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Glucocorticoid antagonism limits adiposity rebound and glucose intolerance in young male rats following the cessation of daily exercise and caloric restriction

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Teich T, Dunford EC, Porras DP, Pivovarov JA, Beaudry JL, Hunt H, Belanoff JK, Riddell MC. Glucocorticoid antagonism limits adiposity rebound and glucose intolerance in young male rats following the cessation of daily exercise and caloric restriction. *Am J Physiol Endocrinol Metab* 311: E56–E68, 2016. First published May 3, 2016; doi:10.1152/ajpendo.00490.2015.—Severe caloric restriction (CR), in a setting of regular physical exercise, may be a stress that sets the stage for adiposity rebound and insulin resistance when the food restriction and exercise stop. In this study, we examined the effect of mifepristone, a glucocorticoid (GC) receptor antagonist, on limiting adipose tissue mass gain and preserving whole body insulin sensitivity following the cessation of daily running and CR. We calorically restricted male Sprague-Dawley rats and provided access to voluntary running wheels for 3 wk followed by locking of the wheels and reintroduction to ad libitum feeding with or without mifepristone (80 $mg \cdot kg^{-1} \cdot day^{-1}$) for 1 wk. Cessation of daily running and CR increased HOMA-IR and visceral adipose mass as well as glucose and insulin area under the curve during an oral glucose tolerance test vs. pre-wheel lock exercised rats and sedentary rats (all $P < 0.05$). Insulin sensitivity and glucose tolerance were preserved and adipose tissue mass gain was attenuated by daily mifepristone treatment during the post-wheel lock period. These findings suggest that following regular exercise and CR there are GC-induced mechanisms that promote adipose tissue mass gain and impaired metabolic control in healthy organisms and that this phenomenon can be inhibited by the GC receptor antagonist mifepristone.

exercise; caloric restriction; glucocorticoid antagonism; glucose intolerance; adiposity rebound

REPEATED BOUTS OF WEIGHT LOSS and weight regain, termed weight cycling, have been associated with a greater risk for developing cardiovascular disease and type 2 diabetes over time (5, 20, 26). Although caloric restriction (CR) and regular exercise reduce body fat content and improve metabolic health (74), a key component to their success is adherence. Following weight loss from CR in overweight or obese individuals, nearly one-half of the weight is regained within one year (16). This weight regain is associated with a marked deterioration in whole body insulin sensitivity, which may occur via increased adipose tissue hypertrophy and hyperplasia, changes in neuroendocrine inputs to adipose tissue (43), and reductions in skeletal muscle insulin sensitivity (32). Although regular exercise attenuates the metabolic drive to regain weight after long-term weight loss in animal models (44), humans tend to relapse toward more sedentary behavior after lifestyle interventions (79). Humans (1, 25, 31, 52, 57, 73) and rodents (13, 32–34, 36, 37) rapidly develop significant insulin resistance, glucose intolerance, and visceral fat growth when increased physical activity or dieting stops. Additionally, the reintroduction to ad libitum feeding following a 1- to 2-wk period of CR (50% of ad libitum feeding), dramatically increases visceral fat mass gains, promotes dyslipidemia, and causes diminished skeletal muscle mitochondrial mass and oxidative capacity (15, 19).

Recently, there has been intensified investigation into unveiling the physiological factors that underlie weight regain, and more specifically visceral adiposity rebound, following weight loss from either dieting or exercise. The key regulatory factors that are believed to promote adiposity recovery and the deterioration in whole body insulin sensitivity include increased appetite and alterations in neuroendocrine signaling as well as alterations in adipokine secretion and stress hormone biology (4, 22, 55). The latter phenomenon is particularly intriguing, since CR in a setting of regular physical exercise may be a combined physiological stressor that sets the stage for adiposity rebound and insulin resistance when the food restriction and exercise stop. In a recent review (43), white adipocytes were highlighted for their role in contributing to weight regain, due in part to alterations in their metabolic and inflammatory characteristics that occur following weight loss, a process that is tightly linked to glucocorticoid (GC) biology. Weight cycling-induced glucose intolerance is paralleled by increased proinflammatory cytokine infiltration within adipose tissue, another process that could be linked to alterations in stress hormones (2). Interestingly, CR-induced weight loss increases GC levels and promotes the adipogenic capacity of human preadipocytes (67).

We hypothesized that increases in visceral adiposity and whole body insulin resistance following the cessation of CR and exercise are related, at least in part, to alterations in GC metabolism centrally and locally. It is already well established that enhanced GC production rates, free cortisol levels, and the prereceptor enzyme 11β-hydroxysteroid dehydrogenase-1

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(11β-HSD1) expression in adipocytes correlate positively with visceral fat mass and insulin resistance (62). 11β-HSD1 acts to convert GCs from their inactive to their active form intracellularly, thereby increasing local GC exposure. The genetic manipulation of 11β-HSD1 expression within adipose tissue induces many features of the metabolic syndrome, including insulin resistance, glucose intolerance, hypertriglyceridemia, and visceral obesity (46, 47). Moreover, the expression, protein content, and activity of 11β-HSD1 are indeed elevated within visceral adipose tissue from obese humans (8, 76) and rodents (39). Paradoxically, we and others have observed that increased physical activity is associated with elevated content and activity of 11β-HSD1 in humans and rodents both systemically (6) and within visceral adipose tissue itself (9, 10, 14). The consequence of this exercise-induced increase in 11β-HSD1 activity/expression is unclear. However, these elevations may predispose an animal to rapid GC-induced adipose tissue mass gain and impaired metabolism when "lifestyle" intervention is ceased. Interestingly, adipose tissue mass gain, which occurs within 1 wk of sedentary behavior in previously active rats (daily wheel running), is associated with an increase in adipocyte number (13, 37). This suggests that hyperplasia, a process highly influenced by GCs (64, 66), is an important mediator underlying adipose tissue rebound. Thus, the rapid adiposity gain following the cessation of weight loss intervention via CR and/or exercise may be facilitated through increased GC exposure through systemic increases in GC levels and via increased 11β-HSD1 expression. This may be a way in which the body combats (or rebounds) adipose tissue mass loss caused by the stress of CR and regular exercise. Additionally, GCs are known to induce lipolysis in differentiated adipocytes and regulate the expression of hormone-sensitive lipase (HSL), adipose triglyceride lipase (ATGL), and lipoprotein lipase (LPL) as well as the phosphorylation status of HSL (11, 21, 81). In the adipose tissue of obese individuals, LPL activity is elevated, and HSL and ATGL expression negatively correlate with extent of hyperinsulinemia, which represents enhanced deposition and impaired breakdown of lipids (29, 50). In contrast, exercise is known to increase the content of HSL and ATGL and lower the content of LPL within adipose tissue. It is unknown whether alterations in these markers contribute to the growth of adipose tissue during weight regain.

In this study, we determined whether GC action contributes, either directly or indirectly, to the early adiposity rebound and metabolic impairments associated with stopping CR and exercise. We hypothesized that, by blocking exposure to GCs upon cessation of CR and regular exercise, less adipose tissue mass will accumulate and the associated metabolic impairments will be prevented. We examined the effect of mifepristone (Korlym), a nonselective GR antagonist, on limiting adiposity rebound and glucose intolerance in young male rats following cessation of CR and regular exercise. The rationale for the use of mifepristone in this study was based primarily on three observations. First, there is a link between excess GC exposure and impairments in metabolic health (46, 47, 62); this can readily be seen in humans with Cushing's syndrome (35) as well as in rodents given GCs exogenously (71). Second, our group and others (6, 9, 10, 14) have observed that an increase in daily physical activity is associated with elevated content and activity of 11β-HSD1, which we believe sets the stage for GC-induced adiposity rebound when exercise ceases. Third, studies investigating the cessation of daily physical activity in rodents demonstrate rapid adipose tissue mass gain (13, 33, 37), but to date no studies have attempted to link this phenomenon to elevations in GC action.

MATERIALS AND METHODS

Ethics Statement

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

Rodent Treatment and Experimental Design

Thirty-six male Sprague-Dawley rats (Charles River Laboratories; 50–75 g upon arrival) were individually housed in an humidity- and temperature- (50% relative humidity, 22–23°C) controlled environment with a 12:12-h light-dark cycle (0800–2000). Rats were randomly divided into the following groups: Sedentary CR (Sed CR), Runners CR (Run CR), placebo post-wheel lock (post-WL), or mifepristone post-WL (n = 8–10) (Fig. 1). We also measured ad libitum food intake in an additional group of sedentary rats, partially incorporated into this study, for 28 days. From *weeks 1* to *3* the Run CR, placebo post-WL, and mifepristone post-WL groups were given 24-h access to voluntary running wheels and were placed on a CR diet of 15 g/day of standard rodent chow (Purina Labdiet 5012, St. Louis, MO). The Sed CR rats were placed on the same CR diet. After 3 wk, the placebo and mifepristone post-WL groups had their running wheels locked and were reintroduced to ad libitum feeding. During the fourth week, the placebo and mifepristone post-WL groups received an oral gavage once daily of either mifepristone (80 mg/kg) or a placebo (vehicle) in a 2 ml/kg solution. The Sed CR and Run CR groups were euthanized at the end of the third week and the mifepristone post-WL and placebo post-WL groups were euthanized at the end of the fourth week.

Mifepristone Administration

First, a stock solution was prepared by dissolving mifepristone into dimethyl sulfoxide (DMSO, 20% wt/vol). A vehicle consisting of 0.5% hydroxypropylmethylcellulose and 0.1% Tween 80 in double-distilled H_2O was used for both the mifepristone and placebo post-WL groups. The mifepristone post-WL group received mifepristone + vehi-

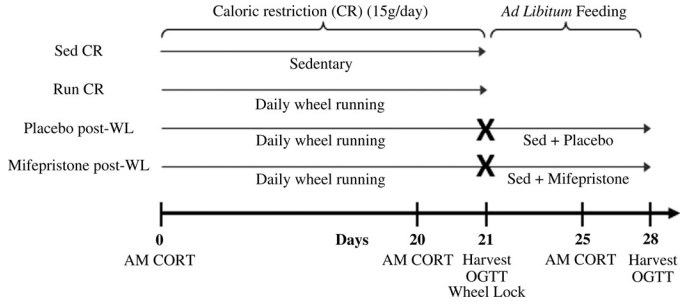

Fig. 1. Experimental timeline. Sprague-Dawley rats were divided into 4 different groups; sedentary caloric restricted (Sed CR), Run CR, placebo post-wheel lock (WL), and mifepristone post-WL. All groups were placed on a CR diet (15 g/day), and all rats except for the Sed CR group were given access to voluntary running wheels 24 h/day for 21 days. Basal (AM) corticosterone (CORT) measurements were taken on *days 0, 20*, and *25*. On *day 21*, placebo and mifepristone post-WL groups had their wheels locked, were reintroduced to ad libitum feeding, and were given daily oral gavage with or without drug (mifepristone). Sed CR and Run CR groups were euthanized on *day 21* and used as pre-wheel lock comparisons. Placebo and mifepristone post-WL groups were harvested on *day 28*, 1 wk following cessation of regular exercise and CR. All groups had an oral glucose tolerance test (OGTT) administered on the day of harvest.

cle and the placebo post-WL group received DMSO + vehicle. The final solutions for both groups contained 20% DMSO in vehicle ± mifepristone. The mifepristone solution was adjusted to provide an 80 mg/kg dose once daily in a 2 ml/kg solution. This dosing strategy was selected based on a murine study in which this dosage attenuated weight gain in a high-fat/high-sucrose diet setting (3). Comparatively, this would be approximately threefold higher than what is prescribed for humans with Cushing's syndrome (25 mg/kg body mass) (12).

Plasma Analysis

Approximately 125 μl of whole blood was collected from saphenous vein bleed to measure fasting plasma insulin (cat. no. 90060; Crystal Chem, Downer's Grove, IL) and basal (resting, AM) corticosterone (cat. no. 07120102; MP Biomedicals, Solon, OH) levels. Nonesterified fatty acids (NEFAs; cat. nos. 999-34691, 995-34791, 991-34891, 993-35191; Wako Diagnostics, Richmond, VA), C-reactive protein (CRP; cat. no. RAB0097; Sigma-Aldrich, Oakville, ON), Leptin (cat. no. RAB0335, Sigma-Aldrich), and adiponectin (cat. no. RAB0004, Sigma-Aldrich) were measured from blood collected during decapitation at the end of the experimental period in midmorning, ~4 h following an OGTT.

Measures Of Insulin Sensitivity and β-Cell Function

Homeostasis model assessment (HOMA) has been described in detail previously (48). Briefly, insulin resistance (HOMA-IR) was calculated as follows: fasted glucose (mM) × fasted insulin (mU/l)/22.5. HOMA for β-cells (HOMA-β) was calculated as follows: [20 × fasted insulin (mU/l)]/[fasted glucose (mM) − 3.5].

Western Blotting

Tissues were homogenized in lysis buffer (135 mM NaCl, 1 mM $MgCl_2$, 2.7 mM KCl, 20 mM Tris base, 1% Triton, 10% glycerol) containing a protease inhibitor cocktail (cat. no. P8340, Sigma-Aldrich) and phosphatase inhibitor cocktail (P0044, Sigma-Aldrich) and were quantified for protein via the Bradford method. Thirty to fifty micrograms of protein lysate from the liver as well as epididymal and subcutaneous adipose tissue was run on a 10% [ATGL, HSL, $pHSL^{Ser660}$, LPL, glucocorticoid receptor (GR), glucose-6-phosphatase (G6Pase), phospho*enol*pyruvate carboxykinase (PEPCK)], or 12% (11β-HSD1) SDS-page gel and transferred to a PVDF membrane (Bio-Rad, Mississauga, ON, Canada). Membranes were blocked in 5% powdered milk and Tris-buffered saline + Tween 20 (TBST) at room temperature for 1 h. Membranes were then incubated overnight in primary antibody at 4°C (ATGL, 1:500, sc-50223, Santa Cruz Biotechnology, Dallas, TX; HSL, 1:1,000, sc-25843, Santa Cruz Biotechnology; $pHSL^{Ser660}$, 1:1,000, cat. no. 4126; Cell Signaling, Beverly, MA; LPL, 1:1,500, ab137821;, Abcam, Cambridge, MA; GR, 1:1,000, sc-8992; Santa Cruz Biotechnology; G6Pase, 1:1,000, sc-25840, Santa Cruz Biotechnology; PEPCK, 1:10,000, sc-32879, Santa Cruz Biotechnology; 11β-HSD1, 1:1,000, cat. no. 10004303, Cayman Chemical, Ann Arbor, MI). The membranes were incubated with goat anti-mouse (1:10,000, ab6789, Abcam) or goat anti-rabbit (1:10,000, ab6721, Abcam) secondary antibodies for 1 h at room temperature. Images were detected on a Kodak In vivo FX Pro imager, and Carestream Image MI SE (v. S.0.2.3.0; Rochester, NY) was used to quantify protein content. Alpha-tubulin (1:40,000, ab7291, Abcam) and β-actin (1:20,000, ab6276, Abcam) were used as loading controls.

Liver Glycogen

Tissue preparation. A section of the left lobe of the liver was cut and plunged into liquid nitrogen until frozen and stored at −80°C until use. On the day of analysis, 25 mg of tissue was weighed and placed in 500 μl of 1 M KOH and placed in a water bath set to 65°C for 1 h and then vortexed thoroughly.

Glycogen analysis with amylo-α-l,4-α-1,6-glucosidase. A volume of 100 μl of the digested sample was aliquoted into 2 tubes. Each tube received 500 μl of 0.2 M acetate buffer pH 4.8 (0.01 M acetic acid: 0.01 M sodium acetate) ± amyloglucosidase (AG, 1 mg/m; Sigma-Aldrich) and was incubated at room temperature overnight. No AG acted as the free glucose control. AG samples were conducted in duplicates. The following day, 1/16 (vol/vol) 5 M NaOH was added to each tube to neutralize the samples. Samples were then briefly vortexed followed by centrifugation for 5 min at 3,000 rpm; 400 μl of the supernatant was then added to a 1.5-ml cuvette. Next, a reagent consisting of 1 mM ATP (Fisher Bioreagents), 0.9 mM NADP (Bioshop), 1.25 unit HK/ml HK/G-6-PDH (Sigma-Aldrich) in triethanolamine hydrochloride (TRA) buffer pH 7.5 (0.3 M TRA, 4.05 mM $MgSO_4$, 120 mM KOH) was added in a volume of 1 ml to each cuvette. After 30 min at room temperature, the glucose formed from the enzymatic breakdown of glycogen was measured using a UV/Visible Spectrophotometer (Ultrospec 2100 Pro) at 340 nm. Absorbance values were adjusted using the optical density 8.89 to obtain values as μmoles glycosil units per milliliter.

Oral Glucose Tolerance Test

An oral glucose tolerance test (OGTT) was administered (1.5 g/kg body mass, 50% dextrose solution) to overnight-fasted animals via oral gavage on the last day of the protocol for each group (Fig. 1). Blood glucose was measured via a handheld glucometer (Bayer, Contour, NY). All rats were euthanized ~4 h following the completion of the OGTT.

Histology

Skeletal muscle (tibialis anterior, TA) from euthanized animals was embedded in tissue freezing medium, frozen in liquid nitrogen, and cryosectioned (10 μm thick). The TA was then stained for succinate dehydrogenase (SDH) activity as modified from Ref. 61. Representative images were chosen for each group from three to four total images. All images were acquired with a Nikon Eclipse 90i microscope and Q-Imaging MicroPublisher with Q-Capture software at ×20 magnification.

Statistical Analysis

All data were analyzed using an appropriate one- or two-way ANOVA with a criterion of $P < 0.05$. A Shapiro-Wilks test was performed to analyze symmetry of the data. All nonparametric data were analyzed using the Kruskal-Wallis test followed by Dunn's test for comparison of groups. All significant differences for one-way ANOVA testing of parametric data were evaluated using Tukey's HSD post hoc test, and two-way ANOVA analysis was performed with a Bonferroni post hoc test (GraphPad Prism v. 6.03). All data are means ± SE.

RESULTS

Mifepristone Attenuates Body Mass and Adipose Tissue Mass Gains Following Cessation of Daily Running and Caloric Restriction

We measured ad libitum food intake in a sedentary, age-matched control group that was partially incorporated into this study, and we observed an average daily food intake of 29.0 ± 1.1 g/day ($n = 8$). The CR of 15 g daily was based on this group (i.e., 50% CR). Daily running with 50% CR for 3 wk significantly increased relative soleus and adrenal mass in the Run CR group vs. all other groups ($P < 0.05$; Table 1). All running groups had significantly lower body mass than the Sed CR group by *day 13* of running. Body mass remained lower in the running animals until WL ($P < 0.05$; Fig. 2*A*). In the

Table 1. *Tissue masses*

	Sed CR	Run CR	Placebo post-WL	Mifepristone post-WL
Final body mass	258 ± 3.80^{ab}	198 ± 4.00^{a}	303 ± 19.3^{b}	277 ± 21.9^{b}
Epididymal fat depot	2.64 ± 0.30^{a}	0.86 ± 0.09^{b}	4.48 ± 0.17^{c}	3.76 ± 0.21^{c}
	0.68 ± 0.08^{a}	0.17 ± 0.02^{b}	1.36 ± 0.06^{c}	1.09 ± 0.06^{d}
Perirenal fat depot	0.42 ± 0.07^{a}	0.20 ± 0.02^{b}	0.91 ± 0.04^{c}	0.64 ± 0.07^{d}
	0.11 ± 0.02^{a}	0.04 ± 0.01^{b}	0.28 ± 0.02^{c}	0.17 ± 0.02^{d}
Inguinal fat depot	1.88 ± 0.22^{a}	0.30 ± 0.06^{b}	3.28 ± 0.32^{c}	2.11 ± 0.20^{a}
	0.48 ± 0.05^{a}	0.06 ± 0.01^{b}	0.88 ± 0.09^{c}	0.58 ± 0.05^{a}
Tibialis anterior	1.97 ± 0.05^{a}	1.95 ± 0.06^{a}	1.78 ± 0.02^{b}	1.79 ± 0.03^{b}
	0.51 ± 0.02^{a}	0.39 ± 0.02^{b}	0.54 ± 0.01^{a}	0.50 ± 0.02^{a}
Plantaris	0.96 ± 0.04^{a}	0.90 ± 0.03^{ab}	0.86 ± 0.02^{b}	0.90 ± 0.02^{ab}
	0.25 ± 0.01^{a}	0.18 ± 0.01^{b}	0.26 ± 0.01^{a}	0.25 ± 0.01^{a}
Soleus	0.45 ± 0.02^{a}	0.54 ± 0.02^{b}	0.45 ± 0.01^{a}	0.45 ± 0.02^{a}
	0.11 ± 0.01^{a}	0.11 ± 0.01^{a}	0.14 ± 0.01^{b}	0.13 ± 0.01^{ab}
Gastrocnemius	5.23 ± 0.13^{a}	4.96 ± 0.12^{ab}	4.67 ± 0.11^{b}	4.86 ± 0.11^{ab}
	1.35 ± 0.04^{a}	1.00 ± 0.04^{b}	1.42 ± 0.05^{a}	1.35 ± 0.05^{a}
Liver	30.2 ± 0.36^{a}	30.7 ± 1.48^{a}	35.9 ± 1.25^{b}	37.9 ± 1.27^{b}
	7.78 ± 0.10^{a}	6.19 ± 0.30^{a}	10.9 ± 0.53^{b}	10.5 ± 0.43^{b}
Adrenals	79.1 ± 3.67^{a}	102.3 ± 5.29^{b}	76.4 ± 2.42^{a}	78.3 ± 2.57^{a}
	20.6 ± 1.01^{a}	20.5 ± 1.21^{a}	22.1 ± 1.21^{a}	21.5 ± 0.40^{a}

All data are means ± SE; $n = 8$–10 for adipose tissue, skeletal muscle, and liver; $n = 3$–4 for adrenals. Final fasted body mass (g). Relative (g/kg body mass) and absolute (g) tissue masses, respectively, for adipose tissue, skeletal muscle, and liver. Relative (mg/kg body mass) and absolute (mg) adrenal mass. Sed, sedentary; CR, caloric restriction; post-wheel lock (post-WL). Different letter indicates a significant difference ($P < 0.05$). All data were analyzed by 1-way ANOVA and Tukey's post hoc test.

post-WL period, the placebo post-WL group (318 ± 8.9 g) had a significantly higher body mass (fed state) than the mifepristone post-WL group (290 ± 6.0 g, $P < 0.05$; Fig. 2*A*). The mifepristone and placebo groups both had significantly greater relative food intake during the post-WL period vs. pre-WL (*day 20*, $P < 0.05$;, Fig. 2*B*). Three weeks of voluntary running significantly reduced relative fat mass in perirenal (0.196 ± 0.02 g), epididymal (0.86 ± 0.09 g), and inguinal (0.30 ± 0.06 g) adipose tissue depots in the Run CR vs. the Sed CR groups (perirenal, 0.42 ± 0.07 g; epididymal, 2.64 ± 0.30 g; inguinal, 1.88 ± 0.22 g, $P < 0.05$; Fig. 2*C, C′, and C′′*). Following the cessation of daily running and CR, there was a significant increase in relative adiposity in the perirenal (0.91 ± 0.04 g), epididymal (4.48 ± 0.17 g), and inguinal (3.28 ± 0.32 g) adipose tissue depots in the placebo post-WL group vs. the Run CR group ($P < 0.05$; Fig. 2*C, C′ and C′′*). This adiposity rebound was significantly attenuated in the perirenal (0.64 ± 0.07 g) and inguinal (2.11 ± 0.20 g) adipose tissue depots with mifepristone treatment post-WL compared with placebo treatment ($P < 0.05$; Fig. 2*C* and *C′′*). For comparison of relative and absolute weights of adipose tissue depots and skeletal muscle, see Table 1. No difference was observed in the total distance that was run between exercising groups during the 3 wk of voluntary running wheel access (Fig. 2*D*).

Mifepristone Reduces 11β-HSD1 but Does Not Alter Markers Of Lipolysis in Visceral Adipose Tissue

Three weeks of daily running increased 11β-HSD1 protein content 5-fold in epididymal fat and 2.5-fold in subcutaneous (inguinal) fat (Run CR vs. Sed CR, $P < 0.05$; Fig. 3, *A* and *G*). 11β-HSD1 remained elevated 1 wk following the cessation of daily running and CR in the placebo post-WL group vs. the Sed CR group in the epididymal depot ($P < 0.05$; Fig. 3*A*). Mifepristone treatment significantly reduced 11β-HSD1 content in epididymal fat during the post-WL period vs. the Run CR and placebo post-WL ($P < 0.05$; Fig. 3*A*). Surprisingly, mifepristone treatment significantly increased 11β-HSD1 content during the post-WL period vs. the placebo post-WL group in the subcutaneous (inguinal) depot ($P < 0.05$; Fig. 3*G*). GR protein content in epididymal fat did not differ between any groups (Fig. 3*B*). The lipolytic markers pHSL^{Ser660}, total HSL, and ATGL were significantly elevated in Run CR vs. the Sed CR group in epididymal fat ($P < 0.05$; Fig. 3, *C–E*). One week of sedentary behavior and ad libitum food intake reduced pHSL^{Ser660} 7-fold, total HSL 2-fold, and ATGL 4-fold vs. the Run CR group ($P < 0.05$; Fig. 3, *C–F*). These lipolytic markers were unaltered by mifepristone treatment (Fig. 3, *C–E*). No significant differences were observed in LPL content (Fig. 3*F*) or fasting plasma NEFAs between any groups (Table 2).

Assessment of Glycogen Content, Glycogenolysis, and Gluconeogenesis

Three weeks of daily voluntary wheel running increased liver glycogen content 6-fold compared with sedentary animals (Run CR vs. Sed CR, $P < 0.05$; Fig. 4*A*). Despite 1 wk of ad libitum food intake following 3 wk of CR plus daily exercise, liver glycogen content was unchanged in the placebo post-WL group vs. the Run CR group but was significantly reduced in the mifepristone post-WL group vs. the Run CR and placebo post-WL groups ($P < 0.05$; Fig. 4*A*). Daily running also significantly increased G6Pase content 2.5-fold vs. the Sed CR group ($P < 0.05$; Fig. 4*B*). No significant differences were observed in G6Pase or PEPCK content in the Run CR, placebo post-WL, or mifepristone post-WL groups (Fig. 4*C*).

Cessation of Daily Running and CR Causes Glucose Intolerance and Insulin Resistance, Which Are Attenuated by Mifepristone

One week of sedentary behavior and ad libitum food intake following 3 wk of daily wheel running and CR impaired glucose tolerance and insulin sensitivity markedly (Fig. 5, *A* and *B*). The glucose area under the curve (AUC) increased

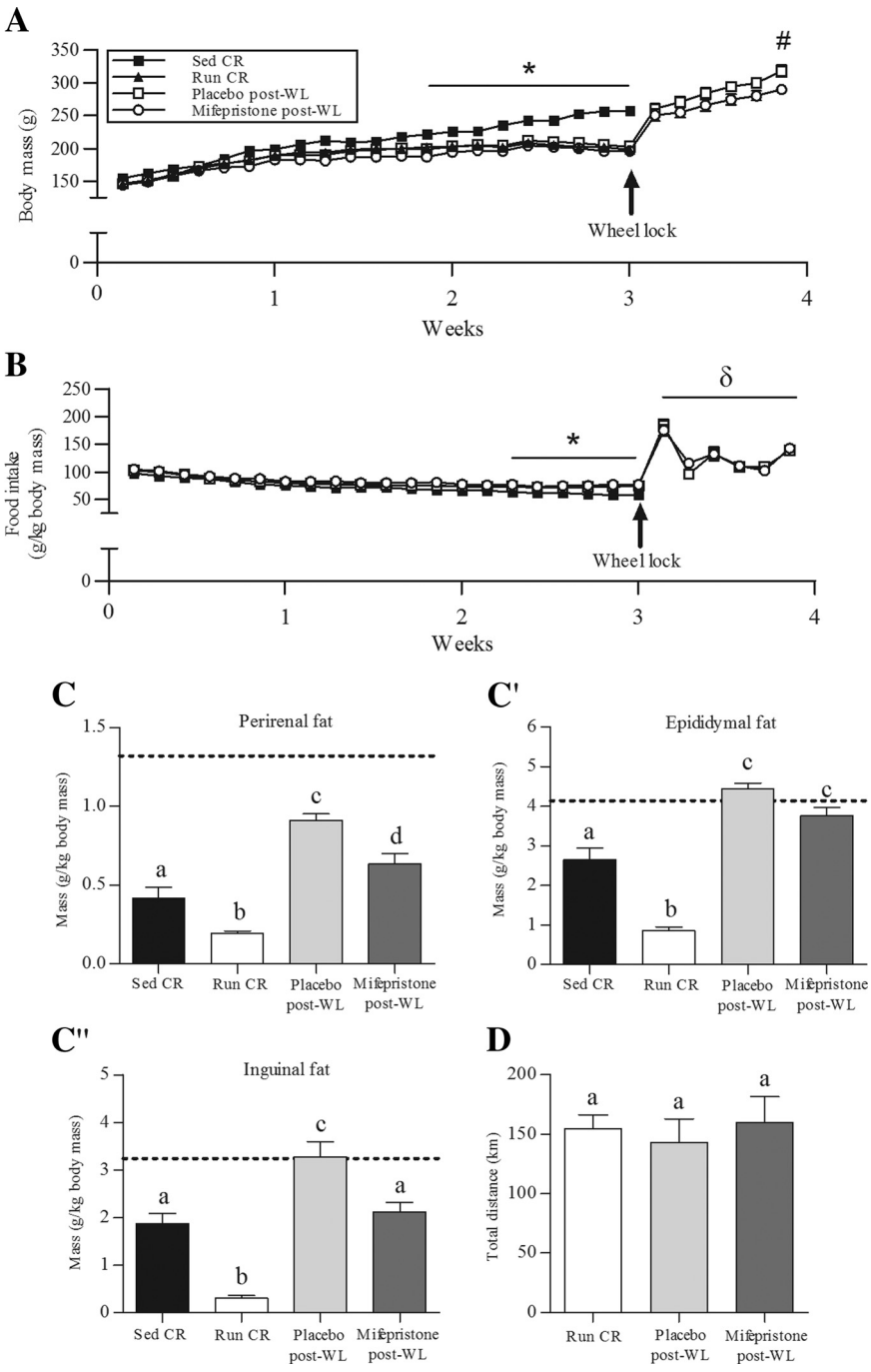

Fig. 2. Body mass, food intake, adipose tissue mass, and running data. *A*: body mass increased over time in all groups. All groups were CR during the 1st 3 wk (15 g/day). At the end of the 3rd wk, placebo and mifepristone post-WL groups had their wheels locked and were reintroduced to ad libitum feeding. Sed CR and Run CR groups were euthanized and used as pre-wheel lock comparisons. *B*: relative daily food intake during the CR (*weeks 1–3*) and ad libitum (*week 4*) periods. *C–C′′*: perirenal, epididymal, and inguinal fat pads were harvested at the end of the 3rd (Sed CR and Run CR) or 4th wk (placebo and mifepristone post-WL). *D*: total running distance throughout protocol in all 3 running groups. Different letter indicates a significant difference ($P < 0.05$). *Sed CR vs. all other groups ($P < 0.05$); #placebo post-WL vs. mifepristone post-WL ($P < 0.05$); δpost-WL (*days 22–27*) vs. pre-WL (*day 21*) in placebo and mifepristone groups. Dotted lines represent an age-matched sedentary group fed ad libitum for 28 days. All data are means ± SE; $n = 8–10$. *A* and *B* were analyzed using 2-way ANOVA with Bonferroni post hoc test; all other graphs were analyzed using 1-way ANOVA and Tukey's post hoc test.

significantly in the placebo post-WL group (238.3 ± 16.3) vs. the Run CR group (133.1 ± 25.8) ($P < 0.05$), and this was prevented with mifepristone treatment (139.2 ± 11.2, $P < 0.05$; Fig. 5*A′*). The placebo post-WL group had a significantly greater insulin AUC (193.2 ± 33.8) vs. the Run CR group (23.4 ± 6.4), and this elevated response, suggesting severe insulin resistance, was attenuated with mifepristone treatment (72.1 ± 9.2) ($P < 0.05$ vs. the placebo post-WL group; Fig. 5*B′*).

There was a significant increase in the placebo post-WL group for fasting glucose (6.12 ± 0.29 mM) and insulin (1.89 ± 0.28 ng/ml) concentrations vs. the Run CR group (4.4 ± 0.22 mM glucose, 0.25 ± 0.05 ng/ml insulin) ($P < 0.05$, Fig. 5*A′′*; $P < 0.01$, Fig. 5*B′′*). These increases were attenuated in the mifepristone post-WL group (4.94 ± 0.19 mM glucose, 0.60 ± 0.11 ng/ml insulin) and were significantly lower than in the placebo post-WL group ($P < 0.05$). HOMA-IR primarily reflects hepatic insulin resistance in the basal state and takes into account the fasted plasma glucose (FPG) and fasted plasma insulin (FPI) values. The placebo post-WL group had a 10-fold greater HOMA-IR vs. the Run CR group (14.88 ± 2.31 vs. 1.46 ± 0.36, respectively), and this increase was largely abolished with mifepristone treatment (3.66 ± 0.77, $P < 0.01$; Fig. 5*C*). HOMA-β measures the basal

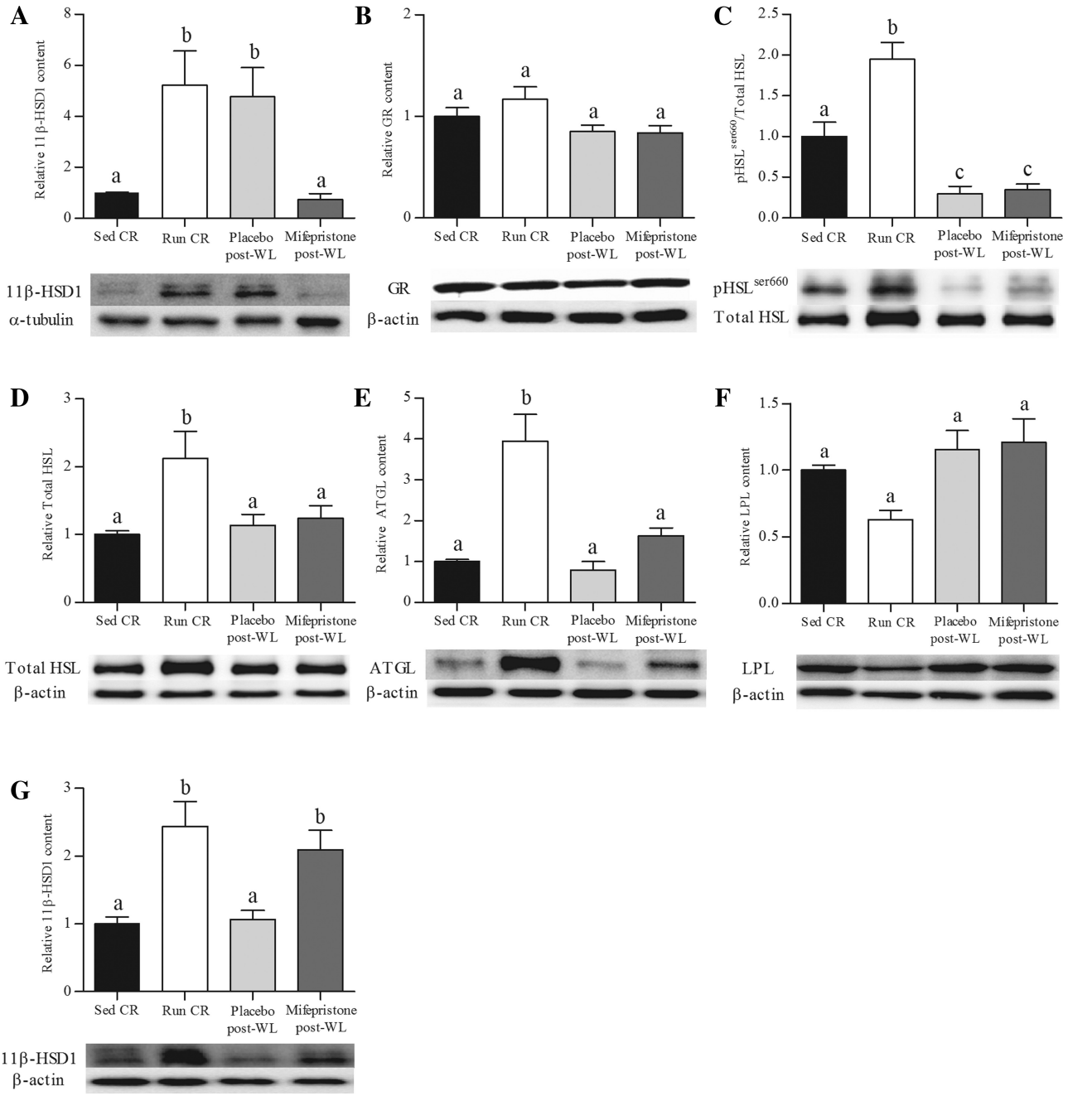

Fig. 3. Adipose tissue glucocorticoid (GC) exposure and lipolytic markers. Relative 11β-HSD1 (*A*) and GC receptor (GR) protein content (*B*) in epididymal fat. Relative phospho-hormone-sensitive lipase (pHSL^{Ser660}; *C*), total HSL (*D*), adipose triglyceride lipase (ATGL; *E*), and lipoprotein lipase (LPL; *F*) protein expression in epididymal fat. *G*: relative 11β-HSD1 content in subcutaneous (inguinal fat). Different letter indicates a significant difference ($P < 0.05$). All data are means ± SE; $n = 4–8$. *A* and *F* were analyzed using Kruskal-Wallis and Dunn's post hoc tests;, all other graphs were analyzed using 1-way ANOVA and Tukey's post hoc test.

insulin secretory function of β-cells and also takes into account FPG and FPI. There was a nonsignificant ($P = 0.06$) increase in relative β-cell function in the placebo post-WL group (1.59 ± 0.23) vs. the Run CR group (0.76 ± 0.20) (Fig. 5*D*). These data indicate that insulin resistance occurs following cessation of daily exercise and CR and that this phenomenon is prevented by 1 wk of daily gavage of mifepristone.

Plasma leptin levels were 2.5-fold higher in the placebo post-WL group vs. all other groups ($P < 0.05$; Table 2). Circulating adiponectin was reduced in the placebo post-WL group vs. the Sed CR group ($P < 0.05$; Table 2), and although the placebo group had the lowest circulating value, there was no statistical difference among the Run CR, placebo-WL, and mifepristone post-WL groups.

Basal Corticosterone Is Increased during Caloric Restriction

Corticosterone measurements were made on *day 0* before the rats were divided into respective groups, and a basal AM value of 117.3 ± 28.3 ng/ml was found for all of the rats (Fig. 6). On

Table 2. *Plasma analytes*

	Sed CR	Run CR	Placebo post-WL	Mifepristone post-WL
NEFA (mM)	0.44 ± 0.04[a]	0.32 ± 0.04[a]	0.47 ± 0.05[a]	0.48 ± 0.04[a]
CRP (μg/ml)	303 ± 22.6[ab]	242 ± 18.6[a]	362 ± 11.3[b]	358 ± 18.5[b]
Leptin (pg/ml)	455 ± 36.1[a]	414 ± 61.5[a]	1217 ± 200[b]	733 ± 83.1[a]
Adiponectin (ng/ml)	727 ± 66.9[a]	655 ± 54.5[ab]	503 ± 66.6[b]	653 ± 59.9[ab]

All data are mean ± SE; n = 7–10. Fasting plasma analytes. CRP, C-reactive protein. Different letter indicates a significant difference ($P < 0.05$). All data were analyzed by 1-way ANOVA and Tukey's post hoc test.

day 20, both Sed CR (390.8 ± 40.5 ng/ml) and the Run CR (336.3 ± 48.2 ng/ml) groups had significantly greater AM corticosterone concentrations vs. *day 0* values ($P < 0.05$). However, there was no additive of effect of exercise on basal circulating corticosterone, as the Sed CR and Run CR groups did no differ significantly on *day 20*. Basal corticosterone was significantly reduced in both the placebo post-WL (48.0 ± 25.8 ng/ml) and mifepristone post-WL group (56.4 ± 18.1 ng/ml) vs. the Sed CR group and all runners on *day 20* ($P < 0.05$; Fig. 6). Despite GC receptor antagonism in the mifepristone post-WL group after cessation of regular exercise and CR, no significant difference in circulating corticosterone levels was observed vs. the placebo post-WL group.

Oxidative Capacity Improves with Exercise but Is Not Altered by Mifepristone

To determine whether the observed differences in glucose tolerance were contributed to by physiological changes in skeletal muscle, oxidative capacity was qualitatively assessed by SDH activity in the TA (Fig. 7). Exercise increased the intensity of staining for SDH, suggesting improved oxidative capacity and mitochondrial content. One week of sedentary behavior and ad libitum feeding did not appear to result in reduced mitochondrial content, as staining intensity in the placebo and mifepristone post-WL groups remained similar to the Run CR group.

DISCUSSION

In this study, 1 wk of physical inactivity and ad libitum feeding, following 3 wk of daily voluntary wheel running and CR, resulted in severe insulin resistance, glucose intolerance and rebound adipose tissue mass gain in healthy young male Sprague-Dawley rats. We also show here that this metabolic dysregulation associated with the cessation of daily exercise and CR was largely abolished with the GC receptor antagonist mifepristone. This suggests that the actions of GCs are linked to early metabolic events that lead to insulin resistance and adipose tissue regrowth after an initial loss of weight induced by CR and daily exercise. The metabolic rescue with mifepristone administration was unrelated to alterations in food intake, adipose tissue lipase content, or hepatic markers of gluconeogenesis and glycogenolysis.

Insulin Resistance after Daily Exercise and CR Is Stopped

Previous studies by Booth and colleagues have demonstrated that insulin resistance rapidly develops (within days) in skeletal muscle (32), but not adipose tissue (33), in their rodent wheel lock model. In this study, cessation of daily running and CR resulted in a two- and eightfold increase in glucose AUC and insulin AUC, respectively, in the placebo post-WL group vs. the Run CR group. Remarkably, fasting insulin and HOMA-IR were seven- and tenfold higher in the placebo post-WL vs. the Run CR group. Mifepristone treatment prevented both hyperglycemia and hyperinsulinemia during the OGTT, reduced fasting blood glucose and insulin levels, and prevented the increase in HOMA-IR vs. the placebo post-WL group. Insulin resistance in the placebo post-WL group occurred despite no change to circulating NEFAs or the key markers of lipolysis in adipose tissue. This suggests that lipid breakdown and delivery into circulation was unrelated to the insulin resistance after wheel lock. Importantly, this hyperinsulinemia induced by the cessation of CR and exercise, paired with maintained adipose tissue insulin sensitivity, may promote rapid adipose tissue regrowth (see below).

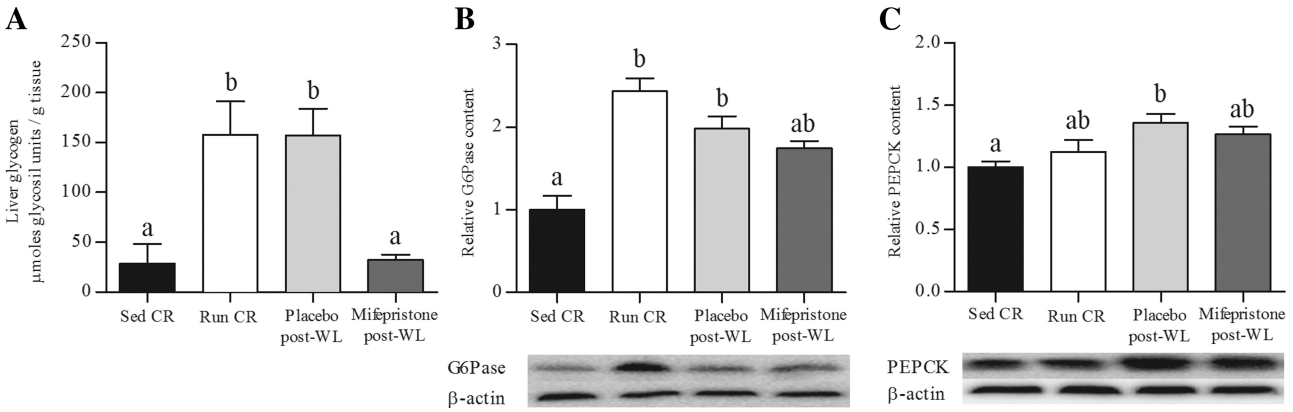

Fig. 4. Assessment of glycogen content, glycogenolysis, and gluconeogenesis. Liver glycogen content (*A*), relative G6Pase (*B*), and relative PEPCK (*C*) protein content. Different letter indicates a significant difference ($P < 0.05$). All data are means ± SE; n = 5–8 All data were analyzed using Kruskal-Wallis and Dunn's post hoc tests.

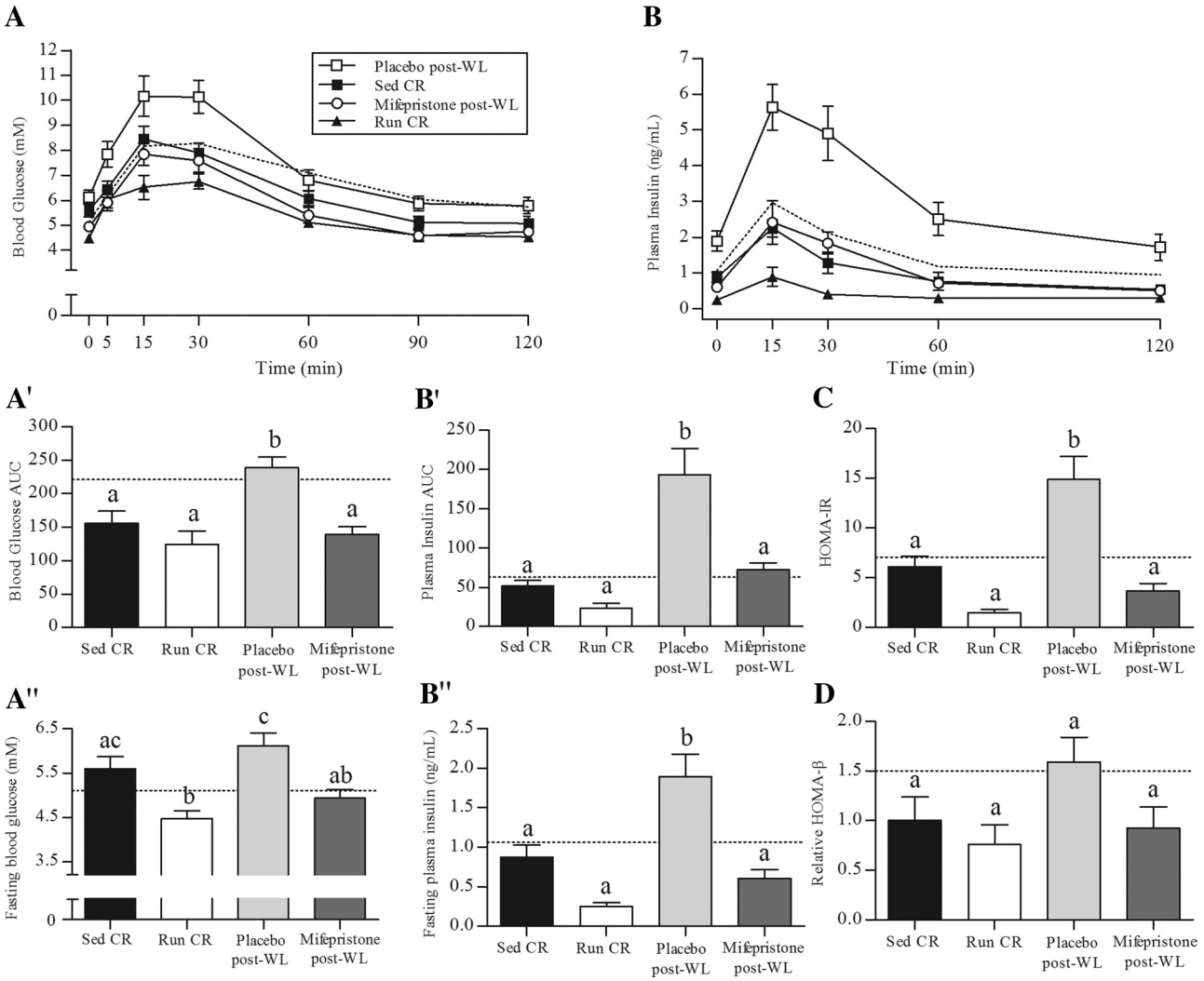

Fig. 5. Glucose tolerance and whole body insulin resistance. All groups had an oral glucose tolerance test (OGTT) (1.5 g/kg body mass) administered at the end of the 3rd (Sed CR and Run CR) or 4th wk (placebo and mifepristone post-WL). *A*: glucose concentrations (mM) in the fasted state ($t = 0$) and at $t = 5$, 15, 30, 60, and 120 min post-oral glucose load. *B*: insulin concentrations (ng/ml) in the fasted state ($t = 0$) and at $t = 15$, 30, 60, and 120 min. *A′* and *B′*: area under the curve (AUC) for glucose and insulin during OGTT was calculated relative to $t = 0$ glucose or insulin value for each individual rat. *A″* and *B″*: fasted glucose and insulin values taken at the end of the 3rd (Sed CR and Run CR) or 4th wk (placebo and mifepristone post-WL). *C*: HOMA-IR was calculated as follows: fasting insulin (mU/l) × fasting glucose (mM)/22.5. *D*: relative HOMA-β (HOMA for β-cells) was calculated as follows: 20 × fasting insulin (mU/l)/fasting glucose (mM) − 3.5. Different letter indicates a significant difference ($P < 0.01$ for *B″* and *C*, $P < 0.05$ for all other graphs). Dotted lines represent age-matched sedentary group fed ad libitum for 28 days. All data are means ± SE; $n = 8$–10. All data were analyzed using 1-way ANOVA and Tukey's post hoc test.

Interestingly, mifepristone treatment was found to have adipose depot-specific effects, as 11β-HSD1 protein content was significantly reduced in the epididymal depot but increased in the inguinal depot relative to the placebo post-WL group. These findings indicate that there may have been depot-specific metabolic consequences of altered 11β-HSD1 content in the placebo post-WL group. It also suggests that 11β-HSD1 content is not directly related to the amount of adipose tissue mass rebound that occurs after exercise and CR are stopped. Overexpression of 11β-HSD1 within adipose tissue increases active intracellular GC levels and can induce visceral obesity, whole body insulin resistance, and glucose intolerance, as well as increased serum NEFAs, triglycerides, and proinflammatory cytokines (46). Indeed, we observed a significant increase in circulating C-reactive protein (CRP) in the placebo post-WL group vs. the Run CR group. No differences were observed between the placebo and mifepristone post-WL groups in CRP levels despite mifepristone lowering epididymal 11β-HSD1 levels. This suggests that the acute change in systemic inflammation did not contribute to the insulin resistance in the placebo post-WL group and that CRP levels are not tightly linked with 11β-HSD1 expression in the epididymal fat pad. However, it was recently found that in wheel-locked rats there is an upregulation of gene transcripts related to immunity, macrophage infiltration, and proinflammation within perirenal adipose tissue (68). Thus, there may be local inflammatory changes in adipose tissue that play a key role in impaired metabolic health in the animals post-wheel lock. Despite the correlation between inflammation, increased 11β-HSD1, and adipose tissue (75, 82), the Run CR group, which had elevated inguinal and epididymal 11β-HSD1 content, had the lowest amount of circulating CRP. This is likely explained by the fact

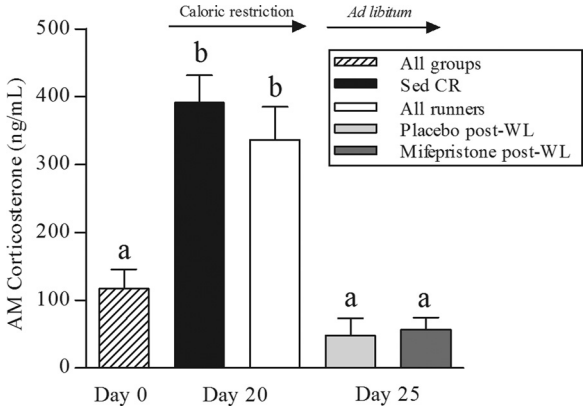

Fig. 6. Basal corticosterone. AM CORT (0800) concentrations were collected on *days 0, 20*, and *25* from the saphenous vein. *Day 0* represents all rats before they were divided into separate groups. *Day 20* is 1 day before Sed CR and Run CR groups were euthanized and 1 day before WL was commenced. *Day 25* represents post-WL period. Different letter indicates a significant difference ($P < 0.05$). All data are means ± SE; $n = 3$–4 for Sed CR and placebo and mifepristone post-WL; $n = 11$–15 for "all groups" and "all runners". Data were analyzed using Kruskal-Wallis and Dunn's post hoc tests.

that exercise training suppresses low-grade inflammation and is associated with improved insulin sensitivity (51, 60). Future studies should further explore this paradoxical relationship among exercise, 11β-HSD1, inflammation, and impaired metabolic health following cessation of exercise and CR.

We observed a threefold increase in circulating corticosterone levels during the pre-WL period in all of the CR rats. Thus, prior to the wheel lock period, there was systemic hypercorticosteronemia, which may have contributed to the deteriorated metabolic profile and rapid adipose tissue mass gain in the placebo rats once ad libitum feeding was resumed. It is important to note that, although the physically active rats had hypercorticosteronemia and elevated inguinal 11β-HSD1 expression relative to the placebo post-WL group, they had substantially better glucose tolerance and insulin sensitivity and less body fat. Previous studies have suggested that highly trained humans can exhibit basal hypercorticosteronemia (40, 77) as well as systemically elevated 11β-HSD1 activity several days after a single bout of endurance exercise (6). The function of the observed elevations in GC exposure within adipose tissue following exercise is unclear. We speculate that it may be one of the mechanisms underlying the body's physiological drive for fat regain after initial fat mass loss. Collectively, the loss of fat mass and improved metabolic efficiency of peripheral tissues induced by CR and daily exercise, mixed with elevated GC exposure within adipose tissue, may become detrimental only once paired with excessive caloric intake and physical inactivity.

GCs are also known for their capacity to stimulate hepatic gluconeogenesis by inducing the expression and increased activity of PEPCK (83). Furthermore, GR antagonism has been shown to suppress hepatic gluconeogenesis (70). To our surprise, this was not the case in our study, where the placebo post-WL group did not differ significantly from the mifepristone post-WL group in either PEPCK or G6Pase content in the liver. This suggests that elevations in basal gluconeogenesis and glycogenolysis did not contribute to the rapid insulin resistance and glucose intolerance in the placebo post-WL group, perhaps because the circulating GC concentrations were normalized 4 days following wheel lock. However, the placebo post-WL group had significantly greater liver glycogen content than the mifepristone-treated rats despite no difference in food intake, suggesting that there was some alteration in liver glycogen metabolism between these groups. In support of this, it has been previously reported that GCs predominantly act to stimulate glycogen synthesis in a high-insulin environment (42). This is consistent with our findings as the placebo post-WL group was hyperinsulinemic, had greater liver glycogen content, and had uninhibited GC action relative to the mifepristone post-WL group.

Adiposity Rebound after Daily Exercise and CR Is Stopped

We observed a four- to tenfold increase in relative adipose tissue mass in perirenal, epididymal, and inguinal fat 1 wk following cessation of CR and daily running. Daily mifepristone treatment in the post-WL period attenuated body mass gain and relative adipose mass in perirenal and inguinal depots and partially attenuated epididymal fat rebound. In fact, the absolute masses of all three fat depots were significantly reduced in the mifepristone post-WL group vs. the placebo-treated animals. Previously, it had been shown that mifepristone treatment reduced adipose tissue mass gain and body weight gain in rodent models of diet-induced obesity (3, 28, 56). In addition, 2–4 wk of mifepristone treatment reduced risperidone- and olanzapine-induced weight gain in healthy men (23, 24). This suggests that GCs play a significant role in contributing to fat deposition and weight gain in these studies.

Paradoxically, GCs appear to be both anabolic and catabolic in adipose tissue (58). Twenty-four hours to 10 days of GC exposure increases lipolysis in differentiated adipocytes (11, 81). Additionally, this elevated rate of lipolysis was abolished by cotreatment with the GR antagonist mifepristone (11, 81). We found a significant increase in total HSL, pHSL^{Ser660}, and ATGL protein content in epididymal adipose tissue in response

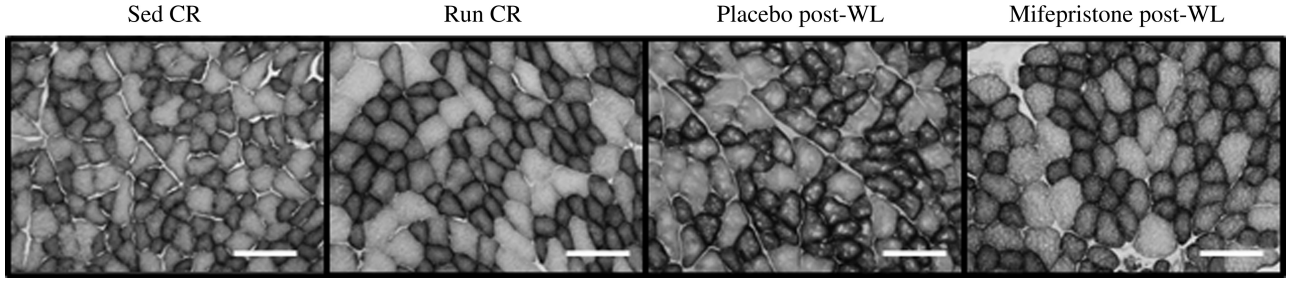

Fig. 7. Oxidative capacity in skeletal muscle. Representative succinate dehydrogenase activity in the tibialis anterior. A ×20 objective was used for all images; scale bar, 100 μm.

to daily voluntary wheel running followed by a two- to fourfold reduction in their content after 1 wk of physical inactivity and ad libitum feeding. However, mifepristone administration did not alter their protein content vs. the placebo post-WL group. It is important to note that GCs are known to have time-, dose-, sex-, and depot-specific regulation of adipocyte lipolysis (41, 58). Thus, differences in the content of these lipolytic proteins may be present in other depots that may have contributed to the differences observed in fat depot weights. In addition, the effects of GCs on lipolysis have been shown to depend on the amount of insulin present. For example, infusion of cortisol enhances or suppresses subcutaneous adipose lipolysis in humans when circulating insulin is "clamped" low (18) or high (69), respectively. Thus, the hyperinsulinemia observed in the placebo post-WL group may explain the lack of lipolytic activity and may have contributed to the greater adipose tissue mass gain in the placebo group compared with the mifepristone group. In addition to being lipolytic in nature, GCs also play a crucial role in stimulating adipogenesis (66). This is supported by our previous data that found that rats treated for 10 days with corticosterone exhibited a 1.5-fold increase in epididymal adipose mass and a 2.5-fold increase in the number of adipocytes per milligram of tissue, despite a significant increase in adipose tissue lipolysis (11). Thus, GCs can concurrently contribute to both the breakdown of lipids and the accumulation of new adipocytes. Interestingly, in studies that tracked the wheel lock period for up to 1 wk, the accrual of epididymal, omental, and retroperitoneal as well as perirenal fat was primarily due to hyperplasia of adipocytes rather than hypertrophy (13, 37). Our finding that mifepristone attenuated fat rebound suggests that the process of adipogenesis during the wheel lock period may be at least partially blocked.

Metabolic Impact of CR

CR by ~50% for 3 wk in humans has been shown to significantly decrease circulating norepinephrine, thyroid stimulating hormone, leptin, insulin, and testosterone levels (54). Although short-term CR is thought to be largely beneficial, this extent of CR for 6 mo can induce anemia, skeletal muscle wasting, dizziness, irritability, lethargy, and depression (30). In more recent years, randomized control trials, which generally reduce caloric intake by 20–30% for 6–12 mo, have observed reductions in circulating insulin, leptin, and TNFα/adiponectin ratio as well as reduced quantity of visceral and subcutaneous fat (27, 63, 78, 80). In rodents, the quantity of circulating leptin, TNFα, and insulin-like growth factor I are negatively correlated to the extent of CR (10–40% reduction) (53). In contrast, corticosterone, which increases in response to 25% CR, is not increased any further by a 50% CR (38). This evidence suggests that most physiological changes induced by CR are sensitive to both the length of time and the percentage of CR. Thus, the metabolic consequences of cessation of CR may also depend on these factors. In addition, the metabolic impairments and adipose tissue rebound in this study may have been more extensive due to pairing cessation of CR with daily exercise. It would be interesting for future studies to investigate whether mifepristone is capable of attenuating impairments induced by CR cessation alone.

Our primary aim was to investigate the role of GCs in contributing to the rapid metabolic impairments and adipose tissue mass gain that occur after regular exercise and CR are stopped. However, it is important to note that the Run CR group had equally good, if not better, glucose tolerance, insulin sensitivity, and lower body fat mass than the mifepristone post-WL group even though the former had systemically elevated GC levels and elevated epididymal 11β-HSD1 content. Thus, exercise with CR should be considered as being effective for promoting insulin sensitivity even if it promotes elevations in GC exposure. Lifelong CR (30–50% less than ad libitum) has been found to increase the lifespan of rodents (45, 49), and physical activity is a primary preventative method against chronic conditions including psychiatric, neurological, metabolic, cardiovascular, pulmonary, and musculoskeletal disorders (7, 59). Therefore, it is important to emphasize the health benefits that can be achieved by exercising regularly and limiting caloric intake.

Limitations and Future Directions

A key limitation of this study was the lack of a precise mechanism underlying the metabolic benefits attributable to mifepristone. Considering the link between adipose tissue inflammation, elevated 11β-HSD1, and a recent study that demonstrated an upregulation of adipose proinflammatory gene transcripts following wheel lock in rats (68), future studies should explore whether a key protective mechanism of mifepristone is through reducing local inflammatory markers such as TNFα, IL-6, and NF-κB or by limiting macrophage infiltration. Conversely, it would be interesting to determine whether administration of an anti-inflammatory compound would yield similar metabolic benefits following wheel lock. For example, curcumin, a component of turmeric, is well known for its anti-inflammatory properties (65, 72) and would be a worthwhile compound to explore. To explore how mifepristone limited adipose tissue mass gain, future studies should explore whether mifepristone limits adipogenesis and lipogenesis in response to cessation of CR and daily running. Unfortunately, other biologically relevant adipose tissue depots, including omental and retroperitoneal fat, were not collected, and, due to technical issues, we were unable to analyze the perirenal adipose tissue that was collected. It would be interesting to note if our 11β-HSD1 and lipolytic marker observations form a consistent difference between visceral vs. subcutaneous depots. Also, the corticosterone measurements in this study were taken from a saphenous vein bleed of rats following the shaving of their hindleg. Circulating corticosterone is acutely sensitive to handling stress in rodents and can decrease over time due to habituation of the sampling technique (17). Thus, any conclusions drawn from this analysis must be made in light of the limitations of this type of sample collection protocol.

Conclusion

In summary, we observed that cessation of daily wheel running and CR in rodents rapidly promotes adipose tissue mass expansion, whole body insulin resistance, and glucose intolerance. This study also demonstrates, for the first time, that the actions of stress hormones may be linked to the deterioration of metabolic health that occurs following cessation of CR and daily exercise. At the very least, many of these

deleterious metabolic effects can be blocked or attenuated by the global GC receptor antagonist mifepristone.

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DISCLOSURES

H. Hunt and J. K. Belanoff are employees of Corcept Theraputics, which develops glucocorticoid receptor ligands for clinical use. Corcept Theraputics provided the mifepristone for this study and financed part of the experiments.

AUTHOR CONTRIBUTIONS

T.T., D.P.P., J.L.B., H.H., J.K.B., and M.C.R. conception and design of research; T.T., E.C.D., D.P.P., J.A.P., and J.L.B. performed experiments; T.T., E.C.D., D.P.P., J.L.B., and M.C.R. analyzed data; T.T., E.C.D., D.P.P., J.L.B., H.H., J.K.B., and M.C.R. interpreted results of experiments; T.T., D.P.P., and M.C.R. prepared figures; T.T. drafted manuscript; T.T., E.C.D., D.P.P., J.A.P., J.L.B., H.H., J.K.B., and M.C.R. edited and revised manuscript; T.T., E.C.D., D.P.P., J.A.P., J.L.B., J.K.B., and M.C.R. approved final version of manuscript.

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