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

Running Title: Exercise prevents hyperglucocorticoidemia in ZDF rats

Key Words: glucocorticoid, stress, hippocampus, type 2 diabetes, wheel running

Jonathan E. Campbell¹, Michael A. Király², Daniel J. Atkinson¹, Anna M. D’souza¹, Mladen Vranic², Michael C. Riddell¹

¹School of Kinesiology and Health Science, Faculty of Health
Muscle Health Research Centre
York University
4700 Keele Street, Toronto, ON, Canada, M3J 1P3

²Departments of Physiology and Medicine
University of Toronto,
1 King’s College Circle, Toronto, ON, Canada, M5S 1A8

Please address correspondence to Dr. Michael C. Riddell,
School of Kinesiology and Health Science, 4700 Keele Street,
Toronto, ON, Canada, M3J 1P3,
Telephone: (416) 736-2100 Ext.40493
E-mail: mriddell@yorku.ca
Abstract

We determined the effects of voluntary wheel running on the hypothalamic-pituitary-adrenal (HPA) axis, and the peripheral determinants of glucocorticoids (GC) action, in male Zucker diabetic fatty (ZDF) rats. Six-week-old euglycemic ZDF rats were divided into baseline (B), sedentary (S), and exercise (E) groups (n= 8-9 per group). B animals were immediately sacrificed, whereas S and E were monitored for 10 weeks. Basal (i.e. AM) GC levels increased 2.3-fold by week 3 in S rats where they remained elevated for the duration of the study. After an initial elevation in basal GC levels at week 1, E rats maintained low GC 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 sacrifice, S had ~40% lower GC receptor (GR) content in the hippocampus, compared to B and E (P<0.05), suggesting that the former group had impaired negative feedback regulation of the HPA axis. Both S and E groups had elevated ACTH compared to B rats indicating that central drive of the axis was similar between groups. However, S, but not E, animals had elevated adrenal ACTH receptor and steroidogenic acute regulatory (StAR) protein content compared with B, suggesting that regular exercise protects against elevations in GCs 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 S compared with B and E (P<0.05). Thus, the gradual elevations in GC 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.
Introduction

Glucocorticoid (GC) excess is characterized by increased central adiposity, insulin resistance, hyperlipidemia and elevated glucose production (3), while in the pancreas, sustained elevations in GCs adversely affect β cells and directly attenuate insulin release (19) – all features that make these hormones potent diabetogenic agents (41). Moreover, GCs are vasoactive and their elevation is an independent risk factor for cardiovascular disease and other diabetes related complications (15). Animal models of both type 1 (12, 14) and type 2 (5, 6, 27) diabetes show elevations in circulating GCs, 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 GCs, either by increased central drive of the HPA axis or by peripheral activation of glucocorticoids through 11β-hydroxysteroid dehydrogenase-1 activity (11βHSD1), 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 phloridzin corrects this hyperactivity (11, 13). Based on these findings, one might assume that the hypercortisolemia that is also observed in animal models of type 2 diabetes (6, 7, 16) is a result of hyperglycemia and relative hypoinsulinemia. Surprisingly, however, no known studies report on the levels of GCs during the progression from prediabetes to T2DM in animal models of this disease.

While several studies have measured GC levels in models of T2DM (6, 7, 16, 42), few have examined the central (brain) and peripheral (adrenal gland and target
tissues) components of GC release and action. Although much of the data point towards 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. These studies suggest that increases in corticotrophin releasing hormone (CRH) sensitivity (6) or adrenocorticotropic hormone (ACTH) sensitivity (4) may account for these changes, 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 if change in basal (resting AM) GC levels in male ZDF rats to determine if the hyperglucocorticoidemia precedes the development of hyperglycemia, or if results after the alteration in glucose homeostasis.

Exercise acutely activates the HPA axis and raises basal GC levels (36). Regular exercise, however, is well known to prevent the development of insulin resistance and to delay the progression towards 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 gland that promote a lower CRH production and reduced adrenal sensitivity to ACTH, respectively (9, 21, 32). Whether these adaptations also exist in the Zucker Diabetic Fatty (ZDF) rat (a rodent model of T2DM that exhibits elevated HPA axis activity) is unknown.
In this study, we set out to determine: 1) if elevations in HPA axis activity precede, or responds to, the development of hyperglycemia in the ZDF rat and 2) if 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 GCs have a causative role in this model of T2DM. 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 weeks of age with initial body weights of 150-175g, and 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, 4.3kcals/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 volitional exercising (“exercise”, 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-hour 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 weeks of age. Sedentary and exercise animals were euthanized 10 weeks later at 16 weeks 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.

**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 15-18h, 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 (ASCENSIA ELITE™ XL Blood Glucose Meter, Bayer, Toronto, Canada). Approximately 100 µl of whole blood was collected into heparinized microvettes (Sarstedt, Montreal, Canada) and the plasma was separated by centrifugation at 400 x g for 1 min and stored at -20°C. Fasting insulin concentrations were analyzed using a rat insulin ELISA assay kit (Crystal Chem Inc, Illinois, USA). Additional blood samples were taken via ‘tail nick’ once per week (Mondays) at 0800h under normal (i.e. non-fasted) conditions for the determination of fed glucose (whole blood), insulin (plasma), and corticosterone (plasma) concentrations. Corticosterone concentrations were analyzed with a commercially available radioimmunoassay (RIA) kit (Medicorp Inc., 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 hours and were then administered an intra-peritoneal injection of 50% dextrose (Abbott Laboratories Limited, Montreal, Canada) at a dose of 2g/kg body weight between 0900h and 1200h. Blood for glucose and insulin levels was collected via tail nick at 30 minute intervals for 2 hours. Blood was immediately centrifuged as previously described and frozen at -20C for subsequent analysis.

Euthanization

As previously mentioned, basal animals were euthanized at 6 weeks of age, whereas sedentary and exercise animals were euthanized at 16 weeks of age. Euthanasia occurred in the morning (0800h to 1000h) to obtain basal plasma hormone levels, within five hours of the last bout of exercise. Trunk blood was collected in EDTA and trasylol coated tubes, immediately centrifuged, and stored at -20C for subsequent hormone analysis. Post-prandial blood glucose was measured with a blood glucose meter. Plasma free fatty acids (FFA) and triglycerides (TG) were determined by an enzymatic colorimetric method (ACS-ACOD; Wako Chemicals, Richmond, VA). Plasma ACTH concentrations were determined by a commercially available RIA (Medicorp Inc., 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 1650 x g for 30 min, and the supernatants were
collected. Protein concentrations were assessed by Bradford method. Seventy-five micrograms of total protein was electrophoretically resolved on either an 8% SDS-polyacrylamide gel (GR), or a 12% SDS-polyacrylamide gel (11βHSD1) and transferred overnight at 20V to PVDF paper. Blots were blocked with 5% BSA in TTBS and then incubated overnight in primary antibody at 4°C (GR: Affinity BioReagents, Cat#:PA1-511A, 1:5000; 11βHSD1:Alpha Diagnostic, Cat#:BHSD11-S). Blots were incubated with the appropriate secondary antibody (Abcam) 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 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 -10ºC, and mounted on SuperFrost Plus Gold slides (Thermo Fisher Scientific). Correct brain orientation was confirmed by hematoxylin staining and architectural examination under a light microscope.

Immunohistochemical (IHC) staining

Slide-mounted tissue sections were air-dried, fixed in 4% paraformaldehyde (Sigma-Aldrich), and permeabilized using either 0.1% (GR, MR) or 0.3% (CRH) Triton X-100 (Sigma). Tissues were then blocked in either 10% normal goat serum (GR, CRH; Vector Laboratories) with 1.5% bovine serum albumin (BioShop) or 10% normal horse serum (MR; Vector Laboratories) with 1.5% bovine serum albumin, and all tissues were incubated in a streptavidin/biotin blocking kit (Vector Laboratories; SP-2002). Sections
were incubated in a humidified chamber at 4°C for 18 hours with their respective primary antibodies (GR: 1:250, Santa Cruz M-20; MR: 1:200, Santa Cruz N-17; CRH: 1:1000, Peninsula Laboratories T-4037) in 1.5% of their respective blocking sera. Signals were detected using either anti-rabbit (GR, CRH) or anti-goat (MR) biotinylated secondary antibodies (Vector Laboratories; BA-1000, BA-9500 respectively) and a TxRed-conjugated streptavidin tertiary antibody (Vector Laboratories; SA-5006). 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 (DAPI; Sigma) before sections were cover-slipped 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 non-specific secondary antibody binding. Slides were visualized 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 not possible. Subsequently, IHC results are based upon 5 animals from each group.

Data analysis

For all experiments, the appropriate t-test, one-way, 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 an ANOVA, a post-hoc analysis using contrasts with a Bonferroni correction factor were performed to determine specific differences. Data are presented as mean ± S.E.M.
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 ± 0.7g and 35.2 ± 0.4 g, respectively). Average daily food intake for the sedentary and exercise groups, over the entire study, was 29.2 ± 0.5 g and 30.0 ± 0.7 g (not significantly different; Table 1). Sedentary animals gained slightly more weight compared to exercise animals throughout the 10 weeks, 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 GC concentrations being higher than sedentary animals during week 1 (p<0.05; Fig. 1A). However, the GC concentrations gradually decreased in the exercise group from week 1 to week 3, and then remained relatively constant until week 10. Contrary to this, GC concentrations gradually increased in the sedentary group from week 1 to week 3, and remained elevated compared to exercise animals throughout the study (p<0.05; Fig. 1A). Both groups had fasting euglycemic levels until week 7, whereupon the sedentary 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 to the basal group for both glucose and insulin (p<0.01; Table 1). The sedentary group also had elevated fed glucose levels compared to basal group at euthanasia (p<0.01; Table 1). Exercise animals had smaller glucose AUC values and fed glucose levels compared to sedentary animals, however, did have the highest AUC for insulin during the IPGTTs (p<0.05; Table 1). Sedentary animals also presented elevated plasma concentrations of TGs and FFAs compared to basal animals at euthanasia (p<0.01; Table 1). Exercise animals did not differ from the basal group in regards to FFAs and had lower levels of TGs compared to 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 to the basal group (p<0.01; Fig. 1B). Exercise animals had less adiposity and more skeletal muscle mass compared to sedentary animals, as shown by lower epididymal and high plantaris weights (p<0.01; Table 1).

Regulation of hypothalamic-pituitary-adrenal axis activity

Exercise prevented the decrease in hippocampal GR receptor protein content seen in sedentary animals. IHC analysis for GR protein in the hippocampus showed a sedentary animals to have a lower signal intensity compared to basal and exercise groups (p<0.05; Fig. 2A-C). Western blot analysis confirmed that GR protein was lower in sedentary animals compared to basal and exercise groups (p<0.05; Fig. 2D). IHC and western blot analysis in the hippocampus for mineralocorticoid receptor showed no differences between groups (CA1 region IHC: B, 4.76±0.385 AU; S, 5.05±0.58 AU; E, 5.41±0.77 AU; P=0.85 Western: B, 100.0±12.1%; S, 92.5±8.6%; E, 93.7±13.2%; P=0.69).
Interestingly, IHC 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 GCs, namely adrenal MC2R and StAR, showed a higher expression for both proteins in the sedentary group compared to the exercise group (p<0.05; Fig. 4B and 4C). In addition, sedentary animals had higher MC2R protein compared to 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 GCs, 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. 5A and 5B). Sedentary animals had higher GR content in epididymal adipose tissue compared to basal animals (p<0.05), though no difference was found between exercise and basal groups (Fig. 5C, left panel). 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 panel).
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 T2DM 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 weeks 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 T2DM.

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 GC action in these disease processes (1, 34). GC induced metabolic complications are ameliorated by adrenalectomy in rodents and reinstated by exogenous GCs (37). Mechanistically, GCs 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 GCs 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 decreased 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 IHC and western 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 to 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 GCs in this rodent model of T2DM mainly through reductions in adrenal sensitivity to ACTH via downregulation of both MC2R and StAR proteins.

Our previous work with ZDF rats has shown that both swim training and intermittent restraint stress help maintain β 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 non-diabetic 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 GC production in ZDF rats. Indeed, the elevations in GCs seen in the exercising group during the first 1-2 weeks in this study may be due, at least in part, to transient increases in adrenal gland sensitivity to ACTH, as we seen previously in healthy rats undergoing training (9). Importantly, however, despite the initial increase in HPA axis activity, sustained exercise for greater than 2 weeks is associated with low basal HPA axis activity in ZDF rats, with similar adaptations occurring in the brain and adrenal gland as observed in non-diabetic exercise-trained rodents (9, 21).

Local amplification of GC action, through increased tissue expression of GR and 11βHSD1, can lead to the development of metabolic complications in the absence of high circulating GCs (28, 29, 31, 44). 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, though a higher expression of GR in the epididymal adipose tissue of the sedentary ZDF rats was found compared with basal rats (Fig. 5C). Increased GC 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 GC 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 GCs since elevations in the prereceptor
enzyme 11βHSD1 is known to increase with both dietary weight loss (40) 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 to 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 GC 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 to 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 T2DM 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.
Table 1 – Animal Characteristics, blood hormone concentrations, and IPGTT results

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<th>Basal</th>
<th>Sedentary</th>
<th>Exercise</th>
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<tr>
<td><strong>Final Body Weight (g)</strong></td>
<td>166.6 ± 3.5</td>
<td>454.0 ± 10.5*</td>
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<td><strong>Average Daily Food Intake (g)</strong></td>
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<td>30.0 ± 0.7</td>
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<td><strong>Epididymal Adipose Weight (g)</strong></td>
<td>0.87 ± 0.06</td>
<td>5.45 ± 0.2*</td>
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<td><strong>Plantaris Weight (mg)</strong></td>
<td>-</td>
<td>108.5 ± 2</td>
<td>141.3 ± 7†</td>
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<tr>
<td><strong>Fasted FFA (µEq/l)</strong></td>
<td>313.4 ± 15.1</td>
<td>816.8 ± 78.4*</td>
<td>324.9 ± 21.7†</td>
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<td><strong>Fasted TG (mM)</strong></td>
<td>1.51 ± 0.09</td>
<td>5.61 ± 0.38*</td>
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<td><strong>Fed Glucose (mM)</strong></td>
<td>6.5 ± 0.2</td>
<td>16.0 ± 2.7*</td>
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<td><strong>IPGTT AUC Glucose (AU)</strong></td>
<td>1104 ± 56</td>
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<td><strong>IPGTT AUC Insulin (AU)</strong></td>
<td>326 ± 63</td>
<td>752 ± 77*</td>
<td>1020 ± 125†</td>
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* - denotes significantly different from Basal group
† - denotes significantly different from Sedentary group
Figure Legends

Figure 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 to the basal group. * - p<0.05; † - p<0.05 vs. basal group. Values are means ± SEM, n=8 for basal and sedentary, n=9 for exercise.

Figure 2 – Hippocampal GR protein
(A) Representative staining of the entire hippocampus regions were for GR and DAPI to confirm the highest GR:nuclei ratio to be in the CA1 regions. (B) Representative images showing hippocampal GR protein staining for basal, sedentary, and exercise groups. (C) Quantification of the relative signal intensity for each group showing a decrease in GR in the sedentary group compared to both basal and exercise (n=5/group). AU = arbitrary units. (D) Western blotting of the hippocampus showed that sedentary animals had decreased hippocampal GR protein compared to basal and exercise groups. * - p<0.05 vs. basal and exercise groups. Values are means ± SEM, n=8 for basal and sedentary, n=9 for exercise.

Figure 3 – Hypothalamic 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. AU = arbitrary units. * - p<0.05 vs. basal and sedentary. Values are means ± SEM, n=5 for all groups.

Figure 4 – Pituitary GR protein, adrenal MC2R and StAR proteins
(A) Western blotting for GR protein in the pituitary showed no group differences. (B) Western blotting for MC2R in the adrenal glands showed increased protein in the sedentary group compared to the basal and exercise groups. (C) Western blotting for StAR in the adrenal glands showed the sedentary animals to have higher expression compared to the exercise animals. The exercise group did not differ from the basal group. * - p<0.05 vs basal and exercise; † - p<0.05 vs sedentary. Values are means ± SEM, n=8 for basal and sedentary, n=9 for exercise.

Figure 5 – GR and 11βHSD1 protein in liver, skeletal muscle, and adipose tissue
Western blotting showed no differences between groups for either GR or 11βHSD1 in the (A) liver and (B) skeletal muscle. (C) The sedentary animals had higher expression of GR protein compared to the basal group in epididymal adipose tissue, whereas the exercise animals did not differ from the basal. The exercise animals showed a trend for higher expression of 11βHSD1 compared to the basal animals (p=0.07), but no differences were found between sedentary and basal groups. * - p<0.05 vs. basal. Values are means ± SEM, n=8 for basal and sedentary, n=9 for exercise.
Figure 6 – Summary of the effects of type 2 diabetes and exercise on the HPA axis in ZDF rats. Sedentary ZDF rats have increased circulating GCs prior to the development of insulin resistance, likely through increased adrenal sensitivity to ACTH. In contrast, exercising ZDF rats maintain low circulating GCs through the maintenance of normal adrenal sensitivity to ACTH. ↓, ↔ and ↑ symbols represent decreases, no change and increases respectively, compared to basal ZDF rats.
References:


14. **Chan O, Inouye K, Vranic M and Matthews SG.** Hyperactivation of the hypothalamo-pituitary-adrenocortical axis in streptozotocin-diabetes is associated with
reduced stress responsiveness and decreased pituitary and adrenal sensitivity.


the sensitivity of glucose transport and metabolism to insulin in rat skeletal muscle. 


Figure 1

A)

B)
Figure 3

A)

B)

C)

![Graph showing relative signal intensity (AU) for Basal, Sedentary, and Exercise conditions.](attachment:image.png)
Figure 4

A) Glucocorticoid Receptor (% Basal)

B) Melanocortin 2 receptor (% Basal)

C) Steroidogenic acute regulatory protein (% Basal)
Figure 5

### Glucocorticoid Receptor

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<td>Basal</td>
<td>Sedentary</td>
<td>Exercise</td>
</tr>
</tbody>
</table>

### 11β Hydroxysteroid Dehydrogenase type 1

<table>
<thead>
<tr>
<th></th>
<th>Liver</th>
<th>Muscle</th>
<th>Adipose</th>
</tr>
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<tbody>
<tr>
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<tr>
<td>11βHSD1</td>
<td><img src="image" alt="image" /></td>
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<tr>
<td>β-actin</td>
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<tr>
<td>11βHSD1 (% of Basal)</td>
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