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Endurance exercise training increases adipose tissue glucocorticoid exposure: adaptations that facilitate lipolysis

Jonathan E. Campbell, Sergiu Fediuc, Thomas J. Hawke, Michael C. Riddell*

School of Kinesiology and Health Science, Faculty of Pure and Applied Science, York University, Toronto, ON, Canada M3J 1P3

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Abstract

Glucocorticoids (GCs) have long been thought to be lipolytic in nature. Recently, however, increased exposure to GCs in insulin-sensitive tissues has been associated with lipid accumulation and metabolic complications, regardless of plasma concentrations. Intracellular GC action is determined by both 11-β hydroxysteroid dehydrogenase type 1 (11βHSD1) and the GC receptor (GR). We hypothesized that exercise training would increase 11βHSD1 and GR protein in adipose tissue, resulting in increased lipolysis. To test the effects of exercise on adipose tissue GR and 11βHSD1 protein, 2 sets of hamsters were trained for 6 weeks: young, diet-induced obese animals and older, overweight animals. Young (6 week old) hamsters, fructose-fed to induce an obese phenotype, and older (6 month old) hamsters were randomly divided into exercising and sedentary groups. Exercise training decreased adipose tissue mass in both fructose-fed and older hamsters. In addition, exercise training increased 11βHSD1 (31.5% ± 15% and 20.0% ± 7%, fructose-fed and older, respectively) and GR (45.6% ± 14% and 61.1% ± 27%, fructose-fed and older, respectively) protein expression in the perirenal adipose depot and increased 11βHSD1 (16.7% ± 7%, $P = .09$) and GR (47.4% ± 19%, $P < .05$) in the subcutaneous adipose depot of the older hamsters. To determine the metabolic effect of increased GC exposure in adipocytes, 3T3-L1 adipocytes were treated with corticosterone for 24 hours; and measures of lipolytic rates were conducted. Low concentrations of GCs (0.01-0.1 μmol/L) increased GR (44.1% ± 18%, $P < .05$) and 11βHSD1 (95.3% ± 24%) protein expression, as well as lipolytic rates (34.6% ± 6%) as measured by glycerol release. The increased lipolysis was blocked by RU486, a GR antagonist, suggesting that the elevated lipolysis was a direct result of GC action. These results suggest that exercise training amplifies the activity of GCs in adipose tissue of overweight animals through alterations in 11βHSD1 and GR despite differences in age and amounts of adiposity. In vitro, GCs are capable of increasing lipolysis, but depend upon the presence of GR. We propose that GCs play a significant role in changing the phenotype of adipose tissue during exercise training, resulting in decreased fat mass.

1. Introduction

Glucocorticoids (GCs) are steroid hormones released from the adrenal glands in response to a variety of physiologic and psychologic stressors. These stress hormones act by binding to cytosolic GC receptors (GRs), resulting in a positive or negative genomic effect [1]. Two major GCs are released from the adrenal glands in humans, cortisol and cortisone, with the former considered active and the latter biologically inactive [1]. In rodents, the equivalent GCs are corticosterone (active) and 11-dehydrocortisone (inactive). 11-β hydroxysteroid dehydrogenase type 1 (11βHSD1) is a prereceptor enzyme that is capable of converting inactive GCs into their active form (ie, cortisone/11-dehydrocortisone into cortisol/corticosterone) [2]. Previous research has shown that tissue-specific overexpression of 11βHSD1 results in a dramatic increase in intracellular concentrations of active GCs despite normal plasma concentrations [3]. Therefore, physiologic activity of GCs is determined not only by circulating concentrations of these hormones but also by the protein levels of both GR and 11βHSD1 [4].

Glucocorticoids have many important physiologic roles, including increasing the amount of energy substrates in the blood during energy deprivation (ie, hypoglycemia) [1]. Whereas the effects in other tissues such as liver or skeletal muscle are more clearly defined, the effect of GCs in adipose tissue is controversial. Glucocorticoids promote preadipocyte differentiation and adipogenesis, whereas inhibition of 11βHSD1 prevents cortisone-induced preadipocyte differentiation [5]. In humans, elevated 11βHSD1 expression in visceral adipose tissue is associated with central obesity and increased fat cell size [6,7]. Mice overexpressing 11βHSD1 under the adipocyte-specific aP2 promoter have higher tissue

* Corresponding author. Tel.: +1 416 736 2100x40493.
E-mail address: mriddell@yorku.ca (M.C. Riddell).

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GC levels and develop central obesity as a result of increased adipocyte size [3]. On the other hand, weight loss via dietary restriction increases 11βHSD1 expression in the adipose tissue of obese persons [8]. Whether this increase in 11βHSD1 with diet is a compensatory response to decreased fat mass or if it helps facilitate increased adipose tissue lipolysis is unclear.

In human [9,10] and rat adipocytes [11], GCs have been shown to increase lipolysis, possibly through increased expression of hormone-sensitive lipase (HSL) [11]. Yet in human adipocytes, GCs are thought to inhibit lipid mobilization in the presence of insulin, thus leading to triglyceride accumulation and retention [12]. Because the density of GR is higher in intraabdominal (visceral) fat than in other fat depots [7], the increased activity of GCs is thought to be accentuated in visceral adipose tissue [13]. Therefore, it may be that the greater GC action observed in central fat depots, in the presence of elevated insulin, results in the accumulation of fat. This increase in abdominal obesity is consistent with some pathologic conditions such as Cushing syndrome and the metabolic syndrome. Indeed, reducing GC exposure in adipose tissue protects from diet-induced obesity in rodents [14,15]. Taken together, these findings suggest that GCs may play a paradoxical role in adipose tissue, causing lipolysis in some situations while facilitating fat accumulation and, thus, obesity in others.

Previous work from our laboratory demonstrates that exercise training in hamsters reduces GR and 11βHSD1 in skeletal muscle and liver tissue, which would decrease GC activity; however, exercise paradoxically increases 11βHSD1 activity in central fat stores [16]. We hypothesize that this increase in 11βHSD1 activity results from increased protein levels and would elevate the action of GCs. This study shows that exercise training results in elevated levels of both 11βHSD1 and GR protein. Importantly, we show that this effect of exercise takes place in both our animal models of excessive adiposity and that both exercising groups have decreased fat mass. Furthermore, we use in vitro experiments to show that GCs are capable of increasing lipolysis and that this is dependent upon the presence of GR. These data show the important role that GCs have during exercise-mediated weight loss.

2. Materials and methods

2.1. In vivo studies

2.1.1. Animals and diets

2.1.1.1. Study 1. To assess the effects of exercise on adipose tissue in a model of diet-induced obesity, 16 young, male Syrian golden hamsters (*Mesocricetus auratus*; Charles River, Montreal, Quebec, Canada), at an initial age of 6 weeks and an initial weight of 87.7 ± 0.84 g (mean ± SEM), were individually housed in clear cages and kept in a temperature- (23°C-25°C) and humidity- (40%-50%) controlled room for a 7-day habituation period. During habituation, the animals were fed standard rodent chow (Purina 5001 Diets, Bethlehem, PA; 4.3 kcal/g metabolizable energy). After this period, the hamsters were randomly assigned to either sedentary or exercise groups. Animals were given a high-fructose diet ad libitum (Dyets, Bethlehem, PA; 60% fructose, 4.1 kcal/g metabolizable energy) to induce visceral obesity and whole-body insulin resistance [17,18]. Exercising animals were individually housed in specialized activity wheel cages (height, 36.4 cm; width, 26.8 cm; depth, 50 cm) with unrestricted, 24-hour access to their wheels (circumference, 108 cm; width, 9 cm). Sedentary animals were housed in similar-sized cages, but without activity wheels. Wheel revolutions, body weight, and food intake were recorded daily. Running distance was calculated daily as the circumference times the revolutions.

2.1.1.2. Study 2. To assess the influence of exercise on adipose tissue of an older, sedentary, overweight model, 16 male Syrian golden hamsters, at an initial age of 6 months and an initial weight of 151.2 ± 3.12 g, were individually housed in a similar environment as previously described for the fructose-fed hamsters for a 7-day habituation period and fed a standard rodent chow. After habituation, the hamsters were randomly assigned to either sedentary or exercise groups. Exercising and sedentary animals were housed in running wheel cages and standard cages, respectively, as previously described for the fructose-fed hamsters. Wheel revolutions, body weight, and food intake were recorded daily. Daily body weight and food intake for these animals have been previously published [19].

All animal experiments were approved by the Animal Care Committee at York University and were conducted in accordance with guidelines set forth by the Canadian Council for Animal Care.

2.1.2. Blood sampling

Nonfasted blood samples were taken via saphenous vein puncture on the first day of every week at approximately 1:00 PM for determination of whole-blood glucose and plasma insulin concentrations. This method of blood sampling causes minimal stress to the animal and has been described previously [20]. Approximately 90 μL of whole blood was collected into heparin-coated microvettes (SARSTEDT, Montreal, Quebec, Canada) and centrifuged at 4°C and 2000 rpm for 5 minutes, and the plasma was frozen and stored at −20°C until further analysis.

2.1.3. Intraperitoneal glucose tolerance test

At the end of the 38 days, intraperitoneal glucose tolerance tests (IPGTTs) were performed on all hamsters after an 18-hour overnight fast. To minimize the effect of stress due to repeated blood sampling, the hamsters were lightly anesthetized with sodium pentobarbital (0.05 g/kg). Previous studies using sodium pentobarbital during glucose tolerance tests report no effect of the anesthesia on glucose dynamics [21]. Before the IPGTT, a fasted blood sample was taken at time 0 minute (8:30 AM), 30 minutes after the

administration of the sedative. Glucose (2 g/kg), dissolved in saline, was then administered intraperitoneally via a 23-gauge needle. Subsequent blood samples were taken at 30, 60, 90, and 120 minutes. All blood samples were collected via saphenous vein puncture in the same method as described above, with the first drop of blood (~15 μL) used for whole-blood glucose measurements and the next 40 μL used for determination of insulin concentration.

2.1.4. Euthanization

After the IPGTT, animals were euthanized by decapitation and adipose tissue (perirenal in young hamsters; perirenal and subcutaneous in old hamsters) and various skeletal muscles were removed, weighed, and snap-frozen on dry ice.

2.1.5. Metabolic hormone concentrations

Fed plasma insulin, leptin, and glucagon hormone concentrations were determined from the final-week saphenous vein blood samples using the Luminex 100 instrument and the LINCOplex Well Plate Assay rat/mouse endocrine panel (LINCO Research, St Charles, MO). Corticosterone concentrations in the plasma collected during the final week were determined with a commercially available radioimmunoassay kit (MP Biomedicals, Montreal, QC, Canada; catalog no. 07-120102).

Plasma insulin collected during the IPGTT was determined using a commercially available enzyme-linked immunosorbent assay kit (Crystal Chem, Downers Grove, IL; catalog no. INSKR020).

2.1.6. Skeletal muscle cytochrome c oxidase activity

Cytochrome *c* oxidase activity in the plantaris muscle was determined as previously described [22]. Enzyme activity was determined as the maximal rate of oxidation of fully reduced cytochrome *c*, measured by the change in absorbance at 550 nm in a microplate reader (ELx800 Universal; BioTek Instruments, Winooski, VT).

2.2. In vitro studies

To assess the role of GCs on adipose tissue 11βHSD1 and GR expression and on rates of adipose tissue lipolysis in vitro, 3T3-L1 cells were grown for 2 days postconfluence in 10% fetal bovine serum (FBS)/Dulbecco modified Eagle medium (DMEM) at 37°C and 5% CO_2. Differentiation was induced with 10% FBS/DMEM containing 0.5 μmol/L isobutylmethylxanthine, 0.25 μmol/L dexamethasone, and 200 pmol/L insulin for 4 days. The cells were then incubated in 10% FBS/DMEM containing 200 pmol/L insulin for 4 days. After this, the cells were incubated in 10% FBS/DMEM until greater than 95% of cells contained lipid droplets (~2-4 days). The medium was changed every other day throughout the entire differentiating period.

2.2.1. GC dose response

After differentiation, 3T3-L1 cells were incubated in a 3% FBS/DMEM containing various concentrations of corticosterone (Sigma, Oakville, ON, Canada, catalog no. C2505) for 24 hours at 37°C and 5% CO_2. After this, the cells were immediately placed on ice and lysis buffer (20 mmol/L Tris, 150 mmol/L NaCl, 1 mmol/L $MgCl_2$, 1 mmol/L $CaCl_2$, 1% Triton X-100, 10% glycerol, pH 7.4) was added. The cells were scraped into separate chilled tubes and centrifuged at 5000 rpm for 30 minutes. The supernatant was removed and stored at −80°C until used. For lipolysis experiments, 3T3-L1 cells were incubated in 3% FBS/DMEM without phenol red containing various concentrations of corticosterone for 24 hours at 37°C and 5% CO_2. In addition to the corticosterone, cells were incubated with either dimethyl sulfoxide or 10 μmol/L RU486 (Sigma, catalog no. M8046), the GR antagonist. A sample of the media was removed at 0 and 24 hours to determine GC-stimulated lipolysis. Lipolysis was determined as the rate of glycerol appearance and expressed as a percentage of control cells. Glycerol concentrations were determined with a commercially available kit (Sigma, catalog no. FG0100). All in vitro data are an average of 3 separate experiments, with 2 replicates per condition for each experiment.

2.2.2. Immunoblotting

This method for protein preparation and quantification has been previously described [16]. Briefly, tissue samples were homogenized to obtain total protein and centrifuged at 5000 rpm for 30 minutes, and the supernatants were collected. Protein concentrations were assessed by Bradford method. Seventy-five micrograms of total protein was electrophoretically resolved on an 8% (GR), 10% (HSL and adipose triglyceride lipase [ATGL]), or 12% (11βHSD1) sodium dodecyl sulfate–polyacrylamide gel and transferred overnight at 20V to polyvinylidene difluoride paper. Blots were blocked with 5% milk in Tris-Tween buffered saline and then incubated overnight in primary antibody at 4°C (GR: Santa Cruz, catalog no. sc-8992, 1:1000; 11βHSD1: Alpha Diagnostic, San Antonio, TX, catalog no. BHSD11-S, 1:1500; HSL: Cell Signaling, catalog no. 4107, 1:1000; ATGL: Cell Signaling, Danvers, MA; catalog no. 2138, 1:1000). Blots were incubated with secondary antibody for 1 hour at room temperature, and hybridization signals were visualized using the Western Lightning Chemiluminescence Reagent Plus kit (PerkinElmer, Wellesley, MA) after exposure to Kodak X-Omat Blue x-ray film (Rochester, NY). β-Actin and glyceraldehyde-3-phosphate dehydrogenase were used as loading controls in tissue and 3T3-L1 cells, respectively.

2.2.3. Data analysis

For all experiments, the appropriate *t* test, or 1- or 2-way analysis of variance was performed to identify significant differences between treatment groups using Statistica 6.0 software (StatSoft, Tulsa, OK), with *P* less than .05 as the criterion. When a significant difference was observed with an analysis of variance, post hoc analysis using contrasts with a Bonferroni correction factor was performed to determine specific differences. Data are presented as mean ± SEM.

3. Results

3.1. In vivo studies

3.1.1. Wheel running causes a transient reduction in body mass gain and an increase in food intake in both fructose-fed and older hamsters

Body weights for the fructose-fed and older hamsters are shown in Fig. 1A. Both exercise groups demonstrated a transient decrease in body weight after the introduction of the running wheel. This occurred from day 1 to day 5 in the fructose-fed hamsters (left panel, $P < .05$) and from day 3 to day 10 in the older chow-fed animals (right panel, $P < .05$). A significant main effect for time was found in the fructose-fed hamsters, but not in the older hamsters. In both groups of hamsters, body mass was similar between exercising and sedentary groups by the end of the experimental period.

In both groups, food intake remained similar between the exercising and sedentary hamsters at the beginning of the study (Fig. 1B). It is important to note that the fructose and standard chow diets were isocaloric. The exercising hamsters began to consume more food than the respective sedentary group after the introduction of the running wheel, and this became statistically different in both groups by day 8 ($P < .05$).

3.1.2. Wheel running causes an increase in skeletal muscle mass and increases mitochondrial oxidative capacity

Both the fructose-fed and older hamsters ran similar distances throughout the protocol, with the average distance for both groups being 11.35 ± 0.13 km/d over the entire protocol.

Exercise training resulted in heavier plantaris weights in the fructose-fed hamsters, but not the older hamsters (Table 1). However, soleus weight was significantly higher in both exercising groups compared with their respective sedentary group ($P < .05$). Cytochrome *c* oxidase activity was approximately 35% higher in exercising hamsters compared with sedentary hamsters ($P < .05$, Table 1).

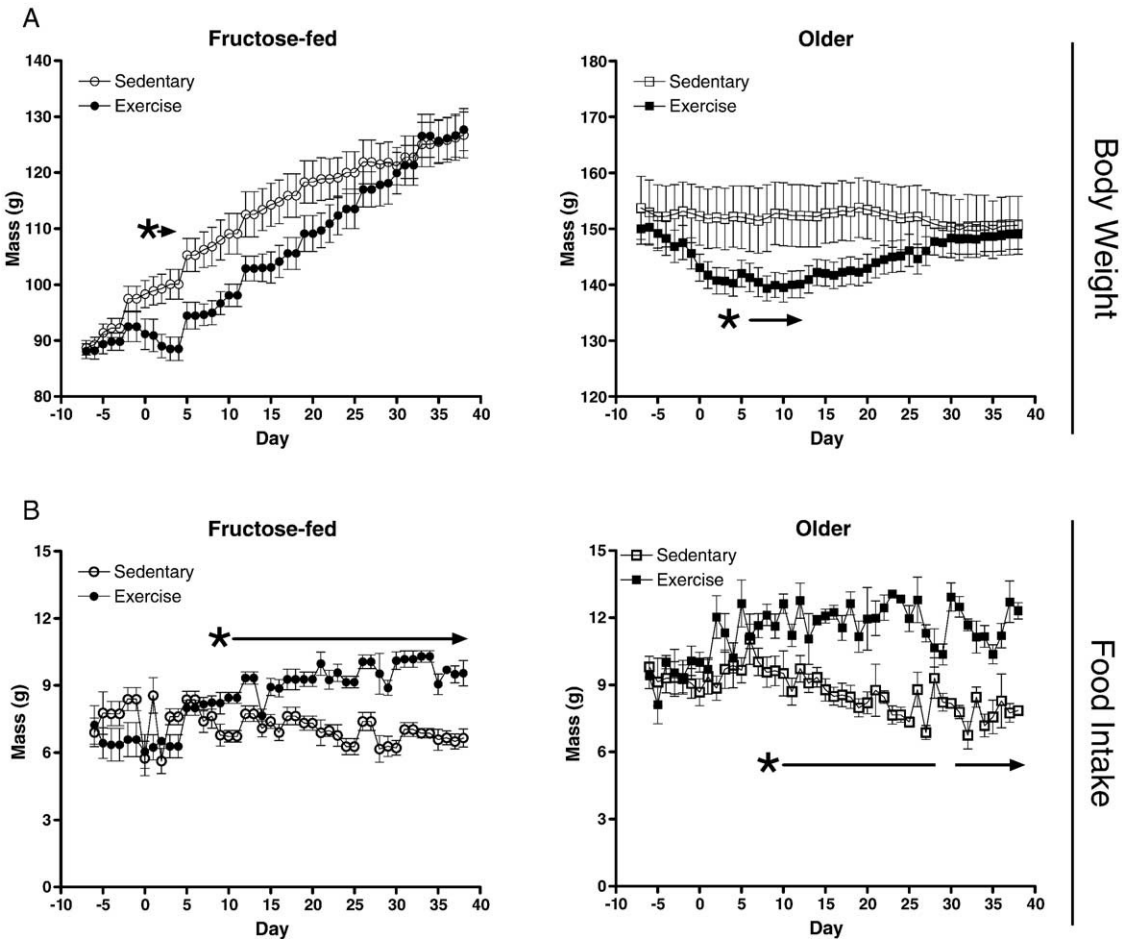

Fig. 1. Daily changes in body weight and food intake throughout the training protocol in fructose-fed and older hamsters. A, The fructose-fed hamsters gained weight throughout the protocol, whereas the older hamsters had stable weights. Both exercise groups experienced a transient decrease in body weight immediately after the introduction of the running wheel. However, exercising hamsters had similar body weight compared with their sedentary group by the end of the 6 weeks. B, Both exercise groups consumed more food compared with their sedentary group after the introduction of the running wheel. This became statistically different at day 8 in both groups. *P less than .05 vs respective sedentary group.

Table 1
Skeletal muscle characteristics and postprandial hormone concentrations

	Fructose-fed sedentary	Fructose-fed exercise	Older sedentary	Older exercise
Skeletal muscle properties				
Plantaris (mg)	28.8 ± 0.9	38.1 ± 1.4*	39.5 ± 1.8	39.1 ± 2.2
Soleus (mg)	14.1 ± 0.8	26.2 ± 1.9*	25.7 ± 0.9	38.8 ± 1.8*
Cytochrome *c* oxidase activity (U/g)	8.05 ± 0.3	10.76 ± 0.9*	6.74 ± 0.3	9.24 ± 0.3*
Postprandial hormones				
Glucose (mmol/L)	4.43 ± 0.3	4.28 ± 0.2	4.66 ± 0.3	4.44 ± 0.2
Insulin (pmol/L)	697.2 ± 59	417.4 ± 65*	210.6 ± 53	176.9 ± 37
Leptin (pmol/L)	124.4 ± 17	59.1 ± 12*	34.0 ± 7	21.0 ± 7*
Corticosterone (ng/mL)	45.8 ± 1.9	45.0 ± 1.9	47.8 ± 2.1	49.2 ± 1.3

* *P* less than .05 vs respective sedentary group.

3.1.3. Wheel running causes a reduction in fed insulin and leptin levels in both fructose-fed and older hamsters

Table 1 shows nonfasted hormone concentrations in the plasma taken during the final week of training. Both exercise groups had lower fed plasma insulin concentrations in comparison with their respective sedentary controls; however, only in the fructose-fed animals did this difference reach statistical significance ($P < .05$). No differences were found with glucagon concentrations in any of the groups (data not shown). Exercising hamsters had significantly lower plasma leptin than sedentary hamsters in both groups ($P < .05$). Exercise had no effect on either basal corticosterone or fed blood glucose concentrations.

3.1.4. Wheel running improved glucose tolerance in fructose-fed young but not older overweight hamsters

Fructose-fed exercising hamsters had lower blood glucose and insulin concentrations compared with the sedentary hamsters throughout the IPGTT ($P < .05$, Table 2). In the older hamsters, the exercise group demonstrated smaller differences in blood glucose and insulin concentrations, with only the baseline and 120-minute insulin time points achieving statistical significance ($P < .05$). An insulin sensitivity index (ISI) ($ISI = 2/[(AUC_{glucose} \times AUC_{insulin}) + 1]$) was calculated based on the areas under the curve for both glucose and insulin during the IPGTT, as has previously been reported [21] (Table 2). Using this formula, both exercise groups had improved insulin sensitivity compared with their sedentary controls ($P < .01$).

3.1.5. Wheel running increases the intracellular determinants of GC action in adipose tissue and lowers adipose tissue mass

To investigate the effects of exercise training on adipose tissue exposure to GCs, Western blotting for both 11βHSD1 and GR was completed in the adipose tissue of the fructose-fed and older hamsters. Fig. 2 shows the perirenal adipose tissue mass and the protein expression for both 11βHSD1 and GR in the fructose-fed hamsters. The exercise group had lower fat mass compared with the sedentary group ($P < .01$), a 46% increase in adipose GR protein expression ($P < .05$), and a 32% increase in 11βHSD1 protein expression ($P = .08$).

Similar to the fructose-fed hamsters, the older exercise group had significantly decreased perirenal adipose mass and increased 11βHSD1 and GR protein expression compared with the sedentary group (20% and 61%, respectively; both $P < .05$). The older exercise group also demonstrated a 47% increase in GR ($P < .05$) and a trend for a 16% increase in 11βHSD1 ($P = .09$) in the subcutaneous adipose tissue (Fig. 3).

3.1.6. Wheel running increases perirenal and subcutaneous adipose tissue HSL and ATGL protein expression

Fig. 4 shows the expression of HSL and ATGL in the perirenal and subcutaneous adipose depots in the older hamsters. The exercise group had increased HSL and ATGL protein expression in both the perirenal and subcutaneous adipose depots, compared with the sedentary group (all $P < .01$). These data suggest that the adipose tissue of the exercising hamsters has an increased potential for lipolytic activity. This may be one potential mechanism for the exercise-induced weight loss seen in these hamsters, which may be mediated through increased GC exposure.

3.2. In vitro studies

3.2.1. GCs alter adipocyte lipolytic rates and the protein expression of 11βHSD1 and GR in a concentration-dependent fashion

To determine a potential mechanism for the increased GR and 11βHSD1 protein expression in adipose tissue, 3T3-L1 adipocytes were incubated in various concentrations of corticosterone for 24 hours (Fig. 5). Lower concentrations (0.01-0.1 μmol/L) of GCs caused an increase in both 11βHSD1 and GR protein levels compared with control

Table 2
Results of the IPGTT: plasma glucose, insulin, and the ISI

	Fructose-fed sedentary	Fructose-fed exercise	Older sedentary	Older exercise
Glucose (mmol/L)				
Baseline	6.6 ± 0.4	6.1 ± 0.3	5.9 ± 0.3	5.6 ± 0.5
30 min	14.4 ± 1.0	11.6 ± 1.4*	12.5 ± 1.6	10.5 ± 0.8
60 min	12.5 ± 0.6	10.2 ± 1.5*	9.9 ± 1.6	9.3 ± 1.5
90 min	9.1 ± 0.9	8.2 ± 1.0*	7.9 ± 0.8	7.4 ± 1.0
120 min	8.0 ± 1.0	6.6 ± 0.5	6.2 ± 0.5	6.0 ± 0.7
Insulin (ng/mL)				
Baseline	3.36 ± 0.4	2.84 ± 0.5	3.33 ± 0.2	2.17 ± 0.2*
30 min	6.49 ± 0.6	4.05 ± 0.6*	3.77 ± 0.4	3.38 ± 0.4
60 min	6.52 ± 0.7	3.89 ± 0.4*	1.97 ± 0.6	1.74 ± 0.3
120 min	4.11 ± 0.2	2.76 ± 0.4*	2.64 ± 0.5	1.39 ± 0.2*
ISI (AU, % sedentary control)	1.0 ± 0.05	1.49 ± 0.10*	1.0 ± 0.1	1.29 ± 0.06*

* *P* less than .05 vs respective sedentary group.

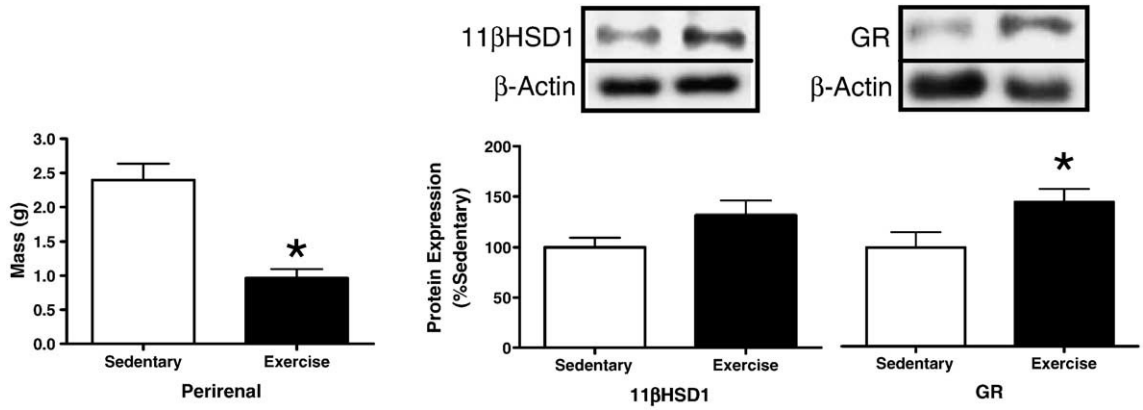

Fig. 2. Perirenal adipose tissue characteristics in the fructose-fed hamsters. The left panel shows that the exercise group had significantly less perirenal adipose mass compared with the sedentary group. The middle and right panels show representative blots and the average densitometry for 11βHSD1 and GR, respectively. The expression of both proteins was increased in the exercise group, suggesting the potential for increased GC action in the perirenal adipose of these animals. A trend was found for 11βHSD1 ($P = .08$); *P less than .05 vs the sedentary group. Average densitometry is expressed as a percentage of the sedentary group.

cells, whereas higher concentrations (1-100 μmol/L) had no effect on 11βHSD1 but caused a decrease in GR protein expression ($P < .05$). These data show that low concentrations of GCs are able to increase 11βHSD1 and GR protein to magnitudes seen in vivo in the exercising hamsters.

To assess the role of GCs on adipose tissue lipolysis, 3T3-L1 adipocytes were incubated in corticosterone for 24 hours with or without RU486, a GR antagonist (Fig. 6). A concentration-dependent increase in lipolysis was found with increasing corticosterone concentrations up to a

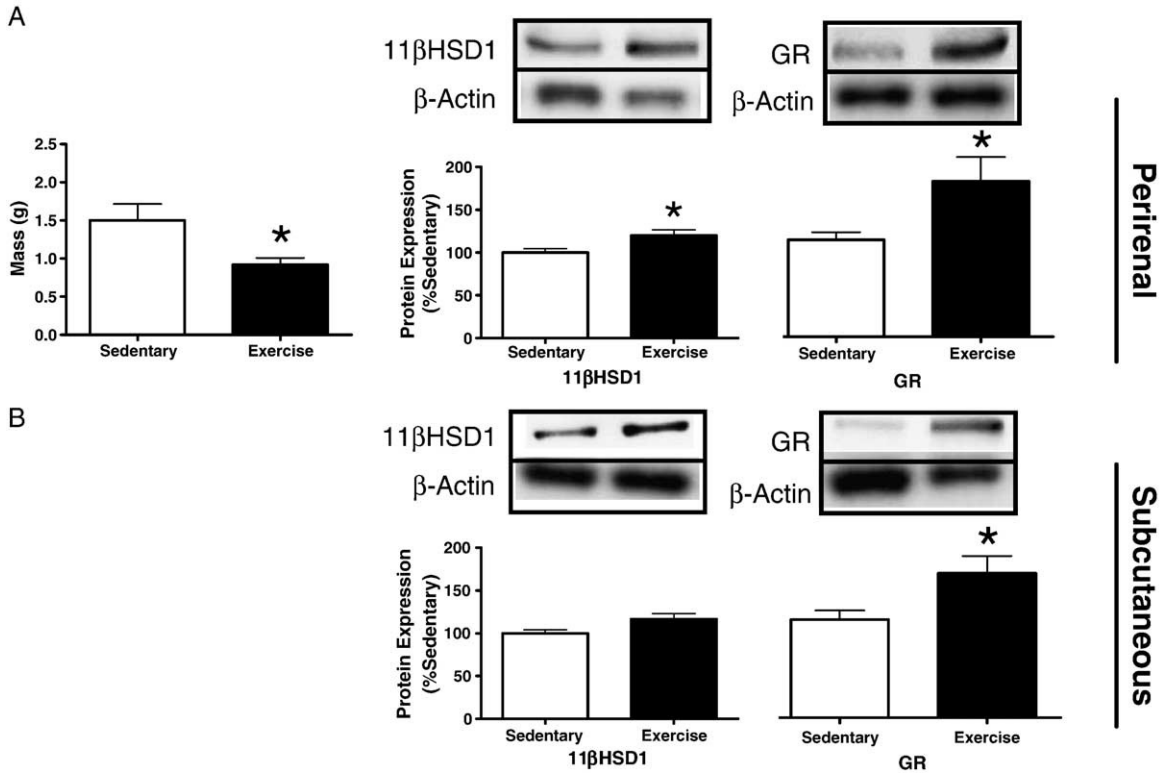

Fig. 3. Perirenal and subcutaneous adipose tissue in the older hamsters. The left panel shows the adipose mass for the perirenal depot; the middle and right panels show the representative blots and average densitometry for 11βHSD1 and GR, respectively. The exercise group had significantly less adipose mass compared with the sedentary group, which was accompanied by increased protein expression for both 11βHSD1 and GR. Furthermore, the exercise group had increased GR and a trend for increased 11βHSD1 ($P = .09$) in the subcutaneous adipose. Similar to the perirenal adipose in fructose-fed hamsters, the adipose depots in older hamsters demonstrated increased potential for elevated GC action. *P less than .05 vs sedentary group. Average densitometry is expressed as a percentage of the sedentary group.

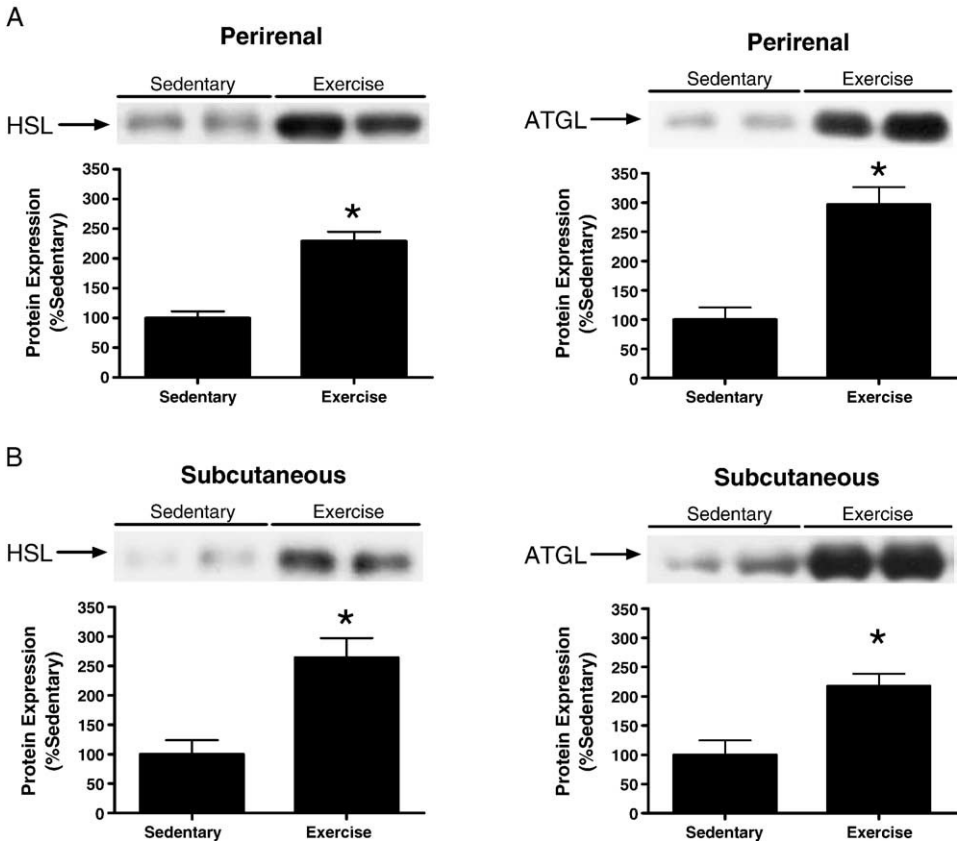

Fig. 4. The expression of HSL and ATGL, the 2 major lipolytic enzymes in adipose tissue, in the perirenal and subcutaneous adipose tissue of the older hamsters. Exercise training was associated with increased ATGL and HSL expression in both the perirenal (A) and subcutaneous (B) adipose depots. This potential increase in lipolytic activity provides a potential mechanism for the exercise-mediated decrease in adiposity. *P less than .01 vs the sedentary group.

maximum of 10 μmol/L (35%, $P < .05$). Higher concentrations of corticosterone did not increase lipolytic rate. Coincubation with RU486 prevented the GC-induced increases in lipolysis at all concentrations over the 24-hour period. This indicates that the effects of GCs on lipolysis are mediated through GR and that the greatest effect is observed at low to moderate concentrations.

4. Discussion

The activity of GCs within cells is dependent largely on the conversion of inactive hormone to biologically active hormone by 11βHSD1 and the expression levels of the hormone receptor GR. Although elevated GCs have largely been associated with negative effects on human health, including central adiposity and insulin resistance, it is interesting to note that exercise results in decreased adiposity and increased insulin sensitivity despite elevations in GCs. To address this apparent disparity in GC action, 2 distinct populations of hamsters with elevated central adiposity that would mimic the human overweight conditions (diet-induced young hamsters and sedentary older hamsters) were exercise trained for 6 weeks.

We demonstrate that exercise training increases the potential for GC action in adipose tissue by increasing the expression of 11βHSD1 and GR. Furthermore, exercise training resulted in an increased expression of HSL and ATGL, 2 key lipolytic enzymes that are thought to be increased by elevations in GCs. To determine the functional consequences of GC action in adipocytes, we undertook in vitro experiments on 3T3 adipocytes. These experiments demonstrate that GCs are capable of increasing lipolysis in a concentration-dependent manner and that this effect is dependent on the presence of GR, as antagonism of this receptor abolishes any change in lipolytic rate. From these data, we believe that, in the situation of exercise training, GC exposure is enhanced in various adipose depots and this up-regulation facilitates an increase in lipolysis.

Our previous research indicated that exercise training increases the activity of 11βHSD1 in the adipose tissue of young, healthy hamsters and that this increase in activity is associated with a reduced adipose mass [16]. Here, we strengthen these findings by showing that exercise increases both 11βHSD1 and GR protein expression in 2 distinct populations of hamsters with elevated central adiposity. Both groups had more adiposity, relative to their body weight,

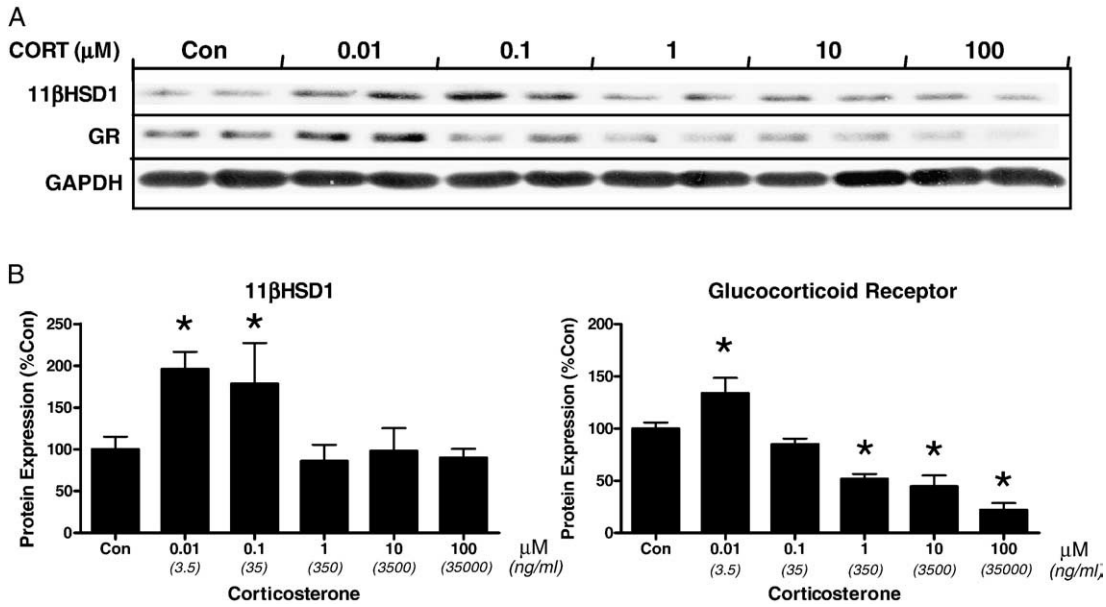

Fig. 5. The effects of GCs on 11βHSD1 and GR protein expression in 3T3-L1 adipocytes. The representative blots (A) and the average densitometry (B) for 11βHSD1 and GR show a dose-response change in protein expression. The 11βHSD1 protein was increased at low concentrations of GCs, but unchanged at high concentrations. The GR protein was increased at low concentrations of GCs, but decreased at high concentrations. This shows that low concentrations of GCs are able to increase 11βHSD1 and GR protein to levels seen in our in vivo studies. Concentrations of corticosterone are presented as micromoles per liter, with approximate nanograms per milliliter provided in parentheses below. Values are an average of 3 separate experiments. *P less than .05 vs control conditions.

compared with the young, healthy hamsters from our previous study [16]. The fructose-fed young hamsters had more intraabdominal fat compared with the overweight older hamsters (Figs. 2 and 3), which likely led to the elevated levels of leptin (Table 1) and decreased insulin sensitivity (Table 2) observed in the sedentary fructose-fed animals compared with the older sedentary animals. Interestingly, both the fructose-fed and older hamsters exercised at approximately the same volume and consequently had similar amounts of perirenal adipose mass and similar glucose tolerance during the IPGTT. Both exercise groups displayed increases in 11βHSD1 and GR protein content, which shows that exercise training has the ability to increase GC action in adipose tissue regardless of diet or age. Although the functional consequence of increased adipose tissue GC exposure is not completely understood, it is tempting to believe that GCs are driving the observed increases in HSL and ATGL found in the exercising hamsters. Indeed, GCs have traditionally been thought to be lipolytic in their activity [10,11,23].

Previous research has shown that exercise is capable of increasing HSL in adipose tissue [24], but no data exist on the relationship between exercise and ATGL. In this study, we show that both key lipolytic enzymes are increased with regular physical activity and we speculate that exercise-associated increases in GCs may be one of the stimuli for this adaptation. Indeed, GCs are capable of increasing HSL messenger RNA in adipocytes [11] and others have shown that GCs can increase ATGL messenger RNA in preadipocytes [25]. Our own unpublished data show that incubating 3T3-L1 adipocytes in GCs increases ATGL protein in a concentration-dependent manner (data not shown). Still, more evidence is needed before a direct link can be made that the elevated concentrations of GCs during exercise cause the increased protein levels of HSL and ATGL that are observed in endurance-trained organisms.

A number of studies suggest that GCs are adipogenic, particularly when circulating insulin levels are also high

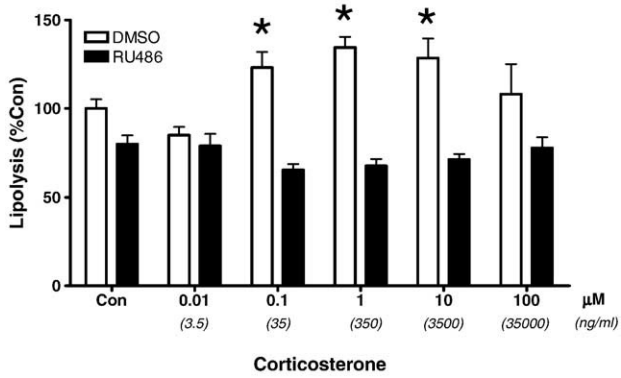

Fig. 6. Glucocorticoids increase lipolysis in a dose-dependent manner, which can be blocked through coincubation with the GR antagonist RU486. Corticosterone increased lipolysis in the dose range of 0.1 to 10 μmol/L. Coincubation with RU486 blocked the lipolytic effects of corticosterone at all concentrations. Lipolysis is an average of 3 separate experiments, expressed as a percentage of control conditions. Concentrations of corticosterone are presented as micromoles per liter, with approximate nanograms per milliliter provided in parentheses below. *P less than .05 vs control conditions (dimethyl sulfoxide).

[3,15,26,27]. Our data here contrast with this notion, however, as exercising animals have repeated exposure to GCs, along with increased intracellular determinants of GC action, but have significantly less adipose mass, suggesting an elevated state of lipolysis rather than lipogenesis. Our observation that fat mass reduction is associated with increased 11βHSD1 and GR expression is in line with the findings by Tomlinson et al [8], who showed that dietary-induced weight loss increases the expression of 11βHSD1 in subcutaneous adipose tissue. It is important to note that the adipogenic effects of GCs are often seen in situations where insulin is also elevated [3,15,27], rather than in humans on dietary restriction [8] or in animal models exposed to exercise training, as is shown here (Table 2) and in our previous study [16]. It may be that, in this situation of low insulin availability, GCs are permitted to stimulate lipolysis and result in the reduced adiposity observed. The relationships among insulin, GCs, and other hormones that influence lipolysis/lipogenesis require a more detailed investigation both in vivo and in vitro.

To identify the direct effects of GCs in adipocytes, we incubated 3T3-L1 cells in increasing concentrations of corticosterone similar to and above the values that we have observed in active animals [28-30]. In these cells, moderate concentrations of corticosterone, similar to the values typically found in exercising animals, increased lipolysis over a 24-hour period. Importantly, we show that the lipolytic action of GCs is dependent upon the availability of GR, as coincubation with a GR antagonist prevented any increase in lipolysis (Fig. 6). We also show that these modestly elevated concentrations of GCs increase the protein content of 11βHSD1 and GR in 3T3-L1 adipocytes. It is interesting to note that lipolysis is still elevated at higher concentrations of GCs despite no increase in 11βHSD1 protein and a progressive decline in GR protein. Unfortunately, because all protein measurements were taken after the 24-hour incubation, we are unable to determine the timing of these events. One possibility is that the GC-mediated events that increase lipolysis (ie, increased ATGL) occur at an earlier time point and remain active through 24 hours. However, down-regulation of 11βHSD1 and/or GR with sustained exposure to the moderate to high concentrations of GCs may take place as a secondary action, perhaps in a negative-feedback manner. In this situation, it is possible to have increased lipolysis despite the observed changes in 11βHSD1 and GR. Indeed, the highest concentration (100 μmol/L) may activate the negative feedback (ie, reduced GR) before the lipolytic actions can take place. This would explain why lipolysis did not continue to increase from the 10- to 100-μmol/L concentrations, where GR protein was the lowest. This situation is likely representative of a pathologic state such as Cushing syndrome, where an organism is chronically exposed to elevated levels of GCs and subsequently develops severe obesity because of a reduction in lipolytic rate.

In summary, we show that 2 different animal models of excessive adiposity undergoing exercise training display increased protein levels of 11βHSD1 and GR in adipose tissue. This adaptation likely results in increased GC action within the adipocyte, which is accompanied by decreases in adipose mass. Our in vitro data show that GCs are capable of increasing lipolysis and that this effect depends on GR. Taken together, we propose that exercise-induced increases in GC action, through elevations in GR and 11βHSD1, increase the lipolytic action in adipose and facilitate a decrease in adiposity.

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