The changes and effect of stress hormone cortisol during extreme diet and exercise
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Thesis

THE CHANGES AND EFFECT OF STRESS HORMONE CORTISOL
DURING EXTREME DIET AND EXERCISE

by

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DEDICATION

I would like to dedicate this work to my parents who continue to support my dreams and also to my new best friend Alex who has made my world a happier place.
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Some people change lives without even knowing it. I have to thank my two thesis readers for agreeing to assist me in putting the final pieces of the paper together. It would not be in the fine state it is in without all your time and input. I would certainly like to acknowledge my advisor Dr. Oberhaus, who made my experience in this program all worth with. Additionally, I have to acknowledge Dr. Davies who is one of the sweetest people I have been fortunate enough to encounter during my time at Boston University.
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ABSTRACT

Cortisol is one of the stress hormones produced as a result of stimulus to the hypothalamus triggering the hypothalamic-pituitary-adrenal axis (HPA). The result of cortisol production after this trigger is to return the body and its relevant systems back to homeostasis. This is a desired state of physiologic equilibrium in the body.

A number of physiological and environmental conditions trigger the HPA pathway. This includes hypoglycemia (low blood sugar), dehydration (low blood volume), exercise, which are considered stress triggers, and changes to the circadian cycle. It is no wonder then that extreme diet and exercise can impact the HPA axis due to the stress caused by such activities or lifestyle choices.

Fortunately, the production of cortisol in response to exercise has been extensively studied. Generally, studies have shown that the level of plasma cortisol levels increase proportionally during high intensity exercise, while a general decrease has been observed during moderate or low intensity exercise (Davis & Few, 1973). In addition, several scientific texts provide substantial information on the correlation between hypoglycemia and cortisol synthesis. This information in addition to other sources has proven useful for assessing the effect of extreme dieting on cortisol production.
The aim of this thesis is to expand on the changes in cortisol caused by extreme exercise and diet as well as elaborate on the physiological effects that these cortisol levels in turn may have.
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LIST OF ABBREVIATIONS

ACTH ................................................................. Adrenocorticotropin Hormone
ADH ............................................................... International Standards Organization
CBG ................................................................. cortisol-binding globulin
CRH ............................................................... Cortisol Releasing Hormones
DNA .............................................................. Deoxyribonucleic Acid
DST ................................................................. Dexamethasone Suppression Test
FFAs ............................................................... Free Fatty Acids
FSH ............................................................... Follicle Stimulating Hormone
GR ................................................................. Growth Hormone
HDL .............................................................. High Density Lipoprotein
HPA ............................................................... Hypothalamic-pituitary axis
IL-1 ................................................................. Interleukin-1
IL-6 ................................................................. Interleukin-6
LDL ............................................................... Low Density Lipoprotein
LH ................................................................. Luteinizing Hormone
LPLs .............................................................. Lipoprotein Lipase
PCOS ............................................................ Polycystic Ovarian Syndrome
POMC .......................................................... pro-opiomelanocortin
TAG .............................................................. Triacyl Glycerol
TNF-α ........................................................... Tumor Necrosis Factor- alpha
VO2 max ...................................................... maximal oxygen consumption
INTRODUCTION

Exercise and diet are becoming an increasingly popular part of one’s lifestyle as public health awareness improves its efforts in promoting fitness and health in our society. According to the national numbers in 2005, at any given time, about 47% of adults in the United States are on a weight loss mission (Tomiyama et al., 2010). The social pressures in addition to the pressure that comes with this new health promotion may be of concern when one considers the extreme efforts that are being made to reach certain fitness or weight goals. Exercising, despite the health gains, generally puts a strain on the body, stimulating changes in cellular and system functions. In addition, severe restriction of caloric intake is one of the most common methods of intentional weight loss (Tomiyama et al., 2010). This exerts a lot of stress on the body especially when the daily energy expenditure outweighs consumption. It is therefore necessary to look at the possible implications of exerting such stress on the body.

Cortisol is known as the main stress hormone produced in the body. This glucocorticoid plays a central role in maintaining blood pressure and energy levels and serves many other functions. Looking at the changes in this hormone’s production and circulating levels not only provides insight on stress levels, but allows one to study the physiological changes that may be resulting from this hormone.

Cortisol is one of the hormones produced in the adrenal gland of the kidney via the HPA axis. Time of day (circadian rhythm), negative feedback inhibition, inflammatory mediators and stress levels are four of the main controllers of this axis that will be discussed further (Kettyle et al., 1998). Several receptors for this hormone exist in
all tissues and its effect is exerted downstream by changing the protein expression within
cells that make up the tissue respective tissues (Kettyle et al., 1998).

Through physiology text and scientific journals, the changes and resulting effects
of cortisol levels due to extreme exercise or dieting will be presented in this thesis. This
thesis begins with a brief overview of the HPA axis for cortisol production, its
metabolism and regulation and the general roles of this stress hormone. We will then
assess the individual effects of exercise and dieting on the HPA axis. Several studies
have been conducted to assess the changes in cortisol and adrenocorticotrophin hormone
(ACTH) production as a result of diet restriction and strenuous exercise. Though several
of them have similar findings, different approaches were used and will be discussed
further. Finally, the effects of the invoked levels of cortisol will then be elaborated with
reference to diseases related to cortisol production.
Cortisol Synthesis:

Figure 1. Structure of cortisol. Image taken from: http://ultimatecityfitness.com/wp-content/uploads/2012/01/cortisol.jpg

Cortisol (figure 1), a glucocorticoid, is one of the steroid hormones produced at the adrenal cortex. Like all steroid hormones, it is synthesized from cholesterol and is able to cross cell membranes. This precursor can either be synthesized within the adrenal cortex or taken up from circulation via the HDL and LDL receptors. Uptake via these receptors involves receptor-mediated endocytosis followed by hydrolysis to produce cholesterol (Arlt & Stewart, 2005).
Once ACTH binds to receptor on the adrenal cortex (figure 2), a regulatory protein then functions to increase the shuffling of cholesterol inside the mitochondrial matrix where the enzymes are localized for cortisol synthesis. The adrenal cortex can be divided into three main functional zones: zona glomerulosa, zona fasciculata and zona reticularis. In the zona glomerulosa, cholesterol is converted to progesterone, which is then converted to cortisol and other glucocorticoids in the zona fasciculata (Arlt & Stewart, 2005).

Figure 2. Zones of the adrenal cortex and hormones. Image taken from: http://www.scielo.br/img/revistas/rbti/v18n1/a14fig01.gif
Cortisol pathway: HPA axis & Stimuli

The neuroendocrine system that is responsible for secretion of cortisol and other glucocorticoids is known as the hypothalamic-pituitary-adrenocortical (HPA) axis (figure 3). This axis is composed of ACTH-releasing neurons in the hypothalamus, the corticotroph cells of the anterior pituitary, the adrenal cortex, and feedback inhibition (Jacobson, 2005).

Figure 3: Components of the hypothalamic-pituitary-adrenal axis. Figure take from http://www.bengreenfieldfitness.com/wp-content/uploads/2013/08/Basic_HPA_Axis.jpeg
When physical or perceived stress occurs in an individual, afferent input signals are sent to the hypothalamus. This stimulates the release of Corticotropin-releasing hormone (CRH), vasopressin and oxytocin from the neurons of the paraventricular hypothalamus. Although all of these play a role in mediating glucocorticoid production, CRH is the predominant factor at this axis (Jacobson, 2005). CRH, a peptide hormone then stimulates the corticotroph cells in the anterior pituitary to up-regulate pro-opiomelanocortin (POMC) gene expression and processing. The cleavage of this gene results in the secretion of Adrenocorticotropic hormone (ACTH) amongst other side products (Jacobson, 2005). The role of this hormone, as the name suggests, is to stimulate production of hormones in the adrenal cortex. Increased levels of ACTH thus bind to the adrenal cortex and cortisol is made and secreted by the cells of the zona fasciculata. High levels of cortisol in turn, inhibit CRH and ACTH production by acting directly on the hypothalamus and anterior pituitary respectively.

Tight regulation of the HPA axis is essential for appropriate response to homeostatic disturbances and prevention of glucocorticoid excess, both of which can have harmful effects. This can result in different types of chronic diseases that will be discussed further in a later section.

Generally, in an unstressed state, HPA activity is governed by circadian rhythm. This is an innate 24-hour clock which governs physiological processes in living things. The amplitude of the observed peaks and troughs of circulating cortisol that make up this natural rhythm are due to the feedback mechanism control from the HPA. In diurnally active humans, peak cortisol levels occur in the early morning after waking, while lowest
levels are observed at the very end of the day. These peaks coincide with the periods of the day in which higher amounts of energy are normally required; possibly for the anticipated workload ahead.

Physical stress is defined by Jacobson as “a situation posing real threat of tissue damage or death if uncorrected” (Jacobson, 2005). This form of stress is able to stimulate the HPA axis via different possible pathways. Hypoglycemia, acidosis, alkalosis are examples of physical stressors. In addition to these forms of stress, another worth noting is stress caused by inflammation. During inflammatory stress, several cytokines are responsible for stimulating HPA activity. These include interleukin 1 (IL-1), interleukin 6 (IL-6) and tumor necrosis factor-α (TNFα).
Cortisol Receptors and mechanism of action

Figure 4: The activation of the glucocorticoid receptor by cortisol. This image was taken from: https://sites.google.com/site/boyswithoutfathers/home/glucocorticoid-receptor
The mineralocorticoid receptors and the glucocorticoid receptors are the two receptors responsible for mediating the effects of endogenous cortisol and the other glucocorticoid hormones. Both receptors are located within their target cells in the cytoplasm (figure 4). Binding of cortisol to the receptors leads to the translocation of the hormone-receptor complex to interact with certain genes through an energy-dependent process. The DNA binding domain interacts with the desired sequence on the target genes (Arlt & Stewart, 2005). Several genes can respond to this glucocorticoid interaction.

Mineralocorticoid receptors bind to the hormones with higher affinity than the glucocorticoid receptors and are found in a limited number of regions such as the colon, kidney and areas of the brain. These regions (tissues) are generally targets of the mineralocorticoid aldosterone.

Mineralocorticoid receptor-Cortisol interactions are inhibited by the activity of the inactivating enzyme type 2 11B-hydroxysteroid dehydrogenase (Jacobson, 2005).

Glucocorticoid receptors however, are expressed more ubiquitously and found in the brain and several peripheral regions. Glucocorticoid receptors predominate the anterior pituitary, while Mineralocorticoid receptors are predominant in the brain, making it highly sensitive to the feedback mechanisms.
Feedback Regulation & Metabolism:

Once secretion is stimulated, the activity of the HPA axis can be regulated via fast and delayed feedback mechanisms. The former works immediately after stress-induced stimulus and is suspected to work directly on the hypothalamic neurons in the PVN (Arlt & Stewart, 2005). Delayed mechanism unlike the rapid, requires protein synthesis and thus often takes more than half an hour to take effect. In addition, the delayed mechanism acts on both the hypothalamus and anterior pituitary (Arlt & Stewart, 2005).

While circulating, 99% of cortisol is bound to a 383 amino acid protein known as cortisol-binding globulin (CBG). CBG levels are said to rise to about three-fold by high estrogen levels. Thus, high levels of estrogen leads to the reduction in cortisol activity and effect since lower levels are able to circulate freely or diffuse into the tissues (Arlt & Stewart, 2005).

The half-life of circulating cortisol is about 1 to 2 hours and its main mode of metabolism is through the activity of the enzyme 11β-HSD1. This interconverting enzyme is responsible for forming cortisone, an inactive hormone from cortisol (Geer et al., 2013). On the other hand, 11β-HSD2 can convert inactive cortisol (cortisone) back into cortisol (figure 5). Alternatively, cortisol may be reduced by a 5β-reductase to form metabolites, which are processed and excreted in urine. As will be discussed later, drastic changes in the levels of these enzymes can therefore lead to drastic changes in ones physiological state.
**Cortisol Functions**

Cortisol has multiple functions in various tissues (figure 6). Generally, its effect on intermediary metabolism is to increase the availability of glucose by decreasing its uptake and increasing its synthesis. This is achieved by acting on several tissues. In the liver, cortisol increases glucose production directly by activating key enzymes such as glucose-6-phosphatase and phosphoenolpyruvate kinase (PEPCK) (Arlt & Stewart, 2005) and indirectly by increasing the activity of other gluconeogenic hormones (Kettyle et al.). Additionally, cortisol increases glycogen synthesis and decreases glycogen mobilization for effective glycogen storage (Arlt & Stewart, 2005).
Cortisol maintains glucose availability peripherally by inhibiting glucose uptake in muscle and tissue. This is achieved effectively by reducing cellular sensitivity to insulin (Geer et al., 2013). In adipose, the breakdown of fat is up-regulated, increasing the plasma levels of free fatty acids (FFAs) and glycerol, the products of fat breakdown. This makes energy sources readily available to keep the body running at times of high demand.

Glucocorticoids also maintain cardiovascular and renal function and thus play a role in maintaining blood pressure. This is can be achieved by down-regulating vasodilators such as Nitric Oxide, preventing blood pressure from falling to a drastically low level. By up-regulating vasoconstrictors such as erythropoietin, blood pressure can be increased. Also, stimulating the renin-angiotensin pathway, glucocorticoids are able to increase vasoconstriction to maintain vascular tone and cardiac output. Cardiac output (the amount of blood pumped by the heart per minute) is also sustained through cortisol’s regulation of the glomerular filtration rate in the kidneys by a similar mechanism of action (Hackbart et al., 2013). Working at the kidneys allows the body to control how much blood volume is lost through excretion.

Glucocorticoids are also responsible for immune suppression and are thus used to treat autoimmune and inflammatory diseases. As will be discussed later, cortisol administration has been found to decrease eosinophil counts, T and B lymphocytes along with their cytokine production, while increasing neutrophil migration (Arlt & Stewart, 2005).
Figure 6: The myriad roles of cortisol. Image taken from http://www.netterimages.com/images/vpy/000/000/09/9025-0550x0475.jpg
Exercising, Stress & Cortisol

One of the mechanisms by which exercise is expected to stimulate the HPA axis is through the stimulation of the chemoreceptors in muscle, which sense the change in hydrogen ion concentration as a result of the lactic acid build-up. Addition, stimulation via baroreceptors occur from hypovolemia, which the volume loss which occurs from dehydration, reducing the systemic pressure (Geer et al., 2014). During intense or prolonged physical exertion these forms of stress would trigger neural reflexes to the hypothalamus, causing the release of CRH. CRH, a peptide hormone, is then able to stimulate the pituitary gland to produce ACTH for secretion. ACTH in turn is able to stimulate cortisol secretion at the adrenal glands. Cortisol then increases glucose availability by inhibiting the effect of insulin, increases vascular tone to maintain blood pressure, and increases glomerular filtration to allow hydrogen ion secretion.

Epinephrine and angiotensin, which are also secreted during exercise are suspected to play a role in the production of cortisol during high-stress situation via ACTH regulation at the HPA (Jacobson, 2005). The two hormones have a synergistic effect during times of stress (Jacobson, 2005).
Figure 7: Effects of cortisol during long-term and short-term stress. Figure taken from [http://www.tammysyoga.co.uk/wp-content/uploads/2013/06/cortisol_stress1335849873182.jpg](http://www.tammysyoga.co.uk/wp-content/uploads/2013/06/cortisol_stress1335849873182.jpg)
Effects of Excess Cortisol

Chronically excessive levels of cortisol are common in diseases such as Cushing’s syndrome. Typically, the resulting features of high glucocorticoid levels include central hyperglycemia, hypokalemia, metabolic alkalosis, hypertension, menstrual irregularities and poor wound healing (figure 7). Due to cortisol’s interplay with various other hormones, excess levels have been found to be associated with myriad defects that involve connective tissue, growth, central nervous and circulatory system defects. These will be discussed in this section.

Approximately 80% of individuals with excess glucocorticoid develop diabetes and a study by Domi in 2011 demonstrated that early diagnosis of diseases that involved hypersecretion of ACTH. As the study suggested, a disease such as Cushing’s disease could be a substantial mode of early prognosis of diabetes (Domi, 2011).

A common symptom observed in individuals with Cushing’s Syndrome is an increase in the ratio of visceral fat to total body fat. This is due to the increased lipogenesis and fatty acid transport to the liver. This preferential site of adipose accumulation could be due to a higher expression of the glucocorticoid receptors in visceral tissue. It is also suspected that the activity of the enzyme lipoprotein lipase (LPL) may be greater in this region. Glucocorticoids such as cortisol are suspected to increase TAG hydrolysis and FA uptake into adipocytes- a process mediated by LPLs. This form of abdominal obesity has been linked to cardiovascular risks, and insulin resistance (Geer et al., 2014).
The glucocorticoid receptor is expressed on the gastrointestinal tract and therefore can have several effects on its state and functions. Excess cortisol acts on the gastrointestinal tract to decrease calcium absorption and increase its secretion this promotes the symptoms of secondary hyperthyroidism (Kettyle et al., 1998). As discovered in several studies including that by Shaker et al., low calcium levels promote osteoclastic activity and inhibit osteoblastic activity. Furthermore, Chiodini et al. found reduced spinal and femoral bone mass in women with Cushing’s syndrome. Subjects with higher levels of cortisol are thus expected to have higher risk of bone fractures (Shaker & Lukert, 2005). In addition, chronically high levels of cortisol have been associated with increased risk of developing peptic ulcer disease.

Cortisol also plays an essential role in fibroblast activity as they regulate the catabolic changes. Excessive levels are known to inhibit the activity of both cells by inhibiting cellular division and DNA synthesis, leading to decreased bone and collagen formation (Kettyle et al., 1998). Additionally, glucocorticoids reduce protein synthesis and cause muscle protein synthesis and reduced gluteal and femoral proximal muscle mass can be observed in patients with Cushing’s syndrome (Arlt & Stewart, 2005).

Also, high levels of cortisol in children can stunt growth due to its ability to down-regulate GH (Kettyle et al., 1998). A study by Kawashima et al. showed that glucocorticoids played a role in the accumulation of growth hormone granules in the cytosol of cells in the anterior pituitary (Vyas & Maatouk, 1996). In addition, studies by Savendahl shows that the growth plate is directly targeted by increased cortisol levels in
children under stress. High stress levels not only stimulate cortisol, but also inflammatory cytokines that stimulate the HPA to up-regulate cortisol production (Kawashima, 2010).

Glucocorticoid secretion has also been known to regulate levels of other anterior pituitary hormones. The expression of TSH and the correlation of its circulating levels with that of glucocorticoids have been studied. Excess glucocorticoids leads to the suppression of TSH synthesis and secretion. Hormones such as cortisol are suspected to act on the hypothalamus-pituitary-thyroid axis. A study by Kakucska et al. investigated the changes in Thyrotropin-Releasing Hormones expression following changes in Corticotropin-Releasing Hormone (Kakucska, 1995).

With the ability to enter the brain, and receptor expression in regions such as hypothalamus, cerebellum, and cortex, high levels glucocorticoids have been associated with varying moods such as depression and euphoria (Kettyle, 1998). Conflicting studies on the effect of excess glucocorticoids on the central nervous system have found cortisol to exacerbate neurodegenerative disorders in addition to some protective effects in the CNS (Smets, 2010). In addition, memory loss and insomnia have also been observed in patients with adrenal hypertrophy (Arlt & Stewart, 2005).

One main reason it is important to investigate the changes and effects of cortisol with stress is the hormone’s ability to regulate other key hormones in the body. Certain studies have shown a possible effect of cortisol on fertility. Glucocorticoids have been found to directly and indirectly reduce the gonadal hormones estrogen and testosterone (Hackbart, 2013). In 2013, Hackbart et al. published their study, which investigated the effect of glucocorticoid-induced insulin resistance on follicle development and ovulation.
In their study dexamethasone, an exogenous glucocorticoid was administered to cows to mimic the effects of cortisol. The resulting effects include hyperinsulinemia and hyperglycemia in subjects. The study was aimed at inducing insulin resistance in the subjects in order to allow further observation of its effect on reproductive hormones and any changes in ovulation. Observations were made using ultrasound to measure the size of ovaries on a daily basis and blood samples to assess hormone and glucose concentrations.

In addition to elevated glucose and insulin levels (see figure 8), inhibition of complete ovulation was observed in all subjects administered dexamethasone (Hackbart, 2013). This study’s findings thus indicate the possible involvement of cortisol and other glucocorticoids in the development of reproductive syndromes such as polycystic ovarian syndrome. Although the focus of the study was to assess the etiology behind PCOS via the two resulting effects of glucocorticoids, it highlights the negative effects that elevated cortisol levels can have.
In addition to the this indirect mode of action, glucocorticoids can directly interrupt signaling and response to insulin at targets tissues such as skeletal muscle and pancreas. Direct and indirect effects on the hypothalamus, pituitary and ovaries are also suspected for the mode of action. The resulting insulin resistance then leads to reduced levels of estrogen. Since some studies have found PCOS to be associated with elevated levels of estrogen rather than reduced, these findings question any correlation between this syndrome and glucocorticoids. In addition, decreased LH was noted, which explained the reduced ovulation.
In order to assess glucocorticoid and HPA changes in response to stress from physical exertion and extreme diets, several studies have used healthy participants who have included athletes of different fitness levels. Additionally, several studies assessing the effect of extreme dieting have looked at the different forms in which diet restriction can take place and interfere with the HPA. For example, some studies, which will be further elaborated below, are follow-ups to compare anorexia nervosa to bulimia since both forms of caloric restriction may have different results. All studies generally involved the withdrawal of blood and/or saliva samples from participants before, during and after exposure. Samples were then measured for cortisol using assays after centrifuging. Generally, exertion studies monitored maximum oxygen consumption to group participants by levels of exertion. The exercise of choice for these studies usually involved running on a treadmill and included a resting-control session.

Additionally, several of these studies included a dexamethasone suppression test (DST). This involved administration of dexamethasone, a glucocorticoid, before extraction of blood samples to assess the efficiency of the negative feedback mechanism directed by the HPA.

In 1973, the effect of exercise on the secretion and metabolism of cortisol was studied by J.D. Few. This was a follow-up study of one previously done by Few and Davies which found a decrease in exogenous cortisol after lower intensity exercise. Cortisol was administered intravenously to subjects who were either at rest, engaging in strenuous exercises, or light-load exercises for one hour. The half-life of exogenous and
changes in concentration of endogenous cortisol was then measured. Increased cortisol secretion occurred after 65 percent of maximal oxygen consumption was reached. This is what was observed in subjects engaging in strenuous exercise. On the other hand, subjects who engaged in light exercise exhibited a decrease in plasma cortisol concentrations.

Duclos et al. in 1997 looked at endurance trained athletes to assess any changes in the sensitivity of the HPA. The investigators were looking for any evidence of adaptation that may have occurred with the repetitive stress that comes with endurance training. Saliva and cortisol levels were measured in this study of endurance-trained athletes 2 hours before and after either resting or strenuous exercise. They observed an increase in ACTH production after any form of exertion in long distance runners. This increase was however not correlated with cortisol levels as would be expected and observed in control/resting participants. Two hypotheses were proposed for this study: The adrenal glands had reduced sensitivity to ACTH and/or the HPA had reduced sensitivity to cortisol levels for effective negative feedback action.

The study used 8 healthy long distance runners with no history of smoking or alcohol abuse. To improve study validity, each subject served as their own control. Subjects reported in at the same time after an overnight fast. They were given a standardized meal 2 hour before the session and consumed only water during the session. To assess ACTH production in the pituitary, a stimulation test using artificial CRH was used. In addition, sensitivity of the adrenal gland was assessed using exogenous ACTH. Blood and saliva samples were then taken at intervals for both these assessments. The
results of the study showed that after the exercise session, ACTH levels were not significantly reduced despite the presence of elevated cortisol from the physical stress. This indicated inefficient negative feedback activity. The CRH stimulation test was able to support this, as it showed a responsive increase in ACTH levels. These levels rose quite similarly as was observed during physical exertion in this study.

As can be seen from figure 9, a significant increase in plasma and cortisol levels was observed. These levels then plateaued after 60 minutes of the post rest session. There was no significant difference in cortisol response for post rest and post exercise sessions. Since no blunted cortisol levels were not attenuated, the result support the hypothesis the sensitivity of the HPA is reduced. This also makes the other hypothesis less plausible adrenal sensitivity to ACTH appears to be normal (Figure 9).
Figure 9: a: Saliva cortisol responses to CRH for post rest and post exercise sessions. b: Plasma cortisol responses to CRH for post rest and post exercise sessions. (Duclos, 1997)
Figure 10: a: Saliva cortisol responses to ACTH for post rest and post exercise sessions. b: Plasma cortisol responses to ACTH for post rest and post exercise sessions. (Duclos, 1997)
One important question that has been posed in several studies is “what exercise intensity produces significant elevation in cortisol levels?” A study by Hill et al. tries to identify the threshold intensity level for glucocorticoid production. Using exercise intensities defined by maximal oxygen uptake (VO₂max), the resulting levels of circulating cortisol are measured to determine which level is able to significantly stimulate the HPA. The investigators acknowledged the potential confounders for this study, which included time of day, the individual’s circadian rhythm, diet and physical fitness.

Hill and colleagues attempted to effectively address this problem by instructing participants to maintain a certain diet before their visits for the entire duration of the experiment. Subjects were also instructed to ensure that their last meal had been had 4 hours prior to checking in at the laboratory for the study.

In their study, blood was collected from subjects before and after the exercises and the percentage change in cortisol and ACTH was measured. An interesting finding was that while high intensity exercise resulted in increased cortisol levels, low intensity exercise rather reduced the level of circulating cortisol.

Studies by Luger et al. in 1987 studied the response of the HPA to the stress caused by physical exercise. One important question this study tried to answer was how if at all do the changes in stress response during strenuous exercise lead to physical fitness. This was investigated using plasma levels of CRH, ACTH, cortisol and lactate as markers using different intensities of treadmill exercise. Trained runners, untrained runners and sedentary subjects were used in the studies and were made to run at 3 different intensity
level with warm-up and cool-down periods. Subjects were also asked to fast at least 6 hours before exercise. Blood from each subject was collected via an intravenous catheter serially minutes before, during and after exercise and markers were then measured using radioimmunoassay.

Although exercises that caused 50 percent of maximum oxygen consumption did not cause elevated cortisol or ACTH levels, activation of HPA was detected for exercise demanding 70 to 90 percent of maximum oxygen consumption in all three groups (Figure 11). CRH concentrations however were below detection limits even at the extreme maximum consumption level.
Figure 11: Mean plasma ACTH, cortisol and lactate in trained, untrained and moderately trained athletes by their maximal oxygen consumption percentages. (Luger et al., 1987)
Studies also found that athletes that were more physically fit (i.e trained runners), produced less lactate acid per workload intensity level but also had a higher basal level of cortisol and ACTH. Despite the resulting elevated basal glucocorticoid levels, the trained runners exhibited attenuated HPA responses during their assigned high intensity exercise (Mastorakos, 2005). It therefore seems from this study’s findings that the effect of constant stress exertion that comes with extreme exercise may not result in excess plasma cortisol levels in individuals who have been physically conditioned for the work load as it may for individuals who have not.

On the other hand, highly trained athletes were reported to have hypercortisolism due to the chronic stress, which may reduce the HPA axis stress response. Several studies seem to have found that in these participants, baseline cortisol levels are higher. It appeared that extreme or strenuous daily exercises promoted adrenal hypertrophy and hypersecretion leading to chronically elevated ACTH levels (Geer at al, 2014). This allows higher levels of cortisol to be attained before a negative feedback signal is sent to the hypothalamus or anterior pituitary.

A not so surprising finding of this study was the association between physical training and percentage of body fat. Not only was the increase in physical correlated with increased maximum levels of oxygen consumption, but a reduction in body weight and percentage of body fat was also observed. The elevated levels of glucocorticoids that were observed during intense exercise induce lipolysis, increasing the levels of circulating Free Fatty Acids. These high levels can reportedly induce insulin resistance.
The effects of this include decreased glucose uptake and increased proteolysis particularly at the skeletal muscle (Geer et al., 2014).

In addition, further investigations by Mastorakos et al. show that high intensity exercise lead to increased production in proinflammatory cytokines such as IL-6. This spike would thus trigger cortisol production by stimulating the HPA axis. The role of glucocorticoid production in this case is to attenuate the cytokine levels. This was validated in a study that showed significantly reduced IL-6 levels in athletes who were administered hydrocortisone prior to their high-intensity training session (Mastorakos, 2005).

Figure 12. Mean ACTH and Cortisol concentrations in three groups of athletes. (Luger et al., 1987)
When evaluating these studies, it is always worth it to consider other contributing factors since there are variables that contribute to cortisol secretion. In 2001, Kanaley et al. investigated the effect of diurnal rhythm on cortisol and growth hormone response to exercise. Sleep and meals were supervised and blood samples taken from moderately trained men. Blood samples were taken 1 hour before and 5 hours after exercise in 5-minute intervals. Investigators found that cortisol secretion in response to exercise, was significantly greater if exercise was performed at 7am than at 7pm (See figure 13). Additionally, baseline cortisol levels were higher at 7am than at 7pm.

Figure 13: Mean Cortisol concentrations in three groups of athletes. (Luger et al., 1987)
Dieting & Cortisol

As can be observed in figure 14, levels of cortisol have been shown to decline after food consumption. This is the expected trend since the role of this stress hormone is to mobilize amino acids and fatty acids in order to synthesize glucose for energy needs (Stull & Rodiek, 1987).

Figure 14: Cortisol levels during 24 hours of a day. Image taken from http://projectfabulousblog.com/wp-content/uploads/2013/05/circadian-rhythm-cortisol.png
An extreme diet in which the daily caloric intake is severely reduced is commonly discouraged for the dangers it poses to our health. As similarly observed in highly trained athletes, subjects with extreme dietary restraint or anorexia nervosa, tend to have mild hypercortisolism (Mastorakos, 2005). Several studies have examined the negative effects of hypoglycemia and weight loss and the roles of cortisol during this physiologically stressful period.

Fichter et al. in 1984 performed a within-subject experimental study in healthy subjects to assess the effects of fasting or weight loss and weight gain on the neuroendocrine system. Participants were taken through four consecutive 3-week phases of baseline, food abstinence, “restoration” and “normal” consumption. The baseline phase required participants to maintain their body weight. The food abstinence phase required participants to consume only water for a total of zero calories per day. The following phase then required participants to gain weight to restore their original weight. The last phase, second baseline phase, was then to maintain this weight. As is evident from figure 15, daily plasma cortisol levels were significantly elevated during the starvation phase (B). In addition, an increase in the half-life of cortisol was evident during this phase, which is consistent with the other studies. The weight gain phase (C) provided evidence that hypercortisolism was reversible once subjects increased caloric intake. This was also a result of the restoration of cortisol’s half-life during this diet phase.
Figure 15: Plasma cortisol levels for the period of 3 weeks for each phase of study. A: baseline B: starvation C: Weight gain D: Return to baseline. (Fichter et al., 1984)
Figure 16: Plasma cortisol levels in the 24 hour day before and after fasting. (Fichter et al., 1984)

Additionally, a Dexamethasone Suppression Test (DST) was performed to assess the HPA responsiveness to cortisol levels. The decrease in the amount of ACTH, and thus cortisol is expected after administration of dexamethasone. As evident from figure 15 for this study, the DST showed normal suppression of cortisol levels during the baseline phases and the weight gain phase. However, during the starvation phase, cortisol suppression was not effective.
Significantly increased average 24-hour plasma cortisol levels were observed during the fasting phase (figure 16). In addition, an increase in the half-life of cortisol was observed along with increased secretion, increasing baseline levels.

In 1989, Fichter et al. went on to evaluate the effect of extreme diets such as Bulimia on the hypothalamo-pituitary-adrenal axes. This study was inspired by other studies on patients with anorexia nervosa that had found increased HPA activity with increased cortisol secretion and decreased cortisol metabolism. Similarly, investigators found that suppression of cortisol was insufficient in the bulimic participants. In their study, a dozen women with eating disorders were compared to a control group. Plasma cortisol levels were assessed overnight along with the close monitoring of dietary intake for the duration of the study.

Measuring caloric intake for bulimic participants may have been the biggest challenge in this study as binge attacks and episodes of vomiting had to be accounted for. To get around this problem, investigators of this study had to see to it that food that was consumed was weighed whenever possible. In addition, they had to trust the patients to accurately report the amount vomited. An estimate was then calculated for total amount actually digested. This then allowed the investigators to create two groups for assessment: one group was labelled the lower caloric intake group, consuming 1443 kcal, while the other group consuming 1943 kcal was the high caloric intake group.

Additionally, the bulimic participants who went for a longer number of days without food and thus a lower number of total calories consumed, showed higher cortisol levels. Similarly, lower fat consumed in the diet was correlated with lower elevated
plasma cortisol concentrations. Endocrine disturbance was observed on a broader scale in this study as prolactin, FSH and LH levels were significantly lower than normal in the bulimic subjects.

Disturbances in other endocrine axes go along with findings in other studies. In cases of extreme dietary restraint as seen with anorexia nervosa, the starvation and thereby reduction in adipose, restricts the levels of the hormones such as leptin. This is a hormone that serves as a good indicator of available energy stores. Normally, leptin suppresses the effect of cortisol in a feedback loop mechanism to restrict appetite and fat deposition (Jacobson). Low leptin levels would then contribute to the high cortisol levels observed during dietary restraint.

A study by Davis et al. showed that inhibiting the cortisol surge that occurs when subjects were hypoglycemic allowed them to preserve the actions of the autonomic nervous system. This indicated that many of the hypoglycemic symptoms including muscle, endocrine and metabolic dysfunction, were due mainly to cortisol (Davis et al., 1997).

McLean et al. performed a study assessing the level of cortisol excretion in healthy premenopausal women under dietary restraint. The study showed that the group with higher dietary restraint had higher stress levels, with increased cortisol excretion (Inder, 1998). The high stress caused CRH circulation, increasing cortisol concentrations, which when excessive, have been found to disturb the reproductive cycle due to the high negative feedback effect on the hypothalamus. Recalling the hormone interactions in
relation to the HPA axis, inhibiting hormone secretion at the hypothalamus would in turn inhibit pituitary hormones, which include LH and FSH.
DISCUSSION

Not all studies have been able to show significant increases in levels of cortisol with exercise at and above 60 % VO2max and it is worth considering why such varying results have been observed. As mentioned earlier, there are several other factors that may influence cortisol circulation such as one’s diet, fitness and circadian rhythm. In addition, one must also consider the interplay of other hormones when it comes to the neuroendocrine system.

The findings from J.D Few’s study highlighted the role of cortisol during exercise as they detected a decrease in plasma levels during low intensity training. At this point, the HPA activity is able to function accordingly with responsive negative feedback mechanism. As cortisol levels are produced and absorbed into target tissue, an overall decrease in levels were detected. In contrast, for the group with over 60 % oxygen consumption (high intensity), the higher cortisol levels were a reflection of the reduced response of HPA as the stress signals cause the negative feedback mechanism to be overwritten.

The changes in cortisol levels observed in the Kanaley et al. study after exercises last between 40 and 130 minutes. They also noticed that if physical exertion occurred after food consumption, the cortisol response to physical stress was reduced. Having a readily available source of energy was thus key for preventing huge spikes in plasma cortisol levels.

Duclos et al. also found an increase in baseline plasma cortisol levels in their endurance-trained athletes after exercise, contributing to the mild hypercortisolism that
was observed. The findings from the CRH and ACTH tests supported the hypothesis of impaired HPA response and thus a retarded negative feedback response. This study also supported the use of saliva samples for measuring cortisol levels as there was a significant correlation between saliva and plasma levels once hormone concentration was below peak levels.

Hill et al. also highlighted the difficulty and importance in attaining an appropriate “resting control” for evaluating cortisol levels before the exercise studies. In addition to keeping the duration of intensities constant, the diet schedule was kept quite similar for most studies. For example, the participants were informed of when their last meals before studies should occur. Also, Hill mentions the importance in standardizing the time of testing to control to account for cortisol variations due to circadian rhythm. For his study in 2008, each participant was made to come in at the same certain time of day to perform their assigned exercises.

Findings from the study by Fichter et al. continue to affirm that serious weight loss can lead to alterations in neuroendocrine activity. Through their study, they further support that in addition to reduced caloric intake, other extreme forms of weight loss would result in pronounced spikes in cortisol levels. Studies showed that severe restriction in calories or starvation, an increase in the basal cortisol levels in a day. This was due to increased secretion and decreased catabolism. Additionally, several subjects failed the DST, showing decreased HPA sensitivity.

For some of these studies it is worth questioning the validity of the findings since the basal cortisol levels were at times used as an index. Since these levels can vary from
individual to individual and within individual even if controls are met, they do not serve as an accurate reference of HPA activity.
CONCLUSION

As several of the studies discovered, physical exertion and extreme diets result in an increase in cortisol levels. These two causes of stress on the body ultimately elevate glucocorticoid levels by interrupting the function of the HPA. As data previously presented showed, the elevated levels are a result of insufficient suppression of ACTH and thus cortisol. By reducing the responsiveness of the HPA to negative feedback of glucocorticoids, the continuous exposure to stress results in increased cortisol secretion.

In addition, several studies showed that not only is cortisol secretion increased with exposure to stress, but the metabolism of cortisol is also decreased with an increase in its half-life. This allowed for circulating levels in plasma to remain high.

As studies by many investigators have shown, the repetitive stress that comes with endurance training results in an adaptation of the HPA. Exercise then provides enough stress to completely override the negative feedback mechanism, resulting in hypercortisolism.

Studies that found that low intensity exercises have the ability to lower cortisol levels were very interesting as they support theories suggesting successful and healthy weight loss through moderate exercise. This appears to be the case as well with dieting as severe restriction of caloric intake only elevates stress levels.

It is certainly worth noting one’s perceived stress in addition to their total cortisol output as this can interfere with goals to lose weight or increase fitness. Since chronic cortisol production has been known to increase fat deposition and cause weight gain in some cases, the right approaches need to be taken for effective and healthy weight loss.
Similarly, with strenuous exercise, endurance trainers exerting a continuous and repetitive amount of physical stress would benefit from taking the right procedures to keep glucocorticoid levels low. This would decrease their vulnerability to muscle tear, ligament injuries, and other debilitating injuries that can interfere with their goals.

Due to cortisol’s essential role in maintaining homeostasis, there are several health risks to be mindful of when this mechanism for regulation is overwritten. As illustrated in figure 7, there are several long-term and short-term effects or stress due to cortisol. Additionally, diseases that are typical with Cushing’s may be observed. Luckily, a change in lifestyle, can reverse some of these symptoms.
FUTURE DIRECTIONS

As this thesis was put together, one question that continued to remain unanswered was how long does the state of hypercortisolism need to be sustained before one can accurately consider the effects of chronic exposure to excessive cortisol levels. For example, assessing the effects of excess cortisol after exercise of high intensity in trained and untrained individuals, a proper definition of the exposure time would be necessary to accurately predict the effects of hypercortisolism in subjects.

Although studies showed the effect of repetitive physical stress on the HPA, more elaborate studies still need to be conducted in participants of varying fitness levels. Studies need to be done to examine the adaptation of the HPA in participants with a sedentary lifestyle and moderately active in addition to the endurance-trained athletes.

Additionally, how does one proceed with this knowledge? Knowing the dangers of hypercortisolism, animal studies may be beneficial in exposing the effects of acute and chronic levels of cortisol on the body. Although several studies show the health risks posed by administration of corticosteroids, more studies need to be targeted at the endogenous levels.

For the health-conscious, more detailed studies on the appropriate intensity level for exercise and caloric consumption for dieting need to be conducted. Currently, several theories and books exist which have yet to be supported by valid experiments.
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EDUCATIONAL BACKGROUND

Boston University School of Medicine, Boston, MA  
Master of Science in Medical Science  
September 2012 - Present

Knox College, Galesburg, IL  
Bachelor of Arts in Biochemistry  
Expected Sept 2014

STUDY ABROAD EXPERIENCE

Medical Biotechnology and Drug Development, Copenhagen, Denmark  
June 2010

• Studied through Danish Institute for Study Abroad Program examining biomedicine, drug discovery and development, and biotechnological tools
• Learned about exploration of the opportunities and challenges biotechnology has for medicine
• Gained knowledge about personalized medicine, biomaterials, stem cells, tissue engineering, etc.; and the Danish pharmaceutical and biotech research community

Program for Medical Practice and Policy, Copenhagen, Denmark  
January-May 2009

• Participated in Danish Institute for Study Abroad Program and was introduced to the important human diseases, their diagnosis and treatment, and to clinical working methods of physicians as practiced at a Danish university hospital
• Taught at Rigshospitalet National University Hospital and Copenhagen University Hospitals (Frederiksberg, Roskilde, Amager)

RESEARCH EXPERIENCE

Tuberculosis Research, Johns Hopkins University  
October 2011- 2012

• Working as a research assistant at the center for tuberculosis
• chemotherapeutic investigations on infected mice
• Harvesting and plating of organs
• Designing projects to investigate efficiency of antibiotics

**Effect of Ethanol on Macrophages**, Knox College Honors Research Program  
**September 2009-June 2010**

- Recommended in a nationally recognized program providing support for advanced independent study.
- Researching the effect of ethanol on LPS-stimulated macrophages
- Assessing enzyme expression using Western Blot and flour imaging and also assessing cytokine secretion using ELISA

**Center for Molecular Medicine**, Karolinska Institutet, Sweden  
**June-August 2009**

- Worked with Ph.D., and M.D. Professor in continuing research of the pathogenesis of Psoriasis
- Perfected lab skills using Acetone fixation and slide preparation of skin tissue
- Used Immunofluorescent labeling and imaging using confocal and axial microscope

**Department of Microbiology, Tumor and Cell biology (MTC)**, Karolinska Institutet, Sweden  
**June-August 2009**

- Worked with research professors in their continuing analysis of the role of Natural Killer (NK) cells in the immune response against the protozoan parasite, Leishmania.
- Performed some analysis in relation a mouse model of cutaneous leishmaniasis.
- Learned how to work under sterile conditions and several other lab skills mentioned below

**PUBLICATIONS**

**Center for Tuberculosis Research**, Johns Hopkins University  
**April 2012**

- Second author in a team project investigating the sterilizing activity of novel combinations lacking first- and second-line drugs in a murine model of tuberculosis.

**LEADERSHIP & VOLUNTEER ACTIVITIES**

**President**, Knox College Harambee African Students’ Club  
**September 2008-June 2010**
• Organize club to create events and fundraisers to promote awareness about the African continent and represent the diversity with which the African students bring

**Member, Knox College Best Buddies**  
*January-June 2010*

• Appointed to a resident of St. Mary’s Square Living Center, home to individuals with developmental disabilities  
• Visited my ‘buddy’ on occasion to develop a friendship through activities such as bowling, poetry writing, etc.

**Observer, Korle Bu Teaching Hospital of Ghana**  
*July-August 2008*

• Viewed various surgeries such as pacemaker insertion and hip replacements in the OR  
• Made ward rounds with residents and also assisted parents watch over younger children at the ICU

**Volunteer, Johns Hopkins Cancer Outpatient Center, Baltimore**  
*July 2011-August 2012*

I spent about two hours at this center every week with cancer patients and their families who have travelled from afar for treatment. The center provides a free meal every Wednesday and I helped organize the event and sit with the patients during the meal to comfort them during this time.

**Volunteer/Observer, OSF St. Mary’s Medical Center, Galesburg**  
*July-August 2008*

• Volunteered in the Emergency Department of the hospital  
• Assisted nurses with guiding patients to rooms or cars, cleaning of hospital room beds, restocking room supplies  
• Observed and learned the regular protocol and codes of the department