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

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ABSTRACT

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 weeks old). Animals were acclimatized to running wheels for 2 weeks, then given a HFD, either wax (placebo) or CORT pellets and split into 4 groups: placebo-SED or -EX and CORT-SED or -EX. After 2 weeks of running combined with treatment, CORT-EX animals had reduced visceral adiposity, increased skeletal muscle type IIb/x fibre area, oxidative capacity, capillary to fibre ratio and insulin sensitivity compared to CORT-SED animals (all p<0.05). Although CORT-EX animals still had fasting hyperglycemia, these values were significantly improved compared to 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.
INTRODUCTION

Sustained elevations in circulating glucocorticoids (GCs) cause major physiological consequences to the body including severe insulin resistance (60) central obesity (50) non-alcoholic 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 weeks, 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 up-regulated mechanisms of skeletal muscle glucose uptake, i.e. GLUT4 translocation (11, 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 signalling 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 weeks of voluntary wheel running with sedentary behaviour 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 signalling 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:* First cohort of animals consisted of forty (40) male Sprague-Dawley rats (Charles River Laboratories, initial mass of 225-250 g, six weeks post-weaned) were individually housed (lights on 12 hour: lights off 12 hour cycle) after one week 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 two week 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 received wax pellets (placebo-EX) and the other group received CORT pellets (CORT-EX) and two sets of SED animals; one group received wax pellets (placebo-SED) and the other group received CORT pellets (CORT-SED). A second cohort of animals (n=20) were utilized to measure incretin levels, muscle histology and western blot data. All animals were placed on an *ad libitum* HFD (see below) following the two-week habituation period and were killed between days 17-22 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-hour 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 two-week 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 x 100 mg; Sigma-Aldrich, Canada, Cat# 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 hour to recover and then placed back in their home cages and were given ad libitum access to a high-fat diet (HFD) and water as previously described (64). This diet consisted of 60% of the total calories from fat and 5.1 kcal per gram (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 hour, blood samples were collected from each animal via saphenous vein for CORT concentration measurements. A small area of hair on the lateral upper hind limb 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, Canada, Cat# 16.443.100) and centrifuged at ~ 9, 500 x g (Eppendorf Mini-Spin Plus, Brinkman Instruments Inc., USA) for 5 minutes, transferred into polyethylene tubes and stored at -80°C until further analysis of CORT concentrations using a radioimmunoassay kit (MP Biomedical, USA). Fed blood glucose
concentrations (mM) were measured on day 7 using a single drop of blood (2 µl) with a handheld glucometer (Bayer Contour, USA), after blood was collected for CORT measurements. This sampling was repeated on day 14 so that CORT concentrations could be assessed two weeks after pellets were implanted.

*Oral glucose and intraperitoneal insulin tolerance test:* Animals were fasted overnight (16 hour) on day 12 and were administered an oral glucose tolerance test (OGTT, 1.5 g/kg body mass) as previously described in (64). Each animal’s fasting blood glucose (time 0) was measured via saphenous vein 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, USA, Cat# 90060). Blood glucose was also measured and recorded for time 5, 15, 30, 60, 90 and 120 minutes post oral gavage. Blood was collected to measure plasma insulin for time 15, 30, 60 and 120 minutes post oral gavage. In separate experiments, total GIP and GLP1 levels were measured at time 0 and 10 min post glucose gavage and analyzed using ELISA kits (Millipore, USA, Cat#EZRMGIP-55K) and (Meso Scale Discovery, MD, USA Cat#K150JVC-1, version 2), respectively. An insulin tolerance test (ITT) was performed on day 16 after an overnight fast by intraperitoneal insulin injection (0.75 units/kg body mass) as previously reported (64). For these tests, ~50 µl of blood was collected to measure glucose concentrations were measured with a glucometer at time 0, 5, 10, 20 and 30 minutes post insulin injection. Non-esterified fatty acid (NEFAs) concentrations were measured from overnight fasted plasma collections, collected before OGTT (NEFA kit, HR Series NEFA-HR, Wako Chemicals, USA). 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 minutes following the oral glucose gavage (previously reported in (6, 32)).
This measurement represents the secretion of insulin in response to an exogenous glucose load (52) Homeostatic Model Assessment for β-cells (HOMA-β) as previously reported in (6, 64) was calculated based on the following equation: 20 x Insulin (μ units·L)/Glucose (mM)-3.5 (68).

Glucose stimulated insulin secretion (GSIS) experiments: All animals were killed by
decapitation between days 17 to 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 re-suspension 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 (Wisent) overnight (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-minute pre-incubation period as previously described (6). Islets were given fresh KREB’s buffer with 2.8 mM glucose + 0.1% BSA for 1 hour at 37°C, 5% CO2. Media was changed to KREBs buffer with 16.7 mM glucose + 0.1% BSA for 1 hour at 37°C, 5% CO2. Immediately following each incubation period, media was 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 x g at 4°C for 10 minutes. Supernatant was collected and stored at -20°C until further analysis of insulin content. All insulin secretion data was normalized to total insulin content. Insulin was measured using radioimmunoassay kit (Millipore, USA).

Histology: Liver and skeletal muscle tissue (tibialis anterior, TA) from euthanized animals were 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 fibre type, SDH activity and capillary to fibre ratio (48) was also completed.

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

**Fibre Type and SDH Analysis:** To identify skeletal muscle fibre type a metachromatic myosin ATPase stain was performed on cross-sections of the TA muscle using a modified protocol (48). Sections were pre-incubated in an acidic buffer (pH = 4.25) to differentially inhibit myosin ATPases within the different fibre types. In this protocol, type I fibres 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 IIb/x. These fibres 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 fibre typing were used for SDH activity determination. Serial sections were used to directly compare levels of SDH in each fibre, to each fibre type. SDH activity was assessed with Adobe Photoshop CS6, converted to greyscale and reported as the average optical density (sixty fibres were counted per muscle section).
greyscale 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 10x magnification.

**Capillary-to Fibre Analysis:** To determine capillary-to-fibre ratio, TA sections (10 µm thick) were fixed with 3.7% para-formaldehyde prior to being stained with fluorescein isothiocyanate-conjugated Griffonia simplicifolia isolectin B4 (1:100; Vector Laboratories, Canada). Sections were viewed using a Zeiss M200 inverted microscope with a 20x objective and images were captured using a cooled CCD camera using Metamorph imaging software. Capillary-to-fibre 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 signalling 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, Canada). Membranes were blocked in 5% powdered milk and Tris-buffered saline with Tween 20 at room temperature for 1 hour. Membranes were then incubated overnight at 4°C with their respective primary antibodies (total AKT, 1:1000, ab47610, Abcam, Toronto, ON; T308, 1:1000, ab5626, Abcam, Toronto, ON; GLUT4, 1:1000, ab65267Abcam, Toronto, ON; FOXO1, 1:1000, Cat #2880, Cell Signaling, USA; PGC1α, 1:1000, Cat #4259, Cell Signaling, USA; total IRS-1, 1:1000, Cat #2382, Cell Signaling, USA). The following morning, the membranes were washed with TBST and incubated with anti-mouse (1:10000, Cat#ab6789, Abcam) or anti-rabbit
(1:10000, Cat#ab6721, Abcam) secondary antibodies for 1 hour 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, New York) was used to quantify protein content. GAPDH (1:10000, ab9484, Abcam, Toronto, ON) 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 0-10 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 to placebo animals (p<0.01, Fig. 2A). No differences were found in relative body mass between CORT-EX and -SED animals on day 10; however, CORT-EX animals had heavier absolute body mass than CORT-SED animals by the termination of treatment (333.5±16.2 vs. 265.9±13.0 g, p<0.05).

Running Distances. On average, CORT-treated runners ran longer distances than placebo runners (6541.7±467 vs. 3308.4±260 m, p<0.05, Fig. 2B). However, this difference was also
noted prior to pellet implant surgery; thereby suggesting that these two groups of rats differed slightly in their preference for wheel running prior to the experimental treatment (CORT vs. wax implantation). When running distances 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 hour on day 7 were higher in all CORT treated groups as compared to 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 to all other animal groups (p<0.05). Levels measured at ~ 0800 h on day 14 were also higher in CORT-treated groups compared to 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 to 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 to CORT-SED animals, although concentrations were still significantly higher when compared to placebo-treated groups (p<0.05, Table 1). CORT-SED animals had ~ 5.4-fold increase in fasting plasma insulin levels compared to placebo-SED and CORT-EX animals had ~ 8-fold increase in fasting plasma insulin levels compared to 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 to all other treatment
groups (p<0.05, Fig. 3A). CORT-EX animals had improved glucose tolerance compared to
CORT-SED animals (p<0.05); however, they still were glucose intolerant when compared to 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 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 to all
treatment groups (p<0.001, 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, Fig. 3B’). Acute
insulin response (AIR) to oral glucose gavage was measured to determine the islet sensitivity to
exogenous glucose challenge (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 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 (Fig. 3D). The r² with all groups included was 0.47 and there was a
significant correlation relationship (p<0.0001) suggesting that higher fasted blood glucose
concentrations were driving higher insulin levels in the CORT-treated animals. It should be
noted that fasting blood 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 (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 amongst treatment groups (Fig. 3E and F). Both incretins tended to be elevated in CORT-treated animals, compared to 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 β-cell function.** HOMA-β was used to calculate basal β-cell function where higher HOMA-β scores indicate greater β-cell function (Fig. 4A). No significant differences in HOMA-β levels were found between CORT-SED and placebo-SED groups, although values tended to be higher in the CORT-SED group compared to the placebo-SED group (p=0.1481). Voluntary wheel running improved β-cell function in CORT animals by ~ 2.5-fold compared to CORT-SED groups (p<0.05, Fig. 4A). It also increased β-cell function in placebo-EX animals compared to placebo-SED animals by a similar extent (p<0.05).

Islets were isolated from each animal upon euthanization (day 17-22) and challenged with low (2.8 mM) and high (16.7 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, Fig. 4B). CORT-EX tended to have lower relative GSIS levels in low glucose media when compared to the CORT-SED group and had significantly lower GSIS in the high glucose media compared to 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 to 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 to CORT-SED group (p<0.05,
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 to placebo-treated
animals (p<0.05) but no differences were found with the addition of exercise in placebo or
CORT-treated animals (Table 2). Relative gastrocnemius mass was lower with CORT-SED
treatment compared to 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 to CORT-EX animals and placebo-SED animals (p<0.05, Table 2). In contrast,
relative epitrochlearis mass, a marker of glycolytic muscle mass, was significantly lower in
CORT-SED animals compared to placebo-SED animals. CORT-EX animals had higher relative
epitrochlearis mass in comparison to CORT-SED animals, with values similar to those observed
in the placebo-treated groups (p<0.01, Table 2).

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

**Muscle oxidative capacity, PGC1α protein, capillary to fibre 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 fibre oxidative potential. The TA from both placebo-EX and CORT-EX treatment groups showed darker SDH staining compared to placebo-EX and CORT-SED muscles (p<0.001, Fig. 6A 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 to their respective SED groups (Fig. 6C).

CORT-treatment alone causes significant reduction in capillary to fibre ratio within skeletal muscle (61). Capillary to fibre ratio was reduced in CORT-SED animals but normalized in CORT-EX animals compared to placebo-treated animals (Fig. 6 D and E). Cross-sectional area, indicating individual muscle fibre size, was lowered in type IIa and IIb/x muscle fibres from the TA in CORT-SED animals (Fig. 6 F and G) and tended to increase with CORT-EX.
treatment. CORT treatment is known to induce atrophic signalling 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. 6 H, p<0.05); however, EX failed to normalize FOXO1 levels.

**Insulin signalling and glucose metabolic protein levels.** Regular exercise is known to stimulate skeletal muscle insulin signalling (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 to CORT-SED animals (Fig. 7A). Total AKT protein content was increased in CORT-EX animals compared to placebo-SED animals (p<0.05, Fig. 7B). However, no differences were found in phosphorylated AKT (T308) or GLUT4 protein expression between any of the treatment groups (Fig. 7C and D).

**Running distance vs. blood glucose, plasma insulin and CORT.** To determine if fasting blood glucose, plasma insulin and CORT are associated with individual running distances in the exercising rats, each value from each exercising animal in both treatment groups was plotted and a linear regression was calculated (data not shown). No significant correlations were found between running distances and fasting blood glucose levels ($r^2=0.083$, $p>0.05$), fasting plasma insulin levels ($r^2=0.023$, $p>0.05$), nor with plasma basal CORT levels ($r^2=0.003$, $p>0.05$).

**Liver lipid staining.** CORT-treated animals increased liver ORO staining compared to placebo-treated animals, indicating more hepatic lipid accumulation (Fig. S2). 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 weeks) reduced visceral adiposity, glucose intolerance, insulin insensitivity and impaired glucose responsiveness. Furthermore, many skeletal 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 when compared to 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 to 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 behaviour 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 weeks, 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. In comparison to 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 to 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 weeks of swim training in ZDF rats compared to SED and lean controls, results which were paralleled by increases in β-cell mass, through increased β-cell proliferation (hypertrophy 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 to placebo-SED islets (Fig. 4). In addition, voluntary wheel running (6 weeks) 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 to 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 when compared to 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 nor hepatic neutral lipid accumulation (Fig. 5A 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 fibres of the CORT-SED animals (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 fibre types of the TA muscle. We suspected that these exercise-mediated improvements could be caused by up-regulation 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 fibre 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 fibre 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 fibres that are less sensitive than fast-twitch fibres 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 fibre 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 suggest 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 IIb/x fibres of the TA muscle with regular exercise and CORT treatment. While it is still not known why GCs target type IIb/x fibres, higher GC receptor expression in these fibres 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 non-genomic (inhibition of insulin
signalling) 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 increase protein synthesis mechanisms (i.e. IGF-1 or mTOR signalling) 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 up-regulation of non-genomic signalling 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 as compared to CORT-SED animals. GCs are known to inhibit P13K/AKT signalling, which down-regulate 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 fibre ratio and shows tendencies to reverse muscle atrophy in type IIb/x fibres 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-signalling 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.
Table 1. CORT, fasted NEFAs, glucose and insulin concentrations.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>CORT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SED</td>
<td>EX</td>
</tr>
<tr>
<td><strong>Day 7 CORT</strong></td>
<td>66.0±17.1</td>
<td>271.3±42.9*</td>
</tr>
<tr>
<td><strong>Day 14 CORT</strong></td>
<td>70.6±12.3</td>
<td>375.0±44.7*</td>
</tr>
<tr>
<td><strong>Fasted NEFAs</strong></td>
<td>0.45±0.1</td>
<td>0.39±0.9</td>
</tr>
<tr>
<td><strong>Fasted Glucose</strong></td>
<td>4.9±0.3</td>
<td>5.1±0.2</td>
</tr>
<tr>
<td><strong>Fasted Insulin</strong></td>
<td>1.24±0.3</td>
<td>1.1±0.5</td>
</tr>
</tbody>
</table>

All values are means ± SE in ng/ml for CORT and insulin. Values are in mM for glucose and NEFAs. The * indicates the mean was statistically significant from placebo-SED, the ** indicates significance from CORT-SED and the # indicates 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.
Table 2. Food intake and body composition.

<table>
<thead>
<tr>
<th></th>
<th>Placebo SED</th>
<th>Placebo EX</th>
<th>CORT SED</th>
<th>CORT EX</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Food intake</strong></td>
<td>0.25±0.02</td>
<td>0.30±0.02</td>
<td>0.45±0.01*</td>
<td>0.44±0.02*</td>
</tr>
<tr>
<td><strong>Epididymal Fat</strong></td>
<td>17.0±1.0</td>
<td>17.0±1.6**</td>
<td>26.9±1.1*</td>
<td>21.8±1.4***</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td>37.4±1.6</td>
<td>37.7±1.6</td>
<td>69.7±2.4*</td>
<td>69.3±6.8*</td>
</tr>
<tr>
<td><strong>Gastrocnemius</strong></td>
<td>4.7±0.2</td>
<td>5.4±0.5</td>
<td>3.7±0.1*</td>
<td>4.2±0.2*</td>
</tr>
<tr>
<td><strong>Tibialis Anterior</strong></td>
<td>1.5±0.04</td>
<td>1.6±0.20</td>
<td>1.35±0.05</td>
<td>1.50±0.1</td>
</tr>
<tr>
<td><strong>Soleus</strong></td>
<td>0.40±0.01</td>
<td>0.45±0.1</td>
<td>0.52±0.03*</td>
<td>0.43±0.02</td>
</tr>
<tr>
<td><strong>Epitrochlearis</strong></td>
<td>0.14±0.01</td>
<td>0.17±0.02</td>
<td>0.11±0.01*</td>
<td>0.20±0.03***</td>
</tr>
</tbody>
</table>

Note: Values are means ± SE in kcal/g of body mass per day for food intake and g tissue/kg of body mass. The * indicates the mean was statistically significant from placebo-SED, the ** indicates significance from CORT-SED and the # indicates 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.
Fig.1. Experimental treatment groups (A) and schematic of experimental design (B). On day -14, all animals were individually housed and divided into one of four groups; placebo-EX, CORT-EX, placebo-SED and CORT-SED (EX, exercise and SED, sedentary). At this time, all EX animals were introduced to running wheels in their cages and allowed 2 weeks to acclimate to the experimental conditions. On day 0, either wax or CORT pellets were subcutaneously implanted into the animals according to the assigned experimental group and all animals were given a HFD (60%) ad libitum. Seven days post pellet implantations (~ 0800 hour), blood samples were taken to measure plasma CORT and blood glucose levels. On day 11 animals were fasted overnight (~ 16 hour) and the following day (12) were administered an oral glucose tolerance test. On day 14 (~ 0800 hour), blood samples were taken again to measure plasma CORT and blood glucose levels. On the evening of day 15, animals were fasted and on day 16 animals were administered an insulin tolerance test. All animals were euthanized between days 17-22 of the experimental protocol.

Fig.2. Body mass relative to pre-surgery mass during pellet treatment (A), absolute daily running distances during pellet treatment (B) and daily running distances relative to pre-surgery running distances (day 2) (C). CORT-treated animals experienced ~ 10-15% reduction in relative body mass throughout the pellet treatment period while placebo-treated animals continuously gained mass from day 2 onward (p<0.05). CORT-EX animals ran further distances per day than placebo-EX animals (p<0.05), however, CORT-EX animals ran more prior to surgery. Pellet implants had no impact on relative running distances. n=7-12. All values are means ± SE.

Fig.3. Glucose tolerance (A and A’), insulin secretion (B and B’), acute insulin responses (AIR, C and D), plasma GIP and GLP1 levels during 0 and 10 min of OGT T (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 to placebo-treated animals and exacerbated with the addition of wheel running (p<0.01). n=7-12 for all above graphs. Acute insulin response (AIR) between basal and 15 minutes post exogenous glucose challenge was elevated with EX 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 (r²=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. The * indicates values are statistically significant from placebo groups and ** indicates significance 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.

Fig.4. β-cell function (A) as measured by HOMA-β, glucose stimulated insulin secretion (GSIS) (B) and insulin sensitivity (C and C’). HOMA-β, an indicator of β-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 mM) and high (16.7 mM) glucose media compared to 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. The * indicates statistical significance from placebo-SED and ** indicates statistical significance from CORT-SED.
Fig. 5. Muscle (A) and liver (B) lipid accumulation was completed through ORO staining. CORT treatment increased lipid deposition in both muscle (tibialis anterior) and liver. There was no effect of exercise to lower lipid accumulation in either tibialis anterior or the liver in CORT-treated animals. Representative images are shown from an n=6.

Fig. 6. Muscle oxidative capacity as measured through succinate dehydrogenase (SDH) histology (A and B), PGC1-alpha protein content (C), capillary to fibre ratio (D and E), cross-sectional area (CSA) in I, IIa, and IIb/x muscle fibre 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 to SED groups. n=5. CORT-SED treatment resulted in a significant reduction in capillary to fibre ratio compared to all treatment groups and CORT-EX animals exhibited greater capillary-to-fibre ratio compared to 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 isolectin-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 fibres. FOXO1 protein content increased with CORT-SED and EX treatment. n=4-5. The * indicates statistical significance from placebo-SED and ** indicates statistical significance from CORT-SED (p<0.05) using two-way ANOVA with Bonferroni’s post hoc test. All values are means ± SE.

Fig. 7. IRS-1 (A), AKT Total (B), AKT phosphorylated T308 (C) and GLUT4 protein content (D) in tibialis anterior (TA) muscle. Exercise tended to increase IRS-1 protein content in placebo and CORT animals. CORT-EX animals had higher AKT Total protein content compared to CORT-SED animals but no differences in AKT phosphorylated T308 protein content were found amongst groups. No differences were found in GLUT4 protein content between the treatment groups. n=4-5. The * indicates statistical significance from CORT-SED (p<0.05) using two-way ANOVA with Bonferroni’s post hoc test. All values are means ± SE.
References


27. Gauthier MS, Couturier K, Charbonneau A and Lavoie JM. Effects of introducing physical training in the course of a 16-week high-fat diet regimen on hepatic steatosis, adipose


Fig. 2.

A

Relative Body Mass

Placebo-SED
Placebo-EX
CORT-SED
CORT-EX

Days

0 2 4 6 8 10

B

Running Distance (m/day)

Pellet Implants

Days

-2 0 2 4 6 8 10

C

Relative Running Distance

Pellet Implants

Days

0 2 4 6 8 10
Fig. 4.
Fig. 6.

A

Placebo-SED  Placebo-Ex

CORT-SED  CORT-Ex

100 µm

B

SCH-Activity Relative Optical Density

Placebo-SED  Placebo-Ex  CORT-SED  CORT-Ex

B1a  B1b/βx

C

PGC1α Total A.U.

Placebo  CORT

PGC1α  GAPDH

D

Placebo-SED  Placebo-Ex

CORT-SED  CORT-Ex

100 µm

E

Capillary Fiber

SED  EX  SED  EX

Placebo  CORT

G

CSA (µm²)

Placebo-SED  Placebo-Ex  CORT-SED  CORT-Ex

H

FOXO1 Total A.U.

Placebo  CORT

FOXO1  GAPDH
Fig. 7