The Combined Effects of Chronic Glucocorticoids and Exercise Training in Peripheral Tissues of Male Sprague Dawley Rats

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ABSTRACT

Although acute elevations in glucocorticoids (GC) are beneficial, prolonged exposure causes detrimental effects including muscle wasting and increased fat. We developed an animal model with an abolished diurnal GC rhythm by elevating basal (AM) levels of GC. We assessed the effects of elevated GC and regular volitional exercise on anthropometric measurements and markers of energy metabolism. Male sprague dawley rats were divided into exercise and sedentary groups and further divided into SHAM controls and corticosterone (CORT) groups. Exercisers had 24-h access to running wheels for 6 weeks while sedentary groups remained in standard rodent cages. All subgroups had free access to food and water. CORT rats received two 150mg corticosterone pellets implanted subcutaneously while SHAM rats received wax pellets. Nadir corticosterone (07 00- 08 00 h) was at least 2-fold higher in CORT groups than SHAMs eliminating the diurnal pattern. Relative to body weight, CORT groups had ~30% more epididymal fat compared with SHAM groups. The visceral fat deposition in sedentary groups was ~50% greater than exercised groups (main effect of exercise, $P<0.05$). Expression of hormone sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) in epididymal fat was elevated in exercised groups (main effect of exercise, $p\leq0.05$) depicting a protective mechanism for reducing visceral adiposity. Bone strength was decreased in CORT treated groups (main effect of CORT, $p\leq0.05$) while the mixed gastrocnemius muscle of CORT groups, had a 70% increase in glucocorticoid receptor a/b isoforms (GRa/b) (main effect of CORT $p\leq0.05$) and an increase of 40% in 11 beta-hydroxysteroid dehydrogenase 1 (11βHSD1) (main effect of CORT $p\leq0.05$) content. This

study proposes a new animal model of chronic GC overexposure (similar to chronic stress or glucocorticoid therapy) and reveals that anthropometric measures are negatively affected by chronically high basal GC levels. Changes occur in the face of chronic GC treatment by altering GR isoform and 11βHSD1 content in muscle; findings which mimic observations in metabolic syndrome patients. We show that regular exercise attenuates visceral fat mass gain associated with chronically elevated basal GC exposure. These findings suggest a therapeutic role for exercise in conditions associated with chronic elevations in GC.

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LIST OF ABBREVIATIONS

11βHSD 1	11 Beta-Hydroxysteroid Dehydrogenase 1
AMPK	Adenosine Monophosphate Activated Protein Kinase
ATGL	Adipose Triglyceride Lipase
ACTH	Adrenocorticotropic Hormone
ANOVA	Analysis of Variance
CGI-58	Comparative Gene-Identification 58
CRH	Corticotrophic Releasing Hormone
CORT	Cortisol/Corticosterone
CBG	Cortisol/Corticosterone Binding Globulin
cAMP	Cyclic Adenosine Monophosphate
COX	Cytochrome C Oxidase
Exercise SHAM	Exer-SHAM
Exercise CORT	Exer-CORT
GIO	Glucocorticoid Induced Osteoporosis
GR	Glucocorticoid Receptor
GRE	Glucocorticoid Response Elements
GCs	Glucocorticoids
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
HSP	Heat Shock Proteins
HSL	Hormone sensitive Lipase
HPA Axis	Hypothalamic Pituitary Adrenal Axis
IMM	Inner Mitochondrial Membrane
IRS-1	Insulin Receptor Substrate 1
IMTGs	Intramuscular Triglycerides
LPL	Lipoprotein Lipase
mRNA	Messenger Ribonucleic Acid
NEFA	Non-Esterified Free Fatty Acids
OGTT	Oral Glucose Tolerance Test
OMM	Outer Mitochondrial Membrane
PVN	Paraventricular nucleus
PPARγ	Peroxisome Proliferator Activated Receptor gamma
PEPCK	Phosphoenolpyruvate Carboxykinase
PI3-K	Phosphoinositide 3 Kinase
POMC	Pro-opimelanocortin
PKA	Protein Kinase A
PKB	Protein Kinase B
RIA	Radioimmunoassay
Sedentary SHAM	Sed-SHAM
Sedentary CORT	Sed-CORT
StAR	Steroidogenic Acute Regularoty Protein
TGs	Triglycerides
T2DM	Type 2 Diabetes Mellitus

INTRODUCTION 1

The term stress was first coined by a researcher called Hans Selye (167). After completing his post doctorate at John Hopkins, Seyle began his research in experimental pathology at McGill university in Montreal. Here, utilizing mice injected with ovarian extracts, he observed that the animals responded in a stereotypical and reproducible fashion (125). He elicited the same response despite the stimulus utilized (cold, hunger, physical trauma) (125). Long before stress hormones were identified, Selye concluded that these stimuli disrupt homeostasis and an organism will try to compensate for the intervention leading to the beginnings of the "General Adaptation Syndrome" (144). Selye is often regarded as a key founder of research in endocrinology and the stress response. His work paved the way for decades worth of research into the stress response including the synthesis and release of adrenocortical hormones.

After successfully isolating the sex hormones progesterone and testosterone in the late 1920s and mid 1930s, researchers focused their attention on the adrenocortical hormones, cortisone and cortisol (68). In 1935, compound E (cortisone) was isolated from bovine by Edward Kendall (68). The new compound proved to be successful in returning strength when injected into adrenalectomized animals (80). Unfortunately, a great deal of tissue was required to produce only a few micrograms of the hormones and so researchers agreed to utilize the compound only on small animals and it was never tried in clinical studies (68).

Catalyzed by a rumor that Nazi air pilots took adrenal extracts to combat low oxygen levels at high altitudes, the United States began a great deal of research into

synthesizing the active adrenal hormones (130). Although the rumor was false, it began what would later be decades of research into the field of glucocorticoids. First, scientists looked to synthesize compound A, later called 11-dehydrocorticosterone, as it had the simplest structure. Although 11-dehydrocorticosterone worked well in animals, it had no effect on humans (68). Attention turned to compound E because it had a similar structure to 11-dehydrocorticosterone. In 1946, Louis Sarett was successful in synthesizing compound E or as it is now referred, cortisone (68). When administered to human patients suffering from rheumatoid arthritis, the results were unprecedented (80, 114). The success of the new hormone treatment was such that by 1948 other unknown diseases with inflammatory symptoms where also successfully treated with cortisone (68, 80).

Unfortunately, the long term side effects of these new powerful drugs were not as positive as the short term benefits experienced by patients. Chronic use of the new steroidal drugs promoted a wide array of negative side effects including water retention, increased gastric acidity and psychosis (68). Since the 1960s, a great deal of research has taken place to produce synthetic glucocorticoids that can mimic the therapeutic benefits provided by adrenal hormones and minimize the undesired side effects of long term use (114). Some of these synthetic drugs include prednisolone, dexamethasone and betamethasone (93). Sixty years after their discovery, adrenal corticosteroid hormones are still utilized in medicine as key drugs to suppress the immune system and treat a number of diseases and ailments. Identifying the links between glucocorticoids and various metabolic pathologies and how exercise and stress influence glucocorticoids secretion

and metabolism have been an active area of investigation since their discovery. This literature review highlights some of the main observations in this active area of study.

LITERATURE REVIEW: 2

2.1 STEROIDGENESIS AND PRODUCTION OF GLUCOCORTICOIDS

Activation of the HPA axis commences when the brain receives an external stimulus interpreted as a stressor. Specifically, parvicellular neurosecretory cells located in the paraventricular nuclei of the hypothalamus receive the external stimulus and begin to release corticotrophic releasing hormone (CRH) into the hypophyseal circulation (89). CRH then travels to the anterior pituitary where it binds its receptor. At the level of the anterior pituitary, CRH enhances the mRNA production of pro-opiomelanocortin (POMC) which is converted to ACTH and released into the systemic circulation (89). ACTH travels to the adrenal glands located above the kidneys and here promotes the synthesis and release of glucocorticoids (Figure 1).

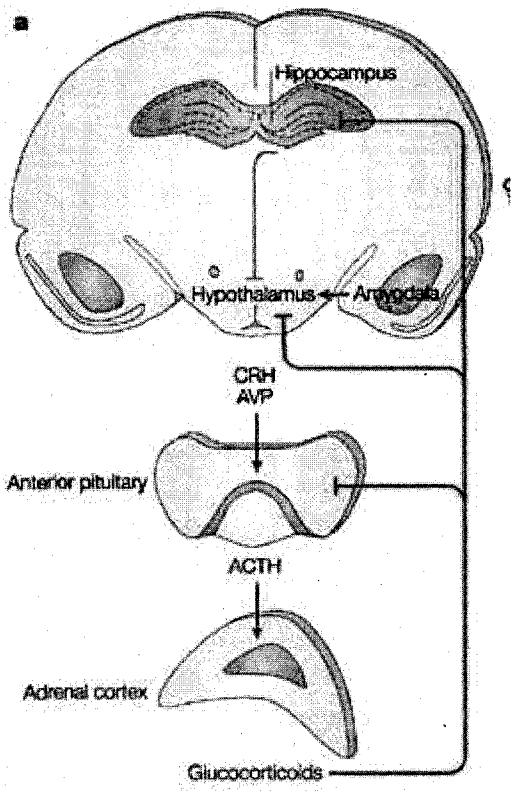

Figure 1: Schematic representation of the hypothalamic pituitary adrenal axis and its regulation *(modified from Sandi, Nat Rev Neurosci 2004 pg 917-930).*

The anatomy of the adrenal gland permits this organ to perform many different endocrine functions. The adrenal gland can be divided into two distinct regions; at the core of the adrenal glands is the adrenal medulla which is responsible for the release of the catecholamine epinephrine during times of stress (136). Surrounding the adrenal medulla is the adrenal cortex. The cortex region of the adrenal gland is further divided into the following layers: Zona Glomerulosa, Zona Recticularis and the Zona Fasciculata. The outer region, the Zona Glomerulosa, is responsible for the production of mineralcorticoids, hormones that help regulate hydration levels in the body (89). The

Zona Recticularis located in the middle is responsible for synthesizing glucocorticoids while the deep layer is crucial for the production of androgens such as dehydroepidosterone which form the origins for sex hormones (89, 136).

At the Zona Recticularis, ACTH released from the anterior pituitary can bind its receptor and begin the process of steroidgenesis. Steroidgenic cells such as those found in the adrenal cortex are capable of producing steroid hormones de novo by utilizing cytoplasmic cholesterol (110). Briefly, insoluble free cholesterol synthesized by the endoplasmic recticulum or released from the breakdown of triglycerides in the lipid droplet can bind to proteins that help transport cholesterol to the outer mitochondrial membrance (OMM) (84). Cholesterol must travel from the OMM to the cholesterol side-chain cleavage enzyme, P450scc, located at the inner mitochondrial membrane where it can be converted to pregnenolone (172). In order to accomplish this conversion, cholesterol utilizes the aid of the steroidogenic acute regulatory protein (StAR) (110). The rate limiting step of steroidgenesis is determined by the conversion of cholesterol to pregnenolone at the inner mitochondrial membrane (84, 172); however, this reaction in itself is limited by the substrate available in the mitochondrial matrix (110). The levels of cholesterol in the matrix are in turn dependent on the activity of StAR. As a result, StAR has attained a great deal of interest as it directly manipulates the production of steroid hormones such as glucocorticoids (84). Once pregnenolone is created from cholesterol it can undergo a series of reactions to produce GC which are released into the circulation.

2.2 CIRCULATING GLUCOCORTICOIDS AND MECHANISM

2.2.1 Determinants of Circulating Glucocorticoids

Stimulation by ACTH causes the adrenal glands to synthesize and release two major types of GC, an active and an inactive form (89). The active form of GC, cortisol in humans and corticosterone in rodents (CORT), is released into the circulation where it is predominantly bound to CORT binding globulin (CBG) (≈90% of CORT bound) (89). The inactive form, cortisone in humans and 11-dehydrocorticosterone in rodents, is soluble in the plasma and thus does not require CBG (89). CORT bound to CBG is unable to enter the cells, whereas the inactive form can freely enter the tissues (64, 107). However, inactive CORT must first be metabolized by the intracellular enzyme 11 beta-hydroxysteroid dehydrogenase (11-βHSD1), which converts it to active CORT. Located in the endoplasmic reticulum, 11-βHSD1 is a protein that is present ubiquitously in the body and plays a key role in metabolic tissues susceptible to insulin's anabolic effects (45).

2.2.2 Diurnal Rhythm of Glucocorticoids

In addition to the stimulus of stress, GC concentrations in the blood are affected by daily circadian rhythms (143). In other words, hormone fluctuations throughout the day occur as a result of an intrinsic program that is about 24 hours long. More specifically, CORT concentrations change as a result of a diurnal pattern which is influenced by the light and dark cycle (89). Nocturnal animals such as rodents have their lowest concentrations of circulating CORT in the morning (nadir) and highest values in the evening when they are active (47). Conversely, humans experience their highest

CORT levels in the early morning in anticipation of the fuel demands required to perform daily activities while the late evenings and early nights present the lowest daily concentrations of CORT (1, 51, 72). Fluctuations in CORT concentrations throughout the 24 hours are important in mobilizing fuels during periods of low food intake and it is also thought that the troughs in the diurnal pattern help protect tissues from experiencing the catabolic effects induced by GC over extended periods of time.

2.2.3 Molecular Regulation of Glucocorticoid Action

GC travel in the circulation to reach various tissues throughout the body. Once present at the tissues, active CORT elicits its effects by binding to the glucocorticoid receptor (GR) located in the cytosol. Alternative splicing generates several GR splice variants, (98)two of which (GRα and β) have been the focus of most studies because of their relative abundance. It is thought that GC bind to GRα and not GRβ (97, 98). For the purpose of this review, GRα will be denoted as "GR" for the rest of this thesis. GR is a protein measuring 94 Kilodaltons that is widely expressed throughout many different tissues. Recently, researchers identified a series of GR isoforms (GRα-A, -B, -C1, -C2, -C3, -D1, -D2 and -D3) (99). Moreover, these same researchers observed that the expression of these isoforms differed among various tissues (99). Some believe that GC' multifaceted actions including immune suppression, substrate mobilization and bone tissue loss may be a result of the various GR isoforms located in differing quantities within tissues (98). More work is required to truly understand the role that the different isoforms of GR play and how they affect tissues when GC concentrations rise.

In the absence of GC, GR's conformation is maintained by heat shock proteins (HSP) (121). When CORT binds the receptor the interaction causes the release of the HSP generating a ligand-receptor complex. The complex dimerizes with a second complex to form a homodimer. This newly formed homodimer then translocates to the nucleus were it binds with glucocorticoid response elements (GRE) on the promoter region of the target genes (75, 78). In this manner GC can promote or inhibit (by transcribing genes for inhibitors) the transcription of proteins and have their physiological effects take place.

2.3 ACUTE METABOLIC EFFECTS OF GLUCOCORTICOIDS

2.3.1 Glucose Metabolism

During times of stress, the body's demand for fuel is increased. This fight or flight response is instantly supported by the release of catecholamines (49) and by the subsequent activation of the HPA axis (24). The release of GC ensues and acutely promotes the mobilization of various fuel sources including glucose, free fatty acids and amino acids. For example, GC help inhibit the anabolic effects of insulin on various tissues. Using the synthetic GC dexamethasone, researchers have been able to minimize glucose uptake into rat primary muscle cells (16). Similarly, in adipocytes the presence of GC prevented insulin mediated glucose uptake in a series of experiments (17, 36, 100).

Perhaps one of the most import roles of GC is their ability to promote gluconeogenesis in the liver. GC can increase the expression of the hepatic enzymes glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (PEPCK) (89, 109).

These are key enzymes involved in hepatic gluconeogenesis, and again highlight the important role GC play in generating glucose during physiological stress. In order to provide fuel for the metabolic demands of the brain, nervous system and active tissues during a stress response the acute rises in GC are crucial.

2.3.2 Lipid Metabolism

Some researchers argue that adipose is also a target tissue for the effects of GC and their ability to provide fuel sources in response to a stressor. Since fat is the most calorie dense macronutrient (137), the human body stores it in order to be utilized later as fuel. Lipids are stored as triglycerides (TGs) in the lipid droplet within adipocytes. When the body's demand for energy is elevated, TGs in the lipid droplet are broken down into glycerol and free fatty acids. The hormonal regulation and molecular signaling of lipolysis has been studied extensively (5, 20, 46, 88). Contrary to basal lipolysis, stimulated lipolysis, as shown in figure 2, causes a net breakdown of TGs (43). Briefly, the catecholamine norepinephrine binds to beta adrenergic receptors on the adipocyte surface (66). These receptors are coupled with G proteins which signal adenyl cyclase to produce cyclic adenosine monophosphate (cAMP) (46). The production of cAMP promotes the activation of protein kinase A (PKA) which in turn activates perilipins and hormone sensitive lipase (3, 25). Perilipins are lipid droplet associated proteins that when activated allow for phosphorylated cystolic HSL to target the lipid droplet (101, 149). Indeed perilipin knock out animal models depict an attenuated lipolytic activity (105, 151).

Until recently, the pathway depicted above was considered the sole mechanism by which lipolysis takes place in a cell. However, new work has provided evidence for a second lipase that plays a crucial role in breaking down the lipid droplet in fat cells. It was recently discovered that, surprisingly, HSL null mice, who were thought to lack the ability to breakdown triglycerides, maintained large elevations in diglycerides (63). Villena et al. helped unravel the mystery of the aforementioned observation when they confirmed the existence of a novel adipocyte TGs lipase called desnutrin and later called adipose triglyceride lipase (ATGL) (161, 176).

Located on the surface of the lipid droplet, ATGL is believed to be the lipase responsible for breaking down a triglyceride to a diglyceride and a nonesterified free fatty acid (176). The further breakdown of a diglyceride to a monoglicyceride and a free fatty acid occurs at a rate that is 10-30 times higher than the hydrolysis of TGs which is the initiating and rate limiting step of lipolysis (62). However, both lipases are crucial for lipolysis to take place. Studies where ATGL function has been lost via gene ablation have demonstrated a decrease in lipolysis in mouse primary cells (176). More importantly, the attenuation in lipolysis was greatly increased in HSL null mice (176) suggesting a synergistic relationship between the two lipases.

To date little is known about ATGL and how it is activated during basal and stimulated lipolysis. Recently, comparative gene-identification 58 (CGI-58) has received some attention as it is a lipid droplet associated protein (173). Scientists have observed that mutations in this protein bring about accumulations in intracellular TGs droplets in most tissues of the human body (91). Utilizing this reasoning, Lass et al. observed that

CGI-58 stimulated in vitro lipolysis and also activated ATGL (90). In addition, these same authors observed that mutated forms of CGI-58 failed to activate ATGL (90). Currently there are no ATGL knock out animal models available but successful creation of this animal model may be required to further elucidate the mechanism by which ATGL is controlled. Together, these findings suggest that the rate of lipolysis is not solely governed by the actions of HSL but rather affected by both the activity of HSL and ATGL. Recently, researchers have observed that both of these enzymes are induced by acute elevations in GC (148, 161). Therefore, some argue that GC promote the release of fatty acids into the circulation as a fuel source by directly affecting HSL and ATGL during times of stress. Although studies exist which support the lipolytic effect of GC (16, 59, 123, 140), a second school of thought contends that GC do not promote lipolysis in adipose tissue. To this effect, Furll and Jackel observed that cows treated with dexamethasone showed lower levels of circulating free fatty acids (50). Similarly, human studies have shown that GC decrease lipolytic activity in abdominal fat cells (71, 133). More recently, Ottoson et al. (118) observed decreases in basal and stimulated lipolysis of human adipose in the presence of cortisol. Given the controversy of GC and the role they play in lipid metabolism, it is evident that more studies are required to understand if GC have an antilipolytic effect on adipose tissue.

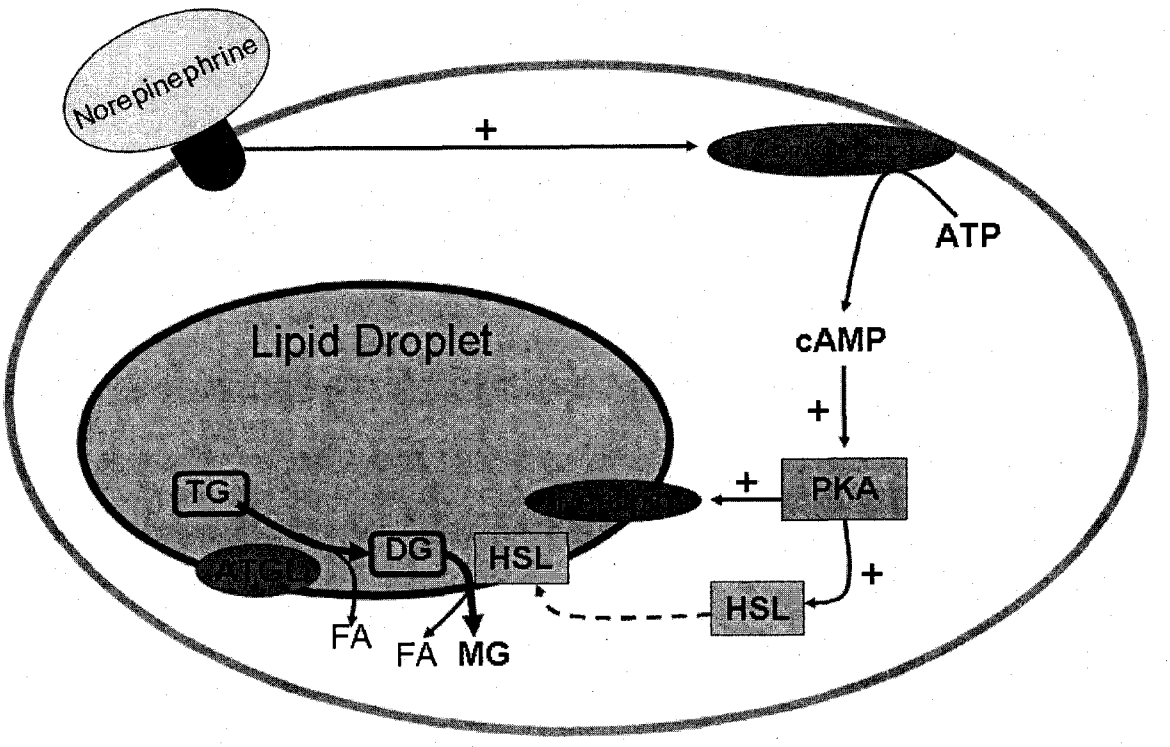

Figure 2: Schematic representation of stimulated lipolysis in an adipocyte

2.3.3 Protein Metabolism

GC also facilitate proteolysis and so are influential in the metabolism of proteins within the body. For instance, doses of GC have been shown to increase the mRNA of proteins involved in the ubiquitin proteosome pathway in rat muscle (8). This is a molecular pathway that is involved in the breakdown of proteins to amino acids. Briefly, proteins destined to be broken down are marked by ubiquination enzymes so that they may be hydrolyzed by 26S proteosome (150). The ubiquitin proteosome pathway is tightly regulated by various factors. Indeed, Bailey et al. observed that a critical balance exists between insulin and GC which regulates proteolysis of skeletal muscle via the ubiquitin and proteosome pathway in rat muscle (9). The increase in proteolysis is beneficial as it allows for an influx of amino acids which can be used for gluconeogenesis

(74). Furthermore, Rannels et al (132) observed that GC treatment reduced synthesis of skeletal muscle by blocking the peptide chain initiation. A decrease in protein synthesis was also seen by Desler et al. (35) in C2C12 cells exposed to increasing concentrations of dexamethasone. Given the multiple sites that GC can target, it is clear that we need to see the systemic manner in which these hormones help maintain adequate substrate levels in the face of a stressor (30).

2.4 THE PATHOLOGY OF HYPERGLUCOCORTICOIDEMIA

2.4.1 Reasons for Hyperglucocorticoidemia

The benefits of acute increases in GC to suppress immune function and mobilize fuels for oxidation and maintain glucose homeostasis have been outlined in the above sections. In marked contrast to the beneficial effects of short term elevations in GC, chronically elevated levels of GC as seen in patients with Cushing's syndrome or the metabolic syndrome (145) can promote numerous deleterious physiological adaptations. These include insulin resistance, increased visceral adiposity, and a decrease in lean body (bone and muscle) mass (22). The following section depicts some of the negative implications that chronically elevated levels of GC can have on various peripheral tissues.

2.4.2 Adipose Tissue

Adipose contains more calories for fuel per gram of weight than protein or carbohydrates (137). However, it is important to note that not all fat depots behave in a similar manner. Compared to subcutaneous fat, intra abdominal adipose tissue has a

higher lipolytic activity (12) and is less influenced by the antilypolytic effects of insulin (128). As such, visceral fat provides an influx of glycerol and free fatty acids that travel to the liver and promote gluconeogenesis and the synthesis of lipoproteins, respectively. Indeed, Kim et al. observed that the removal of visceral fat caused an increase in high density lipoproteins and a decrease in low density lipoproteins, when compared to control animals (83). In human adipocytes, Lundgren et al. confirmed a greater rate of glucose uptake in omental adipocytes when compared to subcutaneous cells (100). Higher glucose transporter isoform 4 (GLUT 4) content in visceral fat is believed to be the main cause for the discrepancy in metabolic activity between the two fat depots. Interestingly, the presence of chronically elevated levels of GC promotes a redistribution of fat depots in the body. Individuals diagnosed with Cushing's syndrome, a disease characterized by chronic elevation in CORT, experience a redistribution of adipose tissue from peripheral to more central fat depots (96). Moreover, patients diagnosed with asthma and rheumatoid arthritis that require chronic use of GC for their immunosuppressant effect also exhibit similar changes in fat distribution (164). This redistribution of adipose has many health implications on the human body. Visceral fat has been associated with a decrease in insulin sensitivity and linked to increases in both the development of type 2 diabetes and the risk of cardiovascular disease (59).

In addition to changing anthropometric measurements, chronic exposure to high levels of circulating GC also directly inhibit the insulin signaling pathway in adipose cells. In human adipocytes, dexamethasone diminished glucose uptake in insulin stimulated omental adipocytes by 50 percent (40 to 20 flow/cell) while the rate of glucose

uptake in the subcutaneous fat cells remained similar to the untreated condition (20 flow/cell) (100). Dexamethasone also decreases the expression of insulin receptor substrate 1 (IRS-1) and protein kinase B (PKB) in omental adipocytes, two key regulators of the insulin signaling pathway (100). These findings mimic the decrease in IRS-1, PKB and phosphorylated PKB expression as a result to dexamethasone treatment observed previously by Buren et al in rat adipocytes (17). More recently, *in vivo* studies utilizing dexamethasone injections in rats confirms the negative effects GC have on various proteins involved in the insulin signaling cascade (16).

The role that the GR expression plays on GC biology in various adipose tissue depots has been investigated, but remains controversial. Some studies suggest a lack of difference between GR expression in visceral and subcutaneous fat (14, 55). However, there is also evidence that supports a higher density of GR in intra-abdominal fat when compared to subcutaneous tissue (122, 147). Conversely, Joyner et al found that premenopasal women have lower GR content in visceral fat than in subcutaneous (77). In this same study the researchers observed that women had less GR expression in visceral fat than male counterparts (77), suggesting a possible mechanism for the gender difference in fat distribution.

Other research has focused on the effects GC have on cell proliferation and differentiation of adipocytes. Utilizing animal models, GC were shown to decrease rat adipose precursor cells (61, 171). Interestingly, research on 3T3 cells and animal primary adipocytes has provided evidence for GC inducing an increase in peroxisome proliferator activated receptor (PPAR γ) mRNA (160). PPAR γ is a dominant activator of fat cell

differentiation, activating adipose specific genes, including those that code for proteins involved in lipid storage and metabolism (154). In support of these findings, Bujalska et al. observed that human preadipocytes treated with cortisol had an increase in transcription of various genes involved in adipocyte differentiation (15). More importantly, the upregulated genes observed by these authors differed between visceral and subcutaneous adipose highlighting depot specific alterations by GC (15). Together these findings depict the multifaceted effect that GC can have on adipose tissue.

2.4.3 Muscle

Muscle atrophy is another common result of chronic hypercortisolemia (95). Muscle mass is dictated by the balance of protein synthesis and breakdown, with muscle atrophy resulting when proteolysis dominates. Acutely, GC stimulate proteolysis in order to utilize the released amino acids for gluconeogenesis during periods such as starvation (7). Extended periods of proteolysis, which can be the result of chronic GC exposure, can decrease muscle mass. Researchers have looked to ubiquitin, a marker of proteolysis, for a possible mechanism. GC increase ubiquitin mRNA content and stimulates protein catabolism in both animal models and cultured muscle cells (47).

In addition to the molecular changes mentioned above, GC also affect substrate availability within muscle tissue. Intramuscular triglycerides (IMTGs) are lipid pools located within the muscle fibers in close proximity to mitochondria (70). Due to their anatomical location, it is believed that IMTGs serve as a quick source of fuel to match the metabolic demands of muscle (56). Interestingly, animal studies utilizing dexamethasone have correlated the elevation in this synthetic glucocorticoid with IMTGs accumulation

(58, 86). These findings are further supported by human studies. Individuals diagnosed with the metabolic syndrome and/or type 2 diabetes (T2DM), two diseases characterized by elevated CORT, have higher levels of IMTGs than controls (113, 127). Various researchers have observed a correlation between IMTGs content and insulin resistance in individuals suffering from T2DM (56). Still others have directly implicated IMTGs in the development of insulin resistance (127). These latter studies investigated the effects of IMTGs accumulation on key steps of the insulin signaling pathway. For instance, the accumulation of IMTGs was shown by Petersen et al. to increase the phosphorylation of serine residues on IRS-1 and so decreasing the phosphorylation of tyrosine residues (127); tyrosine phosphorylation is an important early step in the propagation of the insulin signaling cascade that results in the transport of glucose into the cell. These findings help explain earlier observations by Virkamaki et al. whereby human subjects with elevated levels of IMTGs had lower levels of phosphorylated IRS (162). Together, these results suggest a link between increased circulating GC and increased IMTGs, although to date these observations are correlative and not causative. One study found no change in IMTG levels in dexamethasone treated animals while observing a decrease in muscle lipids of controls (86). However, these animals were Zucker diabetic fatty rats treated with dexmethasone and currently no data exist on IMTGs accumulation in healthy animal models with chronic corticosterone exposure.

Thus far, IMTGs have been described in a diseased state such as T2DM and obesity. However, biopsies taken from endurance trained humans also show increased IMTGs accumulation when compared to sedentary controls (70, 152). Although these

findings may seem perplexing, some researchers have suggested the increased IMTGs content in these two very different populations, exercise trained and type 2 diabetes, may serve different purposes. In patients with type 2 diabetes, the rise in IMTGs content may be a mechanism utilized by the body to remove and store excess free fatty acids circulating in the blood (158). Conversely, IMTGs elevation in athletes may be important as they would provide immediate fuel for a working muscle with a high level of oxidative capacity (56). Hence, although both the diseased and trained populations have elevated IMTGs, the increased rate of beta oxidation is evident only in the trained state (158). Indeed, researchers have observed a reduction of fatty acid oxidation in obese patients and individuals with type 2 diabetes (10, 11, 82). Conversely, in a trained state, the rate of beta oxidation is increased when compared to sedentary controls (73). Although beta oxidation is elevated in trained individuals, Helge et al observed no difference in muscle HSL content or enzyme activity between trained and untrained subjects (65). Being a newly discovered lipase, no data presently exist on the effects of exercise training on muscle ATGL expression. However, Watt et al. observed an inverse relationship between ATGL expression and IMTGs accumulation (163). This same group also found that rodent models of obesity and type 2 diabetes had lower levels of muscle ATGL expressed (163). These findings suggest the possibility that a low ATGL expression may be a key component in promoting the accumulation of IMTGs and thus the development of insulin resistance.

Similar to adipose tissue, chronic elevations in GC negatively affect the insulin signaling pathway at many sites in skeletal muscle. In the presence of GC there is a

decrease in the glucose uptake by the rat soleus muscle (36). The authors of this study attributed the decreased glucose uptake to a diminished translocation of the GLUT 4 transporter. Other researchers have investigated the expression of key proteins involved in the insulin signaling pathway. Specifically, rats injected with GC for 11 days showed a decreased expression of phosphorylated PKB (Ser^{473} and Thr^{308}) (16). Dexamethasone has also been shown to reduce the insulin stimulated activation of phosphoinositide 3 kinase (PI3-K) (141). Therefore, chronic GC elevation both indirectly and directly promote insulin resistance by increasing IMTGs and decreasing proteins involved in the insulin signaling pathway respectively.

2.4.4 Bone

GC may also decrease lean body mass through the breakdown of bone tissue (19). In animal models, elevated levels of GC have led to a decrease in bone formation in the vertebrae as well as long bones of the hind limb (146, 165). These studies reveal that GC affect bone formation by increasing the rate of apoptosis among osteoblasts, the cells responsible for bone formation. This results in decreased bone mineral density, which is seen in patients with chronically elevated levels of CORT (2). These events are believed to be caused by two distinct phases; a rapid early phase and a slower chronic phase. The early phase may be a result of excessive bone resorption that occurs as a result of increased osteoclastogenesis, which is the formation of new osteoclast cells (139). Conversely to osteoblasts, osteoclasts are responsible for the break down of bone. However, it is thought that the early phase does not account for the majority of the decrease in bone mineral density, but rather it is the progressive slower phase. This phase

is characterized by a decrease in the number of cells of the osteoblastic lineage (33, 126) and so a decrease in the rate of bone formation. The end product of this bone density loss has been termed Glucocorticoid Induced Osteoporosis (GIO). The prevalence of GIO on diseased populations dependent on GC therapy proves to be a challenging side effect. New studies have looked to attain the immuno-suppressing effects of GC without the negative effects of the hormones on various tissues of the body, including bone.

2.4.5 Liver

Some studies have also examined the effects of chronic GC levels on the liver. Similar to acute doses, seven days of GC injections have been shown to increase PEPCK mRNA levels in the rat liver (76) and GC have long been shown to increase hepatic glucose production (89, 109). PEPCK is considered the rate limiting enzyme in gluconeogenesis (28) and the upregulation of this enzyme in liver, which increases glucose production, along with the reduction in peripheral glucose uptake into muscle help to explain the hyperglycemic effects of GC *in vivo*. Interestingly, the expression of the PEPCK gene is itself inhibited by the metabolism of glucose via glucokinase (28) hence promoting a negative feedback loop that helps to minimize the GC effect on increasing gluconeogenesis. Importantly, 5 days of dexamethasone treatment increases the expression of AMPK in the rat liver (159), which attenuates the glucose mediated suppression on gluconeogenesis. Utilizing corticosterone pellets, Christ-Cain et al., (23) were successful in increasing AMPK activity in the rat liver after 14 days of treatment. Together these findings suggest a possible mechanism for inadequate glucose homeostasis observed in patients with glucocorticoid excess.

2.5 EXERCISE AND GLUCOCORTICOIDS

2.5.1 Acute Regulations

Exercise is a potent stressor that acts upon the HPA axis resulting in elevations of circulating GC. Animal experiments, using a forced swimming training protocol, have shown an increase in both ACTH and CORT as early as 15 minutes following the onset of exercise (32, 79, 120). However, other stress variables associated with forced swimming may exaggerate the stress response (27, 37). Similarly, forced treadmill running as an exercise protocol makes it difficult to delineate the stress response due to exercise from that due to psychological stress (67). Furthermore, forcing nocturnal animals to exercise during daytime hours, when convenient for the researchers, activates the HPA axis during times that are not reflective of normal human activity patterns. As such, it is imperative to implement voluntary exercise paradigms to accurately gauge the isolated effects of the exercise. Indeed, researchers that have used volitional exercise have witnessed elevations in plasma CORT levels lasting up to two hours after a single bout of exercise (13, 40, 41, 54). This action can be more confidently attributed to exercise alone, rather than the stress associated with forced daytime activities such as swimming or treadmill running with electrical shock.

2.5.2 *Chronic Adaptations*

It is critical that the body returns to homeostasis once the exercise is complete. As mentioned above, after the end of exercise, the HPA axis utilizes a negative feedback mechanism to return plasma GC levels back to basal values. This is accomplished when GC in the circulation bind to GRs in the hypothalamus and the hippocampus, which

inhibits further secretion of the CRH and decreases HPA activity (figure 1). Research has found that exercise training leads to adaptations in the negative feedback potential of the HPA axis. Park et al. (120) utilized forced swimming as an exercise protocol and observed changes to both central and peripheral aspects of the HPA axis. Specifically, GR mRNA in the hypothalamus and the pituitary was transiently decreased while CRH was increased in exercise trained rodents (120). Conversely, recent investigation by Feduic et al., has shown that 4 weeks of volitional exercise has no effect on hippocampus and pituitary gland GR content, suggesting that negative HPA feedback sensitivity is normal in trained rats (47). Interestingly, this same study showed that initially, exercising animals had a greater response to a novel stressor compared with sedentary animals, but this difference disappeared by the fourth week of training. Thus, any changes in central HPA activation or feedback sensitivity may have taken place initially, but were restored by the fourth week. Furthermore, the initial difference in HPA activity occurred despite similar ACTH levels, which suggests that the adrenal gland may be initially more sensitive to ACTH during training. Indeed, a follow up study demonstrated that exercise training is associated with a transitional increase in adrenal sensitivity to ACTH during the first two weeks, but disappears by ~8 weeks of continued training (131). Changes in adrenal sensitivity to ACTH has been observed in ultramarathon runners by Wittert et al. (170). The basal diurnal pattern of the trained marathoners had similar CORT concentrations compared to untrained controls, but the trained group had higher levels of ACTH. The apparent decrease in sensitivity to ACTH with prolonged high volume training may be the result of decreases in the adrenal ACTH receptor or a reduction in the

key rate limiting step of glucocorticoid biogenesis StAR (131), although this requires confirmation. Together, these findings suggest that although exercise may initially increase adrenal sensitivity to ACTH resulting in higher circulating GC concentrations, continued training restores this initial elevation. It is likely that these adaptations are critical in restoring normal diurnal HPA activity in endurance trained organisms to help minimize any potentially deleterious effects of chronically elevated glucocorticoids. To date, no studies have determined if a failure to normalize circulating glucocorticoids negates some of the beneficial effects of regular exercise. It may be that exercise training is able to offset the accumulation in visceral adiposity, and minimize the loss of lean body mass observed with chronic elevations in GC.

2.6 ANIMAL MODELS OF HYPERGLUCOCORTICOIDEMIA

The potent effects of GC on metabolism and in particular, metabolic diseases, has prompted researchers to explore the negative effects of chronic GC using various animal models. One popular method evident in the literature is the use of multiple subcutaneous injections of dexamethasone and other GC over a period of various days (31, 119). Although these studies have encountered interesting findings, some limitations exist; firstly, the daily injections of the treatment themselves pose a confounding variable as they are physical stressors placed on the animal which can activate the HPA axis (34). Furthermore, the use of injections promotes a large bolus of GC to be infused into the animal which is not maintained in the circulation chronically. Hence the sudden rise and drop in GC prevents these models from truly mimicking the physiological conditions of

normal GC secretion (169). Other researchers have looked to avoid the stressful injections by mixing dexamethasone in the drinking water of the animals (26). However, a key drawback to this methodology is the inability to standardize the dosage and timing of glucocorticoids ingested by the animals as it depends on the volume of water consumed. In addition, the use of dexamethasone is not entirely physiological as the affinity to GR by dexamethasone is up to 50 fold greater than CORT (58). In the face of these complications, some researchers have opted to use pellets made of GC which are implanted subcutaneously in the animal (42, 108). Unfortunately, these experiments have not been carried out for longer than 3 weeks and so the long term effects of GC may not have been completely explored. It is also important to note that, to date, no investigations have been published on the physiological adaptations to exercise in an animal model of elevated glucocorticoids.

PURPOSE AND SPECIFIC OBJECTIVES 3

Overall Thesis Purpose

The purpose of this thesis is to investigate the effects of exercise training on peripheral tissues of an animal model with chronically elevated glucocorticoids.

Specific Objectives

Specifically, we first developed a rat model with chronically elevated levels of glucocorticoids by using CORT implants. We next measured, in both trained and untrained animals with or without chronically elevated GC a number of markers of HPA axis activity and insulin sensitivity. Finally, we investigated the interaction between chronic CORT treatment in the presence of exercise training. The parameters measured include:

- Skeletal muscle cytochrome C oxidase activity.
- GR and 11βHSD1 protein expression in skeletal muscle
- ATGL and HSL protein expression in adipose and skeletal muscle
- Lower limb bone strength
- Whole body substrate utilization

Hypotheses

We hypothesize that chronically elevated levels of glucocorticoids, specifically in the nadir phase of the diurnal rhythm, will promote visceral adipose accumulation and reduce muscle size and bone strength. However, we theorize that these negative implications will be attenuated in the presence of exercise training.

4. MANUSCRIPT

Chronic Elevations in Cortiscosterone Eliminate the Diurnal Pattern in Sprague Dawley Rats and Negatively Affect Peripheral Tissues; Exercise Training is Able to Attenuate Some of These Negative Implications

Running title: The Effects of Chronic Glucocorticoid Exposure and Exercise Training on Peripheral Tissues of Male Sprague Dawley Rats.

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Key words: Voluntary exercise, circadian rhythm, glucocorticoids, glucocorticoid receptor, adipose triglyceride lipase, hormone sensitive lipase

CONTRIBUTION BY THE AUTHORS

In this thesis I was involved in the research development with my supervisor (Dr. Riddell), and conducted approximately 90% of all animal work, tissue harvesting, assays and measurements. I was also responsible for all data analysis, initial write up and editing. Jonathan Campbell is a graduate student in Dr. Riddell's laboratory. His assistance in this project included help with the glucose tolerance test, animal euthanization and tissue harvesting. Anna D'souza was a NSERC summer student and a volunteer in Dr. Riddell's laboratory. She helped with recordings of anthopometric measures and tissue harvesting. Anna also sectioned, stained and analyzed intramuscular triglycerides and muscle fiber areas. Martin Butcher is a lab technician at the Henderson Research Center in Hamilton, Ontario and provided the information for bone fractures. Dr. Riddell is the primary investigator and supervisor of this project.

INTRODUCTION

Activation of the hypothalamic-pituatry-adrenal (HPA) axis is one major way the body responds to stress (102). Upon perception of a stressor, corticotropin-releasing hormone (CRH) is secreted from the paraventricular nucleus (PVN) of the hypothalamus into the hypophyseal portal circulation. CRH subsequently acts upon the anterior pituitary gland to promote the secretion and release of adrenocorticotropic hormone (ACTH) into the systemic circulation. ACTH binds its receptor in the adrenal cortex, where it stimulates the synthesis and discharge of glucocorticoids (GC) into the circulation (89). Acute increases in GC are beneficial in elevating concentrations of energy substrates such as carbohydrates and fat in the blood, by means of gluconeogenesis and lipolysis, respectively (89, 129). However, chronic elevations of GC can cause detrimental effects such as muscle wasting, increased visceral adiposity, insulin resistance, and bone thinning (17, 103, 104, 116, 175). These effects are most notably seen in patients with chronic elevations in GC levels due to therapeutic dosages. Furthermore, individuals with type 2 diabetes and/or the metabolic syndrome also display characteristics that closely resemble disregulation of the HPA axis, possibly because of local amplification in GC activity (81). Exercise training is widely thought to be important in preventing many of these metabolic problems improved insulin resistance, decreased visceral adiposity, and increased lean body mass (135, 156, 157, 174). However, exercise is also a potent stimulator of the HPA axis (153) and GC levels tend to remain elevated for hours after the end of exercise (60). Previous work from our laboratory has shown that exercise training is capable of reducing GC action in skeletal muscle and liver tissue in healthy

animals (29), but also increases GC action in adipose tissue (18). This suggests that tissue specific alterations in GC biology occur as a result of training, which may help to protect against the negative effects of elevated GC induced by exercise. However, this research is limited to healthy models, where GC are only elevated periodically, during exercise. To date, no research has focused on the effects of exercise training in a model lacking a diurnal pattern mainly by chronically elevating nadir GC levels. In this study, we use CORT implants in healthy animals to mimic chronic hypercorticosteronemia and determine the effects of exercise training on peripheral tissues. We show that a modest elevation in basal GC concentrations reduces overall growth but increases visceral adipose mass and lowers bone strength. We also show that skeletal muscle is resistant to the elevation in basal GC levels and that regular exercise attenuates many of the detrimental effects of hypercorticostroidemia on adipose tissue mass gain, likely by increasing the key enzymes associated with lipolysis.

METHODS

Animals:

Juvenile, male, Sprague-Dawley rats (age at arrival, 5 weeks: Charles River Laboratories, Montreal, QC, Canada) were singly housed in standard, clear, plastic cages (height: 36.4cm, width: 26.8cm, depth: 50cm) and under standard lighting conditions (12h light and 12h dark cycle, lights on at 0600h). Temperature was maintained between 22-23°C while the humidity of the room remained at 50-60%. Food (Lab Chows, #5012)

and water were available *ad libitum*. Animals were handled daily in order to minimize handling stress on the day of the experiment.

Design:

Upon arrival the animals were randomized into one of four groups: Sedentary SHAM (Sed-SHAM, n=8), Exercise SHAM (Exer-SHAM, n=9), Sedentary CORT (Sed-CORT, n=10) and Exercise CORT (Exer-CORT, n=9). Diurnal glucocorticoid concentrations were measured once per week at the end of each week of training for 6 weeks. At the end of week 6 some animals (3 per group) were placed in a plastic metabolic cage (Windows Oxymax Code #0246-002M Columbus Instruments Ohio, USA) to measure the respiratory exchange ratio and all animals underwent an oral glucose tolerance test.

Corticosterone Pellets

For continuous release of GC, pellets were created using modifications to an established technique (108). Briefly, corticosterone (Sigma-Aldrich, St. Louis, MO, USA Category # C2505) was liquefied using a low flame, poured into a mold manufactured for the pellets, and then allowed to solidify. A scalpel was then used to remove the pellet and trim it to the appropriate mass. Sham wax pellets were also created by following the same procedure with wax in place of CORT. Both the sham and CORT pellets were trimmed to 150 mg. All pellets were stored in clean vials in order to maintain sterility. The size of pellets was determined based on a pilot study (n=4 per group) using 100 mg and 150mg sized pellets.

Voluntary Exercise Model:

After 3 days of acclimation, the Exer-SHAM and Exer-CORT groups received free access to running wheels (1.06m circumference, Harvard Apparatus Inc., Holliston, MA, USA) this was termed day 0. On the 4th day of the protocol, all animals underwent surgery to implant the subcutaneous pellets (day 4). The wheels were removed after surgery for 24hrs and then promptly returned to the Exer-SHAM and Exer-CORT groups for the remainder of the study. A magnetic counter was mounted to each wheel and provided the revolutions following 24 hours of exercise by the animals. The revolutions were recorded daily five days per week. By multiplying the revolutions noted by the circumference of the wheel, the distance ran by each animal was attained. The cages for the sedentary groups remained the same and all cages were changed once per week. All animal experiments were approved by the York University Animal Care Committee, Toronto, Canada.

Surgical Procedures and Physical Parameters:

All animals underwent surgery in order to have two subcutaneous pellets implanted. The pellets were made up of either corticosterone or sham wax pellets. The animals were anaesthetized with 2% inhaled isoflurane. An incision was made in between the scapulae and two pockets for the implants were made at either side of the vertebrae region by blunting subcutaneously. The animals were sutured and allowed to recover in their cages for 24hr. Exer-SHAM and Exer-CORT groups received their running wheels only after the 24hr recovery period. Upon termination of the protocol, all animals had 100µl of blood collected via tail nick into EDTA coated tubes (Sarstadt, Inc., Montreal,

QC, Canada). The rodents then underwent an oral glucose tolerance test (see below) and were promptly euthanized by decapitation. The following tissues were extracted and weighed: Left and right adrenals, left gastrocnemius, plantaris, soleus, the epididymal and subcutaneous fat pads. The left femur and subcutaneous pellets were also excised. All tissues except for the femur and implant pellets were frozen in liquid nitrogen (39) and stored at -80°C until further analysis. The gastrocnemius, plantaris and soleus of the right leg were separated and mounted onto corks using cooled isopentane and tissue freezing medium (Triangle Biomedical Sciences Durham, NC, USA Category # TFM-5). The mounted muscle was then frozen in liquid nitrogen and stored at -80°C to be sectioned later using a cryostat (Cryo-Cyl 120/180/230 LP, Chart Industries Inc. Burnsville, MN USA). Upon extraction, the femur bones were wrapped in parafilm, placed in ice and shipped overnight to the Henderson Research Center in Hamilton, Ontario for analyses.

Diurnal GC Measurements:

Blood samples were obtained at 08:00, 01:00h and 20:00h for diurnal corticosterone concentrations at the end of each of the 6 weeks. During dark phase sampling, the lights were maintained off and the only source of light was a battery operated head lamp (white light).

Approximately 150µl of blood was obtained via tail nick and all samples were collected by the same operator within 90 seconds to minimize activation of the HPA axis. This method has been shown in the literature to not elevate circulating corticosterone levels (52). In order to avoid coagulation of the blood, samples were collected using heparin coated capillary tubes (Sarstadt, Inc., Montreal, QC, Canada), spun in a

microcentrifuge at 14000 rpm for 60 seconds and the plasma was stored at -20°C until assayed. Corticosterone concentrations in the plasma collected during the protocol were analyzed using a commercially available RIA kit (MP Biomedicals, Cat # 07-120102)

Oral Glucose Tolerance Test

The six weeks of the protocol began after the surgery took place (day 4). An oral glucose tolerance test (OGTT) was conducted at the end of the study (day 47) to determine insulin sensitivity. A dosage of 5g/kg of body weight (10 ml/kg of a 50% dextrose solution) was administered orally and whole blood was collected via tail nicks and analyzed for glucose concentrations using a glucometer. Measurements were taken at 0, 30, 40, 50, 60, 70, 80 90, 100 minutes following glucose administration. Values were plotted against time and the area under the curve was determined for each animal. The OGTT was performed as we observed in pilot studies that intraperitoneal injections lacked consistency in results. Furthermore, the OGTT better mimics the manner in which glucose enters the circulation compared to when intraperitoneal injections.

Euthanization

Following the completion of the OGTT, animals were euthanized by decapitation and various muscle and adipose depots were removed, weighed and frozen using liquid nitrogen.

Cytochrome C Oxidase Activity

Cytochrome C Oxidase (COX) activity in the plantaris muscle was determined from euthanized animals, as previously described (57). Enzyme activity was determined

as the maximal rate of oxidation of fully reduced cytochrome c, measured by the change in absorbance at 550nm in a Microplate Reader (ELx800 Universal, Bio-tek instruments).

Non Esterified Free Fatty Acid Analysis

Free fatty acids were quantified using a commercially available assay (HR Series NEFA-HR Code # 999-34691, Wako Pure Chemical Industries Ltd. Richmond, VA USA) measured colorimetrically at 550 nm using a Microplate Reader (ELx800 Universal, Bio-tek instruments).

Western Blot Analysis (GR, 11β-HSD1 in muscle, lipases in muscle and fat depots)

Western blotting technique was utilized to analyze content of 11-βHSD1 and GR in skeletal muscle. Also HSL and ATGL expression were investigated in mixed gastrocnemius and fat depots. Tissues were homogenized in lysis buffer (135mM NaCl, 1mM $MgCl_2$, 2.7mM KCl, 20mM Tris-Base, 0.5mM Na_3VO_4, 10mM NaF, 0.2mM phenylmethylsulfinyl fluoride, 10μg/ml Leupeptin, 1% Triton, 10% Glycerol). Homogenates were centrifuged at 13000 rpm for 20 minutes and the supernatants were collected. For fat tissue this process was repeated a second time in order to obtain a cleaner sample of supernatant. A 10μl aliquot of the homogenate was used to quantify protein using the Bradford method. 75μg of total protein was electrophoretically resolved on an 8% SDS-polyacrylamide gel (muscle GR), 12% SDS gel (muscle 11-βHSD1) and a 10% SDS gel (muscle ATGL and HSL). For ATGL and HSL in fat tissue, 30μg of total protein was electrophoretially resolved in a 10% SDS gel. The protein was then transferred overnight at 20V to PVDF membrane. All blots, except for beta actin, were blocked with 5% BSA in TTBS for 2 hours. B-actin blots were blocked using 5% milk in

TTBS. All blots were then incubated overnight in primary antibody at 4°C (GR: Affinity Bioreagents Cat# PA1-511A, 1:5000, 11β-HSD1: Alpha diagnostic, Cat#:BHSD11-S, 1:5000, ATGL: Cell Signalling Technology Cat# 2138 muscle 1:2000, fat 1:5000, HSL Cell Signalling Technology Cat# 4107, muscle 1:2000, fat 1:5000). After the primary antibodies, the membranes were washed 5 x 10 minutes using TTBS and were then incubated for 1 hour with secondary antibody at room temperature (anti-rabbit conjugate with horseradish peroxidase; 1:10000). Enhanced chemiluminescence detection (Chemiluminescent HRP Substrate Cat# WBKLS0500, Millipore, Billerica MA, USA) and film (CL-X Posture^{TM} Film Cat# PI34091, Thermo Scientific) were used to visualize protein content. An image processing and analysis program (Scion Image) was used to measure the optical density of all the bands.

After the analysis for GR, 11-βHSD1, ATGL and HSL in muscle tissue was completed the membranes were stripped and re-probed with the mouse monoclonal primary antibody to GAPDH (Glyceraldehyde-3-phosphate dehydrogenase, Abcam, Inc., Cambridge, MA, USA) in a 1:10000 ratio. The secondary antibody for GAPDH (Peroxidase labeled anti-mouse, Amersham Biosciences, Corp., Piscataway, NJ, USA) was used in a 1:20000 ratio. GAPDH was used as a loading control for membranes with muscle proteins. Conversely, B-actin (Mouse antibody, BD Biosicences, Missisauga, ON, Canada, Cat # 6126571, 1:5000) was used as a loading control for fat tissue proteins ATGL and HSL content. The secondary used was 1:10000 (anti-mouse, Chemicon USA, Cat # AQ160P).

Muscle Histochemistry

Gastrocnemius muscles mounted and frozen onto corks were cut using a cryostat (Cryo-Cyl 120/180/230 LP, Chart Industries Inc. Burnsville, MN USA). The muscle sections were stored at -20°C until they were all ready to be stained for lipid content and muscle fiber differentiation as noted previous by Koopman et al. (85) and Ogilvie et al. (117) respectively. In order to quantify IMTGs levels and fiber type area the imaging processing program Photoshop was utilized. All quantifications of IMTGs and fiber type areas were conducted by a single operator in order to minimize variability.

Substrate Utilization

Some of the animals from each group (n=3 per group) were randomly selected to measure substrate utilization based on indirect calorimetry. For this, a metabolic plastic cage was employed (Windows Oxymax Code #0246-002M Columbus Instruments Ohio, USA) to measure the respiratory exchange ratio in the animals at rest and active phases of the day.

Bone Strength

Femurs were extracted immediately and placed in ice and shipped overnight to the to the Henderson Research Center in Hamilton, Ontario for analysis. The force required to fracture the bone was measured using an Instron Universal Testing Machine as described previously (124).

Statistical Analysis

Statistical analysis was completed using Statistica 6.0 statistical software. Statistical significance was reached at $p \leq 0.05$. All data are expressed as mean ± standard

error of the mean (SEM). A three way (CORT x Exercise x Time) mixed ANOVA was used to analyze diurnal corticosterone levels, body weights, food consumption, running distance and substrate utilization. In the event an interaction was observed, the ANOVA was decomposed to a two way (CORT x Time) mixed ANOVA. While a two way (CORT x Exercise) factorial ANOVA was used for analyzing muscle and fat tissue weights, bone strength, western blots, fasted insulin levels, free fatty acids, intramuscular triglycerides, muscle fiber type and oral glucose tolerance test. The Tukey's HSD Post-hoc test was used to confirm differences observed in the ANOVA tests. Finally, cytochrome C activity was measured using independent measures t-tests.

RESULTS

Physical parameters:

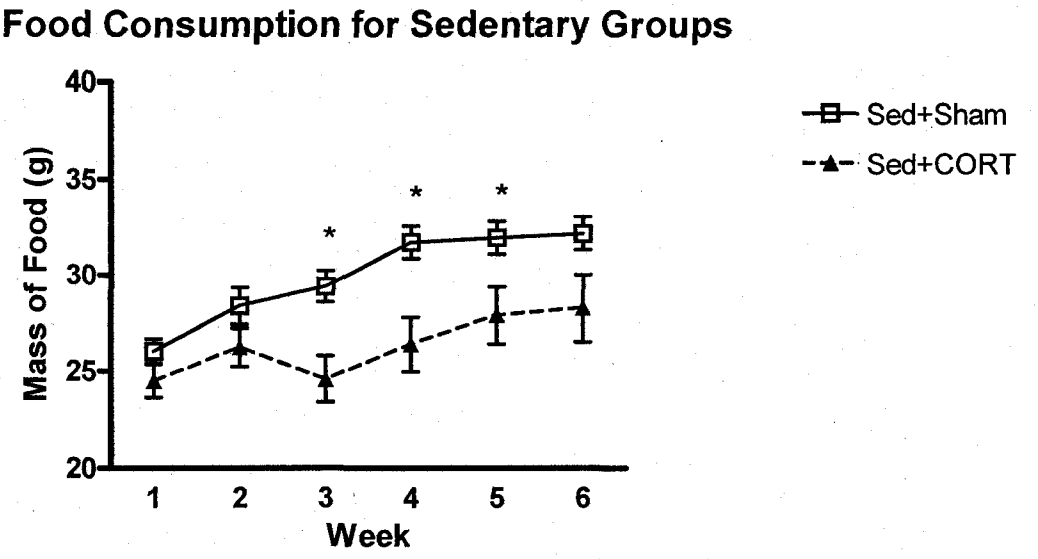
When comparing Sedentary groups, an interaction ($F(5,85) = 2.27$ $p \leq 0.05$) was observed such that Sed-CORT animals ate less food than Sed-SHAM on weeks 3-5 inclusive and a trend ($p=0.07$) was observed in week 6 of the protocol. (Figure 1a). An interaction was also observed in the food intake of the exercised groups ($F(5,85) = 3.01$ $p \leq 0.05$) Figure 1b. Specifically, Exer-CORT animals consumed less *absolute* rat chow than Exer-SHAM rats in weeks 2-4 and 6 while a trend was observed at week 5 ($p=0.08$).

Figure 2a and b depict the body weights of the sedentary and exercised groups respectively. In Figure 2a, an interaction was observed ($F(46, 782) = 4.24$ $p \leq 0.05$) and analysis revealed that Sed-CORT animals were lighter in body weight than Sed-SHAM from day 5 to the end of the protocol. Similarly, in figure 2b Exer-CORT animals

decreased in body weight after the surgical intervention when compared to the Exer-SHAM group ($F(48, 816) = 9.74$ $p \leq 0.05$). Exer-CORT animals were unable to attain body weights comparable to Exer-SHAM rodents by the end of the protocol.

Interestingly, when the food consumed by the sedentary animals was corrected for body mass, a significant CORT by time interaction was evident ($F(5,85) = 6.81$ $p \leq 0.05$) (Figure 3a). Sed-CORT animals showed stunted growth despite eating more food in the first two weeks than Sed-SHAM animals. Exercised groups (Figure 3b) also revealed an interaction ($F(5,85) = 7.00$ $p \leq 0.05$) whereby Exer-CORT animals ate more food despite growing less than Exer-SHAM rodents.

A)



B)

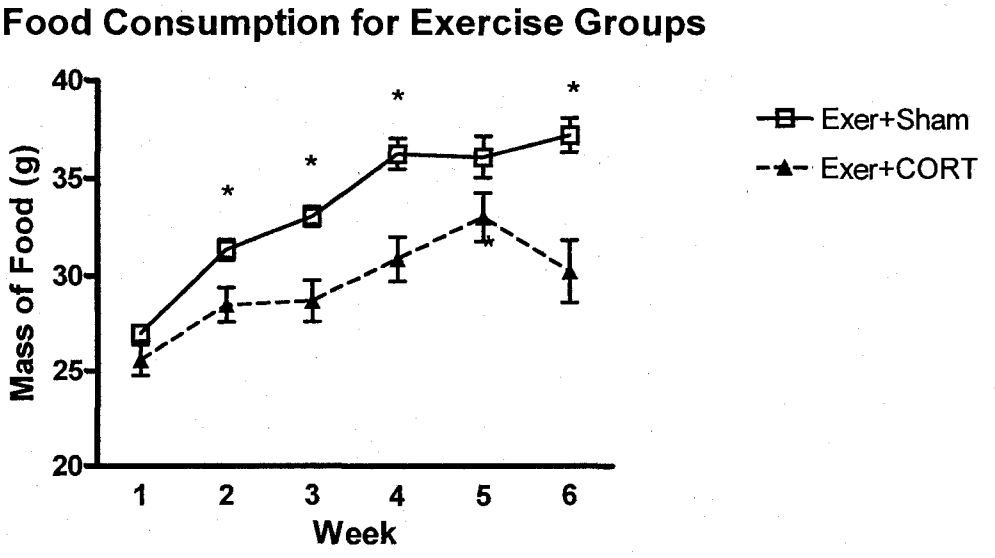
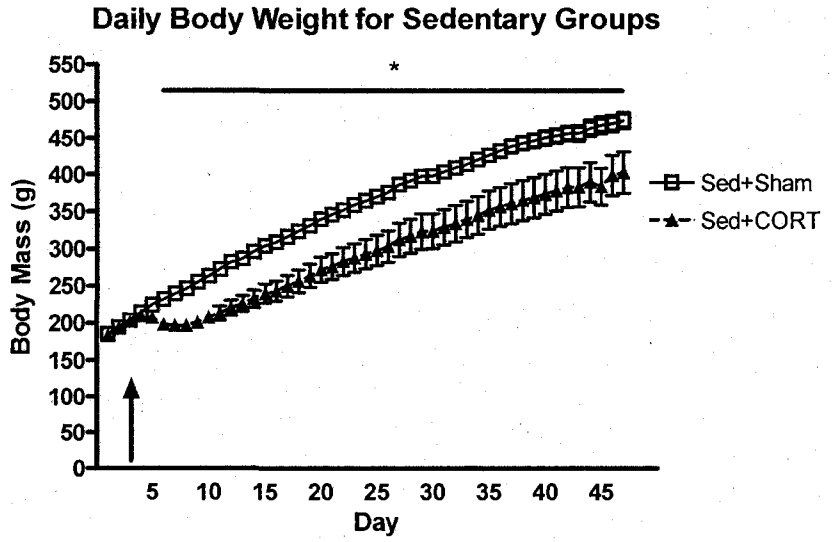


Figure 1: Absolute food consumption for Sed-SHAM (n=8) vs. Sed-CORT (n=10) (A) and Exer-SHAM (n=9) vs. Exer-CORT (n=9) (B). Values are means ± SEM. A Two way mixed ANOVA revealed an interaction (CORT x Time) ($F(5,85)=2.27$, $p \leq 0.05$) and post hoc test showed that Sed-CORT animals ate less food than Sed-SHAM on weeks 3-5 with a trend ($p=0.07$) was observed in week 6 (* denotes $p \leq 0.05$ versus Sed-SHAM). An interaction (CORT x Time) was also evident in exercised groups ($F(5,85) = 3.01$ $p \leq 0.05$) and post hocs confirmed that Exer-CORT animals ate less than the Exer-SHAM group on weeks 2-4 and 6 with a trend ($p=0.08$) on week 5. (# denotes $p \leq 0.05$ versus Exer-SHAM).

A)



B)

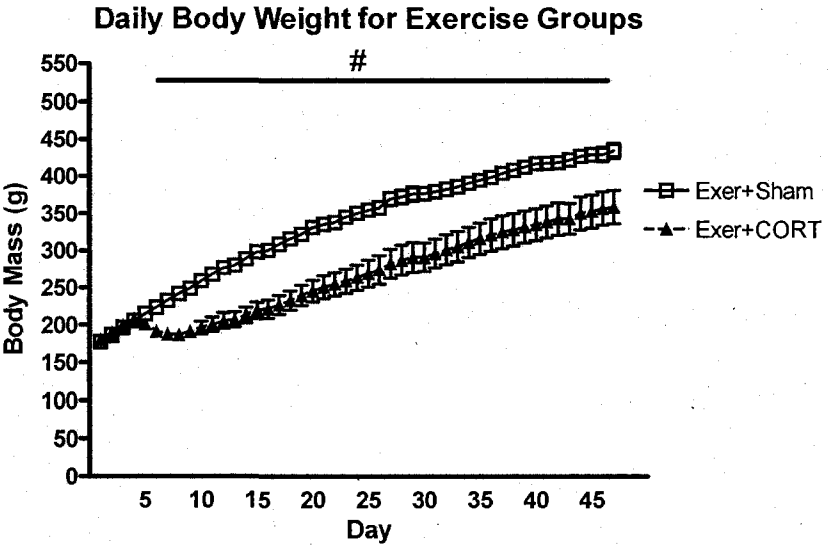
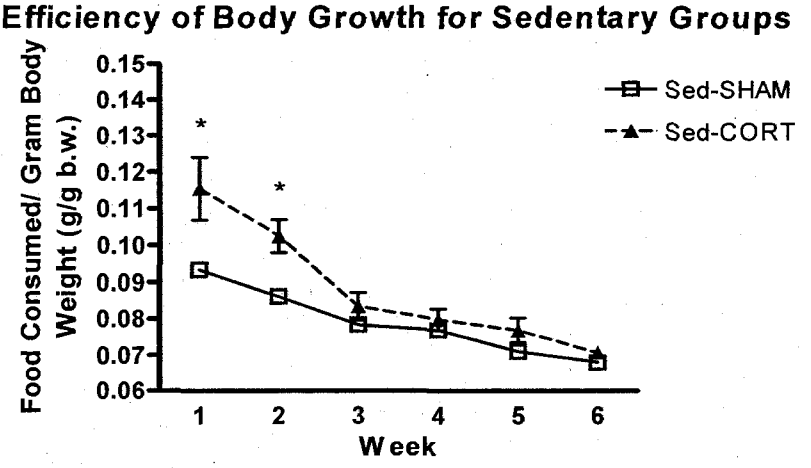


Figure 2: Body weight for Sed-SHAM (n=8) vs. Sed-CORT (n=10) (A) and Exer-SHAM (n=9) vs. Exer-CORT (n=9) (B). Values are means ± SEM. A Two way mixed ANOVA revealed an interaction (CORT x Time) ($F(46, 782) = 4.24$ $p \leq 0.05$) and post hoc test revealed that Sed-CORT animals weighed less than Sed-SHAM from day 5 to the end of the protocol inclusive (* denotes $p \leq 0.05$ versus Sed-SHAM). An interaction (CORT x Time) was also evident in exercised groups ($F(48, 816) = 9.74$ $p \leq 0.05$) whereby the Exer-CORT group weighed less than the Exer-SHAM beginning from the day 5 to the end of the protocol inclusively (# denotes $p \leq 0.05$ versus Exer-SHAM). The arrow indicates surgery day.

A)



B)

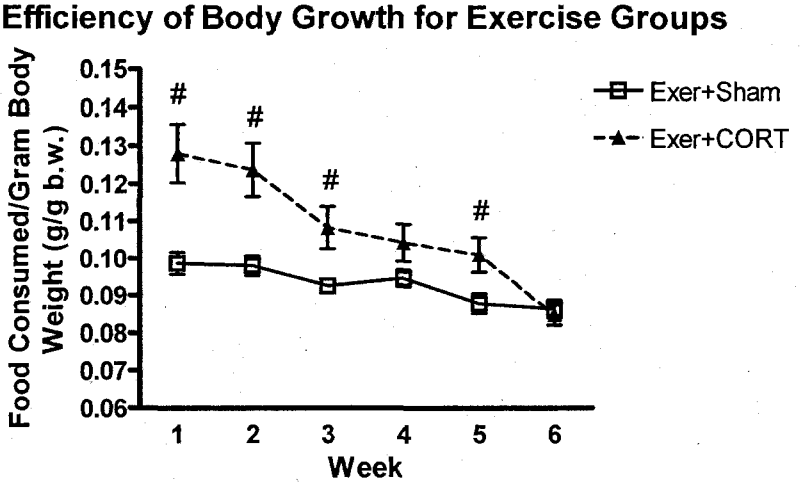


Figure 3: Efficiency of body growth for Sed-SHAM (n=8) vs. Sed-CORT (n=10) (A) and Exer-SHAM (n=9) vs. Exer-CORT (n=9) (B). Values are means ± SEM. A Two way mixed ANOVA revealed an interaction (CORT x Time) ($F(5,85) = 6.81$ $p \leq 0.05$) and post hoc test revealed that Sed-CORT animals had stunted growth despite eating more food on weeks 1 and 2 than Sed-SHAM animals (* denotes $p \leq 0.05$ versus Sed-SHAM). An interaction (CORT x Time) was also evident in exercised groups $F(5,85) = 7.00$ $p \leq 0.05$) whereby the Exer-CORT animals had stunted growth even though they ate more food than Exer-SHAM group (# denotes $p \leq 0.05$ versus Exer-SHAM).

An interaction was observed in running distance ($F(5, 80) = 3.86$ $p \leq 0.05$) (Figure 4), and analysis revealed that in weeks three and four of the protocol, Exer-SHAM animals ran longer distances than Exe-CORT rats. In addition, a trend was observed ($p=0.08$) for higher COX activity in the muscle of Exer-SHAM versus Sed-SHAM animals (Figure 5).

Table 1 displays physical parameters of the animals in the study. These include data for epidydimal fat mass, muscle mass and bone strength. Also included in table 1 are adrenal weights and pellet weights at the end of the protocol. Statistical analysis of the epididymal fat pad corrected for body weight showed that corticosterone pellets promoted an accumulation of epididymal fat ($F(1, 30) = 5.24$ $p \leq 0.05$) while exercised groups had reduced accumulation of adipose mass ($F(1, 30) = 13.1$ $p \leq 0.05$). In the plantaris muscle we observed that CORT treated groups had lighter muscles than sham groups ($F(1, 32) = 5.14$ $p \leq 0.05$). Similarly both Exer-SHAM and Exer-CORT groups experienced decreases in the weight of the plantaris muscle ($F(1, 32) = 4.00$ $p \leq 0.05$). However, when the muscle mass was corrected for total body weight, any differences observed were eliminated. CORT treated animals showed weaker bones as the maximum load required to fracture the femurs was lower in CORT groups than sham groups $F(1, 32) = 22.43$ $p \leq 0.05$) (Table 1). Similar findings were observed even when the diameter and size of the femurs was taken into consideration (data not shown). Investigation of the adrenals revealed that both right and left adrenals weighed less in the CORT groups than the sham controls (data not shown), as expected because of the down regulation in endogenous GC production. Even when corrected for body weight, CORT treated groups

had adrenals that were 35% lighter in weight than sham controls (left ($F(1, 32) = 24.7$ $p \leq 0.05$), right ($F(1, 32) = 37.4$ $p \leq 0.05$). Finally, dissolved CORT pellets were confirmed by weighing the remaining pellet mass at the end of the study. Here we observed that CORT pellets were at least 40% lighter than sham wax pellets at the end of 6 weeks ($F(1, 31) = 28.0$ $p \leq 0.05$).

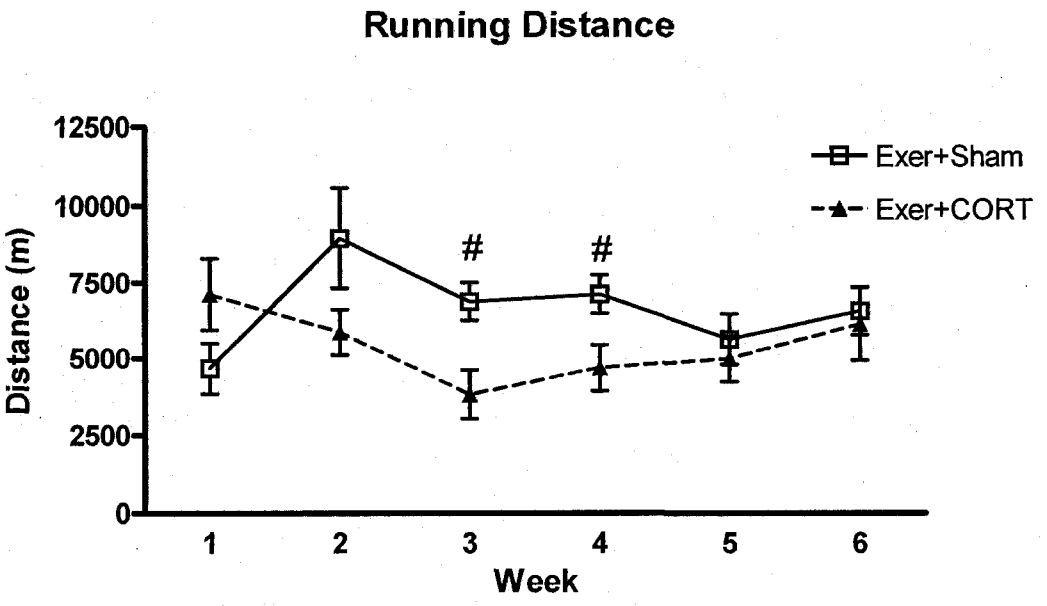


Figure 4: Running distance Exer-SHAM (n=9) vs. Exer-CORT (n=9). Values are means ± SEM. A Two way mixed ANOVA revealed an interaction (CORT x Time) ($F(5, 80) = 3.86$ $p \leq 0.05$). Post hocs tests confirmed that Exer-CORT animals ran less than Exer-SHAM animals in weeks 3 and 4 (# denotes $p \leq 0.05$ versus Exer-SHAM).

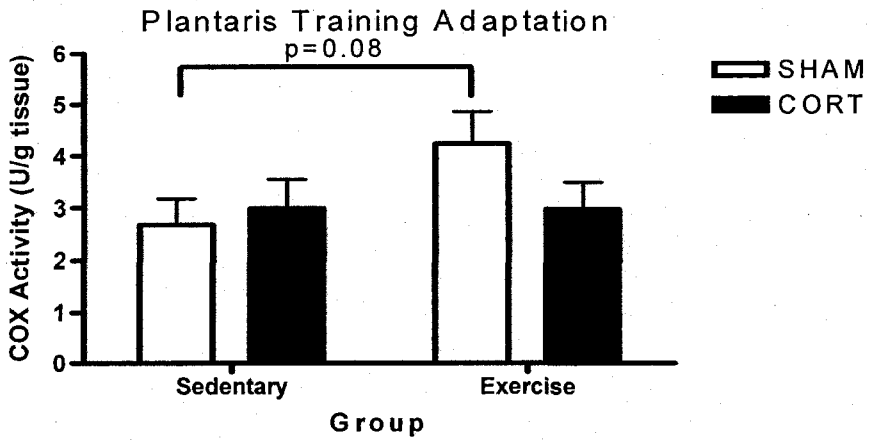


Figure 5: Cytochrome C oxidase (COX) activity for Sed-SHAM (n=7), Sed-CORT (n=10), Exer-SHAM (n=9) and Exer-CORT (n=8). Values are means ± SEM.

Independent measures t-test revealed a trend for higher COX activity in the Exer-SHAM than Sed-SHAM animals (p= 0.08).

Table 1: Physical parameters for Sed-SHAM, Sed-CORT, Exer-SHAM and Exer-CORT animals.

	Sedentary SHAM	Sedentary + CORT	Exercise SHAM	Exercise + CORT	Results
Epididymal Fat/ Total Body Weight (mg/g b.w.)	13.8±0.99	17.0±1.4	8.65±1.5	12.0±1.6	Exercise effect ($p \leq 0.05$) CORT effect ($p \leq 0.05$)
Plantaris Weight (mg)	421.1±23.7	380.7±27.0	386.9±15.8	322.3±23.1	Exercise effect ($p \leq 0.05$) CORT effect ($p \leq 0.05$)
Plantaris/Total Body Weight (mg/g b.w.)	0.904±0.05	0.944±0.03	0.905±0.03	0.903±0.03	NS
Max Load to Fracture (N)	140.2±2.96	117.3±5.14	134.2±5.55	109.5±6.23	CORT effect ($p \leq 0.05$)
LT Adrenal/Total Body Weight (mg/g b.w.)	0.076±0.007	0.051±0.006	0.086±0.006	0.048±0.007	CORT effect ($p \leq 0.05$)
RT Adrenal/Total Body Weight (mg/g b.w.)	0.075±0.007	0.044±0.005	0.077±0.003	0.043±0.005	CORT effect ($p \leq 0.05$)
Combined Pellet Weight (mg)	301.0±0.931	184.2±30.3	303.1±0.951	158.4±30.0	CORT effect ($p \leq 0.05$)

Values are means ± SEM
All measures (except Epididymal and Right adrenal):
Sed-SHAM n=8; Sed-CORT n=10; Exer-SHAM n=9; Exer-CORT n=9
Epididymal fat: Sed-SHAM n=8; Sed-CORT n=8; Exer-SHAM n=9; Exer-CORT n=9
RT Adrenal: Sed-SHAM n=8; Sed-CORT n=9; Exer-SHAM n=9; Exer-CORT n=9

Diurnal Corticosterone Concentrations

Figures 6a-f depict the plasma corticosterone concentrations for the sedentary groups for weeks 1-6 respectively. At all weeks of the protocol, except for week 2 and week 3, interactions for CORT by time were observed (week 1 $F(2, 32) = 4.19$ $p \leq 0.05$; week 4 $F(2, 32) = 4.35$ $p \leq 0.05$; week 5 $F(2, 32) = 4.54$ $p \leq 0.05$; week 6 $F(2, 32) = 10.9$ $p \leq 0.05$). In week 2 a time effect ($F(2, 32) = 3.76$ $p \leq 0.05$) and a CORT effect ($F(1, 16) = 5.59$ $p \leq 0.05$) were observed. Week 3 showed a trend for an interaction ($p = 0.08$) and provided a main effect for time ($F(2, 32) = 5.32$ $p \leq 0.05$). More importantly, the interactions revealed that the diurnal pattern was eliminated in Sed-CORT animals and nadir levels (Figure 8a) were at least 3 fold higher (week 6; Sed-CORT= 115.1±10.6 ng/ml vs. Sed-SHAM= 34.1±10.0 ng/ml) than the Sed-SHAM group.

The plasma corticosterone concentrations for the exercised groups is shown in Figures 7a-f. There was a CORT effect ($F(1, 16) = 8.43$ $p \leq 0.05$) and a time effect ($F(2, 32) = 6.55$ $p \leq 0.05$) in week 1 while in week 2 (Figure 7b) only a time effect was evident ($F(2, 32) = 5.46$ $p \leq 0.05$) indicating that during the second week Exer-SHAM animals experienced elevations in corticosterone that mimicked those of Exer-CORT rodents. Two way (CORT x time) mixed ANOVA tests revealed interactions at weeks 3-6 inclusively (week 3 ($F(2, 32) = 11.0$ $p \leq 0.05$); week 4 ($F(2, 32) = 14.1$ $p \leq 0.05$); week 5 ($F(2, 32) = 7.38$ $p \leq 0.05$); week 6 ($F(2, 32) = 5.09$ $p \leq 0.05$)). Post hoc analysis on the ANOVAs revealed higher levels of corticosterone at the nadir phase in weeks 4 and 5 in

Exer-CORT compared with Exer-SHAM animals, while trends were observed in weeks 3 and 6 ($p= 0.07$, $p= 0.08$ respectively). These findings indicate that the corticosterone circadian rhythm had been disrupted in the Exer-CORT group while the Exer-SHAM animals had a normal diurnal pattern. Specifically nadir levels were chronically elevated in Exer-CORT animals versus Exer-SHAM (Figure 8b).

OGTT, Insulin and Free Fatty acids

Table 2 illustrates the area under the curve for the oral glucose tolerance test in all animals. Our results indicated that exercise training promotes greater efficiency for the removal of glucose from the circulation ($F(1, 32)=13.6$, $p\leq 0.05$). However, no effect was observed for the type of pellet placed in the animals (i.e. CORT vs SHAM). Interestingly, exercise training lowered the concentration of insulin levels ($F(1, 32) = 8.71$ $p\leq 0.05$) but CORT treatment did not have an effect on insulin concentrations (Table 2). Exercised groups also had increased fasted free fatty acid content in the plasma ($F(1, 31) = 7.06$ $p\leq 0.05$) (Table 2).

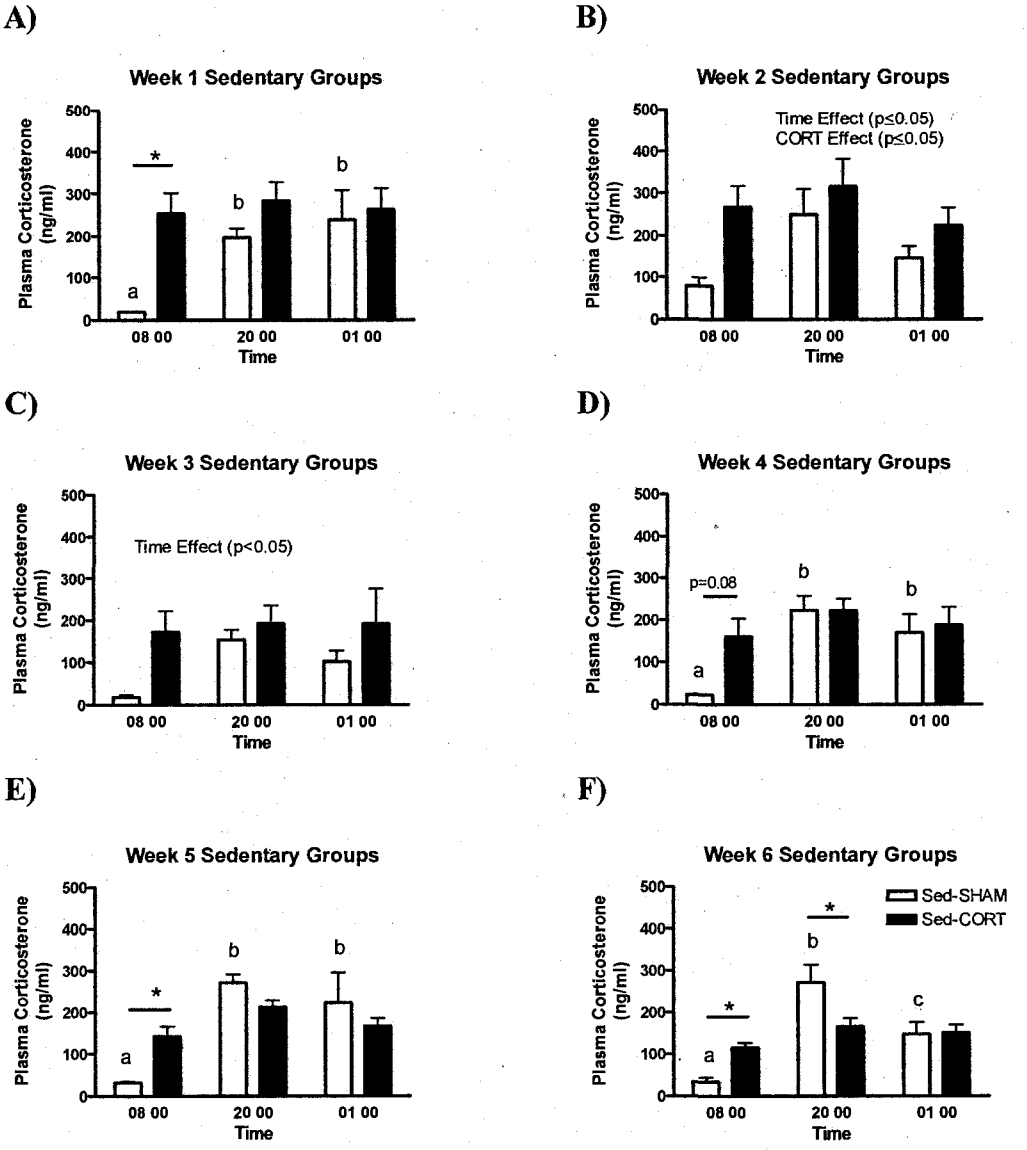


Figure 6: Circadian rhythm of plasma corticosterone between Sed-SHAM (n=8) vs. Sed-CORT (n=10) at the end of each week of training. All values are means ± SEM. A two way mixed ANOVA was performed on data from each individual week followed by post hoc analysis. ANOVA results: A) Week 1, interaction ($F(2, 32) = 4.19$ $p \leq 0.05$); B) Week 2, time effect ($F(2, 32) = 3.76$ $p \leq 0.05$), CORT effect ($F(1, 16) = 5.59$ $p \leq 0.05$); C) Week 3, time effect ($F(2, 32) = 5.32$ $p \leq 0.05$); D) Week 4, interaction $F(2, 32) = 4.35$ $p \leq 0.05$; E) Week 5, $F(2, 32) = 4.54$ $p \leq 0.05$; F) Week 6, $F(2, 32) = 10.9$ $p \leq 0.05$). Post hoc tests revealed that nadir GC levels were elevated and the diurnal pattern was eliminated in Sed-CORT rats in weeks 1, 5 and 6 while a trend was observed in week 4.

Significance between time of day is shown by different letters (a-c), while * = difference between groups. All $p \leq 0.05$

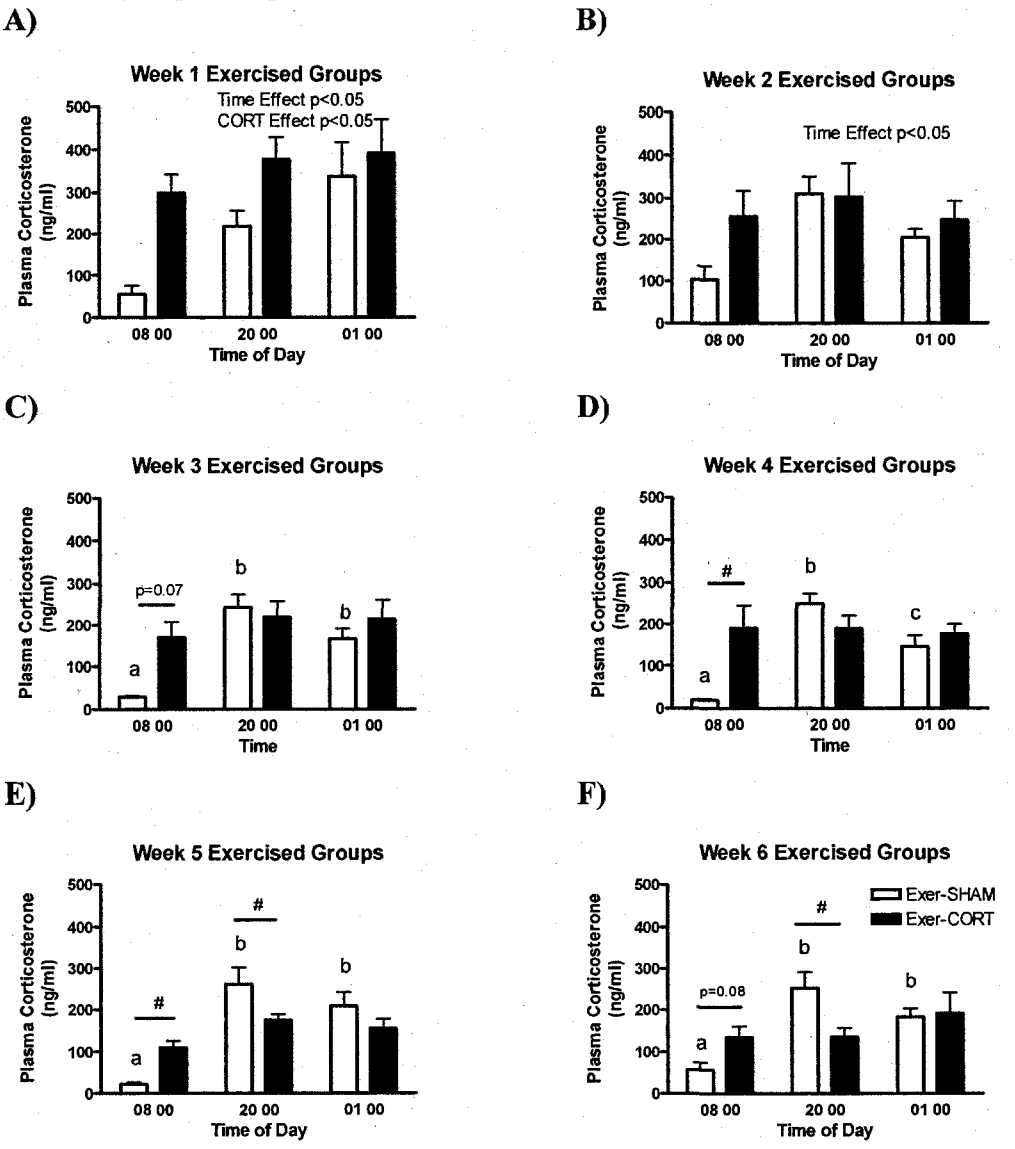
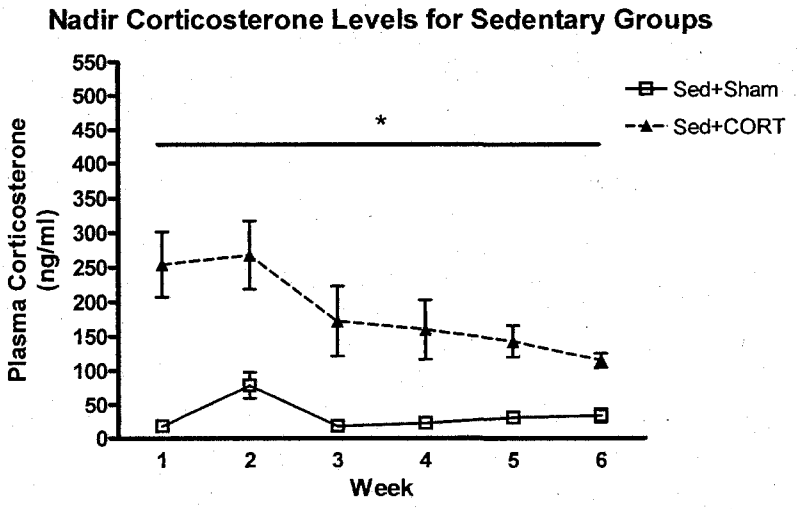


Figure 7: Circadian rhythm of plasma corticosterone between Exer-SHAM (n=9) vs. Exer-CORT (n=9) at the end of each week of training. All values are means ± SEM. A two way mixed ANOVA was performed on data from each individual week followed by post hoc analysis. ANOVA results: A) Week 1, CORT effect ($F(1, 16) = 8.43$ $p \leq 0.05$), time effect ($F(2, 32) = 6.55$ $p \leq 0.05$); B) Week 2, time effect ($F(2, 32) = 5.46$ $p \leq 0.05$); C) Week 3 ($F(2, 32) = 11.0$ $p \leq 0.05$); D) Week 4 ($F(2, 32) = 14.1$ $p \leq 0.05$); E) Week 5 ($F(2, 32) = 7.38$ $p \leq 0.05$); F) Week 6 ($F(2, 32) = 5.09$ $p \leq 0.05$). Post hoc test confirmed that nadir GC levels were elevated and the diurnal pattern was abolished in Exer-CORT rats at the end of weeks 4 and 5 while trends were observed in weeks 3 ($p=0.07$) and 6 ($p=0.08$). Significance between time of day is shown by different letters (a-c), while # = difference between groups. All $p \leq 0.05$

A)



B)

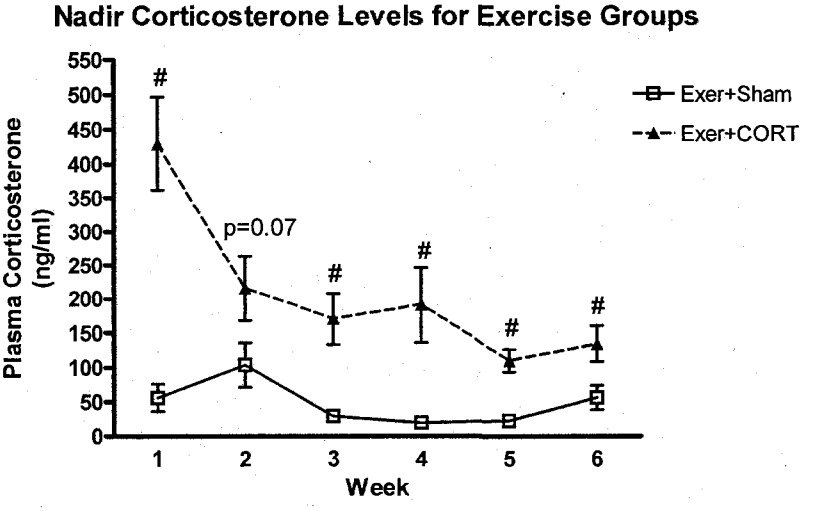


Figure 8: Nadir (08 00) Corticosterone concentration for Sed-SHAM (n=8) vs. Sed-CORT (n=10) (A) and Exer-SHAM (n=9) vs. Exer-CORT (n=9) (B). Values are means ± SEM. A Two way mixed ANOVA revealed an interaction (CORT x Time) ($F(5,80) = 2.41$ $p \leq 0.05$) and post hoc test revealed that Sed-CORT animals had higher basal CORT levels than Sed-SHAM animals at the end of all weeks (* denotes $p \leq 0.05$ versus Sed-SHAM). An interaction (CORT x Time) was also evident in exercised groups $F(5,80) = 4.99$ $p \leq 0.05$) whereby the Exer-CORT animals had higher basal CORT levels than Exer-SHAM rodents at all weeks except at the end of week 2 (# denotes $p \leq 0.05$ versus Exer-SHAM).

Table 2: Oral glucose tolerance test (OGTT), plasma insulin and free fatty acid measures for Sed-SHAM, Sed-CORT, Exer-SHAM and Exer-CORT animals.

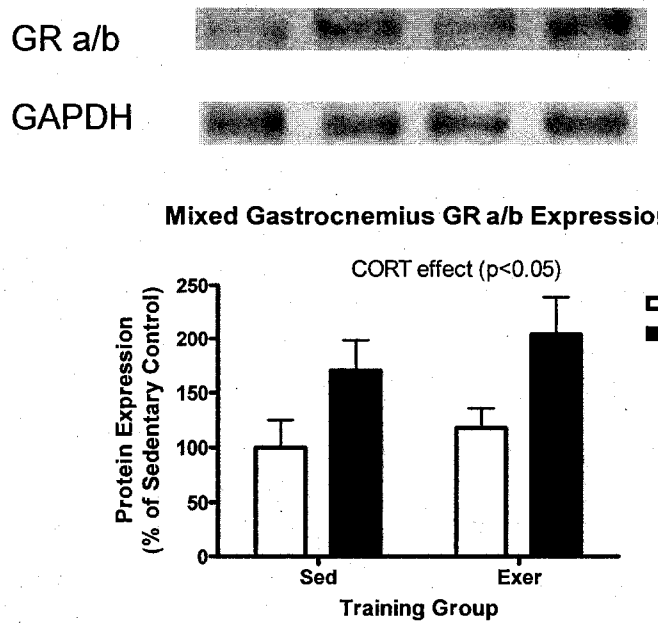
	Sedentary SHAM	Sedentary + CORT	Exercise SHAM	Exercise + CORT	Results
Area Under Curve of OGTT (min*mmol)	837.7±20.9	819.3±20.2	745.8±26.8	694.5±733.5	Exercise effect ($p \leq 0.05$)
Non Esterified Free Fatty Acid Content (mM)	0.843±0.09	0.885±0.06	1.04±0.05	1.04±0.06	Exercise effect ($p \leq 0.05$)
Fasted Plasma Insulin (ng/ml)	1.41±0.24	1.74±0.29	0.800±0.11	0.977±0.24	Exercise effect ($p \leq 0.05$)

Values are means ± SEM
All measures: Sed-SHAM n=8; Sed-CORT n=10; Exer-SHAM n=9; Exer-CORT n=9

Western Blot Analysis

Compared with Sham groups CORT treated groups had a 75% increase in the expression of the GR a/b isoform ($F(1, 32) = 8.27$ $p \leq 0.05$) (Figure 9a). No difference was observed for GR c isoform (Figure 9b) in the mixed gastrocnemius muscle between groups. In regards to the expression of 11-βHSD1, CORT groups had a higher expression of the enzyme when compared to sham groups ($F(1, 32) = 6.74$ $p \leq 0.05$) (figure 10).

A)



B)

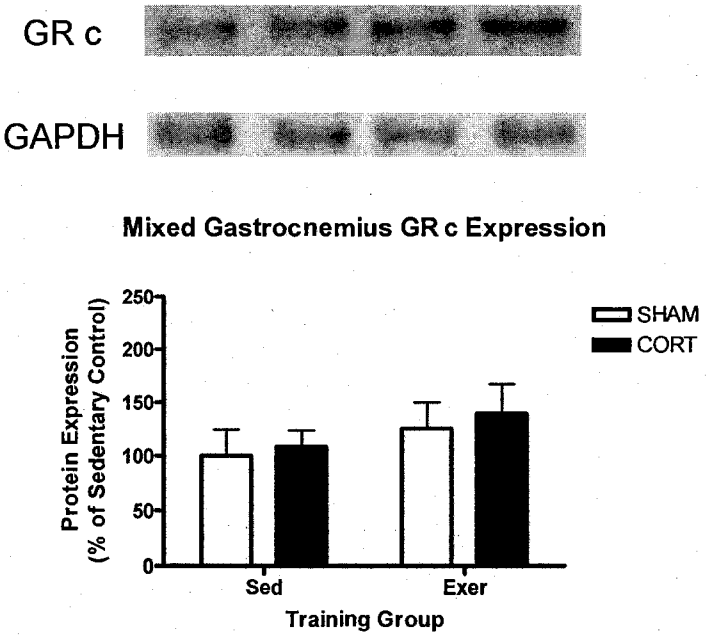
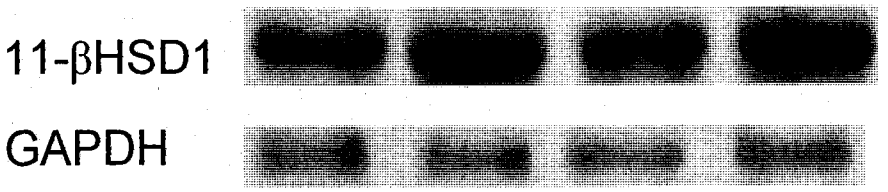


Figure 9: Protein expression of glucocorticoid receptor isoforms a/b (GR a/b) (A) and glucocorticoids receptor isoform c (GR c) (B) in the mixed gastrocnemius muscle of Sed-SHAM (n=8), Sed-CORT (n=10), Exer-SHAM (n=9) and Exer-CORT (n=9). All values are means ± SEM and units are expressed as relative optical density (ROD). A significant CORT effect ($F(1, 32) = 8.27$ $p \leq 0.05$) was seen whereby CORT groups upregulated the expression of GR a/b compared to SHAM groups. Conversely, no change (B) occurred in the expression of GR c.



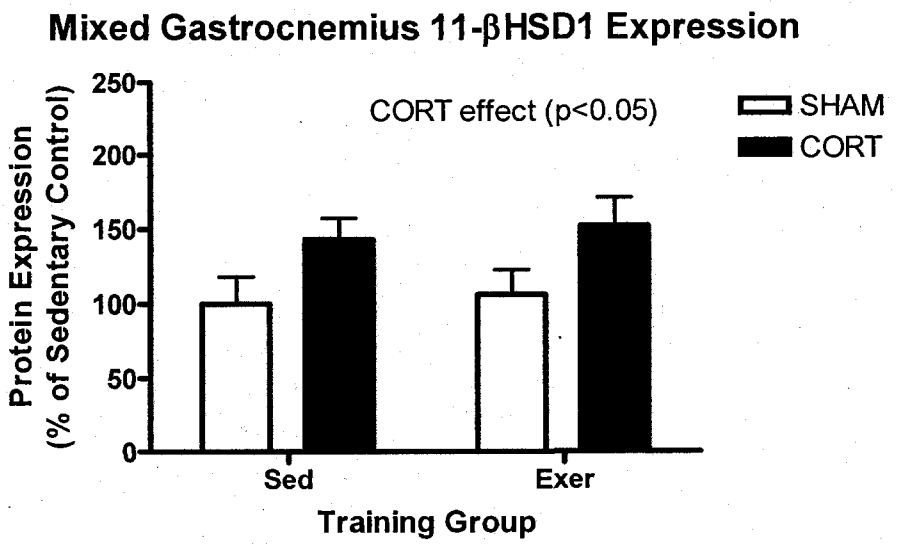
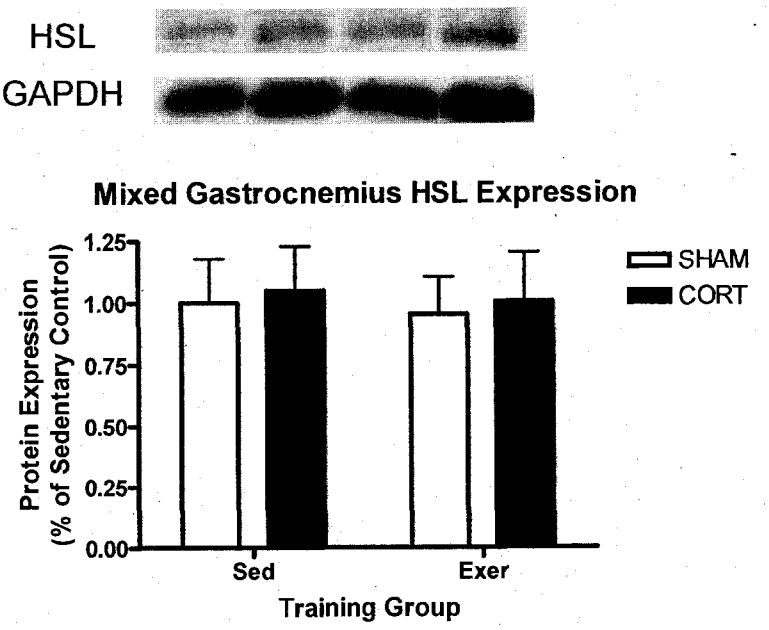


Figure 10: Protein expression of 11 beta-hydroxysteroid dehydrogenase 1 (11-βHSD1) in the mixed gastrocnemius muscle of Sed-SHAM (n=8), Sed-CORT (n=10), Exer-SHAM (n=9) and Exer-CORT (n=9). All values are means ± SEM and units are expressed as relative optical density (ROD). A two way ANOVA on the data provided a significant CORT effect ($F(1, 32) = 6.74$ $p \leq 0.05$) was seen whereby CORT groups upregulated the expression of 11-βHSD1 when compared to SHAM groups.

To investigate tissue specific changes as a result of chronic glucocorticoids, western blotting for HSL and ATGL expression was completed on the gastrocnemius muscle, subcutaneous and epididymal fat pads. HSL and ATGL expression in muscle remained unchanged in the presence of glucocortiods and exercise training (Figure 11a and b). Similar findings were observed in subcutaneous fat tissue (Figure 12a and b). However, epididymal fat displayed increased expression of HSL (Figure 13a) in exercised groups ($F(1, 31) = 8.67$ $p \leq 0.05$). Also exercise tended to increase gastocnemius ATGL expression (Main effect of exercise ($F(1, 31) = 3.73$ $p = 0.06$)) (Figure 13b).

A)



B)

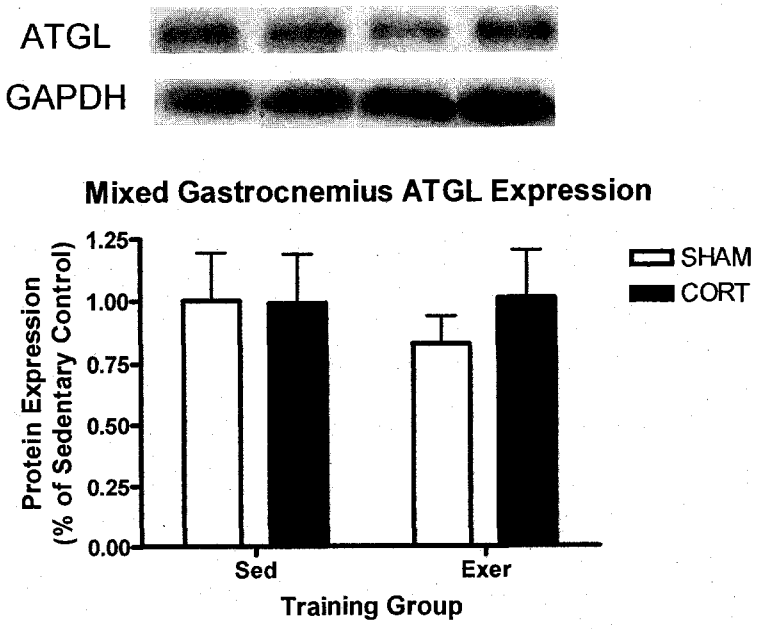
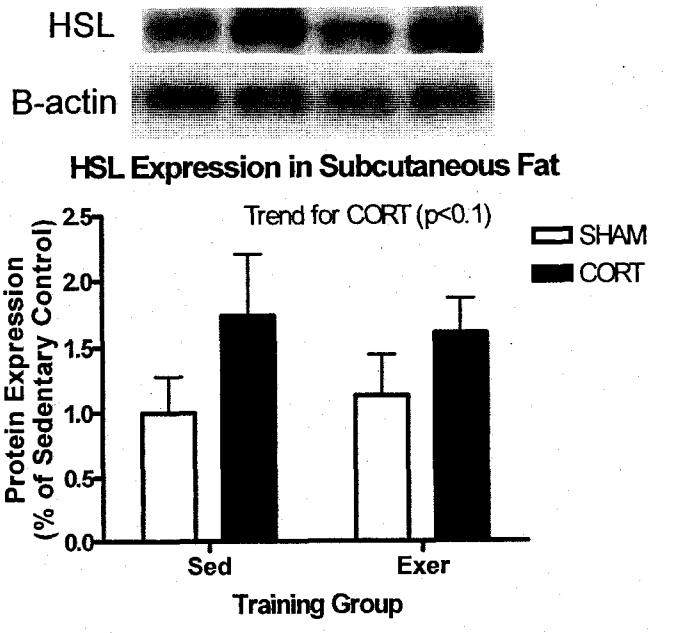


Figure 11: Protein expression of hormone sensitive lipase (HSL) (A) and adipose triglyceride lipase (ATGL) (B) in the mixed gastrocnemius muscle of Sed-SHAM (n=8), Sed-CORT (n=9), Exer-SHAM (n=9) and Exer-CORT (n=8). All values are means ± SEM and units are expressed as relative optical density (ROD). A two way ANOVA on the data revealed no significant differences.

A)



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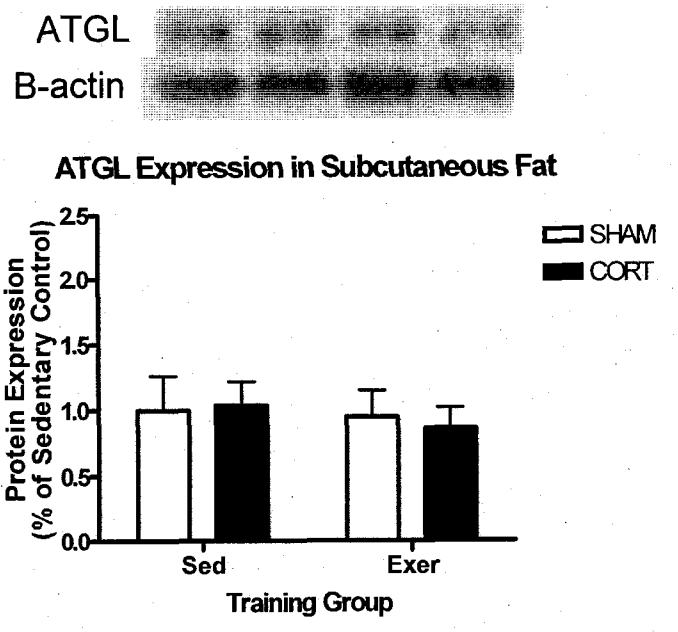
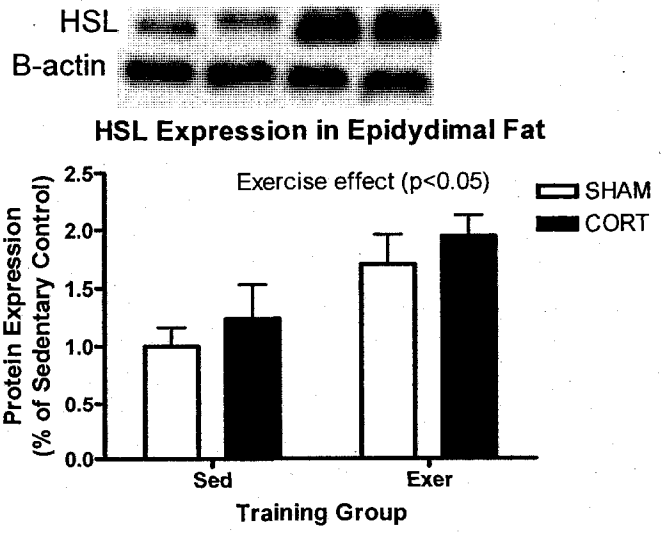


Figure 12: Protein expression of hormone sensitive lipase (HSL) (A) and adipose triglyceride lipase (ATGL) (B) in subcutaneous adipose tissue of Sed-SHAM (n=8), Sed-CORT (n=10), Exer-SHAM (n=8) and Exer-CORT (n=9). All values are means ± SEM and units are expressed as relative optical density (ROD). A two way ANOVA on the data revealed no significant differences.

A)



B)

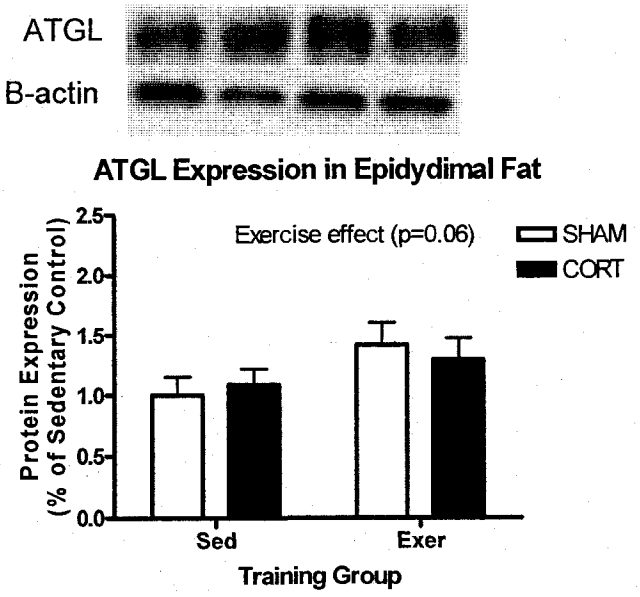
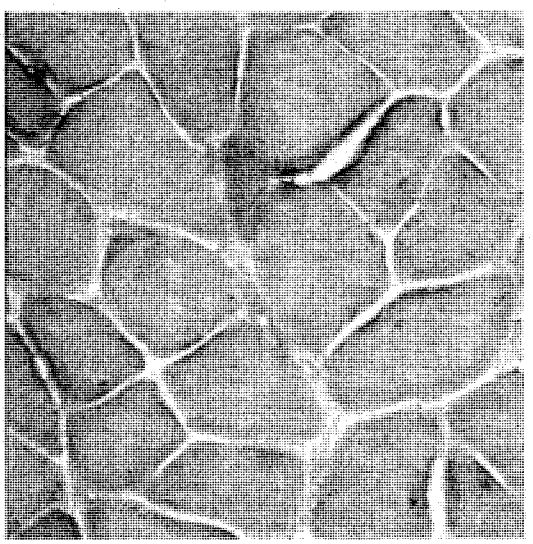


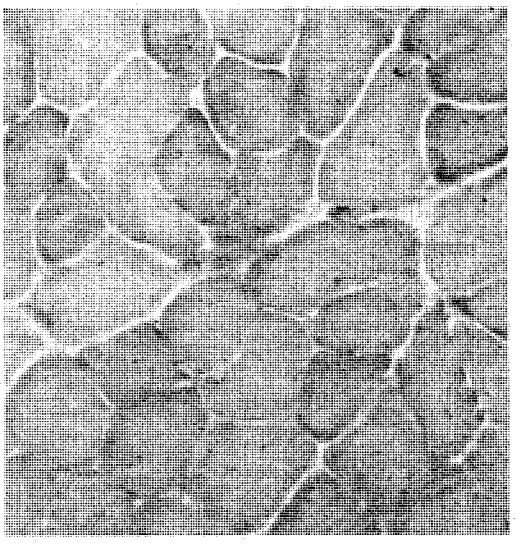
Figure 13: Protein expression of hormone sensitive lipase (HSL) (A) and adipose triglyceride lipase (ATGL) (B) in the epididymal fat pad of Sed-SHAM (n=8), Sed-CORT (n=10), Exer-SHAM (n=8) and Exer-CORT (n=9). All values are means ± SEM and units are expressed as relative optical density (ROD). A two way ANOVA on the data provided an exercise effect ($F(1, 31) = 8.67$ $p \leq 0.05$) such that exercised groups upregulated the expression of HSL when compared to sedentary groups. Analysis of ATGL content revealed a trend for an exercise effect ($F(1, 31) = 3.73$ $p = 0.06$) whereby exercised groups had higher levels of the lipolytic enzyme compared to sedentary groups.

Muscle Histochemistry

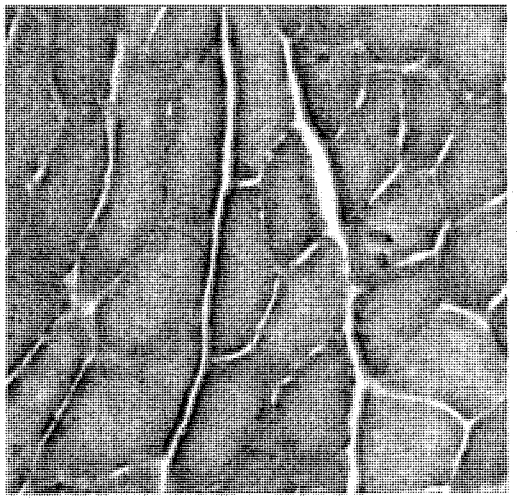
Figure 14 depicts an example of the pictures utilized to quantify the IMTGs in the mixed gastrocnemius muscle. Table 3 shows the results of the Oil Red O analysis for quantification of IMTGs. No difference was observed between the groups in any of the muscle fiber types analyzed within the mixed gastrocnemius. Similarly, neither exercise nor CORT exposure affected any of the fiber type areas found in the mixed gastrocnemius (Table 4).



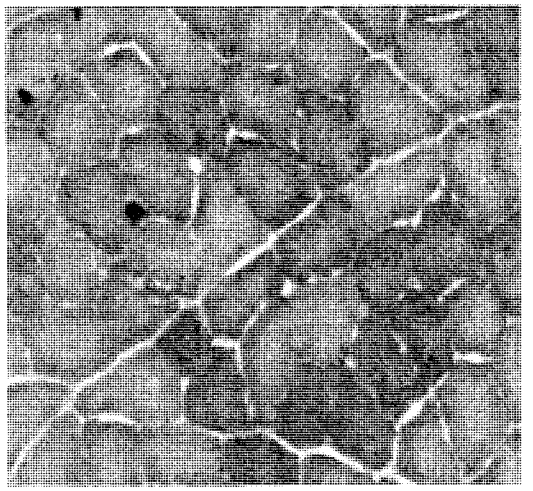
Sedentary SHAM



Sedentary CORT



Exercise SHAM



Exercise CORT

Figure 14: Intramuscular Triglyceride pictures of the mixed Gastrocnemius muscle for Sed SHAM (n=8), Sed-CORT (n=7), Exer-SHAM (n=9), Exer-CORT (n=8) using Oil Red O staining. All values are means ± SEM and units are expressed as optical density. A two way ANOVA on the data revealed no significant differences.

Table 3: Intramuscular triglyceride in mixed gastrocnemius muscle fibers of Sed-SHAM, Sed-CORT, Exer-SHAM and Exer-CORT animals.

	Sedentary SHAM	Sedentary + CORT	Exercise SHAM	Exercise + CORT	Results
Type I Red IMTGs (Index of optic density)	31.9±1.68	39.4±5.98	38.2±2.54	37.0±2.44	NS
Type IIa Red IMGs (Index of optic density)	37.9±1.92	42.5±5.67	41.3±2.94	47.3±5.32	NS
Type IIb/d Red IMTGs (Index of optic density)	30.4±1.56	38.5±6.43	34.7±3.09	35.1±3.15	NS
Type IIb/d White IMTGs (Index of optic density)	43.9±1.85	42.2±5.79	41.7±1.66	46.0±2.09	NS

Values are means ± SEM
All measures: Sed-SHAM n=8; Sed-CORT n=7; Exer-SHAM n=9; Exer-CORT n=8

Table 4: Muscle fiber type areas of the mixed gastrocnemius in Sed-SHAM, Sed-CORT, Exer-SHAM and Exer-CORT animals.

	Sedentary SHAM	Sedentary + CORT	Exercise SHAM	Exercise + CORT	Results
Type I Red Area/Total Area	21.0±0.97	21.8±1.32	21.3±1.29	23.7±1.48	NS
Type IIa Red Area /Total Area	20.6±1.16	20.9±0.79	23.5±0.61	21.9±1.34	NS
Type IIb/d Red Area/Total Area	27.1±0.88	25.6±1.65	26.2±1.28	26.4±0.83	NS
Type IIb/d White/Total Area	31.1±2.56	31.7±2.43	29.0±1.76	28.0±1.55	NS

Values are means ± SEM
All measures: Sed-SHAM n=8; Sed-CORT n=8; Exer-SHAM n=9; Exer-CORT n=8

Substrate Utilization

Due to the limitations of utilizing one metabolic cage, a small subgroup of animals were utilized to determine substrate utilization. Three animals per group were individually monitored in a plastic metabolic cage over a period of 20 hours. A three way (CORT x Exercise x Time) mixed ANOVA was used to determine a time effect ($F(20,180)=1.93$ $p \leq 0.05$) indicating that all animal groups utilized more fat during the *lights on* (basal) than the *lights off* (active) phase (pooled data shown in Figure 15).

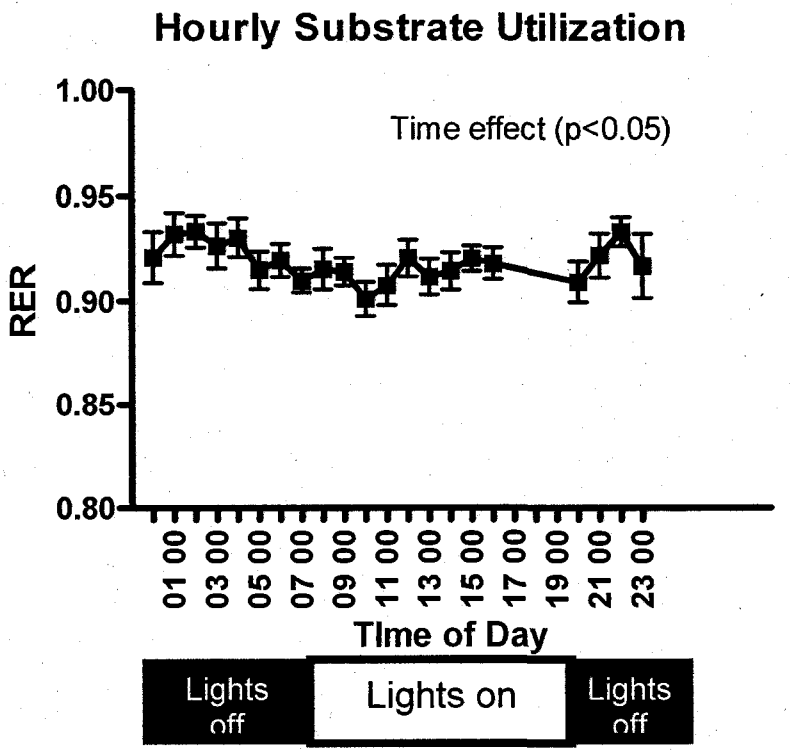


Figure 15: Substrate utilization for Sed-SHAM (n=3), Sed-CORT (n=3), Exer-SHAM (n=3) and Exer-CORT (n=4). All values are means ± SEM. A three way mixed ANOVA revealed a significant time effect ($F(20,180)=1.93$ $p \leq 0.05$) depicting a lower respiratory exchange ratio during the lights on (basal) versus lights off (active) phase. Pooled data.

DISCUSSION

In this study, we used glucocorticoid pellets implanted subcutaneously to abolish the diurnal corticosterone rhythm in young, growing Sprage-Dawley rats. This technique was successful in maintaining elevated basal corticosterone levels for most of the 6 week duration (08 00 in Figures 6, 7 and 8). This is the first time that exogenous corticosterone has been used for metabolic studies lasting longer than 20 days in rodents. Moreover, this study is the first to measure the effects of regular volitional exercise on attenuating the metabolic consequences of elevated glucocorticoids in an animal model. A number of key findings suggest that elevations in basal glucocorticoids promote an increase in central adiposity and that exercise reverses this phenotype. In exercised rodents the rise in HSL and a trend for greater expression of ATGL in epididymal fat provides a mechanism for lowering central adiposity in this animal model. As mentioned above, HSL and ATGL are key regulators of lipolysis in adipose tissue and their increased expression help explain the reduced visceral adiposity seen in all exercised groups. To date, no studies have explored the expression of ATGL in exercised rodents and so we provide novel findings for this newly discovered lypolitic enzyme.

Physical Parameters

Since glucocorticoids are known to have widespread effects on multiple tissues, we measured a number of physical/anthropometric features in this new model of basal hyperglucocorticoidemia. The lower body weight observed in CORT (Figure 2a, b) groups is in agreement with other animal models using exogenous GC treatment (42, 155). Some authors have suggested that a decrease in food intake is responsible for the

decreased growth of GC treated animals (155), as was found in this study (Figure 1). Conversely others have observed an increase in food intake with GC treatment in the presence of weight loss (58). In the present study, absolute values of food intake were lower in CORT compared with SHAM groups (Figure 1a, b and Figure 2a, b). When food consumed was corrected per gram of body weight (Figure 3a, b), as an index of food intake efficiency, interestingly, CORT groups were less efficient at utilizing the food consumed for increasing body weight. It may be that the catabolic nature of increased CORT lowers the metabolic efficiency of ingested food. Some have suggested that GC inhibit the effects of growth hormone on physical maturation and growth (4). The higher levels of visceral fat deposition observed in CORT groups may be explained by an increase in the expression of lipoprotein lipase (LPL) which was not measured in our study. Indeed, dexamethasone treatment of primary rat cells has been shown to promote an increase in LPL activity in visceral adipocytes (6). Similar findings have also been observed using corticosterone (134).

Since increases in visceral fat are known to precede the development of insulin resistance and type 2 diabetes, and because GC are well known to promote insulin resistance, we measured oral glucose tolerance in these animals (Table 2). The oral glucose tolerance test showed that exercise improved the efficiency to tolerate a bolus of dextrose. Surprisingly, we did not observe any differences in glucose tolerance as a result of CORT treatment. These findings suggest that the increase in visceral fat gain and the level of circulating GC were insufficient to promote whole body insulin resistance. Exercise however had a positive effect as it decreased fasted insulin levels in both Exer-

SHAM and Exer-CORT animals. These findings support other studies analyzing exercise trained animal models as well as human studies (112, 166).

Adrenal mass was markedly lower in the CORT vs. SHAM groups, suggesting a decreased production of endogenous corticosterone (Table 1). Indeed, Lesniewska et al. (92) observed a decrease in adrenal size after seven days of dexamethasone treatment. We believe that the elevations of exogenous CORT activated the negative feedback loop of the HPA axis; therefore resulting in a decreased production of ACTH (92) in Sed-CORT and Exer-CORT animals to try and minimize circulating corticosterone levels. Hence the activation of the negative feedback loop effectively decreased or even eliminated the production of endogenous corticosterone in these CORT treated groups. The atrophy of the adrenals and the lower levels of peak (20 00) diurnal corticosterone seen in CORT groups in the last two weeks (Figure 6e, f and Figure 7e, f) lend support to this idea.

Bone strength has clearly been shown to be negatively impacted by increased GC (19, 165). We therefore were interested in measuring the impact of increased basal GC and exercise on markers of bone strength. We found that the max load required to break the femur in situ was lowered with CORT treatment (Table 1). In the present study volitional exercise training was unable to rescue the decrease in bone strength brought upon by CORT treatment. Similarly, Garrel et al. (53) observed that moderate intensity treadmill running was unable to recover the loss of bone density in humans caused by nine days of glucocorticoid treatment. Conversely, exercise protocols that simulated

strength training in rodents have been better equipped at preventing the bone weakness associated with GC treatment (106).

Skeletal Muscle

Glucocorticoids (42, 111) and exercise training (57, 69) have long been thought to influence mitochondrial levels in skeletal muscle. We therefore measured COX activity as an index of mitochondrial content in these animals. Exer-SHAM animals displayed a trend for a 55% increase in COX activity. This increase in COX activity with training however is similar to what we have previously observed in the plantaris muscle with healthy wheel running animals (47). Exer-SHAM animals ran more than Exer-CORT rats on weeks 3 and 4 (Figure 4); hence it is possible that COX activity failed to rise in Exer-CORT animals because the training stimuli were insufficient to promote mitochondrial biogenesis (Figure 5). However running distances of the magnitude seen in our study have previously shown training effects (47). The lack of increased COX activity in Exer-CORT animals may also be a result of decreased mitochondrial efficiency as chronic GC have been shown to decrease oxidative capacity in mitochondria of the plantaris muscle (42, 111). More importantly, Exer-CORT animals displayed crucial phenotypic adaptations that are indicative of volitional exercise training including reduced body weight (47, 131), improved glucose tolerance (18) and decreased visceral adiposity (18, 29). Although the weight of the plantaris muscle was lowered in CORT treated groups, suggesting attenuation in muscle growth, when the weight of the plantaris was corrected for total body weight, this difference disappeared (Table 1). Exercise failed to increase

plantaris muscle mass in both CORT and SHAM treated groups, results that mimic previous findings in healthy rodents (47).

Exercise training also increased the concentration of free fatty acids in animals fasted for 24 hours (Table 2) suggesting a greater reliance on fat metabolism at rest. Unfortunately, the sub segment of animals used in the metabolic cage did not support the increase in fat utilization as a substrate in these CORT treated groups (Figure 15). However, it should be noted that a larger sample size is required to truly confirm the findings of this subgroup of animals. Moreover, free fatty acids (Table 2) were not increased in CORT groups as some authors have previously observed (115, 138). Again, the duration of the protocol may play a role as glucocorticoid treatment has a transient increase in free fatty acids that is attenuated by the 20th day of GC treatment (115). In order to determine if any accumulation of free fatty acids took place in muscle, IMTGs were investigated in all groups. No increases were observed in the IMTGs content in any of the muscle fibers (Table 3) which is consistent with findings by Novelli et al. (115) utilizing dexamethasone injections.

Corticosterone treatment failed to bring about any changes in muscle fiber type area (Table 3). This was surprising considering the loss in body mass that took place in both the Sed-CORT and Exer-CORT groups. Some studies have observed the catabolic effects of GC on muscle fiber area. Specifically, glycolytic fibers seem to be affected negatively by treatment of synthetic GC (48, 94). Our findings may differ from these studies as a result of the methodology used in the protocols. A great deal of studies conducted on the effects of GC in animals have used daily injections to administer GC

treatment (31, 119). The use of needles in these projects poses a severe problem as the injections are a stressor to the animal and so trigger the HPA axis (34). Moreover, the synthetic GC used in these studies do not provide a true physiological paradigm as both dexamethasone and hydrocortisone (synthetic cortisol) are not the main GC produced in a rodent. Based on our data, increases in basal CORT levels do not appear to impact skeletal muscle fiber type size.

Skeletal Muscle Lipase Expression

HSL and ATGL content were investigated in the mixed gastrocnemius muscle (Figure 11a and b). Neither exercise nor glucocorticoid treatment had an effect on HSL content in this muscle. These findings are similar to those reported in both rodent (soleus and extensor digitorum longus muscles) and human studies (vastus lateralis muscle) (44, 65). Interestingly, while the expression of HSL was not affected by exercise, Langfort et al. reported that the activity of the enzyme is increased by muscular contractions (87). Similarly, non significant findings were observed from ATGL western blots but no research currently exists that has studied ATGL expression in skeletal muscle in the face of exercise training. Given its recent discovery, the expression of ATGL has also never been studied in the presence of chronic glucocorticoid treatment.

Skeletal Muscle GR and 11-βHSD1 Expression

Although chronic elevations in the levels of GC did not affect the expression of the GR c isoform, exogenous CORT promoted an increase in GR a/b isoforms in skeletal muscle (Figure 9a). These findings support the idea that differences exist in the expression of these newly discovered isoforms. The difference in GR isoform content in

various tissues may help to explain the multifaceted role that GC play in the body, although this has only been recently speculated (99). Unlike previous findings (29) exercise training (Exer-SHAM and Exer-CORT) did not increase the GR content in skeletal muscle. This discrepancy may be attributed to the animal model utilized. Coutinho et al. (29) made use of hamsters with running distances much greater than the distances ran by our Sprague Dawley rats (Week 4, 20300±800 m/night vs Week 6, (Exer-SHAM 6568±787 m/night; Exer-CORT 6134±1203 m/night) respectively) suggesting that higher endurance training may be required to bring about a reduction of GR content in skeletal muscle.

The rise in GR a/b and 11-βHSD1 expression seen in CORT groups (Figure 10) provide evidence for a feed forward response in the presence of high systemic GC. Indeed, some researchers have suggested that the pathogenesis of the metabolic syndrome may be traced to the elevated GR content seen in these patients' muscle cells (168). However, not all aspects of the metabolic syndrome were present in this model; we did not observe any changes in the glucose tolerance of animals treated with GC as has been observed in other studies (16, 17, 100).

Perhaps the main difference between our study and those mentioned above is due to the use of dexamethasone in the aforementioned experiments while in the present study, corticosterone was utilized. Dexamethasone is a synthetic glucocorticoid that has a much higher (50 times greater) binding affinity to GR than corticosterone (58). Together these findings suggest that a protocol lasting longer than 6 weeks or higher dosages of corticosterone may be required to replicate all observations seen in dexamethasone

animal models and metabolic syndrome patients. In order to attain greater concentrations of GC and sustain these levels in the circulation it is recommended that future studies provide a second surgery on the third week of treatment to replace the partially dissolved CORT pellets.

Finally, Table 5 depicts a brief outline of the findings. In summary, successfully abolishing the corticosterone diurnal pattern and maintaining chronic elevations in GC for most of the six weeks promotes some modest detrimental effects on peripheral tissues. Specifically, visceral adiposity is increased despite having stunted overall weight gain and growth in these animals. In skeletal muscle, chronic exposure to corticosterone promotes a feed forward effect by increasing the expression of GR a/b and 11-βHSD1 which was unaffected by exercise training. The rise in GR and 11-βHSD1 are characteristic of individuals with the metabolic syndrome and/or those on GC therapy. Despite having normal values in the OGTT, many of the adaptations mentioned above mimic the elevations in cortisol levels and visceral fat accumulation seen in type 2 diabetes and the metabolic syndrome.

Table 5. Brief summary of the findings

	Exercise Effect	Corticosterone Effect
Cytochrome C Oxidase	↑ (trend) in SHAM	No change
Epididymal Fat Relative to Body Weight	↓	↑
Absolute Plantaris	↓	↓
Plantaris Relative to Body Weight	No change	No change
Max Load to Fracture	No change	↓
Left Adrenal Relative to Body Weight	No change	↓
Right Adrenal Relative to Body Weight	No change	↓
Combined Pellet Weight	No change	↓
Area Under the Curve of OGTT	↓	No change
Non-Esterified Free Fatty Acids	↑	No change
Plasma Insulin	↓	No change
Intramuscular Triglycerides	No change	No change
Muscle Fiber Type Area	No change	No change
Muscle Glucocorticoid Receptor a/b	No change	↑
Muscle 11 Beta-Hydroxysteroid Dehydrogenase	No change	↑
Muscle Hormone Sensitive Lipase	No change	No change
Muscle Adipose Triglyceride Lipase	No change	No change
Subcutaneous Fat Hormone Sensitive Lipase	↑(trend)	No change
Subcutaneous Fat Adipose Triglyceride Lipase	No change	No change
Epididymal Fat Hormone Sensitive Lipase	↑	No change
Epididymal Fat Adipose Triglyceride Lipase	↑ (trend)	No change

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SUMMARY AND FUTURE DIRECTIONS 5

In this study we investigated the effects of chronically elevated levels of glucocorticoids on various peripheral tissues in the presence or absence of exercise training. Our first objective was to successfully develop an animal model with elevated levels of corticosterone. Our second objective was to analyze various anthropometric measures and markers of energy metabolism in peripheral tissues. To accomplish these objectives, we made use of young, growing Sprague-Dawley rats that were randomly assigned to one of four groups; sedentary SHAM, sedentary CORT, exercise SHAM and exercise CORT. Exercised animals had 24h access to a running wheel for six weeks and all animals had access to water and food *ad libitum*. Food, running distances and body weight were all measured five days per week while blood was collected at three different time points (08 00, 20 00 and 01 00) at the end of each week for corticosterone measurements.

Analysis of nadir corticosterone levels revealed that CORT treated animals indeed had nearly 3-fold elevations in basal corticosterone concentrations, which would be indicative of a moderate level of chronic stress, including a novel exercise training regimen (47), type 2 diabetes mellitus (21) or mild Cushing's Syndrome (38). This model of elevated GC also mimics what would be observed clinically in patients being treated with exogenous corticosteroids (i.e. prednisone). In this model, elevated CORT levels were accompanied by a decrease in body weight gain, a reduction in food intake yet a paradoxical increase in relative epididymal fat mass. On the other hand, with regular exercise, we observed a normalization in epididymal fat mass and a concomitant increase

in hormone sensitive lipase and adipose triglyceride lipase expression in that tissue. We also found in this model, quite surprisingly, that there was increased expression of skeletal muscle GR isoforms a/b and increased expression of the pre receptor enzyme 11-βHSD1 that activates inactive GC to their active form. Together, these tissue specific adaptations to elevations in circulating CORT suggest that a feed forward mechanism exists to increase tissue GC exposure in skeletal muscle. This rise in GC would be expected to worsen the metabolic condition of CORT treated rodents, as has been recently observed in patients with the metabolic syndrome (168). Despite this feed forward increase in muscle GC exposure, we failed to observe any phenotypical effects associated with CORT exposure, as muscle fiber area, IMTG levels, HSL and ATGL levels were all normal in these animals. In addition, oral glucose tolerance was similar between CORT and SHAM animals suggesting that the CORT treated groups were not insulin resistant. The lack of phenotypical effects on mixed gastrocnemius are noteworthy as it suggests that unlike adipose, muscle is resistant to the potentially deleterious effects of elevated CORT. Future work using larger dosages of exogenous corticosterone treatment and a more detailed examination of skeletal muscle phenotype and contractile characteristics could test the hypothesis that skeletal muscle is somewhat resistant to elevations in GC. It is important to note that we did observe a generalized stunted growth in CORT animals suggesting that regulators of protein synthesis and degradation may be modified with this level of chronic corticosterone treatment.

The rise in epididymal fat content with glucocorticoids has been observed previously (142). It would be interesting to confirm this observation in the mesentery fat

depot as this adipose is in direct contact with the viscera. More importantly, the natural question that arises is if CORT is increasing the number of fat cells and/or the size of adipocytes within the central depot. Hence, future work should look to extract primary adipocytes from this animal model to investigate differences that may exist in this tissue.

Finally, the rise in GC seen in this animal model mirrors characteristics seen in a wide variety of clinical populations including type 2 diabetes mellitus, mild cushing's syndrome and the metabolic syndrome. These findings suggest that tissue specific alterations take place in the presence of elevated GC which may be jeopardizing these patients. More importantly, regular volitional exercise can attenuate some of the negative implications associated with chronic glucocorticoids such as visceral fat deposition. Our findings lend support to the benefits of regular exercise as a therapy for chronic diseases characterized by elevated glucocorticoid content.

REFERENCE LIST

1. **Ahn RS, Lee YJ, Choi JY, Kwon HB, and Chun SI.** Salivary cortisol and DHEA levels in the Korean population: age-related differences, diurnal rhythm, and correlations with serum levels. *Yonsei Med J* 48: 379-388, 2007.

2. **Angeli A, Guglielmi G, Dovio A, Capelli G, de Feo D, Giannini S, Giorgino R, Moro L, and Giustina A.** High prevalence of asymptomatic vertebral fractures in post-menopausal women receiving chronic glucocorticoid therapy: a cross-sectional outpatient study. *Bone* 39: 253-259, 2006.

3. **Anthonsen MW, Ronnstrand L, Wernstedt C, Degerman E, and Holm C.** Identification of novel phosphorylation sites in hormone-sensitive lipase that are phosphorylated in response to isoproterenol and govern activation properties in vitro. *J Biol Chem* 273: 215-221, 1998.

4. **Arlettaz A, Portier H, Lecoq AM, Rieth N, De Ceaurriz J, and Collomp K.** Effects of short-term prednisolone intake during submaximal exercise. *Med Sci Sports Exerc* 39: 1672-1678, 2007.

5. **Arner P.** Human fat cell lipolysis: biochemistry, regulation and clinical role. *Best Pract Res Clin Endocrinol Metab* 19: 471-482, 2005.

6. **Ashby P and Robinson DS.** Effects of insulin, glucocorticoids and adrenaline on the activity of rat adipose-tissue lipoprotein lipids. *Biochem J* 188: 185-192, 1980.

7. **Attaix D, Mosoni L, Dardevet D, Combaret L, Mirand PP, and Grizard J.** Altered responses in skeletal muscle protein turnover during aging in anabolic and catabolic periods. *Int J Biochem Cell Biol* 37: 1962-1973, 2005.

8. **Auclair D, Garrel DR, Chaouki Zerouala A, and Ferland LH.** Activation of the ubiquitin pathway in rat skeletal muscle by catabolic doses of glucocorticoids. *Am J Physiol* 272: C1007-1016, 1997.

9. **Bailey JL, Wang X, and Price SR.** The balance between glucocorticoids and insulin regulates muscle proteolysis via the ubiquitin-proteasome pathway. *Miner Electrolyte Metab* 25: 220-223, 1999.

10. **Blaak EE, van Aggel-Leijssen DP, Wagenmakers AJ, Saris WH, and van Baak MA.** Impaired oxidation of plasma-derived fatty acids in type 2 diabetic subjects during moderate-intensity exercise. *Diabetes* 49: 2102-2107, 2000.

11. **Blaak EE and Wagenmakers AJ.** The fate of [U-(13)C]palmitate extracted by skeletal muscle in subjects with type 2 diabetes and control subjects. *Diabetes* 51: 784-789, 2002.

12. **Bolinder J, Kager L, Ostman J, and Arner P.** Differences at the receptor and postreceptor levels between human omental and subcutaneous adipose tissue in the action of insulin on lipolysis. *Diabetes* 32: 117-123, 1983.

13. **Borer KT, Bestervelt LL, Mannheim M, Brosamer MB, Thompson M, Swamy U, and Piper WN.** Stimulation by voluntary exercise of adrenal glucocorticoid secretion in mature female hamsters. *Physiol Behav* 51: 713-718, 1992.

14. **Bujalska IJ, Gathercole LL, Tomlinson JW, Darimont C, Ermolieff J, Fanjul AN, Rejto PA, and Stewart PM.** A novel selective 11beta-hydroxysteroid dehydrogenase type 1 inhibitor prevents human adipogenesis. *J Endocrinol* 197: 297-307, 2008.

15. **Bujalska IJ, Quinkler M, Tomlinson JW, Montague CT, Smith DM, and Stewart PM.** Expression profiling of 11beta-hydroxysteroid dehydrogenase type-1 and glucocorticoid-target genes in subcutaneous and omental human preadipocytes. *J Mol Endocrinol* 37: 327-340, 2006.
16. **Buren J, Lai YC, Lundgren M, Eriksson JW, and Jensen J.** Insulin action and signalling in fat and muscle from dexamethasone-treated rats. *Arch Biochem Biophys* 474: 91-101, 2008.
17. **Buren J, Liu HX, Jensen J, and Eriksson JW.** Dexamethasone impairs insulin signalling and glucose transport by depletion of insulin receptor substrate-1, phosphatidylinositol 3-kinase and protein kinase B in primary cultured rat adipocytes. *Eur J Endocrinol* 146: 419-429, 2002.
18. **Campbell J, Fediuc S, Riddell MC.** Exercise training increases glucocorticoid receptor and 11-beta dehydroxysteroid dehydrogenase-1 in visceral fat of fructose fed hamsters (Abstract). In: *Diabetes*, 2005, p. A263.
19. **Canalis E, Bilezikian JP, Angeli A, and Giustina A.** Perspectives on glucocorticoid-induced osteoporosis. *Bone* 34: 593-598, 2004.
20. **Carmen GY and Victor SM.** Signalling mechanisms regulating lipolysis. *Cell Signal* 18: 401-408, 2006.
21. **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. *Endocrinology* 143: 1761-1768, 2002.
22. **Charmandari E, Kino T, and Chrousos GP.** Glucocorticoids and their actions: an introduction. *Ann N Y Acad Sci* 1024: 1-8, 2004.
23. **Christ-Crain M, Kola B, Lolli F, Fekete C, Seboek D, Wittmann G, Feltrin D, Igreja SC, Ajodha S, Harvey-White J, Kunos G, Muller B, Pralong F, Aubert G, Arnaldi G, Giacchetti G, Boscaro M, Grossman AB, and Korbonits M.** AMP-activated protein kinase mediates glucocorticoid-induced metabolic changes: a novel mechanism in Cushing's syndrome. *Faseb J* 22: 1672-1683, 2008.
24. **Chrousos GP.** The stress response and immune function: clinical implications. The 1999 Novera H. Spector Lecture. *Ann N Y Acad Sci* 917: 38-67, 2000.
25. **Clifford GM, Londos C, Kraemer FB, Vernon RG, and Yeaman SJ.** Translocation of hormone-sensitive lipase and perilipin upon lipolytic stimulation of rat adipocytes. *J Biol Chem* 275: 5011-5015, 2000.
26. **Combaret L, Taillandier D, Dardevet D, Bechet D, Ralliere C, Claustre A, Grizard J, and Attaix D.** Glucocorticoids regulate mRNA levels for subunits of the 19 S regulatory complex of the 26 S proteasome in fast-twitch skeletal muscles. *Biochem J* 378: 239-246, 2004.
27. **Contarteze RV, Manchado FD, Gobatto CA, and De Mello MA.** Stress biomarkers in rats submitted to swimming and treadmill running exercises. *Comp Biochem Physiol A Mol Integr Physiol*, 2007.
28. **Cournarie F, Azzout-Marniche D, Foretz M, Guichard C, Ferre P, and Foufelle F.** The inhibitory effect of glucose on phosphoenolpyruvate carboxykinase gene

expression in cultured hepatocytes is transcriptional and requires glucose metabolism. *FEBS Lett* 460: 527-532, 1999.

29. **Coutinho AE, Campbell JE, Fediuc S, and Riddell MC.** Effect of voluntary exercise on peripheral tissue glucocorticoid receptor content and the expression and activity of 11beta-HSD1 in the Syrian hamster. *J Appl Physiol* 100: 1483-1488, 2006.

30. **Cryer PE, Davis SN, and Shamoon H.** Hypoglycemia in diabetes. *Diabetes Care* 26: 1902-1912, 2003.

31. **Czerwinski SM, Kurowski TG, O'Neill TM, and Hickson RC.** Initiating regular exercise protects against muscle atrophy from glucocorticoids. *J Appl Physiol* 63: 1504-1510, 1987.

32. **Dal-Zotto S, Marti O, and Armario A.** Influence of single or repeated experience of rats with forced swimming on behavioural and physiological responses to the stressor. *Behav Brain Res* 114: 175-181, 2000.

33. **Dalle Carbonare L, Arlot ME, Chavassieux PM, Roux JP, Portero NR, and Meunier PJ.** Comparison of trabecular bone microarchitecture and remodeling in glucocorticoid-induced and postmenopausal osteoporosis. *J Bone Miner Res* 16: 97-103, 2001.

34. **Dalm S, Brinks V, van der Mark MH, de Kloet ER, and Oitzl MS.** Non-invasive stress-free application of glucocorticoid ligands in mice. *J Neurosci Methods* 170: 77-84, 2008.

35. **Desler MM, Jones SJ, Smith CW, and Woods TL.** Effects of dexamethasone and anabolic agents on proliferation and protein synthesis and degradation in C2C12 myogenic cells. *J Anim Sci* 74: 1265-1273, 1996.

36. **Dimitriadis G, Leighton B, Parry-Billings M, Sasson S, Young M, Krause U, Bevan S, Piva T, Wegener G, and Newsholme EA.** Effects of glucocorticoid excess on the sensitivity of glucose transport and metabolism to insulin in rat skeletal muscle. *Biochem J* 321 (Pt 3): 707-712, 1997.

37. **Dishman RK, Bunnell BN, Youngstedt SD, Yoo HS, Mougey EH, and Meyerhoff JL.** Activity wheel running blunts increased plasma adrenocorticotrophin (ACTH) after footshock and cage-switch stress. *Physiol Behav* 63: 911-917, 1998.

38. **Doi M, Sekizawa N, Tani Y, Tsuchiya K, Kouyama R, Tateno T, Izumiyama H, Yoshimoto T, and Hirata Y.** Late-night salivary cortisol as a screening test for the diagnosis of Cushing's syndrome in Japan. *Endocr J* 55: 121-126, 2008.

39. **Droste SK, Gesing A, Ulbricht S, Muller MB, Linthorst AC, and Reul JM.** Effects of long-term voluntary exercise on the mouse hypothalamic-pituitary-adrenocortical axis. *Endocrinology* 144: 3012-3023, 2003.

40. **Duclos M, Corcuff JB, Arsac L, Moreau-Gaudry F, Rashedi M, Roger P, Tabarin A, and Manier G.** Corticotroph axis sensitivity after exercise in endurance-trained athletes. *Clin Endocrinol (Oxf)* 48: 493-501, 1998.

41. **Duclos M, Corcuff JB, Rashedi M, Fougere V, and Manier G.** Trained versus untrained men: different immediate post-exercise responses of pituitary adrenal axis. A preliminary study. *Eur J Appl Physiol Occup Physiol* 75: 343-350, 1997.

42. **Duclos M, Gouarne C, Martin C, Rocher C, Mormede P, and Letellier T.** Effects of corticosterone on muscle mitochondria identifying different sensitivity to

glucocorticoids in Lewis and Fischer rats. *Am J Physiol Endocrinol Metab* 286: E159-167, 2004.
43. **Duncan RE, Ahmadian M, Jaworski K, Sarkadi-Nagy E, and Sul HS.** Regulation of lipolysis in adipocytes. *Annu Rev Nutr* 27: 79-101, 2007.
44. **Enevoldsen LH, Stallknecht B, Langfort J, Petersen LN, Holm C, Ploug T, and Galbo H.** The effect of exercise training on hormone-sensitive lipase in rat intra-abdominal adipose tissue and muscle. *J Physiol* 536: 871-877, 2001.
45. **Espindola-Antunes D and Kater CE.** Adipose tissue expression of 11beta-hydroxysteroid dehydrogenase type 1 in Cushing's syndrome and in obesity. *Arq Bras Endocrinol Metabol* 51: 1397-1403, 2007.
46. **Fain JN and Garcija-Sainz JA.** Adrenergic regulation of adipocyte metabolism. *J Lipid Res* 24: 945-966, 1983.
47. **Fediuc S, Campbell JE, and Riddell MC.** Effect of voluntary wheel running on circadian corticosterone release and on HPA axis responsiveness to restraint stress in Sprague-Dawley rats. *J Appl Physiol* 100: 1867-1875, 2006.
48. **Fimbel S, Abdelmalki A, Mayet MH, Sempore B, Koubi H, Pugeat M, Dechaud H, and Favier RJ.** Exercise training fails to prevent glucocorticoid-induced muscle alterations in young growing rats. *Pflugers Arch* 424: 369-376, 1993.
49. **French DN, Kraemer WJ, Volek JS, Spiering BA, Judelson DA, Hoffman JR, and Maresh CM.** Anticipatory responses of catecholamines on muscle force production. *J Appl Physiol* 102: 94-102, 2007.
50. **Furll M and Jackel F.** [Effects of glucocorticoids on parameters of lipid metabolism, hepatic metabolism, haematological parameters and milk yield in high-yielding cows in early lactation]. *Berl Munch Tierarztl Wochenschr* 118: 247-254, 2005.
51. **Garcia-Prieto MD, Tebar FJ, Nicolas F, Larque E, Zamora S, and Garaulet M.** Cortisol secretary pattern and glucocorticoid feedback sensitivity in women from a Mediterranean area: relationship with anthropometric characteristics, dietary intake and plasma fatty acid profile. *Clin Endocrinol (Oxf)* 66: 185-191, 2007.
52. **Garcia A, Marti O, Valles A, Dal-Zotto S, and Armario A.** Recovery of the hypothalamic-pituitary-adrenal response to stress. Effect of stress intensity, stress duration and previous stress exposure. *Neuroendocrinology* 72: 114-125, 2000.
53. **Garrel DR, Delmas PD, Welsh C, Arnaud MJ, Hamilton SE, and Pugeat MM.** Effects of moderate physical training on prednisone-induced protein wasting: a study of whole-body and bone protein metabolism. *Metabolism* 37: 257-262, 1988.
54. **Girard I and Garland T, Jr.** Plasma corticosterone response to acute and chronic voluntary exercise in female house mice. *J Appl Physiol* 92: 1553-1561, 2002.
55. **Goedecke JH, Wake DJ, Levitt NS, Lambert EV, Collins MR, Morton NM, Andrew R, Seckl JR, and Walker BR.** Glucocorticoid metabolism within superficial subcutaneous rather than visceral adipose tissue is associated with features of the metabolic syndrome in South African women. *Clin Endocrinol (Oxf)* 65: 81-87, 2006.
56. **Goodpaster BH, He J, Watkins S, and Kelley DE.** Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *J Clin Endocrinol Metab* 86: 5755-5761, 2001.

57. **Gordon JW, Rungi AA, Inagaki H, and Hood DA.** Effects of contractile activity on mitochondrial transcription factor A expression in skeletal muscle. *J Appl Physiol* 90: 389-396, 2001.
58. **Gounarides JS, Korach-Andre M, Killary K, Argentieri G, Turner O, and Laurent D.** Effect of dexamethasone on glucose tolerance and fat metabolism in a diet-induced obesity mouse model. *Endocrinology* 149: 758-766, 2008.
59. **Gravholt CH, Dall R, Christiansen JS, Moller N, and Schmitz O.** Preferential stimulation of abdominal subcutaneous lipolysis after prednisolone exposure in humans. *Obes Res* 10: 774-781, 2002.
60. **Grazzi L, Salmaggi A, Dufour A, Gritti A, Lazzaroni M, Bussone G, Nespolo A, and Parati E.** Short and medium-term influence of physical activity on immune parameters. *Int J Neurosci* 71: 267-276, 1993.
61. **Gregoire F, Genart C, Hauser N, and Remacle C.** Glucocorticoids induce a drastic inhibition of proliferation and stimulate differentiation of adult rat fat cell precursors. *Exp Cell Res* 196: 270-278, 1991.
62. **Haemmerle G, Lass A, Zimmermann R, Gorkiewicz G, Meyer C, Rozman J, Heldmaier G, Maier R, Theussl C, Eder S, Kratky D, Wagner EF, Klingenspor M, Hoefler G, and Zechner R.** Defective lipolysis and altered energy metabolism in mice lacking adipose triglyceride lipase. *Science* 312: 734-737, 2006.
63. **Haemmerle G, Zimmermann R, Hayn M, Theussl C, Waeg G, Wagner E, Sattler W, Magin TM, Wagner EF, and Zechner R.** Hormone-sensitive lipase deficiency in mice causes diglyceride accumulation in adipose tissue, muscle, and testis. *J Biol Chem* 277: 4806-4815, 2002.
64. **Hammond GL.** Molecular properties of corticosteroid binding globulin and the sex-steroid binding proteins. *Endocr Rev* 11: 65-79, 1990.
65. **Helge JW, Biba TO, Galbo H, Gaster M, and Donsmark M.** Muscle triacylglycerol and hormone-sensitive lipase activity in untrained and trained human muscles. *Eur J Appl Physiol* 97: 566-572, 2006.
66. **Hellmer J, Marcus C, Sonnenfeld T, and Arner P.** Mechanisms for differences in lipolysis between human subcutaneous and omental fat cells. *J Clin Endocrinol Metab* 75: 15-20, 1992.
67. **Herman JP, Adams D, and Prewitt C.** Regulatory changes in neuroendocrine stress-integrative circuitry produced by a variable stress paradigm. *Neuroendocrinology* 61: 180-190, 1995.
68. **Hillier SG.** Diamonds are forever: the cortisone legacy. *J Endocrinol* 195: 1-6, 2007.
69. **Hood DA and Saleem A.** Exercise-induced mitochondrial biogenesis in skeletal muscle. *Nutr Metab Cardiovasc Dis* 17: 332-337, 2007.
70. **Hoppeler H, Howald H, Conley K, Lindstedt SL, Claassen H, Vock P, and Weibel ER.** Endurance training in humans: aerobic capacity and structure of skeletal muscle. *J Appl Physiol* 59: 320-327, 1985.
71. **Horber FF, Marsh HM, and Haymond MW.** Differential effects of prednisone and growth hormone on fuel metabolism and insulin antagonism in humans. *Diabetes* 40: 141-149, 1991.

72. **Hucklebridge F, Hussain T, Evans P, and Clow A.** The diurnal patterns of the adrenal steroids cortisol and dehydroepiandrosterone (DHEA) in relation to awakening. *Psychoneuroendocrinology* 30: 51-57, 2005.
73. **Hurley BF, Nemeth PM, Martin WH, 3rd, Hagberg JM, Dalsky GP, and Holloszy JO.** Muscle triglyceride utilization during exercise: effect of training. *J Appl Physiol* 60: 562-567, 1986.
74. **Iannoli P, Miller JH, Ryan CK, and Sax HC.** Glucocorticoids upregulate intestinal nutrient transport in a time-dependent and substrate-specific fashion. *J Gastrointest Surg* 2: 449-457, 1998.
75. **Iniguez-Lluhi JA and Pearce D.** A common motif within the negative regulatory regions of multiple factors inhibits their transcriptional synergy. *Mol Cell Biol* 20: 6040-6050, 2000.
76. **Jin JY, DuBois DC, Almon RR, and Jusko WJ.** Receptor/gene-mediated pharmacodynamic effects of methylprednisolone on phosphoenolpyruvate carboxykinase regulation in rat liver. *J Pharmacol Exp Ther* 309: 328-339, 2004.
77. **Joyner JM, Hutley LJ, and Cameron DP.** Glucocorticoid receptors in human preadipocytes: regional and gender differences. *J Endocrinol* 166: 145-152, 2000.
78. **Karin M.** New twists in gene regulation by glucocorticoid receptor: is DNA binding dispensable? *Cell* 93: 487-490, 1998.
79. **Kelliher P, Connor TJ, Harkin A, Sanchez C, Kelly JP, and Leonard BE.** Varying responses to the rat forced-swim test under diurnal and nocturnal conditions. *Physiol Behav* 69: 531-539, 2000.
80. **Kendall EC.** Hormone of the adrenal cortex. *Bull N Y Acad Med* 29: 91-100, 1953.
81. **Kershaw EE, Morton NM, Dhillon H, Ramage L, Seckl JR, and Flier JS.** Adipocyte-specific glucocorticoid inactivation protects against diet-induced obesity. *Diabetes* 54: 1023-1031, 2005.
82. **Kim JY, Hickner RC, Cortright RL, Dohm GL, and Houmard JA.** Lipid oxidation is reduced in obese human skeletal muscle. *Am J Physiol Endocrinol Metab* 279: E1039-1044, 2000.
83. **Kim YW, Kim JY, and Lee SK.** Surgical removal of visceral fat decreases plasma free fatty acid and increases insulin sensitivity on liver and peripheral tissue in monosodium glutamate (MSG)-obese rats. *J Korean Med Sci* 14: 539-545, 1999.
84. **King SR, Ronen-Fuhrmann T, Timberg R, Clark BJ, Orly J, and Stocco DM.** Steroid production after in vitro transcription, translation, and mitochondrial processing of protein products of complementary deoxyribonucleic acid for steroidogenic acute regulatory protein. *Endocrinology* 136: 5165-5176, 1995.
85. **Koopman R, Schaart G, and Hesselink MK.** Optimisation of oil red O staining permits combination with immunofluorescence and automated quantification of lipids. *Histochem Cell Biol* 116: 63-68, 2001.
86. **Korach-Andre M, Gao J, Gounarides JS, Deacon R, Islam A, and Laurent D.** Relationship between visceral adiposity and intramyocellular lipid content in two rat models of insulin resistance. *Am J Physiol Endocrinol Metab* 288: E106-116, 2005.

87. **Langfort J, Ploug T, Ihlemann J, Saldo M, Holm C, and Galbo H.** Expression of hormone-sensitive lipase and its regulation by adrenaline in skeletal muscle. *Biochem J* 340 (Pt 2): 459-465, 1999.
88. **Large V, Peroni O, Letexier D, Ray H, and Beylot M.** Metabolism of lipids in human white adipocyte. *Diabetes Metab* 30: 294-309, 2004.
89. **Larsen PR KH, Melmed S, Polonsky KS.** *Williams textbook of Endocrinology*. Philadelphia: Elsevier Science, 2003.
90. **Lass A, Zimmermann R, Haemmerle G, Riederer M, Schoiswohl G, Schweiger M, Kienesberger P, Strauss JG, Gorkiewicz G, and Zechner R.** Adipose triglyceride lipase-mediated lipolysis of cellular fat stores is activated by CGI-58 and defective in Chanarin-Dorfman Syndrome. *Cell Metab* 3: 309-319, 2006.
91. **Lefevre C, Jobard F, Caux F, Bouadjar B, Karaduman A, Heilig R, Lakhdar H, Wollenberg A, Verret JL, Weissenbach J, Ozguc M, Lathrop M, Prud'homme JF, and Fischer J.** Mutations in CGI-58, the gene encoding a new protein of the esterase/lipase/thioesterase subfamily, in Chanarin-Dorfman syndrome. *Am J Hum Genet* 69: 1002-1012, 2001.
92. **Lesniewska B, Nowak KW, and Malendowicz LK.** Dexamethasone-induced adrenal cortex atrophy and recovery of the gland from partial, steroid-induced atrophy. *Exp Clin Endocrinol* 100: 133-139, 1992.
93. **Li H, He G, Chu H, Zhao L, and Yu H.** A step-wise application of methylprednisolone versus dexamethasone in the treatment of acute exacerbations of COPD. *Respirology* 8: 199-204, 2003.
94. **Livingstone I, Johnson MA, and Mastaglia FL.** Effects of dexamethasone on fibre subtypes in rat muscle. *Neuropathol Appl Neurobiol* 7: 381-398, 1981.
95. **Loeb JN.** Corticosteroids and growth. *N Engl J Med* 295: 547-552, 1976.
96. **Lonn L, Kvist H, Ernest I, and Sjostrom L.** Changes in body composition and adipose tissue distribution after treatment of women with Cushing's syndrome. *Metabolism* 43: 1517-1522, 1994.
97. **Lu NZ and Cidlowski JA.** Glucocorticoid receptor isoforms generate transcription specificity. *Trends Cell Biol* 16: 301-307, 2006.
98. **Lu NZ and Cidlowski JA.** The origin and functions of multiple human glucocorticoid receptor isoforms. *Ann N Y Acad Sci* 1024: 102-123, 2004.
99. **Lu NZ and Cidlowski JA.** Translational regulatory mechanisms generate N-terminal glucocorticoid receptor isoforms with unique transcriptional target genes. *Mol Cell* 18: 331-342, 2005.
100. **Lundgren M, Buren J, Ruge T, Myrnas T, and Eriksson JW.** Glucocorticoids down-regulate glucose uptake capacity and insulin-signaling proteins in omental but not subcutaneous human adipocytes. *J Clin Endocrinol Metab* 89: 2989-2997, 2004.
101. **Marcinkiewicz A, Gauthier D, Garcia A, and Brasaemle DL.** The phosphorylation of serine 492 of perilipin a directs lipid droplet fragmentation and dispersion. *J Biol Chem* 281: 11901-11909, 2006.
102. **Marin MT, Cruz FC, and Planeta CS.** Chronic restraint or variable stresses differently affect the behavior, corticosterone secretion and body weight in rats. *Physiol Behav* 90: 29-35, 2007.

103. **Marinovic AC, Zheng B, Mitch WE, and Price SR.** Tissue-specific regulation of ubiquitin (UbC) transcription by glucocorticoids: in vivo and in vitro analyses. *Am J Physiol Renal Physiol* 292: F660-666, 2007.
104. **Marinovic AC, Zheng B, Mitch WE, and Price SR.** Ubiquitin (UbC) expression in muscle cells is increased by glucocorticoids through a mechanism involving Sp1 and MEK1. *J Biol Chem* 277: 16673-16681, 2002.
105. **Martinez-Botas J, Anderson JB, Tessier D, Lapillonne A, Chang BH, Quast MJ, Gorenstein D, Chen KH, and Chan L.** Absence of perilipin results in leanness and reverses obesity in Lepr(db/db) mice. *Nat Genet* 26: 474-479, 2000.
106. **Matsuo T, Nozaki T, Okamura K, Matsumoto K, Doi T, Gohtani S, and Suzuki M.** Effects of voluntary resistance exercise and high-protein snack on bone mass, composition, and strength in rats given glucocorticoid injections. *Biosci Biotechnol Biochem* 67: 2518-2523, 2003.
107. **Mendel CM.** The free hormone hypothesis: a physiologically based mathematical model. *Endocr Rev* 10: 232-274, 1989.
108. **Meyer JS, Micco DJ, Stephenson BS, Krey LC, and McEwen BS.** Subcutaneous implantation method for chronic glucocorticoid replacement therapy. *Physiol Behav* 22: 867-870, 1979.
109. **Meyer S, Hoppner W, and Seitz HJ.** Transcriptional and post-transcriptional effects of glucose on liver phosphoenolpyruvate-carboxykinase gene expression. *Eur J Biochem* 202: 985-991, 1991.
110. **Miller WL.** Steroidogenic acute regulatory protein (StAR), a novel mitochondrial cholesterol transporter. *Biochim Biophys Acta* 1771: 663-676, 2007.
111. **Mitsui T, Umaki Y, Nagasawa M, Akaike M, Aki K, Azuma H, Ozaki S, Odomi M, and Matsumoto T.** Mitochondrial damage in patients with long-term corticosteroid therapy: development of oculoskeletal symptoms similar to mitochondrial disease. *Acta Neuropathol* 104: 260-266, 2002.
112. **Mondon CE, Dolkas CB, and Reaven GM.** Site of enhanced insulin sensitivity in exercise-trained rats at rest. *Am J Physiol* 239: E169-177, 1980.
113. **Moro C, Bajpeyi S, and Smith SR.** Determinants of intramyocellular triglyceride turnover: implications for insulin sensitivity. *Am J Physiol Endocrinol Metab* 294: E203-213, 2008.
114. **Neeck G.** Fifty years of experience with cortisone therapy in the study and treatment of rheumatoid arthritis. *Ann N Y Acad Sci* 966: 28-38, 2002.
115. **Novelli M, Pocai A, Chiellini C, Maffei M, and Masiello P.** Free fatty acids as mediators of adaptive compensatory responses to insulin resistance in dexamethasone-treated rats. *Diabetes Metab Res Rev* 24: 155-164, 2008.
116. **O'Brien CA, Jia D, Plotkin LI, Bellido T, Powers CC, Stewart SA, Manolagas SC, and Weinstein RS.** Glucocorticoids act directly on osteoblasts and osteocytes to induce their apoptosis and reduce bone formation and strength. *Endocrinology* 145: 1835-1841, 2004.
117. **Ogilvie RW and Feeback DL.** A metachromatic dye-ATPase method for the simultaneous identification of skeletal muscle fiber types I, IIA, IIB and IIC. *Stain Technol* 65: 231-241, 1990.

118. **Ottosson M, Lonnroth P, Bjorntorp P, and Eden S.** Effects of cortisol and growth hormone on lipolysis in human adipose tissue. *J Clin Endocrinol Metab* 85: 799-803, 2000.
119. **Pandya JD, Agarwal NA, and Katyare SS.** Effect of dexamethasone treatment on oxidative energy metabolism in rat liver mitochondria during postnatal developmental periods. *Drug Chem Toxicol* 27: 389-403, 2004.
120. **Park E, Chan O, Li Q, Kiraly M, Matthews SG, Vranic M, and Riddell MC.** Changes in basal hypothalamo-pituitary-adrenal activity during exercise training are centrally mediated. *Am J Physiol Regul Integr Comp Physiol* 289: R1360-1371, 2005.
121. **Patchev VK, Brady LS, Karl M, and Chrousos GP.** Regulation of HSP90 and corticosteroid receptor mRNA by corticosterone levels in vivo. *Mol Cell Endocrinol* 103: 57-64, 1994.
122. **Pedersen SB, Borglum JD, Moller-Pedersen T, and Richelsen B.** Characterization of nuclear corticosteroid receptors in rat adipocytes. Regional variations and modulatory effects of hormones. *Biochim Biophys Acta* 1134: 303-308, 1992.
123. **Pedersen SB, Kristensen K, and Richelsen B.** Anti-glucocorticoid effects of progesterone in vivo on rat adipose tissue metabolism. *Steroids* 68: 543-550, 2003.
124. **Peng TC, Kusy RP, Hirsch PF, and Hagaman JR.** Ethanol-induced changes in morphology and strength of femurs of rats. *Alcohol Clin Exp Res* 12: 655-659, 1988.
125. **Perdrizet GA.** Hans Selye and beyond: responses to stress. *Cell Stress Chaperones* 2: 214-219, 1997.
126. **Pereira RM, Delany AM, and Canalis E.** Cortisol inhibits the differentiation and apoptosis of osteoblasts in culture. *Bone* 28: 484-490, 2001.
127. **Petersen KF and Shulman GI.** Pathogenesis of skeletal muscle insulin resistance in type 2 diabetes mellitus. *Am J Cardiol* 90: 11G-18G, 2002.
128. **Petit F, Bagby GJ, and Lang CH.** Tumor necrosis factor mediates zymosan-induced increase in glucose flux and insulin resistance. *Am J Physiol* 268: E219-228, 1995.
129. **Pilkis SJ and Granner DK.** Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. *Annu Rev Physiol* 54: 885-909, 1992.
130. **Quirke V.** Making British cortisone: Glaxo and the development of corticosteroids in Britain in the 1950s-1960s. *Stud Hist Philos Biol Biomed Sci* 36: 645-674, 2005.
131. **Rakhshani N.** Effects of short- and long-term voluntary exercise training on the stress response and adrenal sensitivity in male sprague dawley rats (submitted 2007).
132. **Rannels SR, Rannels DE, Pegg AE, and Jefferson LS.** Glucocorticoid effects on peptide-chain initiation in skeletal muscle and heart. *Am J Physiol* 235: E134-139, 1978.
133. **Rebuffe-Scrive M, Krotkiewski M, Elfverson J, and Bjorntorp P.** Muscle and adipose tissue morphology and metabolism in Cushing's syndrome. *J Clin Endocrinol Metab* 67: 1122-1128, 1988.
134. **Rebuffe-Scrive M, Walsh UA, McEwen B, and Rodin J.** Effect of chronic stress and exogenous glucocorticoids on regional fat distribution and metabolism. *Physiol Behav* 52: 583-590, 1992.

135. **Rodnick KJ, Haskell WL, Swislocki AL, Foley JE, and Reaven GM.** Improved insulin action in muscle, liver, and adipose tissue in physically trained human subjects. *Am J Physiol* 253: E489-495, 1987.
136. **Rosol TJ, Yarrington JT, Latendresse J, and Capen CC.** Adrenal gland: structure, function, and mechanisms of toxicity. *Toxicol Pathol* 29: 41-48, 2001.
137. **Roth PIaW.** *Core Concepts in Health.* United States of America: The McGraw-Hill Companies, 2002.
138. **Roussel D, Dumas JF, Simard G, Malthiery Y, and Ritz P.** Kinetics and control of oxidative phosphorylation in rat liver mitochondria after dexamethasone treatment. *Biochem J* 382: 491-499, 2004.
139. **Rubin J, Biskobing DM, Jadhav L, Fan D, Nanes MS, Perkins S, and Fan X.** Dexamethasone promotes expression of membrane-bound macrophage colony-stimulating factor in murine osteoblast-like cells. *Endocrinology* 139: 1006-1012, 1998.
140. **Russell ST and Tisdale MJ.** The role of glucocorticoids in the induction of zinc-alpha2-glycoprotein expression in adipose tissue in cancer cachexia. *Br J Cancer* 92: 876-881, 2005.
141. **Saad MJ, Folli F, Kahn JA, and Kahn CR.** Modulation of insulin receptor, insulin receptor substrate-1, and phosphatidylinositol 3-kinase in liver and muscle of dexamethasone-treated rats. *J Clin Invest* 92: 2065-2072, 1993.
142. **Santana P, Akana SF, Hanson ES, Strack AM, Sebastian RJ, and Dallman MF.** Aldosterone and dexamethasone both stimulate energy acquisition whereas only the glucocorticoid alters energy storage. *Endocrinology* 136: 2214-2222, 1995.
143. **Schlechte JA and Hamilton D.** The effect of glucocorticoids on corticosteroid binding globulin. *Clin Endocrinol (Oxf)* 27: 197-203, 1987.
144. **Selye H.** Stress and the general adaptation syndrome. *Br Med J* 1: 1383-1392, 1950.
145. **Sen Y, Aygun D, Yilmaz E, and Ayar A.** Children and adolescents with obesity and the metabolic syndrome have high circulating cortisol levels. *Neuro Endocrinol Lett* 29: 141-145, 2008.
146. **Silvestrini G, Ballanti P, Patacchioli FR, Mocetti P, Di Grezia R, Wedard BM, Angelucci L, and Bonucci E.** Evaluation of apoptosis and the glucocorticoid receptor in the cartilage growth plate and metaphyseal bone cells of rats after high-dose treatment with corticosterone. *Bone* 26: 33-42, 2000.
147. **Sjogren J, Weck M, Nilsson A, Ottosson M, and Bjorntorp P.** Glucocorticoid hormone binding to rat adipocytes. *Biochim Biophys Acta* 1224: 17-21, 1994.
148. **Slavin BG, Ong JM, and Kern PA.** Hormonal regulation of hormone-sensitive lipase activity and mRNA levels in isolated rat adipocytes. *J Lipid Res* 35: 1535-1541, 1994.
149. **Sztalryd C, Xu G, Dorward H, Tansey JT, Contreras JA, Kimmel AR, and Londos C.** Perilipin A is essential for the translocation of hormone-sensitive lipase during lipolytic activation. *J Cell Biol* 161: 1093-1103, 2003.
150. **Taillandier D, Combaret L, Pouch MN, Samuels SE, Bechet D, and Attaix D.** The role of ubiquitin-proteasome-dependent proteolysis in the remodelling of skeletal muscle. *Proc Nutr Soc* 63: 357-361, 2004.

151. **Tansey JT, Sztalryd C, Gruia-Gray J, Roush DL, Zee JV, Gavrilova O, Reitman ML, Deng CX, Li C, Kimmel AR, and Londos C.** Perilipin ablation results in a lean mouse with aberrant adipocyte lipolysis, enhanced leptin production, and resistance to diet-induced obesity. *Proc Natl Acad Sci U S A* 98: 6494-6499, 2001.
152. **Tarnopolsky MA, Rennie CD, Robertshaw HA, Fedak-Tarnopolsky SN, Devries MC, and Hamadeh MJ.** Influence of endurance exercise training and sex on intramyocellular lipid and mitochondrial ultrastructure, substrate use, and mitochondrial enzyme activity. *Am J Physiol Regul Integr Comp Physiol* 292: R1271-1278, 2007.
153. **Tharp GD.** The role of glucocorticoids in exercise. *Med Sci Sports* 7: 6-11, 1975.
154. **Tontonoz P, Graves RA, Budavari AI, Erdjument-Bromage H, Lui M, Hu E, Tempst P, and Spiegelman BM.** Adipocyte-specific transcription factor ARF6 is a heterodimeric complex of two nuclear hormone receptors, PPAR gamma and RXR alpha. *Nucleic Acids Res* 22: 5628-5634, 1994.
155. **Tulipano G, Taylor JE, Halem HA, Datta R, Dong JZ, Culler MD, Bianchi I, Cocchi D, and Giustina A.** Glucocorticoid inhibition of growth in rats: partial reversal with the full-length ghrelin analog BIM-28125. *Pituitary* 10: 267-274, 2007.
156. **Turner LW, Leaver-Dunn D, Dibrezzo R, and Fort I.** Physical Activity and Osteoporotic Fracture Among Older Women. *J Athl Train* 33: 207-210, 1998.
157. **Tyni-Lenne R, Gordon A, Jansson E, Bermann G, and Sylven C.** Skeletal muscle endurance training improves peripheral oxidative capacity, exercise tolerance, and health-related quality of life in women with chronic congestive heart failure secondary to either ischemic cardiomyopathy or idiopathic dilated cardiomyopathy. *Am J Cardiol* 80: 1025-1029, 1997.
158. **van Loon LJ and Goodpaster BH.** Increased intramuscular lipid storage in the insulin-resistant and endurance-trained state. *Pflugers Arch* 451: 606-616, 2006.
159. **Viana AY, Sakoda H, Anai M, Fujishiro M, Ono H, Kushiyama A, Fukushima Y, Sato Y, Oshida Y, Uchijima Y, Kurihara H, and Asano T.** Role of hepatic AMPK activation in glucose metabolism and dexamethasone-induced regulation of AMPK expression. *Diabetes Res Clin Pract* 73: 135-142, 2006.
160. **Vidal-Puig AJ, Considine RV, Jimenez-Linan M, Werman A, Pories WJ, Caro JF, and Flier JS.** Peroxisome proliferator-activated receptor gene expression in human tissues. Effects of obesity, weight loss, and regulation by insulin and glucocorticoids. *J Clin Invest* 99: 2416-2422, 1997.
161. **Villena JA, Roy S, Sarkadi-Nagy E, Kim KH, and Sul HS.** Desnutrin, an adipocyte gene encoding a novel patatin domain-containing protein, is induced by fasting and glucocorticoids: ectopic expression of desnutrin increases triglyceride hydrolysis. *J Biol Chem* 279: 47066-47075, 2004.
162. **Virkamaki A, Korsheninnikova E, Seppala-Lindroos A, Vehkavaara S, Goto T, Halavaara J, Hakkinen AM, and Yki-Jarvinen H.** Intramyocellular lipid is associated with resistance to in vivo insulin actions on glucose uptake, antilipolysis, and early insulin signaling pathways in human skeletal muscle. *Diabetes* 50: 2337-2343, 2001.

163. **Watt MJ, van Denderen BJ, Castelli LA, Bruce CR, Hoy AJ, Kraegen EW, Macaulay L, and Kemp BE.** Adipose triglyceride lipase regulation of skeletal muscle lipid metabolism and insulin responsiveness. *Mol Endocrinol* 22: 1200-1212, 2008.
164. **Wei L, MacDonald TM, and Walker BR.** Taking glucocorticoids by prescription is associated with subsequent cardiovascular disease. *Ann Intern Med* 141: 764-770, 2004.
165. **Weinstein RS, Jilka RL, Parfitt AM, and Manolagas SC.** Inhibition of osteoblastogenesis and promotion of apoptosis of osteoblasts and osteocytes by glucocorticoids. Potential mechanisms of their deleterious effects on bone. *J Clin Invest* 102: 274-282, 1998.
166. **Weiss EP and Holloszy JO.** Improvements in body composition, glucose tolerance, and insulin action induced by increasing energy expenditure or decreasing energy intake. *J Nutr* 137: 1087-1090, 2007.
167. **Weissmann G.** The experimental pathology of stress: Hans Selye to Paris Hilton. *Faseb J* 21: 2635-2638, 2007.
168. **Whorwood CB, Donovan SJ, Flanagan D, Phillips DI, and Byrne CD.** Increased glucocorticoid receptor expression in human skeletal muscle cells may contribute to the pathogenesis of the metabolic syndrome. *Diabetes* 51: 1066-1075, 2002.
169. **Windle RJ, Wood SA, Lightman SL, and Ingram CD.** The pulsatile characteristics of hypothalamo-pituitary-adrenal activity in female Lewis and Fischer 344 rats and its relationship to differential stress responses. *Endocrinology* 139: 4044-4052, 1998.
170. **Wittert GA, Livesey JH, Espiner EA, and Donald RA.** Adaptation of the hypothalamopituitary adrenal axis to chronic exercise stress in humans. *Med Sci Sports Exerc* 28: 1015-1019, 1996.
171. **Xu XF and Bjorntorp P.** Effects of dexamethasone on multiplication and differentiation of rat adipose precursor cells. *Exp Cell Res* 189: 247-252, 1990.
172. **Yago N and Ichii S.** Submitochondrial distribution of components of the steroid 11 beta-hydroxylase and cholesterol sidechain-cleaving enzyme systems in hog adrenal cortex. *J Biochem* 65: 215-224, 1969.
173. **Yamaguchi T, Omatsu N, Matsushita S, and Osumi T.** CGI-58 interacts with perilipin and is localized to lipid droplets. Possible involvement of CGI-58 mislocalization in Chanarin-Dorfman syndrome. *J Biol Chem* 279: 30490-30497, 2004.
174. **Zachwieja JJ, Hendry SL, Smith SR, and Harris RB.** Voluntary wheel running decreases adipose tissue mass and expression of leptin mRNA in Osborne-Mendel rats. *Diabetes* 46: 1159-1166, 1997.
175. **Zakrzewska KE, Cusin I, Stricker-Krongrad A, Boss O, Ricquier D, Jeanrenaud B, and Rohner-Jeanrenaud F.** Induction of obesity and hyperleptinemia by central glucocorticoid infusion in the rat. *Diabetes* 48: 365-370, 1999.
176. **Zimmermann R, Haemmerle G, Wagner EM, Strauss JG, Kratky D, and Zechner R.** Decreased fatty acid esterification compensates for the reduced lipolytic activity in hormone-sensitive lipase-deficient white adipose tissue. *J Lipid Res* 44: 2089-2099, 2003.

Appendices

Running Distance (m)

Exercise SHAM	week 1	week 2	week 3	week 4	week 5	week 6
E1	5881	16787	6852	8772	6407	5705
E2	6623	8539	8527	5286	5564	6625
E3	5477	10338	8216	5765	4607	9842
E4	5475	9368	9129	10678	7261	7029
E5	1794	3933	3596	5037	6973	3451
E6	7635	10496	5845	7327	3263	6817
E7	2243	4828	4563	6236	3015	2749
E8	589	1815	7483	8663	10627	9299
E9	6370	14513	7716	6307	2994	7593
MEAN	4676	8957	6881	7119	5635	6568
SEM	826	1625	622	632	837	787

Exercise CORT	week 1	week 2	week 3	week 4	week 5	week 6
EC1	8961	6081	2903	7765	7170	12597
EC2	9273	10159	2741	4420	4814	2860
EC3	12671	5539	6567	5521	7019	3713
EC4	7894	8369	4913	4880	6284	9609
EC6	1186	5268	2487	2680	3182	6293
EC7	7240	4777	1279	3674	3101	4142
EC8	2977	2300	1241	1363	1389	1933
EC9	4930	4395	4109	3523	3950	4736
EC10	8924	5878	8150	8356	7977	9323
MEAN	7117	5863	3821	4687	4987	6134
SEM	1178	757	786	756	749	1203

Cytochrome C Oxidase

Sedentary SHAM		Sedentary CORT		Exercise SHAM		Exercise CORT	
S1	1.90	SC1	0.91	E1	6.72	EC1	3.06
S2	4.27	SC2	5.72	E2	1.02	EC2	1.99
S3	4.54	SC3	3.54	E3	4.76	EC3	1.38
S4	2.70	SC4	2.85	E4	6.51	EC4	1.48
S6	~	SC5	2.44	E5	4.41	EC6	4.91
S7	2.16	SC6	6.14	E6	2.49	EC8	3.05
S8	2.33	SC7	3.14	E7	3.86	EC9	2.57
S9	0.86	SC8	2.31	E8	3.09	EC10	5.34
		SC9	0.95	E9	5.34		
		SC10	1.95				
MEAN	2.68		2.99		4.24		2.97
SEM	0.50		0.56		0.62		0.52

Epididymal Fat Relative to Total Body Weight (mg/g b.w.)

Sedentary SHAM		Sedentary CORT		Exercise SHAM		Exercise CORT	
S1	8.55	SC1	14.56	E1	6.55	EC1	20.60
S2	12.23	SC2	24.55	E2	6.90	EC2	13.65
S3	12.26	SC3	~	E3	5.23	EC3	6.81
S4	16.70	SC4	12.68	E4	5.55	EC4	9.62
S6	15.61	SC5	15.72	E5	14.84	EC6	9.25
S7	15.42	SC6	~	E6	8.11	EC7	13.58
S8	16.60	SC7	18.13	E7	17.50	EC8	14.55
S9	13.31	SC8	18.27	E8	4.54	EC9	14.75
		SC9	13.30	E9	8.67	EC10	4.89
		SC10	18.53				
MEAN	13.84		16.97		8.65		11.97
SEM	0.99		1.35		1.50		1.60

Absolute Plantaris Weight (mg)

Sedentary SHAM		Sedentary CORT		Exercise SHAM		Exercise CORT	
S1	461.3	SC1	385.9	E1	425.2	EC1	203.7
S2	399.0	SC2	246.2	E2	390.0	EC2	310.8
S3	429.5	SC3	213.7	E3	359.7	EC3	295.8
S4	282.8	SC4	358.7	E4	284.0	EC4	302.0
S6	450.4	SC5	410.8	E5	433.9	EC6	342.3
S7	401.2	SC6	435.6	E6	371.2	EC7	268.4
S8	515.8	SC7	449.5	E7	437.9	EC8	444.6
S9	428.7	SC8	443.9	E8	389.5	EC9	387.1
		SC9	404.1	E9	390.7	EC10	345.9
		SC10	458.5				
MEAN	421.1		380.7		386.9		322.3
SEM	23.7		27.0		15.8		23.1

Plantaris Weight Relative to Total Body Weight (mg/g b.w.)

Sedentary SHAM		Sedentary CORT		Exercise SHAM		Exercise CORT	
S1	1.104	SC1	1.078	E1	0.998	EC1	0.792
S2	0.883	SC2	0.851	E2	1.019	EC2	0.897
S3	0.887	SC3	0.857	E3	0.895	EC3	0.790
S4	0.594	SC4	0.916	E4	0.691	EC4	0.940
S6	1.006	SC5	1.029	E5	0.929	EC6	1.021
S7	0.871	SC6	1.077	E6	0.835	EC7	0.889
S8	0.960	SC7	0.945	E7	0.908	EC8	0.839
S9	0.931	SC8	0.902	E8	0.965	EC9	0.953
		SC9	0.863	E9	0.901	EC10	1.008
		SC10	0.920				
MEAN	0.905		0.944		0.905		0.903
SEM	0.052		0.028		0.033		0.028

Max Load to Fracture Femur (N)

Sedentary SHAM		Sedentary CORT		Exercise SHAM		Exercise CORT	
S1	140.4	SC1	117.9	E1	127.3	EC1	118.7
S2	134.4	SC2	89.9	E2	122.4	EC2	101.1
S3	135.6	SC3	87.2	E3	120.1	EC3	121.7
S4	137.5	SC4	125.8	E4	113.4	EC4	98.0
S6	129.7	SC5	129.9	E5	137.7	EC6	94.8
S7	138.4	SC6	129.7	E6	157.4	EC7	93.6
S8	152.5	SC7	115.6	E7	163.3	EC8	144.1
S9	153.0	SC8	132.4	E8	132.1	EC9	125.2
		SC9	116.6	E9	133.8	EC10	88.4
		SC10	127.6				
MEAN	140.2		117.3		134.2		109.5
SEM	3.0		4.9		5.6		6.2

Left Adrenal Relative to Total Body Weight (mg/g b.w.)

Sedentary SHAM		Sedentary CORT		Exercise SHAM		Exercise CORT	
S1	0.0824	SC1	0.0615	E1	0.0770	EC1	0.0303
S2	0.0449	SC2	0.0263	E2	0.0888	EC2	0.0193
S3	0.0808	SC3	0.0341	E3	0.0802	EC3	0.0363
S4	0.0929	SC4	0.0460	E4	0.0849	EC4	0.0290
S6	0.0484	SC5	0.0301	E5	0.0942	EC6	0.0639
S7	0.0906	SC6	0.0505	E6	0.0828	EC7	0.0526
S8	0.0724	SC7	0.0525	E7	0.0616	EC8	0.0681
S9	0.1077	SC8	0.0577	E8	0.1264	EC9	0.0531
		SC9	0.0843	E9	0.0821	EC10	0.0807
		SC10	0.0686				
MEAN	0.0775		0.0511		0.0864		0.0482
SEM	0.0077		0.0057		0.0058		0.0069

Right Adrenal Relative to Total Body Weight (mg/g b.w.)

Sedentary SHAM		Sedentary CORT		Exercise SHAM		Exercise CORT	
S1	0.0898	SC1	0.0699	E1	0.0704	EC1	0.0264
S2	0.0631	SC2	0.0304	E2	0.0784	EC2	0.0335
S3	0.0357	SC3	0.0321	E3	0.0732	EC3	0.0366
S4	0.0889	SC4	0.0595	E4	0.0681	EC4	0.0455
S6	0.0708	SC5	0.0180	E5	0.0955	EC6	0.0594
S7	0.1010	SC6	0.0425	E6	0.0839	EC7	0.0560
S8	0.0642	SC7	~	E7	0.0633	EC8	0.0378
S9	0.0858	SC8	0.0425	E8	0.0800	EC9	0.0261
		SC9	0.0561	E9	0.0780	EC10	0.0670
		SC10	0.0439				
MEAN	0.0749		0.0439		0.0768		0.0431
SEM	0.0073		0.0053		0.0032		0.0049

Combined Pellet Weight (mg)

Sedentary SHAM		Sedentary CORT		Exercise SHAM		Exercise CORT	
S1	298.9	SC1	246.5	E1	306.4	EC1	2.6
S2	301.8	SC2	0.9	E2	303.4	EC2	236.6
S3	300.4	SC3	17.7	E3	300.6	EC3	191.3
S4	307.0	SC4	245.7	E4	299.6	EC4	77.2
S6	298.8	SC5	233.4	E5	299.0	EC6	80.8
S7	300.1	SC6	168.7	E6	305.5	EC7	176.3
S8	301.3	SC7	232.0	E7	304.0	EC8	251.3
S9	299.8	SC8	235.2	E8	303.0	EC9	143.8
		SC9	200.6	E9	306.6	EC10	265.3
		SC10	261.3				
MEAN	301.0		184.2		303.1		158.4
SEM	0.9		30.3		1.0		30.0

Area Under Curve of Oral Glucose Tolerance Test (min·mmol)

Sedentary SHAM		Sedentary CORT		Exercise SHAM		Exercise CORT	
S1	867.5	SC1	898.0	E1	758.5	EC1	688.0
S2	921.0	SC2	803.5	E2	683.0	EC2	756.5
S3	904.0	SC3	869.0	E3	740.0	EC3	659.0
S4	830.5	SC4	854.5	E4	743.5	EC4	627.5
S6	742.0	SC5	793.0	E5	896.5	EC6	823.5
S7	809.0	SC6	687.5	E6	745.0	EC7	790.0
S8	791.5	SC7	880.0	E7	737.0	EC8	872.5
S9	836.0	SC8	850.0	E8	601.0	EC9	690.0
		SC9	760.5	E9	807.5	EC10	694.5
		SC10	797.0				
MEAN	837.7		819.3		745.8		733.5
SEM	20.9		20.2		26.8		27.2

Non-esterified Free Fatty Acids (mM)

Sedentary SHAM		Sedentary CORT		Exercise SHAM		Exercise CORT	
S1	1.034	SC1	0.992	E1	0.935	EC1	1.027
S2	1.001	SC2	0.915	E2	1.274	EC2	1.019
S3	1.329	SC3	0.913	E3	1.184	EC3	1.195
S4	0.809	SC4	0.672	E4	1.001	EC4	0.802
S6	0.560	SC5	0.838	E5	0.992	EC6	1.204
S7	0.750	SC6	1.155	E6	0.787	EC7	0.983
S8	0.725	SC7	1.127	E7	0.882	EC8	0.721
S9	0.538	SC8	0.880	E8	1.111	EC9	1.116
		SC9	0.730	E9	1.210	EC10	1.314
		SC10	0.626				
MEAN	0.843		0.885		1.042		1.042
SEM	0.094		0.059		0.054		0.064

Plasma Insulin (ng/ml)

Sedentary SHAM		Sedentary CORT		Exercise SHAM		Exercise CORT	
S1	0.533	SC1	2.634	E1	0.807	EC1	0.548
S2	1.270	SC2	0.966	E2	1.041	EC2	0.797
S3	0.647	SC3	1.076	E3	0.418	EC3	1.051
S4	1.076	SC4	1.693	E4	0.508	EC4	1.290
S6	2.545	SC5	1.708	E5	0.782	EC6	0.378
S7	1.818	SC6	2.615	E6	1.459	EC7	0.294
S8	1.424	SC7	0.588	E7	0.837	EC8	2.480
S9	2.032	SC8	3.516	E8	0.857	EC9	1.574
		SC9	1.384	E9	0.493	EC10	0.378
		SC10	1.245				
MEAN	1.418		1.743		0.800		0.977
SEM	0.244		0.288		0.107		0.239

Intramuscular Triglycerides (optical density)

Sedentary SHAM	Type I Red	Type IIa Red	Type IIb/d Red	Type IIb/d White
S1	41.69	48.75	33.21	43.17
S2	33.06	41.85	32.93	42.46
S3	32.96	36.75	29.74	34.77
S4	28.53	32.46	29.78	37.78
S6	27.28	36.02	27.67	47.06
S7	30.89	35.06	29.72	48.77
S8	25.75	31.00	21.84	45.89
S9	34.66	41.04	37.94	51.21
AVERAGE	31.85	37.87	30.35	43.89
SEM	1.67	1.91	1.55	1.83

Sedentary CORT	Type I Red	Type IIa Red	Type IIb/d Red	Type IIb/d White
SC1	20.84	26.22	20.22	14.74
SC2	~	~	~	~
SC3	42.92	45.96	49.88	53.22
SC4	43.55	48.76	43.83	38.44
SC5	58.41	62.45	62.52	42.47
SC6	22.43	24.60	17.94	32.80
SC7	58.50	57.20	48.57	55.61
SC8	~	~	~	~
SC9	~	~	~	~
SC10	28.82	32.64	26.42	58.02
AVERAGE	39.35	42.55	38.48	42.19
SEM	5.98	5.67	6.43	5.79

Intramuscular Triglycerides (optical density)

Exercise SHAM	Type I Red	Type IIa Red	Type IIb/d Red	Type IIb/d White
E1	33.44	38.91	34.44	39.12
E2	28.79	31.40	21.97	43.23
E3	48.86	51.19	48.08	36.98
E4	49.63	55.72	46.17	46.47
E5	40.47	41.69	36.61	47.33
E6	41.90	46.90	40.64	42.11
E7	31.02	35.89	29.04	46.38
E8	37.63	40.66	32.03	41.51
E9	32.12	28.96	22.95	32.17
AVERAGE	38.21	41.26	34.66	41.70
SEM	2.54	2.94	3.09	1.66

Exercise CORT	Type I Red	Type IIa Red	Type IIb/d Red	Type IIb/d White
EC1	44.21	76.56	44.80	47.29
EC2	28.23	33.13	23.84	45.58
EC3	41.51	51.13	39.35	54.28
EC4	47.23	52.98	43.03	50.24
EC6	36.31	54.97	42.21	48.49
EC7	37.02	43.91	36.25	34.11
EC8	~	~	~	~
EC9	31.49	32.30	21.98	44.02
EC10	29.98	33.73	29.52	43.58
AVERAGE	37.00	47.34	35.12	45.95
SEM	2.44	5.32	3.15	2.09

Muscle Fiber Areas (μm^2)

Sedentary SHAM	Type I Red	Type IIa Red	Type IIb/d Red	Type IIb/d White
S1	17.89	19.32	23.27	39.51
S2	23.17	24.99	29.57	22.27
S3	18.00	18.14	28.90	34.96
S4	22.15	16.31	24.85	36.69
S6	18.29	20.16	27.44	34.11
S7	21.70	19.35	24.08	34.88
S8	20.66	21.87	28.84	28.63
S9	26.15	26.71	29.75	17.39
AVERAGE	21.00	20.86	27.09	31.06
SEM	1.03	1.24	0.93	2.71

Sedentary CORT	Type I Red	Type IIa Red	Type IIb/d Red	Type IIb/d White
SC1	0.24	0.18	29.00	29.47
SC2	~	~	~	~
SC3	28.83	23.84	25.44	21.89
SC4	20.70	18.86	21.80	38.65
SC5	20.82	23.17	23.40	32.61
SC6	17.08	19.17	20.53	43.22
SC7	23.57	21.45	28.78	26.21
SC8	~	~	~	~
SC9	17.77	20.34	33.97	27.93
SC10	22.06	22.78	21.58	33.58
AVERAGE	18.88	18.72	25.56	31.69
SEM	2.96	2.73	1.65	2.43

Muscle Fiber Areas (μm^2)

Exercise SHAM	Type I Red	Type IIa Red	Type IIb/d Red	Type IIb/d White
E1	21.52	22.95	22.95	32.58
E2	17.03	24.15	30.14	28.68
E3	26.30	26.40	28.22	19.08
E4	22.66	26.25	27.42	23.67
E5	27.53	22.02	22.64	27.81
E6	21.70	22.58	21.41	34.30
E7	15.78	21.12	31.22	31.87
E8	19.55	22.52	22.36	35.57
E9	19.71	23.76	29.44	27.10
AVERAGE	21.31	23.53	26.20	28.96
SEM	1.29	0.61	1.28	1.76

Exercise CORT	Type I Red	Type IIa Red	Type IIb/d Red	Type IIb/d White
EC1	24.09	23.48	24.56	27.87
EC2	21.64	16.27	31.44	30.64
EC3	31.14	24.41	24.22	20.24
EC4	19.72	26.90	26.88	26.50
EC6	24.17	24.96	26.02	24.85
EC7	27.38	16.98	25.11	30.53
EC8	~	~	~	~
EC9	23.30	20.52	27.49	28.69
EC10	17.90	21.95	25.14	35.00
AVERAGE	23.67	21.93	26.36	28.04
SEM	1.48	1.34	0.83	1.55

Corticosterone Diurnal Pattern (ng/ml)

Sedentary SHAM	Week 1			Week 2			Week 3			Week 4			Week 5			Week 6		
	01 00	20 00	08 00	01 00	20 00	08 00	01 00	20 00	08 00	01 00	20 00	08 00	01 00	20 00	08 00	01 00	20 00	08 00
S1	29.68	188.38	22.19	207.07	88.92	12.88	62.77	154.70	21.73	116.11	409.43	20.36	721.63	254.29	37.22	125.60	481.47	102.26
S2	55.62	143.49	13.51	68.57	286.14	43.94	147.55	124.50	17.98	161.06	211.95	25.83	209.25	199.45	21.48	161.96	286.55	14.45
S3	184.05	168.08	14.81	45.36	120.51	164.47	96.84	294.47	12.55	111.52	213.76	27.04	210.56	267.06	44.28	50.99	231.91	29.63
S4	650.33	231.02	20.64	260.26	336.14	64.67	61.41	187.89	46.86	74.01	176.81	28.62	191.22	363.93	32.69	224.97	229.13	21.49
S6	211.27	245.77	21.33	138.80	270.59	29.81	29.79	136.41	14.85	51.66	65.11	20.60	75.67	351.90	31.83	74.18	335.56	35.12
S7	289.85	307.17	17.33	76.38	572.01	65.21	42.70	155.05	12.94	182.84	265.07	15.27	119.85	248.63	19.38	79.65	53.67	23.95
S8	290.51	150.97	18.62	244.83	55.38	143.11	122.34	92.97	9.58	449.93	195.71	17.48	145.41	218.05	21.06	274.96	266.44	21.44
S9	208.39	142.56	19.10	118.96	269.77	107.14	247.44	82.85	10.08	200.79	248.90	27.15	104.66	266.78	42.46	186.38	287.31	24.23
MEAN	239.96	197.18	18.44	145.03	249.93	78.90	101.36	153.60	18.32	168.49	223.34	22.79	222.28	271.26	31.30	147.34	271.50	34.07
SEM	67.69	20.93	1.09	29.37	58.83	19.19	25.21	23.47	4.32	44.13	34.12	1.77	73.48	20.67	3.47	27.95	42.15	9.98

Sedentary CORT corticosterone Concentration (ng/ml)

	Week 1			Week 2			Week 3			Week 4			Week 5			Week 6		
Sedentary CORT	01 00	20 00	08 00	01 00	20 00	08 00	01 00	20 00	08 00	01 00	20 00	08 00	01 00	20 00	08 00	01 00	20 00	08 00
SC1	514.69	522.33	97.97	121.45	480.36	398.47	117.19	116.11	61.71	225.34	177.60	91.15	114.07	237.88	91.44	98.81	178.18	98.49
SC2	431.61	490.53	516.91	449.02	595.38	441.08	835.06	476.65	521.52	486.74	374.79	481.85	272.69	196.36	248.47	181.62	263.46	151.66
SC3	69.27	186.20	426.95	376.83	682.76	87.75	505.20	395.39	404.70	334.67	326.21	335.97	135.05	98.71	256.10	197.69	172.56	104.15
SC4	20.09	152.43	76.62	72.01	61.14	54.93	45.67	120.79	63.51	93.30	237.17	131.55	235.66	258.03	60.20	192.48	234.76	177.78
SC5	277.59	269.15	213.05	218.33	375.51	261.65	86.99	114.66	103.91	86.23	134.14	122.34	161.38	175.39	189.70	133.17	110.25	118.02
SC6	361.06	408.43	386.28	247.46	259.70	493.98	144.16	165.96	148.01	157.05	228.23	122.34	211.51	251.84	123.92	129.33	78.09	108.88
SC7	198.75	153.88	310.95	348.96	178.59	200.12	44.27	98.56	90.40	83.38	112.57	84.93	105.42	245.86	168.09	83.77	146.47	98.27
SC8	274.55	176.42	172.29	126.74	183.01	392.82	46.00	71.45	107.81	117.64	176.23	80.56	169.95	259.41	143.24	152.61	219.92	144.45
SC9	154.94	238.92	201.81	161.96	190.28	127.03	41.82	232.47	159.84	222.45	154.92	85.82	171.83	220.33	55.75	262.18	166.01	79.12
SC10	331.55	239.47	140.51	117.33	149.70	217.99	50.32	134.35	58.25	72.19	304.69	61.81	88.89	176.81	90.98	78.95	91.20	70.32
MEAN	263.41	283.78	254.33	224.01	315.64	267.58	191.67	192.64	171.97	187.90	222.65	159.83	166.64	212.06	142.79	151.06	166.09	115.11
SEM	49.30	44.04	47.09	40.62	65.94	49.28	84.18	43.22	50.48	42.55	27.80	43.47	18.75	16.16	22.83	18.39	19.48	10.62

Exercise SHAM Corticosterone Concentrations (ng/ml)

	Week 1			Week 2			Week 3			Week 4			Week 5			Week 6		
Exercise SHAM	01 00	20 00	08 00	01 00	20 00	08 00	01 00	20 00	08 00	01 00	20 00	08 00	01 00	20 00	08 00	01 00	20 00	08 00
E1	140.29	21.48	158.95	223.08	259.70	19.75	243.32	119.49	19.67	136.86	268.30	19.15	209.03	185.62	30.42	275.57	106.24	18.21
E2	695.39	175.27	20.66	169.00	188.82	262.74	251.38	344.41	20.33	177.73	330.03	21.39	206.00	291.06	31.99	144.21	210.14	154.94
E3	352.79	158.76	13.94	208.20	545.94	265.81	51.23	261.72	22.43	90.03	116.02	23.57	116.11	208.27	21.86	223.90	191.74	24.97
E4	105.59	232.26	161.46	202.89	259.26	24.84	132.11	159.71	27.87	101.20	245.47	12.02	103.58	75.93	19.77	157.60	124.86	24.32
E5	328.28	242.04	30.99	128.59	328.53	54.16	65.69	135.97	27.36	71.22	278.34	9.78	170.41	359.30	14.30	160.63	425.73	46.97
E6	289.41	277.93	25.10	247.65	184.98	124.25	228.59	200.15	44.82	128.87	177.22	11.38	428.83	182.86	12.75	222.68	273.96	31.22
E7	178.18	145.09	25.33	328.28	452.10	60.55	199.67	319.42	32.77	340.82	262.12	39.01	277.23	452.45	16.12	245.28	346.81	144.87
E8	179.96	287.86	37.57	123.49	278.99	93.73	167.83	310.10	29.51	110.17	240.61	22.42	230.98	226.04	46.93	111.55	194.32	28.26
E9	773.45	428.23	32.10	208.39	297.93	27.13	159.45	338.91	40.77	157.80	337.56	16.97	135.93	376.98	11.11	102.84	400.63	33.15
MEAN	338.15	218.77	56.23	204.40	310.69	103.66	166.59	243.32	29.60	146.08	250.63	19.52	208.68	262.06	22.81	182.69	252.71	56.32
SEM	80.25	37.77	19.78	20.60	39.57	32.39	24.37	30.20	2.91	26.74	23.19	2.96	33.34	39.21	3.89	20.37	38.70	17.90

Exercise CORT corticosterone Concentration (ng/ml)

	Week 1			Week 2			Week 3			Week 4			Week 5			Week 6		
Exercise CORT	01 00	20 00	08 00	01 00	20 00	08 00	01 00	20 00	08 00	01 00	20 00	08 00	01 00	20 00	08 00	01 00	20 00	08 00
EC1	72.68	605.26	333.89	359.42	139.10	152.91	403.13	419.72	281.73	302.81	339.37	271.44	191.40	189.72	217.78	183.71	173.62	257.29
EC2	156.99	180.93	136.01	124.02	162.95	151.49	131.65	122.11	105.05	105.51	106.12	98.73	91.88	106.81	103.52	568.52	103.36	68.53
EC3	245.03	293.10	185.62	139.10	182.91	157.85	118.84	159.08	138.24	287.92	288.59	601.05	108.67	176.36	117.74	131.37	125.39	147.32
EC4	166.91	198.43	320.44	150.67	385.40	15.46	193.41	155.66	114.94	125.57	284.59	164.22	143.77	154.17	139.97	221.35	106.40	227.69
EC6	500.82	424.65	372.66	368.69	822.18	273.29	266.99	330.87	229.61	156.79	142.44	138.50	124.22	164.38	106.95	74.35	71.20	84.02
EC7	531.57	531.97	554.80	479.12	519.95	619.77	470.18	341.36	397.65	138.06	202.24	161.90	131.72	160.22	102.42	70.89	40.13	49.30
EC8	763.47	345.25	170.07	132.58	151.20	245.95	90.84	142.78	40.09	138.83	94.77	56.92	110.57	213.39	37.57	123.23	247.81	197.05
EC9	468.98	551.42	363.10	319.13	253.97	462.58	148.25	194.15	120.40	169.58	152.46	120.88	193.90	251.84	75.08	204.39	164.20	111.46
EC10	619.77	272.67	258.87	161.46	110.12	232.08	111.23	111.58	105.84	167.36	102.01	104.33	298.89	153.93	89.32	146.05	180.07	64.64
MEAN	391.80	378.19	299.50	248.24	303.09	256.82	214.95	219.70	170.40	176.94	190.29	190.88	155.00	174.54	110.04	191.54	134.69	134.15
SEM	79.63	52.45	43.19	44.56	78.79	60.67	45.72	37.77	37.19	23.40	30.88	55.01	21.49	13.67	16.49	50.26	21.11	25.66

Skeletal Muscle- Glucocorticoid Receptor (a/b isoform)

Sedentary SHAM		Sedentary CORT		Exercise SHAM		Exercise CORT	
S1	0.75	SC1	1.06	E1	0.71	EC1	0.86
S2	0.29	SC2	1.01	E2	0.58	EC2	0.94
S3	0.61	SC3	1.23	E3	0.70	EC3	1.32
S4	1.31	SC4	1.11	E4	0.90	EC4	1.34
S6	0.90	SC5	1.17	E5	1.94	EC6	2.54
S7	0.23	SC6	3.19	E6	0.93	EC7	2.51
S8	2.32	SC7	2.84	E7	1.80	EC8	2.04
S9	1.60	SC8	2.72	E8	1.29	EC9	2.78
		SC9	0.90	E9	1.76	EC10	4.05
		SC10	1.85				
MEAN	1.00		1.71		1.18		2.04
SEM	0.25		0.28		0.18		0.35

Skeletal Muscle- Glucocorticoid Receptor (c isoform)

Sedentary SHAM		Sedentary CORT		Exercise SHAM		Exercise CORT	
S1	0.34	SC1	0.72	E1	0.44	EC1	0.87
S2	0.19	SC2	0.66	E2	0.84	EC2	0.88
S3	0.73	SC3	1.00	E3	0.86	EC3	0.60
S4	0.43	SC4	0.40	E4	1.32	EC4	0.45
S6	1.47	SC5	0.95	E5	0.45	EC6	2.45
S7	1.07	SC6	1.46	E6	1.67	EC7	2.71
S8	2.09	SC7	2.10	E7	2.16	EC8	2.19
S9	1.67	SC8	1.24	E8	2.56	EC9	1.23
		SC9	0.99	E9	0.99	EC10	1.18
		SC10	1.35				
MEAN	1.00		1.08		1.25		1.40
SEM	0.24		0.15		0.25		0.28

Skeletal Muscle- 11 Beta-Hydroxysteroid Dehydrogenase 1

Sedentary SHAM		Sedentary CORT		Exercise SHAM		Exercise CORT	
S1	0.48	SC1	0.97	E1	0.51	EC1	0.46
S2	0.14	SC2	1.57	E2	0.78	EC2	1.28
S3	1.17	SC3	1.29	E3	0.92	EC3	0.98
S4	0.75	SC4	0.45	E4	1.11	EC4	2.11
S6	1.45	SC5	1.28	E5	1.19	EC6	1.28
S7	1.00	SC6	1.56	E6	0.61	EC7	1.46
S8	1.67	SC7	1.67	E7	1.17	EC8	2.00
S9	1.33	SC8	1.68	E8	1.07	EC9	2.07
		SC9	1.88	E9	2.21	EC10	2.04
		SC10	1.94				
MEAN	1.00		1.43		1.06		1.52
SEM	0.18		0.14		0.17		0.19

Skeletal Muscle- Hormone Sensitive Lipase

Sedentary SHAM		Sedentary CORT		Exercise SHAM		Exercise CORT	
S1	1.05	SC1	0.55	E1	0.74	EC1	2.24
S2	2.08	SC2	1.03	E2	1.41	EC2	0.71
S3	0.37	SC3	1.59	E3	0.61	EC3	0.69
S4	1.25	SC4	1.07	E4	0.62	EC4	~
S6	0.77	SC5	0.95	E5	0.53	EC6	1.11
S7	0.85	SC6	2.02	E6	1.13	EC7	0.57
S8	0.86	SC7	~	E7	1.64	EC8	0.56
S9	0.77	SC8	1.33	E8	1.44	EC9	1.25
		SC9	0.61	E9	0.47	EC10	0.92
		SC10	0.30				
MEAN	1.00		1.05		0.95		1.01
SEM	0.18		0.18		0.15		0.19

Skeletal Muscle- Adipose Triglyceride Lipase

Sedentary SHAM		Sedentary CORT		Exercise SHAM		Exercise CORT	
S1	0.93	SC1	0.50	E1	0.30	EC1	2.16
S2	1.39	SC2	0.37	E2	1.05	EC2	0.71
S3	0.19	SC3	0.51	E3	0.91	EC3	0.79
S4	0.58	SC4	0.99	E4	0.43	EC4	~
S6	1.55	SC5	0.78	E5	0.96	EC6	0.90
S7	0.52	SC6	2.08	E6	1.02	EC7	0.71
S8	1.08	SC7	~	E7	1.29	EC8	0.40
S9	1.75	SC8	1.81	E8	0.95	EC9	1.37
		SC9	1.06	E9	0.54	EC10	1.04
		SC10	0.81				
MEAN	1.00		0.99		0.83		1.01
SEM	0.19		0.20		0.11		0.18

Subcutaneous Adipose- Hormone Sensitive Lipase

Sedentary SHAM		Sedentary CORT		Exercise SHAM		Exercise CORT	
S1	1.63	SC1	0.67	E1	0.84	EC1	0.56
S2	0.30	SC2	0.58	E2	0.68	EC2	2.17
S3	2.41	SC3	2.76	E3	~	EC3	1.60
S4	0.27	SC4	0.32	E4	2.48	EC4	0.59
S6	1.08	SC5	1.97	E5	0.58	EC6	1.19
S7	1.50	SC6	0.48	E6	2.33	EC7	2.54
S8	0.48	SC7	0.83	E7	0.30	EC8	1.15
S9	0.33	SC8	2.23	E8	1.64	EC9	2.48
		SC9	5.12	E9	0.22	EC10	2.19
		SC10	2.42				
MEAN	1.00		1.74		1.13		1.61
SEM	0.28		0.50		0.30		0.26

Subcutaneous Adipose- Adipose Triglyceride Lipase

Sedentary SHAM		Sedentary CORT		Exercise SHAM		Exercise CORT	
S1	2.33	SC1	1.05	E1	0.87	EC1	0.50
S2	0.51	SC2	0.93	E2	0.89	EC2	1.99
S3	1.99	SC3	2.38	E3	~	EC3	0.92
S4	0.62	SC4	0.62	E4	2.03	EC4	0.92
S6	0.96	SC5	1.65	E5	0.44	EC6	0.60
S7	0.73	SC6	0.42	E6	1.35	EC7	0.71
S8	0.52	SC7	0.65	E7	0.36	EC8	0.37
S9	0.34	SC8	0.86	E8	1.24	EC9	1.06
		SC9	0.90	E9	0.40	EC10	0.66
		SC10	0.92				
MEAN	1.00		1.04		0.95		0.86
SEM	0.26		0.18		0.20		0.16

Epididymal Adipose- Hormone Sensitive Lipase

Sedentary SHAM		Sedentary CORT		Exercise SHAM		Exercise CORT	
S1	0.74	SC1	0.25	E1	0.61	EC1	1.60
S2	0.72	SC2	1.70	E2	1.92	EC2	1.65
S3	0.67	SC3	0.18	E3	~	EC3	2.79
S4	1.53	SC4	1.52	E4	1.47	EC4	1.06
S6	1.18	SC5	0.49	E5	0.86	EC6	2.09
S7	0.61	SC6	2.53	E6	1.86	EC7	2.45
S8	0.73	SC7	0.70	E7	2.06	EC8	2.17
S9	1.83	SC8	1.01	E8	2.88	EC9	1.37
		SC9	2.92	E9	1.97	EC10	2.30
		SC10	1.07				
MEAN	1.00		1.24		1.70		1.94
SEM	0.16		0.31		0.24		0.19

Epididymal Adipose- Adipose Triglyceride Lipase

Sedentary SHAM		Sedentary CORT		Exercise SHAM		Exercise CORT	
S1	1.28	SC1	0.93	E1	0.68	EC1	0.97
S2	1.17	SC2	1.60	E2	2.08	EC2	1.67
S3	0.87	SC3	0.80	E3	~	EC3	1.52
S4	1.81	SC4	1.95	E4	1.81	EC4	1.56
S6	1.03	SC5	0.78	E5	1.15	EC6	1.30
S7	0.84	SC6	1.14	E6	1.54	EC7	0.91
S8	0.55	SC7	1.23	E7	0.71	EC8	0.43
S9	0.44	SC8	0.62	E8	1.82	EC9	1.03
		SC9	1.08	E9	1.59	EC10	2.28
		SC10	0.77				
MEAN	1.00		1.09		1.42		1.30
SEM	0.15		0.13		0.18		0.18

Substrate Utilization

Sedentary SHAM					
Time of Day	S3	S4	S6	MEAN	SEM
20:00	0.9202	0.9322	0.9092	0.9205	0.0066
21:00	0.9337	0.9321	0.9350	0.9336	0.0008
22:00	0.9485	0.9380	0.9306	0.9390	0.0052
23:00	0.9464	0.9187	0.9466	0.9372	0.0093
00:00	0.9508	0.9291	0.9401	0.9400	0.0062
01:00	0.9425	0.9530	0.9377	0.9444	0.0045
02:00	0.9642	0.9301	0.9494	0.9479	0.0099
03:00	0.9601	0.9372	0.9566	0.9513	0.0071
04:00	0.9668	0.9310	0.9441	0.9473	0.0105
05:00	0.9314	0.9157	0.9328	0.9266	0.0055
06:00	0.9366	0.9210	0.9370	0.9315	0.0053
07:00	0.9545	0.9054	0.9297	0.9299	0.0142
08:00	0.9529	0.8899	0.9152	0.9194	0.0183
09:00	0.9422	0.8904	0.8994	0.9107	0.0160
10:00	0.9530	0.8656	0.9471	0.9219	0.0282
11:00	0.9318	0.8776	0.9257	0.9117	0.0171
12:00	0.9330	0.8674	0.9047	0.9017	0.0190
13:00	0.9682	0.8924	0.9065	0.9224	0.0233
14:00	0.9432	0.9039	0.9118	0.9196	0.0120
15:00	0.9484	0.8877	0.9457	0.9273	0.0198
16:00	0.9660	0.9153	0.9322	0.9378	0.0149

Substrate Utilization

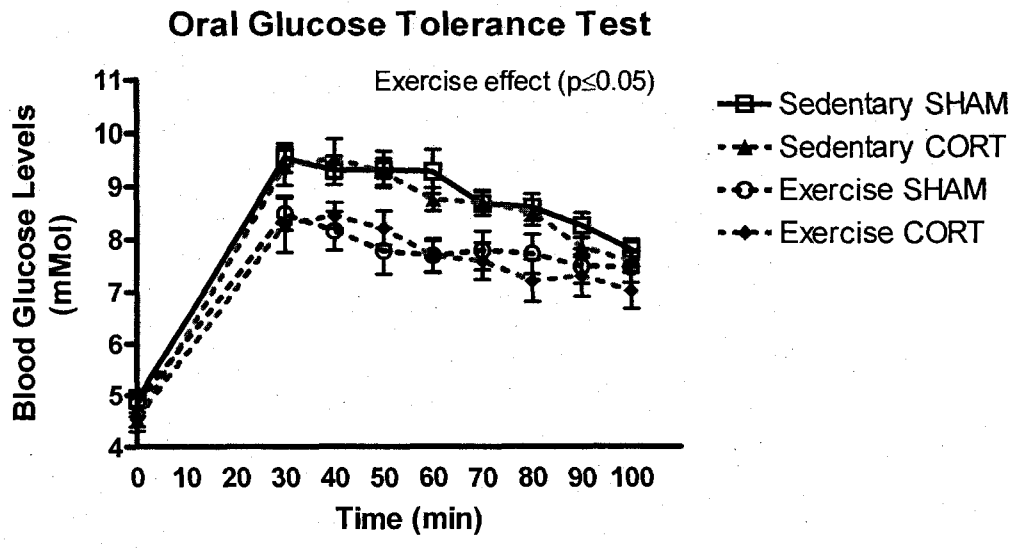
Sedentary CORT					
Time of Day	SC1	SC6	SC7	MEAN	SEM
20:00	0.9549	0.9098	0.9341	0.9329	0.0130
21:00	0.9595	0.9264	0.9432	0.9430	0.0096
22:00	0.9617	0.9259	0.9394	0.9424	0.0104
23:00	0.9591	0.9225	0.9172	0.9329	0.0132
00:00	0.9434	0.9282	0.9205	0.9307	0.0067
01:00	0.9099	0.9472	0.9065	0.9212	0.0130
02:00	0.9537	0.9304	0.9131	0.9324	0.0117
03:00	0.9571	0.9228	0.9180	0.9326	0.0123
04:00	0.9546	0.9275	0.9100	0.9307	0.0130
05:00	0.9248	0.9150	0.8714	0.9037	0.0164
06:00	0.9373	0.9081	0.9227	0.9227	0.0084
07:00	0.9102	0.9022	0.9062	0.9062	0.0023
08:00	0.9127	0.9220	0.9174	0.9174	0.0027
09:00	0.8922	0.9103	0.9012	0.9012	0.0052
10:00	0.8700	0.8938	0.8819	0.8819	0.0069
11:00	0.8833	0.8954	0.8893	0.8893	0.0035
12:00	0.9166	0.9388	0.9277	0.9277	0.0064
13:00	0.9123	0.9233	0.8852	0.9069	0.0113
14:00	0.9063	0.9403	0.9081	0.9182	0.0110
15:00	0.9141	0.9372	0.9227	0.9246	0.0067
16:00	0.9276	0.9343	0.9208	0.9276	0.0039

Substrate Utilization

Exercise SHAM					
Time of Day	E1	E2	E3	MEAN	SEM
20:00	0.9254	0.9126	0.8757	0.9046	0.0149
21:00	0.9502	0.9261	0.8811	0.9191	0.0202
22:00	0.9421	0.9338	0.9210	0.9323	0.0061
23:00	0.9380	0.7604	0.9164	0.8716	0.0559
00:00	0.9522	0.8491	0.9330	0.9115	0.0316
01:00	0.9703	0.9010	0.9422	0.9378	0.0201
02:00	0.9533	0.9150	0.9380	0.9354	0.0111
03:00	0.9448	0.9281	0.9234	0.9321	0.0065
04:00	0.9590	0.9287	0.9270	0.9382	0.0104
05:00	0.9460	0.9376	0.8974	0.9270	0.0150
06:00	0.9488	0.9388	0.9105	0.9327	0.0115
07:00	0.9315	0.9357	0.9089	0.9254	0.0083
08:00	0.9429	0.9430	0.9200	0.9353	0.0076
09:00	0.9405	0.9323	0.9240	0.9323	0.0048
10:00	0.9525	0.9309	0.9093	0.9309	0.0125
11:00	0.9390	0.9361	0.9333	0.9361	0.0017
12:00	0.9388	0.9317	0.9246	0.9317	0.0041
13:00	0.9413	0.9328	0.9243	0.9328	0.0049
14:00	0.9406	0.9396	0.9385	0.9396	0.0006
15:00	0.9343	0.9351	0.9359	0.9351	0.0005
16:00	0.9431	0.9375	0.9319	0.9375	0.0032

Substrate Utilization

Exercise CORT						
Time of Day	EC1	EC6	EC9	EC10	MEAN	SEM
20 00	0.8167	0.8822	0.9374	0.9111	0.8868	0.0260
21 00	0.8211	0.9009	0.9614	0.9158	0.8998	0.0292
22 00	0.8695	0.9168	0.9721	0.9316	0.9225	0.0212
23 00	0.8546	0.9311	0.9773	0.9319	0.9237	0.0254
00 00	0.8598	0.9152	0.9676	0.9409	0.9209	0.0230
01 00	0.8791	0.9424	0.9780	0.9438	0.9358	0.0206
02 00	0.8881	0.9293	0.9700	0.9416	0.9323	0.0170
03 00	0.8434	0.9290	0.9679	0.9305	0.9177	0.0263
04 00	0.8742	0.9075	0.9731	0.9416	0.9241	0.0214
05 00	0.8675	0.9168	0.9450	0.9292	0.9146	0.0167
06 00	0.8756	0.8849	0.9348	0.9356	0.9077	0.0160
07 00	0.8777	0.8879	0.9141	0.9251	0.9012	0.0111
08 00	0.8765	0.8517	0.9204	0.9490	0.8994	0.0218
09 00	0.8967	0.8895	0.9471	0.9082	0.9104	0.0128
10 00	0.8716	0.9072	0.9091	0.8857	0.8934	0.0090
12 00	0.9199	0.9060	0.9504	0.8524	0.9072	0.0205
13 00	0.9302	0.8903	0.9255	0.8534	0.8998	0.0179
14 00	0.9176	0.9097	0.8931	0.8521	0.8931	0.0146
15 00	0.9063	0.9243	0.9207	0.8755	0.9067	0.0111
16 00	0.8968	0.9248	0.9000	0.8656	0.8968	0.0121


Oral Glucose Tolerance Test
Exercise effect (p≤0.05)
Blood Glucose Levels (mMol)
11
10
9
8
7
6
5
4
0 10 20 30 40 50 60 70 80 90 100
Time (min)
Sedentary SHAM
Sedentary CORT
Exercise SHAM
Exercise CORT