Regular exercise prevents the development of hyperglucocorticoidemia via adaptations in the brain and adrenal glands in male Zucker diabetic fatty rats

Jonathan E. Campbell,1 Michael A. Király,2 Daniel J. Atkinson,1 Anna M. D’Souza,1 Mladen Vranic,2 and Michael C. Riddell1

1School of Kinesiology and Health Science, Faculty of Health, Muscle Health Research Centre, York University, and 2Departments of Physiology and Medicine, University of Toronto, Toronto, Ontario, Canada

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Regular exercise prevents the development of hyperglucocorticoidemia via adaptations in the brain and adrenal glands in male Zucker diabetic fatty rats. Am J Physiol Regul Integr Comp Physiol 299: R168–R176, 2010. First published April 14, 2010; doi:10.1152/ajpregu.00155.2010.—We determined the effects of voluntary wheel running on the hypothalamic-pituitary-adrenal (HPA) axis, and the peripheral determinants of glucocorticoids, in male Zucker diabetic fatty (ZDF) rats. Six-week-old euglycemic ZDF rats were divided into Basal, Sedentary, and Exercise groups (n = 8–9 per group). Basal animals were immediately killed, whereas Sedentary and Exercising rats were monitored for 10 wk. Basal (i.e., ~0900 AM in the resting state) glucocorticoid levels increased 2.3-fold by week 3 in Sedentary rats where they remained elevated for the duration of the study. After an initial elevation in basal glucocorticoid levels at week 1, Exercise rats maintained low glucocorticoid levels from week 3 through week 10. Hyperglycemia was evident in Sedentary animals by week 7, whereas Exercising animals maintained euglycemia throughout. At the time of death, the Sedentary group had ~40% lower glucocorticoid receptor (GR) content in the hippocampus, compared with the Basal and Exercise groups (P < 0.05), suggesting that the former group had impaired negative feedback regulation of the HPA axis. Both Sedentary and Exercise groups had elevated ACTH compared with Basal rats, indicating that central drive of the axis was similar between groups. However, Sedentary, but not Exercise, animals had elevated adrenal ACTH receptor and steroidogenic acute regulatory protein content compared with the Basal animals, suggesting that regular exercise protects against elevations in glucocorticoids by a downregulation of adrenal sensitivity to ACTH. GR and 11β-hydroxysteroid dehydrogenase type 1 content in skeletal muscle and liver were similar between groups, however, GR content in adipose tissue was elevated in the Sedentary groups compared with the Basal and Exercise (P < 0.05) groups. Thus, the gradual elevations in glucocorticoid levels associated with the development of insulin resistance in male ZDF rats can be prevented with regular exercise, likely because of adaptations that occur primarily in the adrenal glands.

GLUCOCORTICOID EXCESS IS CHARACTERIZED by increased central adiposity, insulin resistance, hyperlipidemia, and elevated glucose production (3), while in the pancreas sustained elevations in glucocorticoids adversely affect beta cells and directly attenuate insulin release (19)—all features that make these hormones potent diabetogenic agents (41). Moreover, glucocorticoids are vasoactive, and their elevation is an independent risk factor for cardiovascular disease and other diabetes-related complications (15). Human and animal models of both type 1 (12, 14) and type 2 (5, 6, 27) diabetes show elevations in circulating glucocorticoids, supporting the hypothesis that hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis is tightly coupled to the pathophysiology of both forms of the disease. Evidence is also mounting that elevations in glucocorticoids, either by increased central drive of the HPA axis or by peripheral activation of glucocorticoids through 11β-hydroxysteroid dehydrogenase-1 (11βHSD1) activity, plays a pathological role in the development of the metabolic syndrome (1). We have shown previously that hyperglycemia resulting from streptozotocin-induced diabetes increases the activity of the HPA axis, and that normalizing glucose with either insulin or chloridrazine corrects this hyperactivity (11, 13). Based on these findings, one might assume that the hypercortisolemia that is also observed in type 2 diabetes (6, 7, 16) is a result of hyperglycemia and relative hypoinsulinemia. Surprisingly, however, no known studies report on the levels of glucocorticoids during the progression from prediabetes to type 2 diabetes in animal models of this disease.

While several studies have measured glucocorticoid levels in models of type 2 diabetes (6, 7, 16, 40), few have examined the central (brain) and peripheral (adrenal gland and target tissues) components of glucocorticoid release and action. Although much of the data point toward increased HPA activity with hyperglycemia (6, 27), the results are not consistent (2) and the mechanism(s) behind the observed increase in HPA activity in these rodent models are also unclear. Some studies suggest that increases in corticotrophin-releasing hormone (CRH) sensitivity (6) or ACTH sensitivity (4) may account for the observed increases in circulating glucocorticoid levels, while others have shown decreased negative feedback following a dexamethasone suppression test (6, 10), suggesting abnormalities at the level of the hippocampus or hypothalamus. To our knowledge, no study has profiled the time course of change in basal (~0900 AM in the resting state) glucocorticoid levels in male Zucker diabetic fatty (ZDF) rats to determine whether the hyperglucocorticoidemia precedes the development of hyperglycemia or whether it results after the alteration in glucose homeostasis.

Exercise acutely activates the HPA axis and raises basal glucocorticoid levels (36). Regular exercise, however, is well known to prevent the development of insulin resistance and to delay the progression toward type 2 diabetes in both humans with prediabetes (26) and in rodent models of the disease (17, 23, 24, 35, 38). We have recently shown that adaptations exist in healthy rodents that normalize a transiently elevated activity of the HPA axis within days to weeks after the start of training (9, 21, 32). We attribute this restoration in HPA axis activity in healthy rats to adaptations in the hypothalamus and adrenal...
can prevent, or at least delay, the development of hypercortisolemia. These novel findings are important as they illustrate new mechanisms for how regular exercise induces positive adaptations, primarily in the adrenal gland, which promote a lower CRH production and reduced adrenal sensitivity to ACTH, respectively (9, 21, 32). Whether these adaptations also exist in the ZDF rat (a rodent model of type 2 diabetes that exhibits elevated HPA axis activity) is unknown.

In this study, we set out to determine 1) whether elevations in HPA axis activity precede, or respond to, the development of hyperglycemia in the ZDF rat and 2) whether regular exercise, which is known to prevent hyperglycemia in this animal model, prevents the central and peripheral hyperactivity of the HPA axis that is associated with disease development. We show that sedentary ZDF rats have elevations in HPA axis activity that precedes the development of hyperglycemia, suggesting that glucocorticoids have a causative role in this model of type 2 diabetes. Furthermore, we show that regular exercise induces positive adaptations, primarily in the adrenal gland, that lowers hypercortisolemia. These novel findings are important as they illustrate new mechanisms for how regular exercise can prevent, or at least delay, the development of hypercortisolemia and its associated metabolic disturbances.

RESEARCH DESIGN AND METHODS

Animals. Male ZDF rats were obtained from Charles River Laboratories (Saint-Constant, Quebec, Canada) at 5 wk of age with initial body weights of 150–175 g, were individually housed in clear cages, and kept in a temperature (23–25°C)- and humidity (40%–50%)-controlled room for a 7-day habituation period. The animals were given standard rodent chow (Purina 5001, 4.3 kcal/g metabolizable energy) and water ad libitum throughout the study duration. Following the habituation period, rats were randomly assigned to one of three groups: Basal (n = 8), Sedentary (n = 8), or Exercise (volitional exercising, n = 9). Exercise animals were individually housed in specialized activity wheel cages (height: 36.4 cm, width: 26.8 cm, depth: 50 cm) with unrestricted, 24-h access to their wheels (circumference: 108 cm, width: 9 cm). Basal and Sedentary animals were housed in similarly sized cages, but without activity wheels. Wheel revolutions, body weight, and food intake were recorded daily. Running distance was calculated as the circumference times recorded revolutions. Basal animals were euthanized following the habituation period at 6 wk of age. Sedentary and Exercise animals were euthanized 10 wk later at 16 wk of age. All experiments were approved by the Animal Care Committee of the Faculty of Medicine at the University of Toronto in accordance with regulations set forth by the Canadian Council for Animal Care.

Table 1. Animal characteristics, blood hormone concentrations, and IPGTT results

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Sedentary</th>
<th>Exercise</th>
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<tbody>
<tr>
<td>Final body weight, g</td>
<td>166.6 ± 3.5</td>
<td>454.0 ± 10.5*</td>
<td>426.4 ± 6.4*</td>
</tr>
<tr>
<td>Average daily food</td>
<td></td>
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<tr>
<td>intake, g</td>
<td>29.2 ± 0.5</td>
<td>30.0 ± 0.7</td>
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<tr>
<td>Epididymal adipose</td>
<td></td>
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<tr>
<td>weight, g</td>
<td>0.87 ± 0.06</td>
<td>5.45 ± 0.2*</td>
<td>4.10 ± 0.2†</td>
</tr>
<tr>
<td>Plantaris weight, mg</td>
<td>108.5 ± 2</td>
<td>141.3 ± 7†</td>
<td></td>
</tr>
<tr>
<td>Fasted FFA, μEq/l</td>
<td>313.4 ± 15.1</td>
<td>816.8 ± 78.4*</td>
<td>324.9 ± 21.7†</td>
</tr>
<tr>
<td>Fasted TG, mM</td>
<td>1.51 ± 0.09</td>
<td>3.61 ± 0.38*</td>
<td>3.74 ± 0.29†</td>
</tr>
<tr>
<td>Fed glucose, mM</td>
<td>6.5 ± 0.2</td>
<td>16.0 ± 2.7*</td>
<td>5.1 ± 0.3†</td>
</tr>
<tr>
<td>IPGTT AUC glucose, AU</td>
<td>1104 ± 56</td>
<td>2141 ± 144*</td>
<td>1376 ± 125†</td>
</tr>
<tr>
<td>IPGTT AUC insulin, AU</td>
<td>326 ± 63</td>
<td>752 ± 77*</td>
<td>1020 ± 125††</td>
</tr>
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Values are means ± SE. FFA, free fatty acids; TG, triglycerides; IPGTT, intraperitoneal glucose tolerance test; AUC, area under the curve. *Significantly different from basal group; †significantly different from sedentary group.

Blood sampling. The glycemic profile and glucose tolerance of these animals have been published elsewhere (25). Once per week (Thursdays), the rats were fasted overnight, for 15–18 h, after which blood samples were taken via a venous tail nick for glucose and insulin concentrations. The first drop of blood was used to measure glucose concentrations using a blood glucose monitor (model ASCENSIA ELITE XL blood glucose meter; Bayer, Toronto, Canada). Approximately 100 μL of whole blood were collected into heparinized microvetttes (Sarstedt, Montreal, Canada), and the plasma was separated by centrifugation at 400 g for 1 min and stored at −20°C. Fasting insulin concentrations were analyzed using a rat insulin ELISA assay kit (Crystal Chem, Downers Grove, IL). Additional blood samples were taken via tail nick once per week (Mondays) at 0800 h under normal (i.e., nonfasted) conditions for the determination of fed glucose (whole blood), insulin (plasma), and corticosterone (plasma) concentrations. Corticosterone concentrations were analyzed with a commercially available RIA kit (Medicorp, Montreal Canada).

Intraperitoneal glucose tolerance test. All groups were subjected to an intraperitoneal glucose tolerance test (IPGTT) 3 days prior to euthanasia. Rats were fasted overnight for 15–18 h and were then administered an intraperitoneal injection of 50% dextrose (Abbott Laboratories, Montreal, Canada) at a dose of 2 g/kg body wt between 0900 h and 1200 h. Blood for glucose and insulin levels was collected via tail nick at 30-min intervals for 2 h. Blood was immediately prepared for analysis by centrifugation at 400 g for 1 min and stored at −20°C. Fasting insulin concentrations were analyzed using a rat insulin ELISA assay kit (Crystal Chem, Downers Grove, IL). Additional blood samples were taken via tail nick once per week (Mondays) at 0800 h under normal (i.e., nonfasted) conditions for the determination of fed glucose (whole blood), insulin (plasma), and corticosterone (plasma) concentrations. Corticosterone concentrations were analyzed with a commercially available RIA kit (Medicorp, Montreal Canada).

Fig. 1. Weekly measurements of corticosterone and plasma ACTH concentrations at euthanasia. A: corticosterone concentrations in Sedentary animals were elevated by week 3, whereas corticosterone in Exercising animals decreased from week 1 to week 3 and remained low thereafter. B: ACTH concentrations at euthanasia were elevated in both Sedentary and Exercising animals compared with the Basal group. Values are means ± SE. *P < 0.05, †P < 0.05 vs. Basal group; n = 8 for Basal and Sedentary groups, n = 9 for Exercise group.
centrifuged as previously described, and frozen at −20°C for subsequent analysis.

**Euthanization.** As previously mentioned, Basal animals were euthanized at 6 wk of age, whereas Sedentary and Exercise animals were euthanized at 16 wk of age. Euthanasia occurred in the morning (0800 h to 1000 h) to obtain basal plasma hormone levels, within 5 h of the last bout of exercise. Trunk blood was collected in EDTA and trasylol-coated tubes, immediately centrifuged, and stored at −20°C for subsequent hormone analysis. Postprandial blood glucose was measured with a blood glucose meter. Plasma free fatty acids and triglycerides were determined by an enzymatic colorimetric method (ACS-ACOD; Wako Chemicals, Richmond, VA). Plasma ACTH concentrations were determined by a commercially available RIA (Medicorp, Montreal Canada).

**Immunoblotting.** This method for protein preparation and quantification has been previously described (8) with some modifications. Briefly, tissue samples were homogenized to obtain total protein, centrifuged at 1,650 g for 30 min, and the supernatants were collected. Protein concentrations were assessed by the Bradford method. Seventy-five micrograms of total protein were electrophoretically resolved on either an 8% SDS-PAGE (GR), or a 12% SDS-PAGE (11βHSD1) and transferred overnight at 20 V to polyvinylidene difluoride paper. Blots were blocked with 5% BSA in Tween-20 Tris-base sodium and then incubated overnight in primary antibody at 4°C (GR: 1:5,000, cat. no. PA1–511A; Affinity BioReagents and 11βHSD1, cat. no. BHSD11-S; Alpha Diagnostic International, San Antonio, TX). Blots were incubated with the appropriate secondary antibody (Abcam) for 1 h 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 α-tubulin were used as a loading controls (Abcam).

**Tissue cryosectioning.** Brains were mounted on annular discs using tissue-freezing medium (Triangle Biomedical Sciences), sectioned to 10 microns in a refrigerated microtome (ThermoShandon Cryotome) at −10°C, and mounted on SuperFrost Plus Gold slides (Thermo Fisher Scientific). Correct brain orientation was confirmed by hematoxylin and eosin staining and architectural examination under a light microscope.

**Immunohistochemical staining.** Slide-mounted tissue sections were air-dried, fixed in 4% paraformaldehyde (Sigma-Aldrich), and perme-
abilized using either 0.1% [GR, mineralocorticoid receptor (MR)] or 0.3% (CRH) Triton X-100 (Sigma). Tissues were then blocked in either 10% normal goat serum (GR, CRH; Vector Laboratories, Burlingame, CA) with 1.5% BSA (BioShop) or 10% normal horse serum (MR; Vector Laboratories) with 1.5% BSA, and all tissues were incubated in a streptavidin/biotin blocking kit (cat. no. SP-2002; Vector Laboratories). Sections were incubated in a humidified chamber at 4°C for 18 h with their respective primary antibodies (GR: 1:250, cat. no. M-20; Santa Cruz Biotechnology, Santa Cruz, CA; MR: 1:200, cat. no. N-17; Santa Cruz Biotechnology; CRH: 1:1,000, cat. no. T-4037; Peninsula Laboratories) in 1.5% of their respective blocking sera. Signals were detected using either anti-rabbit (GR, CRH) or anti-goat (MR) biotinylated secondary antibodies (cat. nos. BA-1000 and BA-9500, respectively; Vector Laboratories), and a Texas Red-conjugated streptavidin tertiary antibody (cat. no. SA-5006; Vector Laboratories). Autofluorescence was reduced by sequential incubation in solutions of 0.3% Sudan Black (BioShop) in 70% ethyl alcohol, and CuSO4/NH4Ac (Acros Organics and BioShop, respectively). Nuclei were stained with 4,6-diamidino-2-phenylindole (Sigma) before sections were coverslipped with Fluoromount (Sigma) to preserve fluorescence. For all sections, a negative control was incubated with PBS instead of primary antibody to determine the degree of nonspecific secondary antibody binding. Slides were visualized by using a fluorescence-equipped microscope (Nikon Eclipse 90i), and image overlays were performed using Adobe Photoshop CS software. Signal intensities were recorded in Photoshop and expressed as arbitrary units (AU). Some samples were significantly damaged by the freezing process, leading to freeze fractures and making analysis impossible. Subsequently, immunohistochemical results are based upon five animals from each group.

**Data analysis.** For all experiments, the appropriate t-test, or one- or two-way ANOVA was performed to identify significant differences between treatment groups using Statistica 6.0 software, with \( P < 0.05 \) as the criterion. When a significant difference was observed with ANOVA, a post hoc analysis using contrasts with a Bonferroni correction factor was performed to determine specific differences. Data are presented as means ± SE.

Fig. 3. Hypothalamic corticotrophin-releasing hormone (CRH) protein. **A:** representative DAPI staining to confirm the location of the hypothalamus. **B:** representative images showing CRH (red) and nuclei (blue) for Basal, Sedentary, and Exercise groups. **C:** quantification of the relative signal intensity for each group showing no difference in CRH protein between the Basal and Sedentary groups, and an elevation in CRH in the Exercise group. Values are means ± SE. *\( P < 0.05 \) vs. Basal and Sedentary groups; \( n = 5 \) for all groups.
RESULTS

Food intake, body weights, and running distance. Food intake did not differ between groups at any time point. Sedentary and Exercise animals gradually increased food intake from week 1 (23.5 ± 0.7 g and 22.3 ± 0.6 g, respectively) to week 10 (34.5 ± 0.7 g and 35.2 ± 0.4 g, respectively). Average daily food intakes for the Sedentary and Exercise groups over the entire study were 29.2 ± 0.5 g and 30.0 ± 0.7 g (not significantly different; Table 1). Sedentary animals gained slightly more weight compared with Exercise animals throughout the 10 wk and were statistically heavier at the end of the study (P < 0.05; Table 1). Average daily running distances for the exercising animals began at 3.4 ± 0.2 km/day during week 1, peaked at 6.5 ± 0.5 km/day during week 6, and then slowly declined to 4.5 ± 0.7 km/day during week 10.

Weekly corticosterone and glycemia. As noted earlier, weekly fed and fasted blood glucose values in these animals have been previously reported (25). Exercise initially caused elevations in HPA activity, with glucocorticoid concentrations being higher than Sedentary animals during week 1 (P < 0.05; Fig. 1A). However, the glucocorticoid concentrations gradually decreased in the Exercise group from week 1 to week 3 and then remained relatively constant until week 10. Contrary to this, glucocorticoid concentrations gradually increased in the Sedentary group from week 1 to week 3 and remained elevated compared with Exercise animals throughout the study (P < 0.05; Fig. 1A). Both groups had fasting euglycemic levels until week 7, whereupon the Sedentary group developed hyperglycemia, while the Exercise animals remained euglycemic for the remainder of the experimental period [P < 0.05; data reported previously (25)].

End point glucose tolerance, tissue weights, and blood chemistry analysis. Exercise prevented the impaired glucose tolerance seen in the Sedentary animals. During the IPGTT, the Sedentary group had a higher area under the curve (AUC) compared with the Basal group for both glucose and insulin (P < 0.01, Table 1). The Sedentary group also had elevated fed glucose levels compared with the Basal group at euthanasia (P < 0.01; Table 1). Exercise animals had smaller glucose AUC values and fed glucose levels compared with Sedentary animals; however, Exercise animals did have the highest AUC for insulin during the IPGTTs (P < 0.05; Table 1). Sedentary animals also presented elevated plasma concentrations of triglycerides and free fatty acids compared with Basal animals at euthanasia (P < 0.01; Table 1). Exercise animals did not differ from the Basal group in regards to free fatty acids and had lower levels of triglycerides compared with the Sedentary group, although still higher than the Basal (P < 0.05; Table 1). Both Sedentary and Exercise groups had higher plasma ACTH concentrations compared with the Basal group (P < 0.01; Fig. 1B). Exercise animals had less adiposity and more skeletal muscle mass compared with Sedentary animals, as shown by epididymal and plantaris weights (P < 0.01; Table 1).

Regulation of HPA axis activity. Exercise prevented the decrease in hippocampal GR receptor protein content seen in Sedentary animals. Immunohistochemical analysis for GR protein in the hippocampus showed Sedentary animals to have a lower signal intensity compared with Basal and Exercise groups (P < 0.05; Fig. 2, A–C). Western blot analysis con-

![Image of graphs](http://ajpregu.physiology.org/10.22033.5 on June 29, 2017)

Fig. 4. Pituitary GR protein, adrenal melanocortin type 2 receptor (MC2R), and steriodogenic acute regulatory (StAR) proteins. A: Western blot analysis for GR protein in the pituitary glands showed no group differences. B: Western blot analysis for MC2R in the adrenal glands showed increased protein in the Sedentary group compared with the Basal and Exercise groups. C: Western blot analysis for StAR in the adrenal glands showed the Sedentary animals to have higher expression compared with the Exercise animals. The Exercise group did not differ from the Basal group. Values are means ± SE. *P < 0.05 vs. Sedentary group; †P < 0.05 vs. Basal and Exercise groups; ‡P < 0.05 vs. Sedentary group; n = 8 for Basal and Sedentary groups, n = 9 for Exercise group.

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firmed that GR protein was lower in Sedentary animals compared with Basal and Exercise groups ($P < 0.05$; Fig. 2D). Immunohistochemical and Western blot analysis in the hippocampus for mineralocorticoid receptors (MR) showed no differences between groups (CA1 region immunohistochemical: Basal, 4.76 ± 0.385 AU; Sedentary, 5.05 ± 0.58 AU; Exercise, 5.41 ± 0.77 AU, $P = 0.85$; Western blot analysis: Basal, 100.0 ± 12.1%; Sedentary, 92.5 ± 8.6%; Exercise, 93.7 ± 13.2%, $P = 0.69$). Interestingly, immunohistochemical analysis of the hypothalamus showed CRH protein to be highest in the Exercise group ($P < 0.05$; Fig. 3). Western blot analysis for GR protein in the pituitary gland revealed no group differences (Fig. 4A). Western blot analysis for regulatory proteins for the production of glucocorticoids, namely adrenal melanocortin type 2 receptor (MC2R) and steriodogenic acute regulatory (StAR), showed a higher expression for both proteins in the Sedentary group compared with the Exercise group ($P < 0.05$; Fig. 4, B and C). In addition, Sedentary animals had higher MC2R protein compared with Basal animals ($P < 0.05$; Fig. 4B), but only a trend was found for elevations in StAR between these two groups ($P = 0.08$; Fig. 4C).

Glucocorticoid action in peripheral tissues, expression of GR and 11βHSD1. To determine the effects of insulin resistance and exercise on peripheral tissue exposure to circulating glucocorticoids, skeletal muscle (mixed quadriceps), liver, and adipose tissues were probed for GR and 11βHSD1 protein content. No group differences were found for GR and 11βHSD1 levels in the skeletal muscle or liver tissues (Fig. 5, A and B). Sedentary animals had higher GR content in epididymal adipose tissue compared with Basal animals ($P < 0.05$),
although no difference was found between Exercise and Basal groups (Fig. 5C, left). Furthermore, a trend was found for elevated 11βHSD1 content in the adipose tissue of Exercise animals compared with Basal animals (P = 0.07; Fig. 5C right).

DISCUSSION

This study shows that there is a gradual increase in Basal HPA axis activity in Sedentary male ZDF rats that precedes their development of hyperglycemia. We also show that the elevations in HPA activity in this rodent model of type 2 diabetes coincide with increased adrenal cortical proteins, which, in turn, increase adrenal sensitivity to ACTH. In contrast to sedentary behavior, we show that regular exercise prevents hyperglucocorticoidemia for at least 10-wk duration, all the while maintaining euglycemia, likely through reduced adrenal sensitivity to ACTH. These novel findings indicate a new potential mechanism for the prevention of hyperglycemia in this rodent model of type 2 diabetes.

Researchers and clinicians have long postulated that stress hormones may be involved in the development of type 2 diabetes and that exercise may be beneficial for stress reduction. Indeed, the close phenotypic parallels between the metabolic syndrome and cortisol excess (e.g., Cushing’s syndrome) indicate a common underlying role for glucocorticoid action in these disease processes (1, 34). Glucocorticoid-induced metabolic complications are ameliorated by adrenalectomy in rodents and are reinstated by exogenous glucocorticoids (37). Mechanistically, glucocorticoids promote visceral adiposity (29), increase free fatty acid release (33), elevate liver glucose production (22), and exacerbate muscle insulin resistance (20).

Despite these previous research findings, to our knowledge, no study has previously shown that increased activity of the HPA axis proceeds, and thus potentially contributes to, the onset of hyperglycemia and insulin resistance in ZDF rats. Our study clearly demonstrates that elevations in glucocorticoids occur prior to the development of glucose intolerance and may facilitate the subsequent onset of hyperglycemia in the ZDF rat. In Sedentary rats, hypercortisolemia was evident by week 3, whereas hyperglycemia emerged on week 7, likely a result of diminished insulin production in the face of elevated insulin resistance (23).

We show the mechanisms associated with elevations in HPA activity in Sedentary ZDF rats appear to be a combination of several factors, including: 1) reduced negative feedback regulation of the axis as a result of diminished hippocampal GR content; 2) increased central drive of the axis, as evidenced by increased ACTH levels; and 3) increased adrenal sensitivity to ACTH demonstrated by increased StAR and MC2R protein content. It has been previously shown that obese Zucker rats have reduced MR content, which would also potentially contribute to the dysregulation of the HPA axis (30). In our study, both immunohistochemical and Western blot analysis showed no difference between groups. We also show that although exercise maintains normal hippocampal GR protein (indicating the continuance of adequate negative feedback compared with Sedentary animals), exercising animals also have elevations in both hypothalamic CRH protein content and circulating ACTH levels. Therefore, we conclude that regular exercise prevents elevations in circulating glucocorticoids in this rodent model of type 2 diabetes, mainly through reductions in adrenal sensitivity to ACTH via downregulation of both MC2R and StAR proteins (Fig. 6).
Our previous work with ZDF rats has shown that both swim training and intermittent restraint stress help maintain beta cell mass and prevents (or at least delays) the onset of type 2 diabetes (4, 5, 23, 24). Furthermore, we have previously demonstrated that wheel running exercise in healthy nondiabetic rodents causes initial hyperactivity of the HPA axis that is followed by a complete restoration to basal states (9, 21). Importantly, we found that the initial increase in HPA activity following the onset of exercise is the result of a transient increase in adrenal sensitivity to ACTH (9). We extend our findings in this study by showing that regular exercise elicits a similar mechanism for reduced glucocorticoid production in ZDF rats. Indeed, the elevations in glucocorticoids seen in the Exercising group during the first 1–2 wk in this study may be due, at least in part, to transient increases in adrenal gland sensitivity to ACTH, as we have seen previously in healthy rats undergoing training (9). Importantly, however, despite the initial increase in HPA axis activity, sustained exercise for >2 wk is associated with low basal HPA axis activity in ZDF rats, with similar adaptations occurring in the brain and adrenal gland as observed in nondiabetic exercise-trained rodents (9, 21).

Local amplification of glucocorticoid action, through increased tissue expression of GR and 11βHSD1, can lead to the development of metabolic complications in the absence of high circulating glucocorticoids (28, 29, 31, 42). Therefore, we probed insulin target tissues for GR and 11βHSD1 in Basal, Sedentary, and Exercise-trained rats. We found no differences in skeletal muscle or liver tissues, although a higher expression of GR in the epididymal adipose tissue of the Sedentary ZDF rats was found compared with Basal rats (Fig. 5C). Increased glucocorticoid action in visceral adipose tissue induces the metabolic syndrome through the development of central obesity and dyslipidemia (29). Thus, exercise training may also be protecting against the development of insulin resistance by lowering glucocorticoid exposure in adipose tissue and subsequently preventing dyslipidemia, as was observed in the Exercise group (Table 1). A reduction in GR content with training may also be important in lowering adipose tissue exposure to reactivated glucocorticoids since elevations in the prereceptor enzyme 11βHSD1 is known to increase with both dietary weight loss (39) and with exercise (8, 18).

It is important to note some of the limitations of our study. First, exercise training reduced adipose tissue mass, which in turn, leads to improvements in insulin sensitivity (35). The exercising ZDF rats in our study also experienced an attenuated gain in fat mass compared with the Sedentary animals (Table 1), making it difficult to delineate the contributions of the HPA axis from the effects of decreased adiposity on the prevention of hyperglycemia. Although a calorically restricted group of Sedentary ZDF animals could have been added to the experimental design to help tease out the effects of decreased fat mass on diabetes development caused by exercise, we have previously shown that modest caloric restriction also activates the HPA axis in Sedentary ZDF rats (5), which would make it difficult to compare this group to the Exercising group. Another important limitation to our study is that we propose that exercise training lowers adrenal sensitivity to ACTH, thus resulting in lower glucocorticoid levels, although we did not directly measure adrenal sensitivity per se. However, we have previously shown that the protein levels of StAR and MC2R are directly associated with the sensitivity of the adrenals to ACTH in trained and untrained rats by using exogenous ACTH challenge (9).

**Perspectives and Significance**

This study is the first to show that hyperactivity of the HPA axis and elevated plasma glucocorticoids precedes the development of, and thus may contribute to, hyperglycemia in ZDF rats. Furthermore, we demonstrate that exercise training is capable of attenuating these alterations in the HPA axis, allowing for maintenance of normal plasma glucocorticoids in this model of type 2 diabetes development. These data reveal novel neuroendocrine mechanisms for the beneficial effects of exercise on the management of the stress axis, which may aid in the prevention of type 2 diabetes.

**GRANTS**

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**DISCLOSURES**

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

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