (*P < 0.05) which was further demonstrated ex vivo in isolated islets. We conclude that voluntary wheel running in rats improves, but does not fully normalize, the metabolic profile and skeletal muscle composition of animals administered CORT and HFD.

SUSTAINED ELEVATIONS in circulating glucocorticoids (GCs) cause major physiological consequences to the body including severe insulin resistance (60), central obesity (50), nonalcoholic fatty liver disease (23), and hyperglycemia (41). Both rodent (15, 37) and human (13, 18, 41) studies of type 2 diabetes mellitus (T2DM) show a strong correlation between hypercortisolemia and the development of T2DM. Examination of possible therapeutic options to ameliorate GC-induced metabolic conditions is of utmost importance as exogenous GCs are among the most commonly prescribed anti-inflammatory and immunosuppressive medications (56).

Our laboratory has developed an animal model of rapid-onset diabetes induced through treatment with exogenous corticosterone, (CORT, the primary active GC in rodents) and a high-fat diet (HFD, 60% calories from fat) (6, 7, 64). We found that modest elevations in CORT, comparable to peak diurnal levels observed in healthy rodents, when combined with a HFD, result in severe hyperglycemia and hyperglucagonemia, despite hyperinsulinemia and increased pancreatic β-cell mass. These results are largely due to extreme whole body insulin insensitivity and impaired insulin responsiveness to oral glucose challenge (6). Recently, we have also demonstrated that this diabetic phenotype can be corrected by the administration of a nonspecific GC receptor II antagonist, RU486, without modification to the animal’s diet (7). However, RU486 is also highly selective for the progesterone receptor (34) and may present complications with pregnancy and other undesirable side effects. To date, other more selective GR antagonists have failed to completely reverse the diabetic phenotype in GC-treated animals (7). Thus alternative therapies for reducing the metabolic effects of hypercortisolemia are needed.

Regular exercise in diabetic-prone rats can improve glucose homeostasis by increasing whole body insulin sensitivity (39), β-cell function, (37) and by lowering circulating basal (non-stimulated) GC levels (15, 38). Interestingly, in Zucker Diabetic Fatty (ZDF) rats, the prevention of hyperglycemia with regular exercise coincides with the prevention of basal hyperglucocorticoidemia (15). This may indicate that some of the metabolic advantages of exercise in diabetes prevention and treatment occur through a reduction in chronic GC elevation, thereby limiting the GC action at the tissue level. Little is known about the effect of voluntary exercise on a model of exogenously administrated GCs, as acute exercise would not be expected to lower GC concentrations (25). Interestingly, dexamethasone-treated Wistar rats that underwent treadmill training for 8 wk showed restoration of muscle glycogen levels and improved muscular atrophy, despite sustained insulin resistance (4), thereby suggesting exercise training may offer some protection against hyperglucocorticoidemia.

Voluntary exercise normalizes glucose tolerance and islet glucose sensitivity (8) through upregulated mechanisms of skeletal muscle glucose uptake, i.e., GLUT4 translocation (12, 67), oxidative capacity (47) and pancreatic islet function (22). In this rodent model of elevated GCs and high fat feeding, we hypothesized that voluntary exercise would alleviate hyperglycemia, impaired muscle insulin signaling, and skeletal muscle wasting while helping to preserve β-cell function. As elevated GCs are caused by exogenous treatment in our model, voluntary exercise would not be expected to lower GC concentrations sufficiently to promote full metabolic recovery. To examine the metabolic effects of exercise in this rodent model, we compared the effects of ~2.5 wk of voluntary wheel running with sedentary behavior on various markers of energy metabolism including 1) whole body glucose homeostasis,
2) body composition and skeletal muscle tissue histology, 3) in vivo and ex vivo islet responsiveness to glucose challenge, and 4) skeletal muscle atrophic/insulin signaling proteins.

**METHODS**

This study was carried out in accordance with the recommendations of the Canadian Council for Animal Care guidelines and was approved by the York University Animal Care Committee (2013-6).

**Animals.** The first cohort of animals consisted of 40 male Sprague-Dawley rats (Charles River Laboratories, initial mass of 225-250 g, 6 wk postweaned) were individually housed (12:12-h lights on:lights off cycle) after 1 wk of acclimatization to room temperature (22–23°C) and humidity (50–60%-controlled facilities. Animals were initially divided equally into either sedentary (SED) or wheel running exercise (EX) groups (day −14) for a 2-wk habituation phase, and then further subdivided into either CORT- or placebo-treated groups (i.e., 4 groups in total with 10 in each subgroup). Thus the four experimental groups consisted of two sets of EX animals, one group receiving wax pellets (placebo-EX) and the other group receiving CORT pellets (CORT-EX); and two sets of SED animals, one group receiving wax pellets (placebo-SED) and the other group receiving CORT pellets (CORT-SED). A second cohort of animals (n = 20) was utilized to measure incretin levels, muscle histology, and Western blot data. All animals were placed on an ad libitum HFD (see below) following the 2-wk habituation period and were killed between days 17 and 22 to accommodate for islet isolation experiments.

**Experimental design.** A timeline of the experimental protocol is shown in Fig. 1. Each EX animal was placed into specialized rodent cages with 24-h access to a running wheel (Harvard Apparatus), while SED animals were housed in standard cages. Each wheel was equipped with a magnet and sensor that was wired to an electronic counter. Wheel revolutions were counted each time the magnet passed the sensor and the numbers on the counters were recorded and reset to zero daily. The wheel revolutions were collected daily and were multiplied by the wheel circumference (106 cm) to estimate the running distances of each animal (previously reported in 16). During the 2-wk cage habituation period, rodents were given standard rodent chow (Purina chow 5012) and water ad libitum.

**Pellet surgeries.** On day 0, each rat received a subcutaneous implantation of either corticosterone (CORT) pellets (4 × 100 mg; Sigma-Aldrich, cat. no. C2505) or wax pellets (placebo), as previously described (64). Immediately following surgery, rats were placed into standard sterile cages with Chow and water for 24 h to recover and then placed back in their home cages and were given ad libitum access to a HFD and water as previously described (64). This diet consisted of 60% of the total calories from fat and 5.1 kcal/g (D12492, Research Diets); this diet was maintained until the end of the experimental protocol.

**CORT sampling.** As in previously published protocols (6, 23, 64) on day 7, at ~0800, blood samples were collected from each animal via saphenous vein for CORT concentration measurements. A small area of hair on the lateral upper hindlimb was shaved with an electric razor and wiped with a thin layer of petroleum jelly. Once the saphenous vein was located, a sterile 25-gauge needle was used to puncture the vein and whole blood was collected in lithium-heparin-coated microvette capillary tubes (Sarstedt, cat. no. 16.443.100) and centrifuged at ~9,500 g (Eppendorf Mini-Spin Plus, Brinkman Instruments) for 5 min, transferred into polyethylene tubes and stored at −80°C until further analysis of CORT concentrations using a radio-immunoassay kit (MP Biomedical).Fed blood glucose concentrations (mM) were measured on day 7 using a single drop of blood (2 μl) with a handheld glucometer (Bayer Contour), after blood was collected for CORT measurements. This sampling was repeated on day 14 so that CORT concentrations could be assessed 2 wk after pellets were implanted.

**Oral glucose and intraperitoneal insulin tolerance test.** Animals were fasted overnight (16 h) on day 12 and were administered an oral glucose tolerance test (OGTT, 1.5 g/kg body mass) as previously described (64). Each animal’s fasting blood glucose (time 0) was measured via saphenous vein blood bleed (~50 μl) using a sterile needle (25G) and glucometer (Bayer One Touch) and plasma was also collected for later analysis of insulin concentrations via an ELISA 96-well kit (Crystal Chem, cat. no. 90060). Blood glucose was also measured and recorded at 5, 15, 30, 60, 90 and 120 min post oral gavage. Blood was collected to measure plasma insulin for time 15, 30, 60 and 120 min post oral gavage. In separate experiments, total gastric inhibitory polypeptide [also known as glucose-dependent in-
sulnatriotrophic polypeptide (GIP) and glucagon-like peptide 1 (GLP1) levels were measured at time 0 and 10 min post glucose gagage and analyzed using ELISA kits (Millipore, cat. no. E2RMGIP-55K) and (Meso Scale Discovery, cat. no. K1501VC-1, version 2), respectively. An insulin tolerance test (ITT) was performed on day 16 after an overnight fast by intraperitoneal injection injection (0.75 units/kg body mass) as previously reported (64). For these tests, ~50 μl of blood was collected to measure glucose concentrations with a glucometer at time 0 and 5, 10, 20, and 30 min post injection. Nonesterified fatty acid (NEFAs) concentrations were measured from overnight fasted plasma collections, collected before OGTT (NEFA kit, HR Series NEFA-HR, Wako Chemicals). Glucose and insulin area under the curve (AUC) was measured relative to the lowest fasting glucose and insulin levels of a placebo-SED animal. The acute insulin response (AIR) was determined between the difference in basal plasma insulin (fasting insulin levels) and 15 min following the oral glucose gagage (previously reported in Refs. 6, 32). This measurement represents the secretion of insulin in response to an exogenous glucose load (32). Homeostatic Model Assessment for β-cells (HOMA-β) as previously reported in (6, 64) was calculated based on the following equation: $20 \times \text{insulin (μU/ml)/glucose (mM)} - 3.5$ (Ref. 68).

Glucose-stimulated insulin secretion (GSIS) experiments. All animals were killed by decapitation between days 17 and 22 of the experimental protocol, in a counterbalanced fashion, and islet isolations were carried out as previously reported (6). Collagenase pancreas digestion was followed by Histopaque-1077 (H8889, Sigma-Aldrich, Canada) pellet suspension followed by resuspending in Krebs buffer (125 mM NaCl, 4.7 mM KCl, 1.2 mM, 5 mM NaHCO3, 2.5 mM CaCl2, 2.4 mM MgSO4, 10 mM HEPES, 0.5% BSA, pH 7.4). Islets were hand selected and cultured in filtered RPMI buffer with 1% BSA and 0.1% streptomycin for 24 h at 37°C, 5% CO2. Islets were separated into a 12-well culture plate (6–10 islets/well in 3 batches) and given a 30-min preincubation period as previously described (6). Islets were given fresh Krebs buffer with 2.8 mM glucose + 0.1% BSA for 1 h at 37°C, 5% CO2. Media was changed to Krebs buffer with 16.7 mM glucose + 0.1% BSA for 1 h at 37°C, 5% CO2. Immediately following each incubation period, media were collected, centrifuged, and stored at −20°C for further analysis. At the termination of experiments, islets were placed in 1 ml cold lysis buffer (acid-ethanol solution), sonicated (15 s), and centrifuged at ~13,500 g for 10 min. Supernatant was collected and stored at −20°C until further analysis of insulin content. All insulin secretion data were normalized to total insulin content. Insulin was measured using radioimmunoassay kit (Millipore).

Histology. Liver and skeletal muscle tissue (tibialis anterior, TA) from euthanized animals was embedded in tissue freezing medium, frozen in liquid nitrogen, cryosectioned (10 μm thick), and stained with Oil Red O (ORO) for neutral lipid content as previously described (40). Staining for fiber type, SDH activity and capillary-to-fiber ratio (48) was also completed.

**ORO staining.** Liver and TA sections were fixed with 3.7% formaldehyde for 1 h at room temperature while an ORO solution composed of 0.5 g ORO powder (Sigma-Aldrich) and 100 ml of 60% triethyl phosphate (Sigma-Aldrich) was mixed and filtered. Following fixation in 3.7% formaldehyde, slides were immersed in filtered ORO solution for 30 min at room temperature. Slides immediately underwent five washes with ddH2O, were allowed to dry for 10 min, and were stained with Perlmount (Sigma-Aldrich). Liver and TA images were acquired at 20× and 10× magnifications, respectively, using a Nikon Eclipse 90i microscope (Nikon) and Q-imaging MicroPublisher 3.3 RTV camera with Q-capture Software.

**Fiber type and SDH analysis.** To identify skeletal muscle fiber type a metachromatic myosin ATPase stain was performed on cross-sections of the TA muscle using a modified protocol (48). Sections were preincubated in an acidic buffer (pH = 4.25) to differentially inhibit myosin ATPases within the different fiber types. In this protocol, type I fibers appear dark blue, type IIa appear very light blue, and type IIb and IIx are not apparent from each other and are thus classified as IIbX. Type IIb fibers appear bluish-purple. A representative image of the TA for each group was acquired for analysis. Succinate dehydrogenase (SDH) activity was then assessed using histochemical analysis and expressed in relative optical density to placebo-SED (63). The same muscle regions of the TA that were used for fiber typing were used for SDH activity determination. Serial sections were used to directly compare levels of SDH in each fiber with each fiber type. SDH activity was assessed with Adobe Photoshop CS6, converted to grayscale, and reported as the average optical density (sixty fibers were counted per muscle section). The grayscale is evaluated on a range of completely black (set as zero units) to white (set at 255 units). All images were acquired with a Nikon Eclipse 90i microscope and Q-Imaging MicroPublisher with Q-Capture software at 10× magnification.

**Capillary-to-fiber analysis.** To determine capillary-to-fiber ratio, TA sections (10 μm thick) were fixed with 3.7% paraformaldehyde prior to being stained with fluorescein isothiocyanate-conjugated Grifonia simplicifolia isoflcitin B4 (1:100; Vector Laboratories). Sections were viewed using a Zeiss M200 inverted microscope with a 20× objective, and images were captured using a cooled CCD camera using Metamorph imaging software. Capillary-to-fiber counts were averaged from 5–7 independent fields of view per animal by a blinded observer.

**Western blot analysis.** We quantified protein expression for key determinants of skeletal muscle insulin signaling including total IRS-1, total AKT, pAKT (T308), GLUT4, FOXO1, and PGC1α to assess the atrophic and mitochondrial biogenesis pathways. Western blot analysis was carried out according to previously published work (6). In brief, 50 μg of protein lysate from the TA was run on a 10% [total AKT, pAKT (T308), GLUT4, FOXO1] or 6% (total IRS-1 and PGC1α) SDS-page gel, and proteins were transferred to a PVDF membrane (Bio-Rad). Membranes were blocked in 5% powdered milk and Tris-buffered saline with Tween 20 at room temperature for 1 h. Membranes were then incubated overnight at 4°C with their respective primary antibodies (total AKT, 1:1,000, ab47610, Abcam, Toronto, Ontario, Canada; T308, 1:1,000, ab5626, Abcam; GLUT4, 1:1,000, ab65267, Abcam; FOXO1, 1:1,000, cat. no. 2880, Cell Signaling; PGC1α, 1:1,000, cat. no. 4259, Cell Signaling; total IRS-1, 1:1,000, cat. no. 2382, Cell Signaling). The following morning, the membranes were washed with TBST and incubated with anti-mouse (1:10,000, cat. no. ab6789, Abcam) or anti-rabbit (1:10,000, cat. no. ab6721, Abcam) secondary antibodies for 1 h at room temperature. Membranes were then washed and imaged. Images were detected on a Kodak In vivo FX Pro imager and molecular imaging software (Carestream Image MI SE, version S.0.2.3.0, Rochester, NY) was used to quantify protein content. GAPDH (1:10000, ab9484, Abcam) was used as a loading control for all proteins.

**Statistical analysis.** All data are represented as means ± SE, with a criterion of $P < 0.05$ and $P < 0.001$, and were assessed as stated using two-way ANOVAs as a means of statistical significance. Individual differences were calculated using Bonferroni’s post hoc test unless stated as using a Duncan’s post hoc test (Statistica 6.0 software).

**RESULTS**

**Body mass.** Relative body mass was reported between 0 and 10 days as metabolic profiling (OGTT, ITT) of these animals in a fasted state was performed on days 11–17. All animals had similar body mass prior to pellet surgery (day 0, ~350 g). Both placebo-treated groups gained mass after pellet surgery from day 0 to 10 (Fig. 2A). However, on experiment termination day (days 17–22) placebo-SED animals tended to weigh more than placebo-EX animals (472.7 ± 14.4 vs. 438.7 ± 31.5 g). Similar to previously reported findings (6, 7, 23, 64), both CORT-treated groups lost body mass after pellet surgery compared with placebo.
were expressed relative to day −2 (i.e., before surgery), CORT treatment, per se, did not appear to influence running distances significantly (Fig. 2C).

Plasma CORT, fasting NEFAs, glucose, and insulin concentrations. As expected, CORT levels measured at ~0800 on day 7 were higher in all CORT-treated groups compared with placebo-treated animals (P < 0.05, Table 1) and similar to our previously published results (64). Placebo-SED animals had the lowest CORT levels compared with all other animal groups (P < 0.05). Levels measured at ~0800 on day 14 were also higher in CORT-treated groups compared with placebo-SED and not significantly impacted by exercise in the CORT-treated groups. On day 14, CORT levels in placebo-EX animals were higher than in placebo-SED animals and similar to what was observed in the CORT-SED group, but still lower than the CORT-EX animals (P < 0.05, Table 1). Fasted NEFA levels were higher in CORT-treated animals compared with placebo animals (P < 0.05, Table 1), while CORT-EX animals tended to have lower fasting NEFAs than CORT-SED animals. Placebo-SED animals tended to have higher fasting NEFAs than placebo-EX animals, although this difference failed to reach statistical significance.

Fasting blood glucose levels prior to oral glucose challenge were ~4-fold higher in CORT-SED animals than in placebo-SED (P < 0.05, Table 1). CORT-EX animals had lower fasting blood glucose levels compared with CORT-SED animals, although concentrations were still significantly higher compared with placebo-treated groups (P < 0.05, Table 1). CORT-SED animals had ~5.4-fold increase in fasting plasma insulin levels compared with placebo-SED, and CORT-EX animals had ~8-fold increase in fasting plasma insulin levels compared with placebo-EX animals (P < 0.05, Table 1). CORT-EX animals also tended to have higher fasting plasma insulin levels than CORT-SED animals, although these values did not achieve statistical significance.

Oral glucose tolerance test. CORT-SED animals had markedly elevated blood glucose levels before and during the oral glucose tolerance challenge compared with all other treatment groups (P < 0.05, Fig. 3A). CORT-EX animals had improved glucose tolerance compared with CORT-SED animals (P < 0.05); however, they still were glucose intolerant compared with the two placebo groups (P < 0.001, Fig. 3A'). Interestingly, placebo-EX rats had similar fasting glucose and glucose tolerance as placebo-SED rats, suggesting that exercise failed

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Table 1. CORT, fasted NEFAs, glucose, and insulin concentrations

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<th>Placebo</th>
<th>CORT</th>
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<tr>
<td></td>
<td>SED</td>
<td>EX</td>
</tr>
<tr>
<td>Day 7 CORT</td>
<td>66.0 ± 17.1</td>
<td>271.3 ± 42.9*</td>
</tr>
<tr>
<td>Day 14 CORT</td>
<td>70.6 ± 12.3</td>
<td>375.0 ± 44.7*</td>
</tr>
<tr>
<td>Fasted NEFAs</td>
<td>0.45 ± 0.1</td>
<td>0.39 ± 0.9</td>
</tr>
<tr>
<td>Fasted glucose</td>
<td>4.9 ± 0.3</td>
<td>5.1 ± 0.2</td>
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<tr>
<td>Fasted insulin</td>
<td>1.24 ± 0.3</td>
<td>1.1 ± 0.5</td>
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All values are means ± SE in ng/ml for cortisone (CORT) and insulin. Values are in mM for glucose and nonesterified fatty acids (NEFAs). *Mean was statistically significant from placebo-sedentary (SED). #Significance from CORT-SED. *Significance from placebo-exercised (EX). All analysis was carried out by a two-way ANOVA with a Tukey’s post hoc, where P < 0.05; n = 7–12.
Fig. 3. Glucose tolerance (A and A’), insulin secretion (B and B’), acute insulin responses (AIR, C and D), and plasma gastric inhibitory polypeptide (GIP) and glucagon-like peptide 1 (GLP1) levels during 0 and 10 min of oral glucose tolerance test (OGTT) (E and F). Blood glucose levels were elevated with CORT-SED treatment ($P < 0.001$) and voluntary wheel running decreased glucose intolerance in CORT-treated animals. Plasma insulin levels post oral glucose challenge were elevated with CORT treatment compared with placebo-treated animals and exacerbated with the addition of wheel running ($P < 0.01$); $n = 7–12$ for all above graphs. AIR between basal and 15 min post exogenous glucose challenge was elevated with EX-treated and reduced in SED-treated animals ($n = 6–12$). Higher fasting plasma insulin concentrations were correlated with higher fasting blood glucose concentrations in both CORT-EX and SED groups ($r^2 = 0.47$, $P < 0.0001$); $n = 6–12$. CORT animals had higher fasting and stimulated GIP and GLP1 levels than placebo groups; $n = 4–5$. Statistically significant difference *from placebo groups and **from CORT-SED ($P < 0.05$) using a two-way ANOVA with Bonferroni’s (A’, B’, E, and F) and Duncan’s (C) post hoc tests. All values are means ± SE except in D, where values are individual.
to improve glucose homeostasis in these high-fat fed animals. Plasma insulin concentrations were highest in CORT-EX animals before and during the oral glucose tolerance challenge compared with all treatment groups \((P < 0.001, \text{Fig. 3B})\). CORT-SED animals also had increased levels of plasma insulin at baseline, but levels failed to respond to oral glucose challenge \((P < 0.01, \text{Fig. 3B'})\). Acute insulin response (AIR) to oral glucose gavage was measured to determine the islet sensitivity to exogenous glucose challenge \((\text{Fig. 3C})\). CORT-SED animals had a negative AIR to glucose challenge, indicating impaired islet responsiveness \((P < 0.05)\), as has been recently demonstrated in this model \((6)\). Voluntary wheel running in both placebo- and CORT-treated animal groups increased AIR by ~2-fold above placebo-SED animals \((P < 0.05)\). To determine the influence of basal blood glucose levels on plasma insulin concentrations in each treatment group, these parameters were plotted against each other \((\text{Fig. 3D})\). The \(r^2\) with all groups included was 0.47 and there was a significant correlation relationship \((P < 0.0001)\) suggesting that higher blood glucose levels were driving higher insulin levels in the CORT-treated animals. It should be noted that fasting glucose and fasting plasma insulin values in the placebo animals did not deviate markedly, clustering at around 3.5–5.5 mM of glucose and less than 4 ng/ml of insulin, while the CORT treated animals had much higher (and wider) ranges in both fasting glucose and insulin levels \((\text{Fig. 3D})\). Total GIP and GLP1 plasma levels were measured at 0 and 10 min during a separate OGTT to determine differences in insulin secretion among treatment groups \((\text{Fig. 3, E and F})\). Both incretins tended to be elevated in CORT-treated animals compared with placebo animals. However, there were no differences found in GIP or GLP1 levels between CORT-EX and SED groups at 0 or at 10 min post gavage.

**In vivo assessment of \(\beta\)-cell function.** HOMA-\(\beta\)-cell function, as measured by HOMA-\(\beta\), was increased with voluntary wheel running in placebo and CORT-treated animal groups. CORT-SED group had higher insulin secretion in low \((2.8 \text{ mM})\) and high \((16.7 \text{ mM})\) glucose media compared with placebo-treated groups. Voluntary exercise normalized GSIS in CORT-treated animals; \(n = 3–7\) per group. Insulin sensitivity measured by the response to an insulin bolus was improved with voluntary wheel running in CORT-treated animals \((P < 0.05)\); \(n = 7–12\). Statistical significance *from placebo-SED and **from CORT-SED \((P < 0.05)\) using two-way ANOVA with Duncan’s \((B)\) and Bonferroni’s \((C)\) post hoc tests. All values are means \(\pm\) SE.

Islets were isolated from each animal upon euthanization \((\text{days 17–22})\) and challenged with low \((2.8 \text{ mM})\) and high \((16.7 \text{ mM})\) glucose containing media. We found that in both low and high glucose media, CORT-SED islets had higher relative glucose-stimulated insulin secretion (GSIS) than all the other groups \((P < 0.05, \text{Fig. 4B})\). CORT-EX tended to

![Fig. 4. Beta-cell function (A) as measured by HOMA-\(\beta\), glucose stimulated insulin secretion (GSIS) (B), and insulin sensitivity (C and C'). HOMA-\(\beta\), an indicator of \(\beta\)-cell function, was increased with voluntary wheel running in placebo and CORT-treated animal groups. CORT-SED group had higher insulin secretion in low \((2.8 \text{ mM})\) and high \((16.7 \text{ mM})\) glucose media compared with placebo-treated groups. Voluntary exercise normalized GSIS in CORT-treated animals; \(n = 3–7\) per group. Insulin sensitivity measured by the response to an insulin bolus was improved with voluntary wheel running in CORT-treated animals \((P < 0.05)\); \(n = 7–12\). Statistical significance *from placebo-SED and **from CORT-SED \((P < 0.05)\) using two-way ANOVA with Duncan’s \((B)\) and Bonferroni’s \((C)\) post hoc tests. All values are means \(\pm\) SE.](image-url)
have lower relative GSIS levels in low glucose media compared with the CORT-SED group and had significantly lower GSIS in the high glucose media compared with the CORT-SED group.

An insulin tolerance test was conducted in vivo to measure insulin sensitivity primarily in the skeletal muscle (Fig. 4C). CORT-SED animals had the highest glucose AUC levels compared with all treatment groups \((P < 0.05\), Fig. 4C\). Voluntary wheel running reduced glucose AUC in CORT-EX animals indicating improved peripheral insulin sensitivity \((P < 0.05)\).

**Food intake and body composition.** As expected, relative food intake tended to be slightly higher in placebo-EX animals than placebo-SED animals, although it was not significant \((P > 0.05\), Table 2\). There was no significant difference in relative food intake between CORT-SED and -EX animals; however, there was a main effect of pellet treatment across groups with the CORT-treated animals having a higher relative food intake than placebo-treated animals \((P < 0.05)\). Visceral adiposity, as measured by relative epididymal fat pad mass, greatly increased in CORT-treated animals but was lower in CORT-EX compared with CORT-SED group \((P < 0.05, \text{Table}\ 2)\). There were no differences in visceral adiposity between placebo-SED and placebo-EX groups. Relative liver mass was increased with CORT treatment compared with placebo-treated animals \((P < 0.05)\), but no differences were found with the addition of exercise in placebo or CORT-treated animals \(\text{Table}\ 2\). Relative gastrocnemius mass was lower with CORT-SED treatment compared with placebo-treated animals \((P < 0.05)\) and tended to be higher with CORT-EX treatment. No differences were found with relative TA mass between any treatment groups. Relative soleus mass, a marker of oxidative muscle mass, was higher in CORT-SED animals compared with placebo-EX animals \((P < 0.05)\), but no differences were found with the addition of exercise in placebo or CORT-treated animals \(\text{Table}\ 2\). Relative epitrochlearis mass was a marker of glycolytic muscle mass, was significantly lower in CORT-SED animals compared with placebo-SED animals. CORT-EX animals had higher relative epitrochlearis mass compared with CORT-SED animals, with values similar to those observed in the placebo-treated groups \((P < 0.01, \text{Table}\ 2)\).

**Muscle lipid staining.** ORO staining of TA muscle sections was used to visually examine lipid accumulation in all treatment groups \(\text{Fig.}\ 5\). Lipid staining appeared to be higher in placebo-EX animals compared with placebo-SED animals, as expected since endurance exercise generally increases muscle lipid storage in healthy rats. CORT-treated animals had increased lipid staining compared with placebo-treated animals, indicating more lipid accumulation in the muscle, and no obvious differences were observed between CORT-SED and CORT-EX groups.

**Table 2. Food intake and body composition**

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<tr>
<td></td>
<td>SED</td>
<td>EX</td>
</tr>
<tr>
<td>Food intake</td>
<td>0.25 ± 0.02</td>
<td>0.30 ± 0.02</td>
</tr>
<tr>
<td>Epididymal fat</td>
<td>17.0 ± 1.0</td>
<td>17.0 ± 1.6†</td>
</tr>
<tr>
<td>Liver</td>
<td>37.4 ± 1.6</td>
<td>37.7 ± 1.6</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>4.7 ± 0.2</td>
<td>5.4 ± 0.5</td>
</tr>
<tr>
<td>Tibialis anterior</td>
<td>1.5 ± 0.04</td>
<td>1.6 ± 0.20</td>
</tr>
<tr>
<td>Soleus</td>
<td>0.40 ± 0.01</td>
<td>0.45 ± 0.1</td>
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<tr>
<td>Epitrochlearis</td>
<td>0.14 ± 0.01</td>
<td>0.17 ± 0.02</td>
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Values are means ± SE in kcal/g of body mass per day for food intake and g tissue/kg of body mass. *Mean was statistically significant from placebo-SED. †Significance from CORT-SED. #Significance from placebo-EX. All analysis was carried out by a two-way ANOVA with a Tukey’s post hoc, where \(P < 0.05;\ n = 7–12\).
Muscle oxidative capacity, PGC1α protein, capillary-to-fiber ratio, cross-sectional area, and FOXO1 protein levels. To determine the effects of exercise training, the TA muscle of representative animals from each treatment group was stained for SDH activity (Fig. 6, A and B). SDH is an oxidative complex bound to the inner mitochondrial membrane of skeletal muscle cells and is responsible for oxidizing succinate to fumarate in the citric acid cycle (31). Strong SDH staining correlates with higher muscle fiber oxidative potential. The TA from both placebo-EX and CORT-EX treatment groups showed darker SDH staining compared with placebo-EX and CORT-SED muscles (P < 0.001, Fig. 6, A and B). In type I and IIa muscle sections, CORT-SED animals had lighter SDH staining than all other treatment groups (P < 0.05, Fig. 6, A and B). PGC1α is a known protein marker for mitochondrial biogenesis (70) and was measured to help explain the observations of impairments in SDH staining in CORT-SED animals. Although data failed to reach statistical significance, PGC1α tended to be elevated in both EX groups compared with their respective SED groups (Fig. 6C).

CORT-treatment alone causes significant reduction in capillary-to-fiber ratio within skeletal muscle (61). Capillary-to-fiber ratio was reduced in CORT-SED animals but normalized in CORT-EX animals compared with placebo-treated animals (Fig. 6, D and E). Cross-sectional area, indicating individual muscle fiber size, was lowered in type IIa and IIb/x muscle fibers from the TA in CORT-SED animals (Fig. 6, C and D) and tended to increase with CORT-EX treatment. CORT treatment is known to induce atrophic signaling pathway leading to muscle wasting (42). Therefore, FOXO1 protein levels were measured as an indicator of muscle degradation. CORT treatment alone increased FOXO1 protein content (Fig. 6H, P < 0.05); however, EX failed to normalize FOXO1 levels.

Insulin signaling and glucose metabolic protein levels. Regular exercise is known to stimulate skeletal muscle

Fig. 6. Muscle oxidative capacity as measured through succinate dehydrogenase (SDH) histology (A and B), PGC1-alpha protein content (C), capillary-to-fiber ratio (D and E), cross-sectional area (CSA) in I, IIa, and IIb/x muscle fiber types (F and G), and FOXO1 protein content (H). Muscle oxidative capacity was increased in all placebo- and CORT-EX muscle types; n = 6. In type I and IIa muscle sections, CORT-SED had lower oxidative capacity. PGC1-alpha tended to be higher in EX treated animal groups compared with SED groups; n = 5. CORT-SED treatment resulted in a significant reduction in capillary-to-fiber ratio compared with all treatment groups and CORT-EX animals exhibited greater capillary-to-fiber ratio compared with CORT-SED animals (P < 0.05, n = 6–9), demonstrating that CORT-induced capillary rarefaction is prevented by volitional exercise. This assay stained for capillaries via *Griffonia Simplicifolia* isoelectin-fluorescein and image color was inverted to enhance muscle fiber visualization. CSA tended to normalize with exercise in CORT-treated animals in IIb/x muscle fibers. FOXO1 protein content increased with CORT-SED and EX treatment; n = 4–5. Statistical significance *from placebo-SED and **from CORT-SED (P < 0.05) using two-way ANOVA with Bonferroni’s post hoc test. All values are means ± SE.
insulin signaling (29); therefore protein markers of these pathways were analyzed. Voluntary wheel running increased total IRS-1 protein content in placebo animals by ~2-fold and tended to increase total IRS-1 in CORT-EX animals compared with CORT-SED animals but no differences in AKT phosphorylated T308 protein content were found among groups. No differences were found in GLUT4 protein content between the treatment groups; \( n = 4\)–5. *Statistical significance from CORT-SED (\( P < 0.05 \)) using two-way ANOVA with Bonferroni’s post hoc test. All values are means ± SE.

Liver lipid staining. CORT-treated animals increased liver ORO staining compared with placebo-treated animals, indicating more hepatic lipid accumulation (Fig. 5). There were no differences in ORO staining between CORT-SED and -EX groups as well between placebo-SED and -EX groups.

DISCUSSION

The effect of voluntary wheel running was assessed to determine if exercise has the ability to reduce, or offset, the detrimental metabolic outcomes typically observed in rodent models of exogenous GC treatment. In this study, we demonstrate that voluntary exercise (for ~2.5 wk) reduced visceral adiposity, glucose intolerance, insulin insensitivity, and impaired glucose responsiveness. Furthermore, many skeletal
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muscle specific abnormalities such as oxidative capacity, atrophy and capillary rarefaction associated with sustained GC treatment were improved with voluntary exercise. These novel findings suggest that regular exercise is able to promote healthier metabolic function, even in situations of hypercortisolemia, i.e., Cushing’s syndrome or patients treated with exogenous GCs.

Generally, GCs decrease absolute body mass but increase relative fat mass, an outcome that appears to be dependent upon animal model/type, dosing, and duration of treatment (19, 35, 36, 46, 53, 71). Much of the loss in total mass is speculated to be due to reductions in skeletal muscle mass, although body length (or height) is also reduced if GCs are elevated in young growing animals (64) or in human adolescence (1). In this study, and in our previously published studies (6, 7, 61, 64), we found that CORT treatment induces a dramatic (~50%) reduction in total body mass compared with placebo-treated animals, and this effect was not mitigated by a high-fat diet. Normally, voluntary wheel running in healthy young animals lowers body mass gain (16, 25, 71) and reduces adiposity in obesity-prone animals (9, 49). However, in our study, voluntary exercise did not cause a further reduction in body mass nor did it prevent the body mass loss that is typically observed in CORT-treated animals. Indeed, both CORT-SED and -EX-treated groups had similar body masses throughout the 10 days of treatment (Fig. 2A), although it was obvious that the exercising animals had much less adipose mass and greater skeletal muscle mass (i.e., altered body composition) compared with the CORT-SED animals (Table 2). Some evidence indicates that the lower body mass observed in GC-induced models is the result of reduced food intake (54). Others suggest that GCs increase the preference for foods with high-fat content, overriding the anorexigenic effects of insulin (20) and leading to larger fat accumulation. We found that CORT-treated animals, regardless of being SED or EX, consumed more calories relative to body mass than the placebo-treated animals (~1.5-fold change, Table 2). Although exercise improved body composition (decreased adiposity and increased skeletal muscle mass) in the CORT-treated animals, possibly due to greater running distances than placebo animals (Fig. 2), the growth impairments and changes in eating behavior accompanying CORT treatment were seemingly unaffected by exercise.

Exercise training and adaptations to restraint stress reduce hyperglucocorticoidemia and prevent diabetes development in ZDF rats (5, 37) and in other insulin-resistant rodent models (45). In this study, and in our previously published work (6, 64), we show that elevated GCs induce severe glucose intolerance, hyperinsulinemia, impaired β-cell function, and severe insulin resistance. Here, we show that daily voluntary wheel running, for ~2.5 wk, improves glucose intolerance and insulin responsiveness to oral glucose challenge in GC-treated high-fat fed rats (Fig. 3). However, increased fasting and stimulated insulin levels were observed. Compared with other published work on dexamethasone treatment and treadmill exercise (4), we found that CORT-EX animals had elevated fasted and stimulated insulin levels during the glucose challenge compared with all other treatment groups (Fig. 3B).

In an attempt to explain the higher levels of circulating insulin in CORT-treated animals, we measured plasma incretin hormone (GIP and GLP1) levels, which are known to stimulate insulin secretion upon glucose ingestion (14) and are also known to be elevated with CORT treatment (33). In our study, CORT treatment increased GIP and GLP1 levels in both the basal and glucose-stimulated states, but there were no definitive distinctions found between SED and EX treatments. As such, we conclude that the improvements in insulin responsiveness to oral glucose feeding in the CORT-exercising animals cannot be attributed to differences in incretin secretion. However, similar to our observation of increased GIP and GLP-1 with CORT treatment in general, others have shown increased fasting insulin levels after 11 wk of swim training in ZDF rats compared with SED and lean controls, results which were paralleled by increases in β-cell mass, through increased β-cell proliferation (hyperplasty and hyperplasia) mechanisms (37). Although β-cell mass was not measured directly in this study, we do demonstrate greater AIR and HOMA-β levels with CORT exercise treatment (Fig. 4), suggesting that our exercise model was sufficient to improve β-cell function possibly via proliferation or β-cell-specific pathways.

We have recently shown that CORT-SED treatment impairs β-cell responsiveness to glucose in vivo but not ex vivo (6). Regardless of exercise protocol, endurance training (swimming, voluntary wheel running, treadmill running) results in reduced insulin release in isolated healthy rodent islets (2, 26, 66, 73, 74) and more specifically in the β-cell itself (24). Our findings are in line with these previous observations, since placebo-EX animals tended to have lower GSIS compared with placebo-SED islets (Fig. 4). In addition, voluntary wheel running (6 wk) improves GSIS in ZDF rat islets as well as prevents complete depletion of insulin stores within the islet (22). Our study is the first to show that voluntary exercise lowers relative GSIS in CORT-treated animals; however, it does not normalize to that of placebo-treated SED animals. Our results differ somewhat from those found in active ZDF rats, as GSIS was elevated in high glucose and palmitate media compared with inactive ZDF rat islets (22). This may be because ZDF rats have larger compensatory β-cell mass in response to exercise causing higher insulin levels.

GCs help to regulate adipose tissue by promoting mechanisms of differentiation and ectopic fat distribution (50). In this model of elevated GCs and HFD, there is an excessive accumulation of visceral fat that is ~1.5-fold higher in CORT-SED animals compared with placebo treatment (Table 2), which parallels previous work (10, 17, 55, 64). Exercise is known to help reduce fat mass gain (21, 28, 65) even in instances of high-fat feeding (27, 28). In the present study, we demonstrate that voluntary wheel running is sufficient to attenuate visceral fat accumulation in CORT/HFD-treated animals, but not muscle or hepatic neutral lipid accumulation (Fig. 5, A and B, respectively). Perhaps this may explain why we demonstrate some relief from GC-induced abnormalities with exercise but not complete reversal of the various features of insulin resistance and glucose intolerance.

GCs are well known to induce muscle atrophy (46), likely through negative protein balance due to increased protein degradation and decreased protein synthesis (30). Both GCs and diabetes advance muscle atrophy by targeting fast-twitch (type II), glycolytic muscle (57), and reducing muscle mitochondrial content (43). In this model of CORT/HFD, we found lower skeletal muscle oxidative capacity, as measured by SDH staining, in type I and type II fibers of the CORT-SED animals.

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(Fig. 6, A and B). This impaired oxidative capacity, along with the obvious muscle atrophy and capillary rarefaction, likely contributes to the poor glycemic control and skeletal muscle lipid accumulation in these animals. Similar to previous findings (47), we found that regular exercise improved muscle oxidative capacity in all fiber types of the TA muscle. We suspected that these exercise-mediated improvements could be caused by upregulation of PGC1α, a transcription factor that turns on oxidative metabolic proteins that contribute to greater mitochondrial biosynthesis (51) and slow-twitch (i.e., type I) muscle fiber formation (44). We found that EX treatment tends to increase PGC1α protein levels in the TA muscle indicating that greater levels of oxidative capacity may be due to elevated mitochondrial biosynthesis and possibly more slow-twitch fiber recruitment, helping to utilize higher levels of plasma glucose. In addition, it is a continued observation in our model that relative soleus muscle mass increases with CORT and HFD treatment (Table 2). The soleus muscle is composed of slow-twitch fibers that are less sensitive than fast-twitch fibers under conditions of atrophy (reviewed in 59), and increased compensation by the soleus muscle may result. Interestingly, CORT-EX treatment normalized soleus muscle mass, indicating decreased compensation, possibly elicited by the activation of oxidative muscle fiber types with voluntary exercise.

One of the most novel aspects of this study was the finding that CORT-EX treatment was able to induce angiogenesis by increasing the number of capillaries in a given area of muscle thereby evading CORT-induced capillary rarefaction, which is a hallmark of GC treatment (61). The increased capillarization suggests that exercise improves nutrient and insulin delivery to skeletal muscle in GC-treated animals, and that this adaptation may contribute to the augmentation in glucose tolerance and muscle insulin sensitivity detected in these animals. In line with this, we also observed a tendency for improved cross-sectional area in the type Ibx/x fibers of the TA muscle with regular exercise and CORT treatment. While it is still not known why GCs target type Ibx/x fibers, higher GC receptor expression in these fibers may be a likely mechanism (62). Indeed, skeletal muscle-specific GC receptor knockout animals are resistant to GC-induced muscle atrophy (69). However, these knockout studies are not entirely conclusive, as they do not allow determination of exact mechanisms of GC-induced atrophy. Therefore, it remains that either genomic (atrophic genes, i.e., FOXO1) or nongenomic (inhibition of insulin signaling) pathways are likely contributors to muscle wasting with GC treatment. In this study, we confirm increases in FOXO1 protein levels in CORT-treated animals (Fig. 6H); however, unlike previous literature (72) regular exercise was unable to normalize FOXO1 levels. It is possible that although these FOXO1 levels were not normalized in the CORT-EX animals, voluntary wheel running was sufficient to stimulate increased protein synthesis mechanisms (i.e., IGF-1 or mTOR signaling) independent of elevated FOXO1 levels (reviewed in 3). Although IGF-1 was not measured in this study, we have previously measured and found lower plasma IGF-1 levels in CORT-SED animals (unpublished observations) indicating less activation of the growth pathways. We suggest that regular exercise in CORT-treated animals may promote higher levels of IGF-1 overriding protein degradation pathways, such as FOXO1. Moreover, we recognize that we used whole muscle homogenates to measure FOXO1 protein levels, and these methods may not have allowed us to specifically capture true nuclear FOXO1 translocation. The upregulation of nongenomic signaling pathways, such as IRS-1 and AKT, may help to reverse GC-induced muscle atrophy mechanisms and improve glucose intolerance (57). We demonstrate increased protein levels of total AKT, as well as the tendency to increase total IRS-1 protein levels, in CORT-EX animals compared with CORT-SED animals. GCs are known to inhibit PI3K/AKT signaling, which downregulate protein synthesis pathways (58) and increase muscle catabolism, in addition to propagating insulin resistance through impaired GLUT4 translocation (29). Together, we conclude that voluntary exercise is an adequate stimulus to improve skeletal muscle oxidative capacity, capillary-to-fiber ratio, and shows tendencies to reverse muscle atrophy in type Ibx/x fibers in situations of elevated GCs and HFD. These improvements in skeletal muscle composition are most likely mediated via the atrophic mechanisms beyond FOXO1 protein expression, as insulin-signaling proteins (i.e., GLUT4 and IRS-1) remained relatively unaffected.

In summary, we have shown that elevated CORT exposure, in conjunction with a HFD, induces severe hyperglycemia, hyperinsulinemia, and muscle wasting along with impaired insulin response and islet glucose sensitivity, features that can all be attenuated with volitional activity. Our study shows that voluntary exercise attenuates symptoms of T2DM induced by elevated CORT and HFD and provides evidence that exercise can independently influence tissues even when the exercise-associated reductions in basal GC levels are prevented. Exercise intervention may be an ideal form of rehabilitation in individuals with hypercortisolemia or Cushing’s syndrome as these individuals suffer from hyperglycemia, insulin insensitivity, muscular atrophy, and increased central obesity. Therefore, these individuals may benefit from the positive effects of regular exercise that may help to control or attenuate symptoms of diabetes development.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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