Interrelationship of Diet, Visceral Adipose Tissue and Cortisol in Pre-menopausal Female Runners

Thesis

Presented in partial fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

By

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ABSTRACT

Background: The interaction of diet and serum cortisol has been researched in endurance athletes, producing conflicting results. Higher serum cortisol levels have also been well documented in several disease states, but the data is not available for a more physically active, healthy population.

Objective: This project was a secondary analysis of a female runner study to assess dietary macronutrients, as well as visceral adipose tissue, in relation to morning serum cortisol values.

Participants/Setting: Final analysis included 100 premenopausal recreational female runners between the ages of 18 – 50 who had been running at least 15 miles per week for the immediate 6 weeks preceding data collection. A standard three-day food diary was used to collect dietary data and the Bouchard physical activity grid was used to estimate energy expenditure. Fasting serum cortisol was collected between 7am and 9am to reflect typical early morning levels. In order to allow a more relevant view of carbohydrate, values in grams/kilogram were stratified into two groups based on activity level¹: “Carbohydrate Inadequate” (n = 52) and “Carbohydrate Adequate” (n = 48).

Energy availability was defined as energy intake minus energy expended in exercise normalized for fat free mass.² Full body GE Lunar iDXA³ scans, with additional analysis using the GE CoreScan version 15 software⁴ were used to estimate visceral adipose tissue.

Statistical Analyses: Final data was collated from the parent study, and the data and variables of interest were compared using quantitative analysis. An independent
samples t-test was performed to on serum cortisol in relation to carbohydrate adequacy groups. Univariate analyses, with linear and quadratic models, were used to analyze serum cortisol in relation to carbohydrate, protein and fat, respectively. Similarly, a univariate analysis, with a linear and quadratic model, was performed on serum cortisol in relation to visceral adipose tissue. Finally, a regression was performed to on serum cortisol in relation to visceral adipose tissue and energy availability. A priori statistical significance for all tests was set at $p \leq 0.05$.

**Results:** The results show that the carbohydrate inadequate group had a significantly lower serum cortisol than the carbohydrate adequate group. Both group means were within the normal range.\textsuperscript{5} Macronutrient analysis for carbohydrate, protein and fat failed to demonstrate significance as predictors for serum cortisol. However, visceral adipose tissue proved to be a significant predictor for serum cortisol. The correlation was the opposite of what was expected in that serum cortisol increased as visceral adipose tissue decreased. Analysis of visceral adipose tissue, controlled for energy availability, was not a significant predictor for serum cortisol. Future research should examine diet, serum cortisol and visceral adipose tissue among a more active population with a wider range of visceral adipose tissue.
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Major Field: Allied Medicine
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<td>HPA</td>
<td>Hypothalamic Pituitary Adrenal</td>
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<td>VAT</td>
<td>Visceral Adipose Tissue</td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic Hormone</td>
</tr>
<tr>
<td>PCOS</td>
<td>Polycystic Ovarian Syndrome</td>
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<tr>
<td>CT</td>
<td>Computed Tomography</td>
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<tr>
<td>WHR</td>
<td>Waist-to-Hip Ratio</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>AHA</td>
<td>American Heart Association</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<td>AT</td>
<td>Adipose Tissue</td>
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<tr>
<td>L4</td>
<td>Lumbar 4</td>
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<tr>
<td>L5</td>
<td>Lumbar 5</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>cm</td>
<td>Centimeters</td>
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<tr>
<td>kg/m²</td>
<td>Kilograms per meters squared</td>
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<td>FAT</td>
<td>Female Athlete Triad</td>
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<td>RED-S</td>
<td>Relative Energy Deficiency in Sport</td>
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<td>LEA</td>
<td>Low Energy Availability</td>
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<tr>
<td>IOC</td>
<td>International Olympic Committee</td>
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<tr>
<td>EA</td>
<td>Energy Availability</td>
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<tr>
<td>EEE</td>
<td>Exercise Energy Expenditure</td>
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<td>EI</td>
<td>Energy Intake</td>
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<tr>
<td>FFM</td>
<td>Fat Free Mass</td>
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<tr>
<td>kcal/kg</td>
<td>Kilocalories per kilogram</td>
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<tr>
<td>FFQ</td>
<td>Food Frequency Questionnaire</td>
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<tr>
<td>RDA</td>
<td>Recommended Daily Allowance</td>
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<td>ACSM</td>
<td>American College of Sports Medicine</td>
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<tr>
<td>g/kg</td>
<td>Grams per kilogram</td>
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<td>Abbreviation</td>
<td>Description</td>
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<td>RMR</td>
<td>Resting Metabolic Rate</td>
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<td>g</td>
<td>Grams</td>
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<tr>
<td>kcal</td>
<td>Kilocalories</td>
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<tr>
<td>RQ</td>
<td>Respiratory Quotient</td>
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<td>LDL</td>
<td>Low-Density Lipoprotein</td>
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<tr>
<td>HDL</td>
<td>High-Density Lipoprotein</td>
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<tr>
<td>μg/dL</td>
<td>Micrograms per deciliter</td>
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<tr>
<td>nmol/L</td>
<td>Nanomole per liter</td>
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<tr>
<td>IMTG</td>
<td>Intramyocellular Triglyceride</td>
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<tr>
<td>FFA</td>
<td>Free Fatty Acid</td>
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<tr>
<td>g/kg BW</td>
<td>Grams per kilogram Body Weight</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<td>CHO/kg</td>
<td>Carbohydrates per kilogram</td>
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<tr>
<td>lbs</td>
<td>Pounds</td>
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<tr>
<td>in³</td>
<td>Inches cubed</td>
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<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
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Chapter 1: Introduction

Endurance exercise, particularly running, is a popular form of activity among recreational and competitive athletes. Within this group, a lean physique is commonly considered ideal and, despite the high calorie demand of distance running, some runners employ behaviors such as increasing exercise or decreasing caloric intake, in an attempt to maintain or lose weight. Caloric restriction can lead to low calorie balance as well as inadequate intake of dietary micro- and macronutrients. Over time, this imbalance between calorie expenditure and calorie intake can result in physical abnormalities including reduced energy, poor bone density and hormone alterations.

Hormone balance is a highly fluid and complex system. One such hormone that is integral to exercise and recovery is cortisol; a highly variable glucocorticoid that can vary in response to physiological stress, psychological stress and the presence of disease. During and after a physiological stress such as endurance exercise, cortisol aids in maintaining blood glucose levels and remodeling tissue leading to an adaptation that essentially increases fitness and acclimates to reduce the response to similar future exercise. Adequate calorie and dietary macronutrient intake help attenuate cortisol and the stress response following exercise, ensuring proper recovery. However, in pursuit of a leaner physique, runners may reduce calorie and macronutrient intake enough to adversely impact, or exacerbate, the stress response following exercise. In the absence of adequate dietary intake for recovery, stress hormones, such as cortisol, may remain elevated for a greater period of time following exercise.
The hypothalamic pituitary adrenal (HPA) axis is the neuroendocrine pathway that regulates cortisol secretion within the body.\textsuperscript{14} Maintenance of cortisol within the appropriate range is linked to normal homeostasis, but chronically elevated or depressed levels can indicate a disruption in the HPA axis.\textsuperscript{15} This area of the brain is also known to regulate reproductive and thyroid functions so it is a hub of hormonal control.

In less active individuals, HPA axis dysfunction and chronically elevated levels of cortisol are associated with accumulation of visceral adipose tissue (VAT).\textsuperscript{16} VAT is thought to be more metabolically active than subcutaneous fat, and has more potentially adverse consequences on health.\textsuperscript{17–19} The link between high levels of cortisol and increased VAT are still uncertain; however, these characteristics are present in several disease states where more VAT is common. This intra-abdominal pattern of body fatness and increase in VAT is closely linked to chronic health conditions including diabetes and cardiovascular disease.\textsuperscript{9,18} While elevated cortisol and visceral fat have been identified in an overweight population\textsuperscript{20}, a similar relationship has yet to be established in a more active, apparently healthy, population. The link between dietary macronutrient intake, the stress response and body fat distribution should be investigated in a more active population.

**Aims**

This analysis evaluated visceral adipose tissue (VAT) in relation to serum cortisol levels and dietary macronutrient intake in adult female recreational runners. This study also determined if dietary macronutrient composition and the presence of VAT was correlated to higher serum cortisol values as they are in a more sedentary population. Specifically, this analysis answered the following questions:
1. Does a correlation exist between dietary macronutrient content and serum cortisol values in recreational female runners?
   
a) Does the level of dietary carbohydrate intake (based on amount of daily exercise as defined by current recommendations) correlate with serum cortisol levels?
   
b) Does a pattern exist between the amount of dietary carbohydrate, in grams/kilogram, and serum cortisol values in female runners?
   
c) Does a pattern exist between the amount of dietary protein, in grams/kilogram, and serum cortisol values in female runners?
   
d) Does a pattern exist between the amount of dietary fat, in grams, and serum cortisol values in female runners?

2. Do higher serum cortisol values coincide with greater visceral adipose tissue in this cohort of recreational female runners?
Chapter 2: Review of the Literature

While exercise is a generally healthy activity, it presents a unique physiological stress to the body. Some of the physiological responses to exercise, such as an increase in cortisol secretion, are similar to those seen in certain disease states, albeit to a much lesser extent. Increases in circulating cortisol, regulated by the hypothalamic-pituitary-adrenal (HPA) axis as part of a negative feedback loop, are typically temporary following exercise; however, disrupted HPA axis function can result in abnormal levels of cortisol. Dietary intake before, during, and after exercise plays a major role in the body’s stress response and adaptation to endurance exercise stress.21–23 A review of the literature regarding endurance exercise, differing dietary macronutrient intake and alterations in the stress response and circulating hormones will contribute to the analysis of the data.

Hypothalamic Pituitary Adrenal Axis

When examining dietary adequacy and the stress response, macronutrient intake and activity level, represent one part of the puzzle to be considered. Stress, whether physical or psychological, stimulates neuroendocrine pathways. The hypothalamic-pituitary-adrenal (HPA) axis is a neuroendocrine system that plays a crucial role in the stress response and adaptation to stressors.14 Responses to the various types of acute stress in humans are well known, and adaptation following exercise is characterized by the increased activity of the HPA axis, resulting in increased concentrations of adrenocorticotrophic hormone (ACTH) thus cortisol.24,25
In the presence of a stressor, the body mobilizes energy to mount a physiological response to the potential threat. A stage of adaptation follows, where the physiological response declines. In an environment of continued stress, whether environmental or nutritional, the body may reach a stage of exhaustion. This state is often characterized by hormone imbalances and a diminished ability to mount the appropriate stress response. When this chain of events is disturbed or exaggerated by chronic overactivity or physiological stress in the absence of proper recovery, the body’s ability to adapt to stressors may be limited. Therefore, it is possible that even in seemingly healthy and active individuals, long-term physiological stress, such as that resulting from inadequate nutrition or frequent exercise, may result in a chronic hormonal imbalance like that found in overtraining syndrome.

Figure 1 – HPA Axis Hormone Regulation Scheme

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Figure 1, shown above, demonstrates the complex interaction and hormonal regulation that is controlled by the HPA axis. Each component of the axis is responsible for maintaining physiologic functions as well as interacting and remaining in equilibrium with the other HPA axis components. Endurance exercise in particular appears to activate the HPA axis to release cortisol. Alterations in cortisol secretion during maximal exercise, over a period of increased training, implies diminishing adrenocortical activity and a decreased ability to respond to exercise stress.

Bobbert et al investigated HPA axis alterations in response to marathon training in a population of healthy recreational male and female distance runners. Participants were ages 25 – 66 and training at similar volumes within gender groups. Fasting blood draws were completed 5 times (6 weeks pre-marathon, 10 days pre-marathon, 2 days post marathon, 10 days post-marathon and 6 weeks post-marathon) over the study in order to track progressive changes over a longer period of time and in relation to changes in training volume before and after the marathon. Several hormone values were measured, although a low dose (1milligram) dexamethasone suppression test was the primary measure of HPA axis sensitivity. Results from this suppression test demonstrated that the HPA axis was more responsive (ie: better able to decrease cortisol) in the measurements taken 10 days and 6 weeks after the marathon when subjects had reduced training volume by nearly 65% to promote recovery. Although no measures of dietary intake were included in this study, these results indicate that high volume endurance training can decrease the HPA axis’s ability to respond to variations in hormones and possibly hamper its ability to regulate the diurnal rhythm of hormone release as would be expected in a healthy individual. This provides one example of the link between training in an exhausted state and disturbances in the HPA axis and
cortisol rhythm; however, nutritional status warrants greater attention as a component of the analysis.

**Cortisol**

Cortisol is a glucocorticoid secreted by the adrenal cortex, whose major functions include development and regulation of the stress response and the immune system.\(^\text{10}\) As the predominant glucocorticoid in humans, cortisol also plays an important role in the metabolism of glucose and other nutrients.\(^\text{10}\) Specifically, in response to physiological stress of exercise, cortisol plays a role in adaptation and the remodeling of tissue.\(^\text{31}\) In a healthy state, cortisol follows a circadian rhythm similar to the sleep-wake rhythm, with higher values in the morning and relatively lower values in the evening.\(^\text{32}\) This circadian rhythm naturally promotes a healthy pattern of sleep and waking as demonstrated in the graph below by Weitzman et al.\(^\text{5}\)

Fluctuations in cortisol occur in response to environmental or physiological stressors. In response to a bout of exercise in a healthy individual, cortisol increases and may remain elevated for hours after exercise is completed.\(^\text{8,34}\) This predicted increase in the stress hormone helps maintain blood glucose levels during exercise and aids the body in recovering after exercise is completed.\(^\text{30,35,36}\) Cortisol and its natural fluctuations are integral to adapting and responding to stressors.

Following stimulation of HPA, cortisol should trend back toward pre-stress levels. This is accomplished through a negative feedback loop. In normal conditions, accumulation of cortisol following stress acts to reduce the release of the glucocorticoid and reduce circulating levels in the body.\(^\text{26}\) The diagram illustrates the inhibitory effect of cortisol on each component of the HPA axis.
The current literature suggests that dietary macronutrient composition has the potential to alter cortisol levels in relation to exercise, and possibly, over a longer duration.\textsuperscript{38,39,11,40,41} Without proper amounts of carbohydrate to replenish glycogen and maintain blood glucose following exercise, cortisol is secreted in order to maintain blood glucose.\textsuperscript{31,42} This is achieved through muscle proteolysis, amino acid oxidation and gluconeogenesis.\textsuperscript{43} A reduction in blood glucose level has been linked to HPA activation, increased release of adrenocorticotropic hormone (ACTH) and cortisol in addition to other abnormalities.\textsuperscript{8,27} Interactions between exercise, macronutrient fueling and HPA axis activity could have a potentially large acute and chronic impact on homeostasis.
Clearly the HPA axis is a complex and intricately intertwined regulatory system. Imbalances for any reason can lead to irregular function of the entire axis. Over time, unusual, or extreme, environmental and physiological conditions can cause shifts in the normal circadian rhythm of cortisol in an individual. For instance, cortisol values can remain chronically elevated in athletes exposed to increased physiological stress as a result of overtraining. On the other hand, while the HPA axis may become somewhat suppressed during periods of high volume training, patterns in cortisol levels can produce conflicting trends. In the study by Bobbert et al, recreational marathoners demonstrated better HPA axis function once training volume decreased. However, serum cortisol remained consistent and within the normal range throughout the study.

The fluctuation in cortisol following a period of exercise in healthy individuals, under normal conditions, makes it difficult to determine the long-term implications of

Figure 3 – HPA Axis/Cortisol Negative Feedback Loop

![Diagram of the HPA Axis/Cortisol Negative Feedback Loop]
elevated cortisol levels as a result of increased stress. Physiological stress has the potential to cause chronically elevated or chronically depressed cortisol. Analysis of serum cortisol in relation to variations in dietary macronutrient intake and level of physical activity may lend insight into the influence of nutrition on these endurance adaptations.

Abnormally elevated cortisol values can also be present in certain disease states where the normal rhythm of the HPA axis is disrupted. Polycystic Ovarian Syndrome (PCOS) and Cushing’s syndrome are two conditions that often present with elevated levels of cortisol. While Cushing’s syndrome provides an extreme example of the effect of cortisol hypersecretion, it is useful in demonstrating some of the negative ramifications of prolonged elevation of cortisol. Assessment of body composition in women with glucocorticoid excess and Cushing’s syndrome has demonstrated significantly greater android fat mass and lower lean body mass compared to healthy women. These conditions, involving endocrine malfunction and chronically elevated levels of circulating cortisol are related to significant increases in mortality due to comorbidities including diabetes and cardiovascular disease. Cortisol is an integral hormone in the regulation of stress and normal body function; however, variations in normal secretion can lead to adverse health consequences.

While the mechanism of pathophysiology has yet to be clearly elucidated, chronically elevated cortisol is implicated in a greater distribution of fat to the visceral depot. In addition to elevated cortisol secretion, both PCOS and Cushing’s Syndrome are commonly associated with obesity and an increase in VAT. Research has demonstrated that premenopausal women with Cushing’s syndrome experienced a significant decrease in plasma and serum cortisol and VAT following treatment as determined by a computed tomography (CT) scan. The link between serum cortisol
and VAT is clearly present in certain disease states; however, it is more difficult to evaluate this relationship in apparently healthier individuals.

Obesity and the subsequent presence of an inflammatory state is a known contributor to many diseases including diabetes, hypertension and cardiovascular disease. Cortisol rhythm and changes in adipose tissue distribution may play a role in contributing to these chronic diseases.\textsuperscript{9,18,51} Data in obese, premenopausal females, demonstrates an association between urinary and serum cortisol and central adiposity indicated by higher waist-to-hip ratio (WHR).\textsuperscript{52} A prolonged elevation of cortisol could be detrimental to long-term health. There is considerable evidence from clinical, to cellular and molecular studies that elevated cortisol, particularly when combined with secondary inhibition of sex steroids and growth hormone secretions, causes accumulation of fat in visceral adipose tissues as well as metabolic abnormalities.\textsuperscript{17} Similar to PCOS, female endurance athletes may experience menstrual disturbances and dyslipidemia\textsuperscript{53} that are likely related to a heightened cortisol response to diet and training. Exploration of cortisol relative to dietary macronutrients, energy availability and volume of exercise may demonstrate important correlations to these maladaptations and long-term health consequences.

**Visceral Adipose Tissue (VAT)**

Adipose tissue is a metabolically active organ containing mature adipocytes, preadipocytes, vascular, neural and immune cells and is responsible for modulating many of these obesity-related risk factors.\textsuperscript{18} Specifically, regional differences in fat distribution are important in determining the risk of developing these obesity-related diseases, with an increased focus on an android (central) fat pattern.\textsuperscript{50,54}

Abdominal adipose tissue can be divided into two types of adipose tissue by a membranous layer of fascia.\textsuperscript{56} Visceral adipose tissue is contained behind this layer of
fascia, closer to vital organs. In particular, visceral adipose tissue is metabolically active and appears to be important for the pathogenesis of insulin resistance, dyslipidemia, glucose intolerance, hypertension and cardiovascular risk. Physiological conditions related to accumulation of VAT are linked to several disease states where overweight and obesity is common. It remains to be determined if relatively higher VAT is associated with the same disease conditions in a more active population.

The metabolic syndrome is a condition that demonstrates the link between VAT and chronic health issues. Metabolic syndrome is a constellation of risk factors for cardiovascular disease, type II diabetes mellitus, stroke and kidney disease. Symptoms of metabolic syndrome include insulin resistance, dyslipidemia, central obesity, hypertension, glucose intolerance and increased atherosclerotic disease as defined by the World Health Organization (WHO) and the American Heart Association (AHA). In 2010, nearly one-fifth of the US population could have been classified as having metabolic syndrome, with a greater percentage of women being affected by the syndrome. Population data from 1999 to 2010 demonstrated that the prevalence of central obesity in the US increased from 45% up to 56%, particularly in females. The details of metabolic syndrome are beyond the scope of this paper, however, central adiposity as a risk factor for the syndrome demonstrates the potential health risks associated with the presence of VAT.

It is widely accepted that overweight and obesity increase the risk of long-term health conditions. Additionally, chronically elevated circulating cortisol and the accumulation of VAT are each detrimental to health, and may also lead to chronic health conditions. However, the link between elevated cortisol and an increase VAT, in particular, may contribute more to the development of these chronic health concerns than an increase in weight alone. Therrien et al found that obese subjects with an
accumulation of visceral adipose tissue demonstrated increased awakening cortisol levels.\textsuperscript{57} Furthermore, visceral fat distribution in both lean and obese subjects has been associated with an increase in cortisol secretion following physical and psychological stressors.\textsuperscript{16,20,45,58} To date, no research has attempted to identify patterns between cortisol and visceral adipose tissue in endurance athletes. It remains to be seen if interactions between exercise stress, awakening serum cortisol and macronutrient intake in recreational runners produces fat distribution patterns similar to those seen in sedentary, overweight individuals.

**Measurement of VAT**

Since the distribution of adipose tissue, not just overall weight, is important to determining health risks, the method of measurement is of critical importance. Anthropometric measurements, such as skinfolds and circumferences, are inexpensive and easily portable\textsuperscript{59}, making them an attractive option. However, body circumferences can give inaccurate estimates of body fat because muscle, connective tissue, bones and body water are included in these measurements in addition to the body fat. Skinfolds can provide approximate measure of subcutaneous adipose tissue in the different regions of the body. Research investigating the correlation of different abdominal measurements demonstrated that anthropometric measurements show a stronger correlation with total adipose tissue and subcutaneous adipose tissue, than for visceral adipose tissue.\textsuperscript{60} While relatively easy and cost-effective, neither of these measurement methods can accurately differentiate between visceral and subcutaneous adipose tissue.

Magnetic resonance imaging (MRI) and CT scan are more accurate, but costly, methods of determining amounts of subcutaneous and visceral adipose tissue.\textsuperscript{60} Both of these methods/machines are also frequently used in the hospital, making them difficult to schedule for use. MRI and CT assessment of VAT yield a 2-dimensional
measurement of adipose tissue (AT) area in cm² based on a single slice analysis of the MR or CT image taken from the lumbar-4 and lumbar-5 (L4 and L5) intervertebral space.60,61 It is possible to create a volumetric measurement of VAT using MRI and CT62; this can be done by performing several single slice analyses in order to build a 3-dimensional picture of the adipose tissue. While this method provides a better picture of abdominal adipose tissue, it is also more time-consuming and, when using CT, requires additional exposure to radiation.60,61 A total-body MRI has the potential to provide a more complete picture of AT distribution; however, this measurement takes over 30 minutes to complete.61 Despite the fact that MRI and CT typically only account for AT present in a single slice of the abdomen, and that a CT uses a relatively high amount of radiation, CT is the current gold standard for measurement of internal organs and tissues.61

Recently, a new method of quantifying VAT using the iDXA has been developed. This method, called CoreScan, is software developed by GE healthcare for use with the GE Lunar iDXA. It provides a relatively easier and lower radiation means of estimating visceral adiposity. Recently approved by the US Food and Drug Administration (FDA), the GE CoreScan is an algorithm for the volumetric estimation of VAT.63 The iDXA VAT measurement has been validated using volumetric CT as the reference standard.62 CoreScan is able to detect the width of the subcutaneous adipose tissue (SAT) layer on the lateral aspects of the abdomen, and the anteroposterior (AP) thickness of the abdomen in order to compute VAT within the android region.62 This region is generally around 10 cm in height and extends from the iliac crest towards the chin.62 The GE CoreScan algorithm has been validated for use in adults ages 18-90 with BMIs in the range of 18.5-40kg/m².62 In this validation data, volumetric CT was compared to CoreScan iDXA measurements in 124 men and women. The 95% confidence interval for the mean of the differences between CT and DXA visceral fat volume was −96.0 to
−16.3 cm³ with a Bland–Altman bias of +67 cm³ for females and +43 cm³ for males. A large range of BMIs was used to validate this technology. This provides useful guidelines for analysis in the general population, but may prove less applicable in an active, relatively normal BMI research population such as included in this study. However, studies have indicated that the precision of this algorithm in identifying VAT would be useful in research examining android fat distributions in a variety of subjects.⁴,⁶⁴ The active individuals included in this study may possess lower VAT due to regular physical activity; however, the CoreScan algorithm allows for a very specific, new evaluation of body composition of the visceral fat compartment. This new technology will be useful in assessing visceral fat among our recreational female runners.

**Physiology of Endurance Exercise**

Recreational running among females is a particularly popular means of exercise and the cardiovascular and general health benefits of endurance training are well documented,⁶⁵–⁶⁷ however, exercise presents a unique stress to the homeostasis of the body. The degree of the stress response is dependent on numerous variables including environment and nutritional status. Adaptations achieved through consistent exercise reduce the physical demand of future exercise on the body’s homeostatic limits.³¹ These physiological adaptations allow for changes that lead to increases in fitness following consistent exercise.

**Female Athlete Triad and Relative Energy Deficiency in Sport**

Despite increased energy requirements, energy restriction is common in females who undertake endurance exercise as a means of maintaining or losing weight.²²,³⁸ The negative effects of chronic low energy availability (LEA) are outlined in the most recent International Olympic Committee (IOC) position paper: Relative Energy Deficiency in Sport (RED-S), and by the more established Female Athlete Triad (FAT) as positioned
by the American College of Sports Medicine (ACSM). The three components outlined by the triad are 1) low energy availability, with or without disordered eating, 2) menstrual dysfunction, and 3) low bone mineral density. The triad is well established in the literature as a spectrum along each component. However, the more recent RED-S proposes that low energy availability has numerous maladaptive physiological responses in addition to the three triad components. RED-S is defined as energy deficiency relative to the balance between dietary intake and energy expenditure in physical activity, activities of daily living and physiological functions. RED-S proposes that chronic energy deficiency “is no longer only a triad, but rather a syndrome that affects many aspects of physiological function, including metabolic rate, menstrual function, bone health, immunity, protein synthesis and cardiovascular and psychological health”. This energy deficiency is the likely outcome of under-consumption of both micro- and macronutrient levels below adequate amounts for adaptations to, and recovery from, exercise.

**Energy Availability**

Energy availability (EA) is the amount of dietary energy remaining after accounting for the energy spent in exercise, and is the basal energy available for all other physiological processes. EA is calculated by subtracting exercise energy expenditure (EEE) from dietary energy intake (EI), divided by (adjusted for) kilograms of fat free mass (FFM).

\[
EA = \frac{(EI - EEE)}{FFM}
\]

Logically, exercising individuals will have greater energy expenditure and, therefore, require a greater energy intake in order to maintain appropriate energy availability for metabolic processes. Chronic deficits in energy availability are associated with metabolic suppression. Furthermore, adequate energy intake is necessary for optimal performance and recovery, and ensures that the body can
maintain normal physiological function. Accordingly, low EA can lead to numerous adverse health conditions in addition to poor exercise performance especially when the deficit occurs over an extended period of time.

The current literature states that female athletes should aim for 45 kilocalories/kilogram fat free mass (kcal/kg FFM) per day of energy intake to ensure optimal energy availability for all physiological functions. Research by Loucks compared exercising and non-exercising women to establish an estimated threshold reflecting when hormone activity is suppressed in relation to low EA. Low EA is defined as 30kcal/kg FFM, and represents the lower threshold on this spectrum. The intermediate range of 30 – 45kcal/kg FFM is considered suboptimal for female athletes and can have adverse effects on body functions. Chronically low EA has the potential to disrupt HPA axis and hormone activity, potentially causing long-term health concerns.

**Methods of Determining Energy Availability**

EA in exercise includes dietary energy intake (EI) minus energy expended in exercise (EEE) and normalized by fat free mass (FFM). Several measurement methods exist for EI, EEE and FFM. Accurate estimation of these three components is critical for overall accuracy of EA and its relation to the variables being measured in this study.

Several collection methods exist for measuring EI. These include self-reported three-day food record, food frequency questionnaire (FFQ) or 24-hour recall. Accuracy of EI measurement is a delicate balance between ensuring a collection period which is long enough to reflect habitual intake, while also making data recording or recall as easy as possible to attain high compliance. Along these lines, previous research has demonstrated that diet recording for longer than three days decreases compliance. A three-day food record has also been shown to be a reliable tool to estimate dietary intake compared to a FFQ. The most highly recommended method to date is a
combination of several 24 hour recalls and a FFQ\textsuperscript{73}; however this is a time consuming, expensive, and has a high participant burden. In order to assess total dietary nutrient composition and caloric intake, all dietary data from food records of recalls must be accurately entered into a nutrient analysis program. A nutrition analysis program such as the ESHA Food Processor SQL is a valid tool for nutrient analysis in a research setting.\textsuperscript{72}

Numerous measurement devices are available to estimate EEE. Examples of some of these devices include accelerometers, heart rate monitors and activity questionnaires. Cost plays a large role in this area of data collection, as heart rate monitors and accelerometers are relatively expensive and therefore difficult to use in large studies. The Bouchard physical activity grid is a self-report questionnaire that is more cost-effective and has been validated and demonstrated a strong linear relationship with the Tritrac accelerometer.\textsuperscript{74,75} The grid divides a 24-hour day into 15-minute intervals and subjects report their average activity intensity on a scale of 1 to 9, with a 6 or greater indicating exercise.\textsuperscript{74} The Bouchard grid offers examples of activities at every intensity level on the scale so that individuals can accurately rate themselves. Each level of the scale is related to METS, so this tool can be used to estimate calories when combined with body weight.

**Metabolic Fuel for Endurance Exercise**

Fat and carbohydrate are the primary fuel sources used by muscle during aerobic exercise. Intramyocellular triglycerides (IMTGs) and plasma free fatty acids (FFAs) are the primary sources of fat, while muscle glycogen and blood glucose are the major sources of carbohydrates provided to working muscle.\textsuperscript{22,76} Proper provision of these substrates is necessary for adaptation and maintenance of homeostasis following endurance exercise.
Carbohydrate

Carbohydrates provide fuel for normal physiological function and for physical activity. Depending on the intensity of exercise, carbohydrates are utilized in varying amounts. Adequate carbohydrate intake is generally the first macronutrient considered for optimal recovery from endurance exercise and inadequate carbohydrate intake has been specifically implicated in overtraining syndrome.44,79,80

A diet providing adequate calories and consisting of appropriate amounts of both macro- and micronutrients is crucial to adaptation and recovery from the stress of exercise. For females engaged in exercise for 30-60 minutes/day, 3-4 times/week, the general carbohydrate recommendation is 3-5 grams per kilogram body weight per day (g/kg BW/day) according to the International Olympic Committee (IOC).1 Those involved in moderate- to high-intensity volume, 2-3 hours/day, 5-6 times/week would require as much as 6-8 g/kg BW/day.1,78 This relatively high carbohydrate intake is usually recommended due to the role of carbohydrates in maintaining glycogen stores during endurance exercise.78 Other authors have suggested that carbohydrate status is best considered in terms of “low carbohydrate availability” or “high carbohydrate availability”.38 These terms address whether total daily intake and timing of carbohydrate in relation to exercise, maintains an adequate supply of carbohydrate substrate for the muscle and CNS (high availability), or whether carbohydrate fuel sources are depleted for the daily exercise program (low availability).38 While total daily intake and nutrient timing are potentially valuable aspects of assessing dietary adequacy in athletes, current recommendations for overall dietary carbohydrate consumption are measured in g/kg BW/day.

Given the historically integral role of carbohydrate, several studies support the importance of carbohydrate to adaptations for endurance exercise. Indeed, increased
muscle glycogen content has been noted among well trained athletes following short
term increases in carbohydrate intake.\textsuperscript{81} Additionally, some studies have demonstrated
increases in resting stress hormone levels, such as cortisol, following endurance
exercise training on a low carbohydrate diet.\textsuperscript{11,13,39,82,83} It remains to be seen if this
increase in cortisol secretion has the same long term impact on body fat distribution in
apparently healthy, active individuals, as is seen in disease states marked by elevated
cortisol.

Studies examining the effects of endurance exercise while consuming diets
differing in macronutrient composition have also demonstrated alterations in cortisol
values post exercise. Mitchell, et al\textsuperscript{82} included 10 men completing a glycogen-depleting
bout of cycling followed by 48 hours of either a high carbohydrate (8.0g CHO/kg) or low
carbohydrate (0.5g CHO/kg) isocaloric diet.\textsuperscript{82} Subjects completed a VO\textsubscript{2max} and serum
blood draw at baseline then completed a glycogen-depleting bout of cycling followed by
the dietary intervention. Food was provided for all subjects to ensure adherence to high
or low carbohydrate diet. After the 48-hour dietary intervention, subjects completed a 60-
minute ride at 75% VO\textsubscript{2max}. Blood samples were taken before exercise, immediately after
exercise, as well as 2 and 24 hours after exercise. Cortisol values of all of the subjects
were significantly elevated in the low carbohydrate group compared with the high
carbohydrate group immediately following exercise. The low carbohydrate group also
had higher serum cortisol 2 and 24 hours post-exercise although these values were not
significantly greater than serum cortisol in the high carbohydrate group. While this was a
relatively short dietary intervention, the acute depletion of glycogen stores in this study
may give insight into the potential impact of a chronically carbohydrate restricted diet.

Furthermore, Slivka et al\textsuperscript{84} tested differences in hormonal response to exercise in
a state of negative energy balance while ingesting either caffeine plus carbohydrate, or
caffeine alone.\textsuperscript{84} The study demonstrated that the ingestion of caffeine alone, during exercise, increased salivary cortisol. However, the increase in cortisol was nearly eliminated when carbohydrate was co-ingested with caffeine. Additional literature has demonstrated a markedly higher magnitude of cortisol following endurance exercise when individuals consumed a very low carbohydrate diet (<10\% of dietary intake) versus a normal or high carbohydrate diet.\textsuperscript{39,40} In the context of endurance exercise, carbohydrate has been well associated with attenuating the cortisol response.

In another trial, Costa et al\textsuperscript{11} used a 6-day dietary intervention to investigate the impact of dietary carbohydrate content, with an increased exercise load, in 32 male Olympic and Ironman triathletes. Baseline \( \text{VO}_2\text{max} \) and salivary cortisol was completed along with a 7-day food diary. The athletes were randomly divided into a self-selected carbohydrate (SS, \( n = 16 \)) intake or a high carbohydrate (H-CHO, \( n = 16 \)) intake. The SS group continued to consume their regular diet, which was reflective of a typical endurance athlete’s diet (58\% carbohydrate, 18\% protein and 24\% fat), while the H-CHO group was provided with a 12g/kg carbohydrate diet for the 6-day intervention. In addition to their normal training routine, subjects completed an hour long run at 70\% \( \text{VO}_2\text{max} \) to provide an increase in training load. Salivary cortisol was measured pre- and post- exercise, as well as the morning after, on days 1, 4 and 6. The SS and H-CHO groups had similar cortisol before the intervention, however the SS group had a significant increase in salivary cortisol pre-exercise to morning after (days 1, 4 and 6), and pre- to post-intervention. On the contrary, the H-CHO group had a decrease in cortisol following the intervention although it was not significant. Even more importantly, the H-CHO group demonstrated a significantly greater reduction in morning after cortisol values when compared to the SS group.\textsuperscript{11} This is significant because the high carbohydrate group experienced the expected increases in cortisol immediately after
exercise, but appeared to be better equipped, perhaps in terms of metabolic substrates, to return cortisol back toward baseline levels. Carbohydrate consumption during exercise attenuates rises in cortisol for non-fatiguing bouts of exercise.\textsuperscript{39,83}

While there are some studies in female endurance athletes, a great deal of the current literature was performed in male subjects. More research is needed among female populations. Still, the existing research demonstrates different physiological responses, including alterations in cortisol secretion, to differing levels of carbohydrate intake in endurance athletes.

**Protein**

Protein is currently the only dietary macronutrient with a recommended dietary allowance (RDA), established by the U.S. Food and Nutrition Board in 1989.\textsuperscript{85} The RDA of 0.8 grams/kilogram of body weight is in reference to the general population and does not include special recommendations for physically active individuals. Research since the establishment of the RDA has indicated that increased protein intake, to a point, is beneficial for active individuals and athletes.

For endurance exercise, early and landmark research compiled by Peter Lemon estimated that protein intake should be approximately 1.2 – 1.4 grams per kilogram, body weight (g/kg BW) to maintain nitrogen balance.\textsuperscript{86} Higher protein requirements of at least 1.2 g/kg BW are supported by guidelines established by both the International Olympic Committee (IOC) and the American College of Sports Medicine (ACSM).\textsuperscript{1,7} Increased protein is likely needed due to increased amino acid oxidation and increased muscle protein synthesis to minimize and repair any damage from prolonged aerobic exercise.\textsuperscript{86} As well, when female runners are eating to achieve weight loss, there may be a slightly higher protein requirement to maintain lean mass.\textsuperscript{86}

Protein intake plays a role in post-exercise recovery, however, its impact on
cortisol and the stress response varies. Studies investigating the relationship between short-term increases in protein ingestion, endurance exercise and the stress response have found inconclusive variations in cortisol. The long-term implications of dietary protein content on the stress response and recovery from endurance exercise require further research, and should consider the blend of other macronutrients.

A small study conducted by Rowlands et al. in 10 endurance-trained male cyclists explored the short-term effect of varying dietary protein on cortisol in response to endurance exercise. The participants were equal in physical and training characteristics as well as VO$_{2\max}$. For the trial, the research team took baseline cortisol measurements, a three-day food diary and VO$_{2\max}$ test. Subjects then completed high-intensity intervals until exhaustion. Following the test, half of the men were provided a low protein (0.8g/kg), high carbohydrate diet, while half received a higher protein (1.2g/kg), high carbohydrate diet to consume for the next 2 days. Carbohydrate and calories between groups were not significantly different throughout the study. After the 2-day dietary intervention, the cyclists completed the same bout of exercise to exhaustion. While the high protein group reported lower perceived stress during exercise, serum cortisol values measured post-exercise and the morning after were not significantly different in either group. This is a very short dietary intervention, but it does support the lack of a solid link between protein and cortisol in endurance athletes as well as the value of higher dietary protein in the recovery phase of exercise.

Another study in trained cyclists consuming a 6g/kg carbohydrate, and either a high protein (3g/kg) or normal protein (1.5g/kg), isocaloric diet, over a 3 week block of normal, intensified and recovery training fails to provide a strong link between much higher dietary protein and cortisol. Cyclists in each group (n = 8 for both) completed a baseline fasting blood draw and VO$_{2\max}$ followed by three weeks of training and dietary
intervention. After three weeks, subjects completed another VO$_{2\text{max}}$ test. Final data showed no significant difference in serum cortisol measured immediately after exercise or the following morning. However, similar to the previous study, the higher protein group reported lower perceived exertion during the intense training phase.$^{87}$ The literature surrounding dietary protein and cortisol in endurance exercise points to the potential benefits in perceived exertion, but fails to provide a consistent link between protein and cortisol. Still, many of these studies are relatively short and performed among small groups of male athletes, which limits generalizability. More research is needed among female athletes.

**Fat**

In addition to carbohydrate and protein, dietary fat content also plays an important role in training and recovery for endurance exercise. As mentioned previously, fat provides fuel for endurance exercise in the form of intramyocellular triglycerides and plasma free fatty acids.$^{22,76}$ In comparison to carbohydrate and protein intake recommendations, which are considered in terms of grams/kilogram of body weight, fat intake is generally assessed as a percentage of overall dietary composition. Traditionally, the recommended adequate dietary fat intake for endurance athletes ranges from 25-30% of total dietary intake.$^{22}$

While fat is important for endurance athletes, some research has demonstrated that female runners with restrictive eating patterns, compared to those meeting energy requirements, tend to consume adequate carbohydrate and protein, while significantly limiting fat intake.$^{90}$ This particular study by Tomten and Høstmark assessed dietary intake and resting hormone status in 10 women with regular menstrual function, and 10 women without regular menstrual function. The groups were similar in training volume, physical characteristics and previous race performance levels. However, the women with
irregular menstrual status consumed an average of 600 fewer kcals per day, and were in negative energy balance according to a 3-day food diary and resting metabolic rate (RMR).\textsuperscript{90} While irregular menstrual status can be indicative of hormonal abnormalities\textsuperscript{2}, this group of female runners demonstrated fasting serum cortisol values that did not differ significantly between groups despite the reduced calorie and fat intake within the restricted-eating group.\textsuperscript{90} Fat restriction, among individuals attempting to achieve weight loss in particular, may be attributed to the relative caloric density of fat (9 kilocalories/gram (kcal/kg)) versus carbohydrate and protein (4kcal/kg).\textsuperscript{91}

A study with healthy and highly fit recreational male and female runners compared serum stress hormone levels in response to endurance exercise and after a 4-week dietary intervention. Venkatraman et al\textsuperscript{92} had subjects consume a 15% (of daily energy) fat diet (n = 7 women, 7 men), 30% fat diet (n = 9 women, 6 men) or 40% fat diet (n = 8 women, 6 men) for four weeks. Protein was held constant at 15% of dietary intake, energy intake was matched for body mass and activity level, and meals with the appropriate macronutrient distribution were provided over the intervention. Subjects had serum hormones measured at baseline, then before and after a VO\textsubscript{2max} test at the end of the dietary intervention. Subjects completed food diaries for the duration of the study. While some meals were provided, the food records shown from their study table (figure 4), demonstrate an average increase of 400kcal and 10g of protein with increasing dietary fat content.

Final data showed that serum cortisol levels after dietary intervention and before the VO\textsubscript{2max} test were significantly higher among men and women who consumed the 40% fat diets. All serum cortisol levels were significantly higher after the endurance run compared to baseline levels on all diets (p < 0.004). The post-exercise plasma cortisol level also significantly increased with increasing dietary fat (15% F vs. 40% F = p <
From a performance standpoint, it is interesting to note that runners consuming the 30% fat diet were able to run significantly longer after the intervention, while those in the 15% and 40% fat groups did not experience the same improvement. Resting data collection from pre- to post-intervention highlights the fact that the 15% fat group maintained serum cortisol levels but had no improvement in exercise time and the 40% fat group had both elevated serum cortisol and no improvement in exercise time, while the 30% fat group maintained serum cortisol and was able to exercise longer. This performance and serum cortisol data seems to support the current recommendation that endurance athletes consume 25-30% of total energy from dietary fat. It may also be important to remember that the carbohydrate in these diets may have been too high to allow for adaptation to fat as the primary fuel in a muscle system where carbohydrate is the physiological preference.

Another study in eleven healthy, endurance-trained male athletes of similar fitness levels demonstrated that a 3 day high fat (HF) vs. a 3-day low fat (LF) diet had little impact on cortisol values after a 3-hour endurance ride. Participants had fasting baseline values drawn and then completed this randomized crossover design following
two types of diet: 1) LF – 0.5 g fat/kg body weight (BW) per day for 2.5 days, or 2) HF – 0.5 g fat/kg BW per day for 1 day followed by 3.5 g fat/kg BW per day for 1.5 days. Carbohydrate and protein were kept within appropriate ranges at 7g/kg BW and 1.2g/kg BW respectively. The data showed that cortisol remained within normal limits and was not significantly different between diet groups\(^3\). These studies provide insight into the relationship between macronutrients by demonstrating that adequate carbohydrate is important while fat has relatively less influence when it is not displacing carbohydrate in the diet.

However, both of the previous studies demonstrate a common limitation in diet, and particularly fat, manipulation in that it is difficult to keep calories constant between groups so that the effect of fat may be assessed with similar carbohydrate and protein intake. Both of these studies led to higher overall calorie intake on the high fat diets, which could impact hormone levels. This hurdle remains as one of the limiting factors among studies assessing the outcomes of a high fat diet. In addition, the long-term adaptation to various diet manipulations should be considered, as any new diet manipulation likely requires a period of adaptation in terms of body stress.

Recently, the literature surrounding dietary fat content in endurance athletes has expanded to evaluate the possibility of using fat as a primary fuel source during exercise by limiting carbohydrate in the diet. In the scope of the traditional diet, where the majority of the energy intake comes from carbohydrates, a high fat diet has been discouraged due to the potential link to many chronic diseases\(^{94,95}\). In particular, a diet high in saturated fat has been implicated in abnormalities in mineral absorption and utilization and several chronic disease states\(^{94,96}\). While these have been the traditional concerns regarding dietary fat content, research over the past decade has become increasingly supportive of the potential benefits of high fat diets in place of the typical high
carbohydrate diet. These high fat diets would differ from those discussed in that they would limit carbohydrate long enough for the body to adapt before evaluating the associated cortisol levels.

Recommendations for high fat diets are typically avoided because of the belief that this will lead to an increase in saturated fat intake and subsequent adverse health outcomes. Indeed, increasing fat intake while continuing to consume the same amount of carbohydrate and protein will likely lead to a calorie and nutrient surplus. However, research in both normal weight and overweight individuals has supported the potential benefit of a high fat, restricted carbohydrate diet for improving health. Intriguing benefits from this type of dietary modification, in the absence of any exercise intervention, include decreases in body fat and serum insulin, increases in lean body mass and improved glycemic control. A carbohydrate-restricted, high fat diet is also receiving additional attention for its potential usefulness among endurance athletes.

As previously discussed, the traditional endurance athlete’s diet includes a high proportion of carbohydrates to maintain glycogen stores for exercise performance. However, high fat, carbohydrate-restricted diets have become increasingly popular in an attempt to increase fat oxidation, thereby sparing muscle glycogen during endurance exercise; this concept is referred to as “fat adaptation” or ketoadaptation. The adaptation piece of this diet refers to the fact that these metabolic changes in substrate use do not happen immediately and require an adjustment period. When carbohydrate is restricted for a short period of time (< 1 week), muscle glycogen becomes depleted and endurance performance suffers. However, research among endurance athletes has demonstrated that a high fat, carbohydrate-restricted diet can trigger alterations in metabolic substrate utilization within a period of just 2 weeks. When used properly,
these diets are not energy restricted in endurance athletes, as is commonly the case with low carbohydrate diets\textsuperscript{105}; they reduce carbohydrate to 10-15\% of caloric intake, maintain protein at an adequate level and increase dietary fat to meet energy needs.\textsuperscript{102,105}

Once fat adaptation occurs, endurance athletes appear to experience improvements in performance as well as decreased exercise stress and improvements in some lipid values. Phinney et al\textsuperscript{104,105} demonstrated that cyclists consuming an isoenergetic high fat diet, compared to a traditional high carbohydrate diet, demonstrated improvements in cycling time to exhaustion, decreased respiratory quotients (RQ) and decreased muscle glycogen utilization after a four week dietary intervention. In addition to performance improvements, Thompson et al\textsuperscript{106} noted better low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglyceride levels among endurance runners consuming a high fat diet after a 14-day intervention. In this study, the runners consumed isoenergetic diets composed of either 69\% carbohydrate or fat. While both groups had lipid and triglyceride levels above the average sedentary individual, the high fat diet group had a more positive result\textsuperscript{106}. This line of research demonstrates the potential influence of dietary fat on both health and performance in endurance athletes and the general population. Additionally, the relatively recent focus on fat adaptation, illustrates the potential for confounding factors when using short-term diet histories or protocols to assess the impact of dietary macronutrients. It will be interesting to see how the long-term keto-adapted diet fares in terms of cortisol stimulation as this line of research continues.

Clearly the physiological effects of individual dietary macronutrients are difficult to assess due to the fact that each component of diet, and total energy intake, are inextricably intertwined and physiological manifestations can rarely be attributed to just
one. However, macronutrient recommendations for athletes can provide a range of intakes with which to assess dietary adequacy. Exploring the recommended macronutrient levels in relation to hormonal and physical alterations can provide valuable insight into how we might modify dietary recommendations in the future.
Chapter 3: Methods

This project is a retrospective analysis of a primary study on adult female runners, and thus the methods and data collection are limited to the previously executed protocol. The original study was set up to identify evidence of the female athlete triad in recreational female runners (IRB approved protocol 2009H0177). The study team recruited 125 women, ages 18 and older, whom were running at least 15 miles per week for the immediate 6 weeks, and had no pre-existing thyroid, adrenal or bone issues. Several recruitment venues including running clubs, fliers given at a science museum and local road races in Columbus, Ohio, were used to reach potential participants. Due to inaccuracy of self-reported menstrual status around menopausal years, complete data in the original study was evaluated from 101 premenopausal women. This narrowed the ages of the premenopausal women to between 18 and 50 years of age. Similarly, these women had no known thyroid abnormalities, health issues or medications that would adversely influence bone mass.

A standard written three-day food record was used to collect dietary intake data from each of the female runners. Following submission of the food record, one of three researchers reviewed the dietary data with the participant to fill in missing information and ensure the highest possible accuracy level. After collection, data was entered into the professional grade ESHA Food Processor SQL version 10.2 and triple checked by different researchers to determine dietary intake. The data were compiled and variables, including total kilocalories and grams of carbohydrate, protein and fat, were exported to an excel spreadsheet.
Energy expenditure and duration of usual exercise were estimated using the Bouchard physical activity tool.\textsuperscript{74,75} The Bouchard physical activity grids were counted and triple checked by different research assistants, then frequency counts were entered into an excel spreadsheet and calories expended were calculated per tool instructions. Exercise energy expenditure (EEE) was determined using the self-reported Bouchard physical activity grid, where a level 6 or higher is considered exercise.\textsuperscript{58} The average frequency of 6 or higher was multiplied by 15 minutes to estimate usual duration of exercise, in minutes, to be used in determining adequacy of carbohydrate intake.

Collection of morning serum cortisol was completed in a fasted state, after an overnight rest, to reflect the serum cortisol values.\textsuperscript{59} Serum cortisol collection was completed between 7am and 9am, with an occasional appointment at 9:30 or 10am. Data collection times were coordinated with the normal waking schedule of individual participants so that serum cortisol values would reflect typical early morning values for each subject. Normal serum cortisol concentrations are higher in the early morning, ranging from 10 to 20 \( \mu g/dL \) (275 to 555 nmol/L).\textsuperscript{60,61} The samples were analyzed at the Wexner Medical Center laboratory using competitive chemiluminescent immunological reaction with para-magnetic particles as the solid phase, and acridinium ester as the chemiluminescent label.\textsuperscript{62} Values were recorded in \( \mu g/dL \).

As previously mentioned, the GE Lunar iDXA was used in this study for body composition and partitioning of the VAT became possible with the additional CoreScan\textsuperscript{107} software. A two-compartment model of body composition was estimated by the GE lunar iDXA densitometer to determine fat mass and fat free mass (FFM) for participants.\textsuperscript{3,108} The iDXA is a relatively easy, low radiation means of measuring body composition, and is recognized as one of the more accurate measurements of fat and fat free mass.\textsuperscript{3,108} The same operator conducted and analyzed all iDXA scans to ensure
accuracy of the data. The iDXA protocol required height and weight measurements, where heights were taken using a stadiometer (nearest cm) and weights were taken with a Healthometer scale (to the nearest tenth of a pound). Use of the iDXA is central to this analysis of body composition variables. The lean body mass (including bone) was used in the energy availability calculation.

For the current project, total body scans were reanalyzed using the GE EnCore version 15 software to determine the amount of visceral adipose tissue. CoreScan provides VAT measurements in both mass (lbs) and volume (in$^3$). Studies validating the CoreScan algorithm used volumetric CT and iDXA VAT; therefore, volumetric measurements of VAT were chosen for use in this study. VAT volume and body fat measurements obtained from the iDXA scan were compiled and entered into the excel spreadsheet containing all other data.

**Statistical Analysis of Research Questions**

The compiled data was imported into SPSS software program for analysis. Statistical methods are described below:

1. **Does a pattern exist between dietary macronutrient content and morning serum cortisol values in recreational female runners?**
   
a) **Does the level of dietary carbohydrate intake (based on amount of daily exercise as defined by current recommendations) correlate with serum cortisol levels?**

Carbohydrate is the only macronutrient with specific recommendations in grams/kilogram/day, based on the hours of daily exercise. According to the American College of Sports Medicine (ACSM), “athletes” should consume 6 – 10g/kg/d, while the International Olympic Committee (IOC) recommends 6 – 10g/kg/d for endurance exercise of 1 – 3 hours per day. Therefore, in an alternate analysis, the relationship of
cortisol and carbohydrate status was examined, considering the minutes of physical activity completed per day as determined using data from the Bouchard data.

For the analysis, Bouchard Grid variables ranked 6 and up were transformed to hours to determine the amount of time devoted to exercise each day. After determining the time of exercise per day, carbohydrate adequacy was categorized in terms of the ACSM and IOC guidelines. Participants exercising for less than 1 hour were classified as consuming adequate carbohydrate if they were consuming at least 3g/kg BW/d and they were classified as consuming inadequate carbohydrate if they consumed less than 3g/kg BW/d. Participants exercising for over 60 minutes were classified as consuming adequate carbohydrate if they were consuming at least 6g/kg BW/d and they were classified as consuming inadequate carbohydrate if they consumed less than 6g/kg BW/d. Cortisol was treated as a continuous variable in order to determine the relationship between adequate or inadequate carbohydrate intake and serum cortisol levels. Cortisol levels were compared between groups using a standard independent samples t-test. While this examines the data similar to the procedure in question 1, it does allow a broader look at the same data.

<table>
<thead>
<tr>
<th>Exercise Time:</th>
<th>0</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 1 hour</td>
<td>Carb/kg &lt; 3</td>
<td>Carb/kg ≥ 3</td>
</tr>
<tr>
<td>More than 1 hour</td>
<td>Carb/kg &lt; 6</td>
<td>Carb/kg ≥ 6</td>
</tr>
</tbody>
</table>

Table 1 - Carbohydrate Adequacy by Time of Exercise

b. Does a pattern exist between the amount of dietary carbohydrate, in grams/kilogram, and serum cortisol values in female runners?

In order to analyze the data set, carbohydrate intake (reported in grams/kilogram body weight) and serum cortisol will be treated as continuous variables. To assess the
variables, a univariate model was used to determine the potential correlation of carbohydrate intake and serum cortisol.

\[
\text{Cortisol (μg/dL) = Dietary Carbohydrate (grams/kilogram)}
\]

c. Does a pattern exist between the amount of dietary protein, in grams/kilogram, and serum cortisol values in female runners?

Similarly, protein intake and serum cortisol were analyzed as continuous variables. A univariate model including grams/kilogram of protein and micrograms/deciliter of serum cortisol was used to model the data.

\[
\text{Cortisol (μg/dL) = Dietary Protein (grams/kilogram)}
\]

d. Does a pattern exist between the amount of dietary fat, in grams, and serum cortisol values in female runners?

As with carbohydrate and protein, fat intake and serum cortisol will be assessed as continuous variables. Fat will be analyzed in total grams, instead of grams/kilogram, as dietary recommendations for fat are not given in grams/kilogram.

\[
\text{Cortisol (μg/dL) = Dietary Fat (grams)}
\]

2. Do higher serum cortisol values coincide with greater visceral adipose tissue in female runners?

Analysis will treat VAT volume (in³) and serum cortisol (μg/dL) as continuous variables and will be analyzed using a regression model. Energy availability (EA) is a potential confounding variable; therefore, it will be included in the regression equation as an independent variable.

\[
\text{Cortisol (μg/dL) = VATvolume (in³) + Energy Availability (EA)}
\]
Chapter 4: Results

The final data analyzed in this study included 100 premenopausal female runners, excluding one subject found to have significant thyroid issues during the parent study. The average female in this nested study was nearly 33 years old, 64 inches in height and weighed 130 pounds with 25% body fat. Despite the fact that the study population consisted of female runners, these women demonstrated a fairly large range of weight and body fat percentage. Demographic data is detailed in table 2.

<table>
<thead>
<tr>
<th></th>
<th>Mean (± SD)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>32.7 (+/- 7.77)</td>
<td>18.40</td>
<td>49.50</td>
</tr>
<tr>
<td>Weight (lbs)</td>
<td>130.1 (+/- 17.32)</td>
<td>89.20</td>
<td>203.60</td>
</tr>
<tr>
<td>Height (in)</td>
<td>64.6 (+/- 2.43)</td>
<td>57.50</td>
<td>71.30</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>25.22 (+/- 5.22)</td>
<td>14.60</td>
<td>42.90</td>
</tr>
</tbody>
</table>

Table 2 - Demographic Data for 100 Premenopausal Female Runners

In addition to a wide range of weight and percent body fat, these female runners also demonstrated a large range of daily activity and calories consumed. According to the data collected, our average woman was consuming 1,890 calories per day. However, this average woman was also expending an average of 2,915 calories throughout the day, with about 550 of those calories expended during exercise. Similarly, our average participant had an EA of 32.6, classifying her as consuming just enough calories to fuel adequately, but not optimally, for her activity level.
### Table 3 - Energy Balance Data

<table>
<thead>
<tr>
<th></th>
<th>Mean (+/- SD)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories Consumed</td>
<td>1890.7 (+/ 436.83)</td>
<td>812.36</td>
<td>2916.63</td>
</tr>
<tr>
<td>Minutes of Exercise</td>
<td>77.7 (+/- 34.51)</td>
<td>15.00</td>
<td>180.00</td>
</tr>
<tr>
<td>Avg. Daily Calorie Expenditure</td>
<td>2915.0 (+/- 442.84)</td>
<td>1983.68</td>
<td>4168.03</td>
</tr>
<tr>
<td>Avg. Daily Calories Expended Exercising</td>
<td>554.7 (+/- 264.78)</td>
<td>61.85</td>
<td>1410.95</td>
</tr>
<tr>
<td>Energy Availability</td>
<td>32.6 (+/- 12.61)</td>
<td>7.30</td>
<td>57.50</td>
</tr>
</tbody>
</table>

### Table 4 - Macronutrient Consumption Chart

<table>
<thead>
<tr>
<th></th>
<th>Mean (=/- SD)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate (g)</td>
<td>250.7 (+/- 67.42)</td>
<td>96.57</td>
<td>399.57</td>
</tr>
<tr>
<td>Carbohydrate (g/kg)</td>
<td>5.8 (+/- 1.73)</td>
<td>2.09</td>
<td>10.28</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>82.0 (+/- 22.40)</td>
<td>40.46</td>
<td>158.06</td>
</tr>
<tr>
<td>Protein (g/kg)</td>
<td>1.9 (+/- 0.46)</td>
<td>0.86</td>
<td>3.20</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>62.7 (+/- 20.34)</td>
<td>22.79</td>
<td>119.39</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>29.6 (+/- 5.41)</td>
<td>19.30</td>
<td>42.0</td>
</tr>
</tbody>
</table>

### Carbohydrate Adequacy and Serum Cortisol

As previously described, carbohydrate adequacy was determined based the amount of exercise and current sports nutrition guidelines. Carbohydrate adequacy included two groups where a group “0” was inadequate and group “1” was adequate. According to categorization by these guidelines, less than half of our study population was consuming the recommended amount of carbohydrates to support their activity level.
Mean serum cortisol measurements were compared across the two groups of carbohydrate adequacy (0, 1) using an independent samples t-test. Levene’s statistic was examined to ensure similar variances between groups ($p = 0.36$). The inadequate carbohydrate group had a mean serum cortisol of 17.39 μg/dL while the adequate carbohydrate group had a mean of 19.79 μg/dL. The t-test indicated a significant difference ($p = 0.042$) in the mean serum cortisol measurements between the inadequate and adequate carbohydrate groups. When we looked further at the carbohydrate adequacy groups, it was interesting to note the number of women within each group who were outside the normal serum cortisol range of 10 - 20 μg/dL. Among the carbohydrate adequate women, 15 had high serum cortisol, while only 1 female fell beneath the low end of the acceptable cortisol range. In comparison, the carbohydrate inadequate group had 19 women with high serum cortisol and 6 who displayed low levels.

**Individual Macronutrient Intake and Serum Cortisol**

To explore the relationship of diet to serum cortisol, carbohydrate, protein and fat intake were analyzed. Initially, we intended to include energy availability in this analysis because considering overall energy status may be important when attempting to look at the impact of individual macronutrients intake. However, using an ANOVA to analyze cortisol in relation to macronutrient intake and energy availability produced unstable
<table>
<thead>
<tr>
<th></th>
<th>t-test for Equality of Means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t</td>
</tr>
<tr>
<td>Cortisol</td>
<td></td>
</tr>
<tr>
<td>Equal var.</td>
<td>-2.06</td>
</tr>
<tr>
<td>assumed</td>
<td></td>
</tr>
</tbody>
</table>

Table 6 - Independent Samples Test: Serum Cortisol Comparison for Carbohydrate Adequacy Groups

Figure 5 - Box Plot for Cortisol (μg/dL) by Carbohydrate Adequacy Group
models. In these models, energy availability demonstrated a high collinearity with the individual macronutrient being examined. In other words, because the individual macronutrients were part of total energy intake, and therefore indirectly contributed to EA, both values cannot be included together in the analysis. After identifying this pattern, we removed energy availability and considered each macronutrient related to serum cortisol as a univariate model.

**Carbohydrate Intake and Serum Cortisol**

In addition to analyzing cortisol in relation to carbohydrate adequacy, we wanted to further assess cortisol values related to carbohydrate intake as a continuous variable in grams or carbohydrate per kilogram of body weight. Using a univariate model, where carbohydrate intake (g/kg) was used to predict serum cortisol (μg/dL), the scatterplot hinted at a potential quadratic relationship. We considered both linear and quadratic lines of best fit. Table 6 demonstrates that the analysis using a quadratic model improved the fit ($r^2 = 0.048$), as well as the significance of the model ($p = 0.090$), compared to the linear model ($r^2 = 0.019$ and $p = 0.175$). If this model was significant, the quadratic relationship indicates that cortisol was lower when carbohydrate intake was both on the low and the high end of the range. Those subjects with a carbohydrate intake in the middle of the range tended to have higher morning serum cortisol values. It is important to note that this model failed to reach significance ($p = 0.090$).

<table>
<thead>
<tr>
<th>Equation</th>
<th>Model Summary</th>
<th>Parameter Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2$</td>
<td>$F$</td>
</tr>
<tr>
<td>Linear</td>
<td>0.019</td>
<td>1.871</td>
</tr>
<tr>
<td>Quadratic</td>
<td>0.048</td>
<td>2.463</td>
</tr>
</tbody>
</table>

Table 7 - Linear and Quadratic Models for Carbohydrate and Serum Cortisol
Similar to carbohydrate, protein intake (g/kg) was analyzed using a univariate model to assess its predictability for serum cortisol (μg/dL); both linear and quadratic lines of best fit were considered. Neither the linear (p=0.317), nor the quadratic models (p=0.504) demonstrated significance. The lack of a significant relationship is easily seen in the scatterplot for cortisol and protein intake.

Figure 6 - Scatterplot of Carbohydrate (g/kg) and Serum Cortisol (μg/dL)
Table 8 - Linear and Quadratic Models for Protein and Serum Cortisol

<table>
<thead>
<tr>
<th>Equation</th>
<th>R Square</th>
<th>F</th>
<th>df1</th>
<th>df2</th>
<th>Sig.</th>
<th>Constant</th>
<th>b1</th>
<th>b2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear</td>
<td>0.010</td>
<td>1.013</td>
<td>1</td>
<td>98</td>
<td>0.317</td>
<td>16.120</td>
<td>1.296</td>
<td></td>
</tr>
<tr>
<td>Quadratic</td>
<td>0.014</td>
<td>0.691</td>
<td>2</td>
<td>97</td>
<td>0.504</td>
<td>11.582</td>
<td>6.272</td>
<td>-1.286</td>
</tr>
</tbody>
</table>

Figure 7 - Scatterplot of Protein (g/kg) and Serum Cortisol (μg/dL)
Fat Intake and Serum Cortisol

Similar to protein and carbohydrate intake, dietary fat in grams, and serum cortisol (μg/dL) were examined as a univariate model and considered using both linear and quadratic lines of best fit. The quadratic model demonstrated a slightly better fit in explaining a little more of the variability ($r^2=0.021$ vs. the linear model $r^2=0.003$). However, neither the linear ($p=0.601$), nor the quadratic models ($p=0.359$) demonstrated that fat intake was a significant predictor of serum cortisol.

<table>
<thead>
<tr>
<th>Equation</th>
<th>Model Summary</th>
<th>Parameter Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R Square</td>
<td>F</td>
</tr>
<tr>
<td>Linear</td>
<td>0.003</td>
<td>0.276</td>
</tr>
<tr>
<td>Quadratic</td>
<td>0.021</td>
<td>1.035</td>
</tr>
</tbody>
</table>

Table 9 - Linear and Quadratic Models for Fat and Serum Cortisol

Figure 8 - Scatterplot of Fat (g) and Serum Cortisol (μg/dL)
### Table 10 – Linear and Quadratic Models for Visceral Adipose Tissue and Serum Cortisol

<table>
<thead>
<tr>
<th>Equation</th>
<th>Model Summary</th>
<th>Parameter Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2$</td>
<td>F</td>
</tr>
<tr>
<td>Linear</td>
<td>.051</td>
<td>5.233</td>
</tr>
<tr>
<td>Quadratic</td>
<td>.062</td>
<td>3.208</td>
</tr>
</tbody>
</table>

**Serum Cortisol, Visceral Adipose Tissue and Energy Availability**

The initial analysis of visceral adipose tissue as a predictor for serum cortisol indicated a significant relationship in both the linear ($p = 0.024$) and quadratic ($p = 0.045$) model. Given the lower $p$-value for the linear model, as well as the apparent influence of a single data point for the quadratic model, the linear model is more significant. The scatterplot of this data demonstrates the low VAT measured within our study population, where several women had no measurable VAT. Indeed, in the linear model, the increase per unit of VAT is very small (0.19) for changing serum cortisol. The scatter plot also shows that serum cortisol crosses the $y$-axis at 19.57 (near the high cusp of normal) and increases as VAT decreases. Given that this is the opposite of what we would expect to see, additional analysis included EA in the model.

When we included EA in the model as a categorical variable (3 groups) using an ANOVA, the model to evaluate whether VAT can significantly predict cortisol is significant ($r^2 = 0.073$ and $p = 0.026$). At the risk of giving those categories too much weight where there are many values close to the cut-offs, we also chose to add EA as a continuous variable in the same model.
When EA was modeled as a continuous variable in a linear model with VAT to predict cortisol, it was not statistically significant (p = 0.778). Additionally, we assessed EA as a quadratic variable in the model to examine VAT volume controlled for EA as a predictor for serum cortisol. However, when EA was analyzed as a quadratic variable in the overall model, the model p-value was improved to indicate a potential trend (p=0.059), but as a quadratic variable EA (modeled with VAT) was still not a significant predictor for serum cortisol (p = 0.123 and 0.129).
<table>
<thead>
<tr>
<th>Model</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Regression</td>
<td>174.404</td>
<td>1</td>
<td>174.404</td>
<td>5.233</td>
</tr>
<tr>
<td></td>
<td>Residual</td>
<td>3265.951</td>
<td>98</td>
<td>33.326</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>3440.355</td>
<td>99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Regression</td>
<td>254.995</td>
<td>3</td>
<td>84.998</td>
<td>2.562</td>
</tr>
<tr>
<td></td>
<td>Residual</td>
<td>3185.360</td>
<td>96</td>
<td>33.181</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>3440.355</td>
<td>99</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. Dependent Variable: Cortisol  
b. Predictors: (Constant), VATvolume  
c. Predictors: (Constant), VATvolume, EA, EA<sup>2</sup>  

**Table 11 - Linear Regressions to Predict Serum Cortisol**

<table>
<thead>
<tr>
<th>Model</th>
<th>p</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant) VATvolume</td>
<td>0.024</td>
<td>B: 19.575</td>
<td>Std. Error: .733</td>
<td>Beta: -.225</td>
<td>t: 26.715</td>
</tr>
<tr>
<td>(Constant) VATvolume</td>
<td>0.059</td>
<td>B: 14.548</td>
<td>Std. Error: 3.430</td>
<td>Beta: -.219</td>
<td>t: 4.242</td>
</tr>
<tr>
<td>EA</td>
<td>.343</td>
<td>Std. Error: .220</td>
<td>Beta: .733</td>
<td>t: 1.558</td>
<td>Sig: .123</td>
</tr>
<tr>
<td>EA&lt;sup&gt;2&lt;/sup&gt;</td>
<td>-.005</td>
<td>Std. Error: .003</td>
<td>Beta: -.721</td>
<td>t: -1.532</td>
<td>Sig: .129</td>
</tr>
</tbody>
</table>

**Table 12 - Stepwise Analysis of VATvolume, EA and EA<sup>2</sup> as Predictors of Serum Cortisol**
Chapter 5: Discussion

The purpose of this study was to evaluate morning serum cortisol levels in relation to dietary macronutrient intake, and specifically carbohydrate adequacy, based on endurance sports nutrition guidelines. Additionally, we explored the interrelation between visceral adipose tissue and morning serum cortisol values in recreational female runners with and without controlling for energy availability. The results of this study could provide insight into alterations in serum cortisol and VAT that may occur in response to dietary energy and macronutrient content in recreational female runners.

**Carbohydrate Intake and Serum Cortisol**

Within the realm of endurance activities, carbohydrate is crucial in fueling the physiological functions to support optimal training and performance, and International sports nutrition guidelines go into great depth defining the appropriate amount of carbohydrate for activities of varying duration and intensity. In addition to fueling endurance activities, appropriate carbohydrate intake has demonstrated a key role in maintaining a normal cortisol rhythm as part of the stress response to exercise. Given the traditional significance of carbohydrate for endurance exercise, our study assessed carbohydrate and serum cortisol from two perspectives: carbohydrate adequacy for activity level and average daily carbohydrate intake, in grams/kilogram of body weight.

The IOC Sports Nutrition Guidelines define adequate carbohydrate in terms of duration of the activity, where individuals exercising for less than an average of 1 hour per day should consume 3-5 g/kg BW of carbohydrate while those exercising for an
average of an hour or more should consume 6-10 g/kg BW carbohydrate. Our results demonstrated that, once divided into inadequate and adequate carbohydrate groups based on these guidelines, less than half of our female runners were fueling adequately with carbohydrate.

Adequate carbohydrate maintains muscle and liver glycogen stores, which are essential to performance before, during and after endurance exercise. Along these lines, carbohydrate ingestion during bouts of prolonged aerobic exercise has proven effective at attenuating rises in serum cortisol immediately after activity. This is logical considering that cortisol functions to maintain blood glucose in the absence of adequate carbohydrate during exercise. While cortisol fluctuations during, and immediately after exercise are important, it is the chronic alterations in the circadian rhythm that may be more indicative of HPA axis dysfunction. Literature on long-term cortisol rhythm in endurance athletes is relatively scarce due to the complexity of controlling diet and activity, as well as monitoring cortisol over an extended time period.

When exploring morning serum cortisol in response to carbohydrate adequacy, our findings demonstrated that serum cortisol was significantly greater in the carbohydrate adequate women. In an alternate analysis, we examined serum cortisol measurements in relation to carbohydrate intake in grams/kilogram of body weight (as a continuous variable). The results of this analysis demonstrated that the relationship was not significant. However, our scatterplot did demonstrate a trend toward a quadratic relationship between serum cortisol and g/kg carbohydrate. In this model, increasing carbohydrate was accompanied by an increasing cortisol up to a point where the higher carbohydrate appeared to attenuate the cortisol level. While this is merely a trend, it does bear mentioning as a potential pattern that deserves further research. It is
important to remember how many of the runners had very low VAT as it would undoubtedly strengthen the analysis to have a larger range of VAT.

As previously discussed, cortisol fluctuates in a circadian rhythm where values are typically highest in the morning; research defines the normal morning cortisol range as 10 – 20 μg/dL.\(^5\) According to these guidelines, while the carbohydrate adequate females displayed higher morning cortisol values, neither the adequate, nor the inadequate carbohydrate group had mean values outside of the normal morning serum cortisol range. However, when looking at individuals, it is interesting to note that 19 women in the inadequate carbohydrate group, versus 15 women in the adequate carbohydrate group, had serum cortisol values above the normal range. Similarly, 6 women in the inadequate carbohydrate group compared with just 1 woman in the adequate carbohydrate group, displayed serum cortisol levels below the acceptable range.

In another study, Lane et al\(^{13}\) investigated overtraining by measuring changes in cortisol in response to low (n = 8) vs. high carbohydrate (n = 12) diets and three consecutive days of training in. Test subjects completed baseline fasting serum cortisol, which was similar between groups. They were randomly assigned to a carbohydrate group and completed a food diary over five days of the diet intervention. On the first three days, subjects completed 60 minutes of exercise at 70% VO\(_{2\text{max}}\) and had serum cortisol measured pre- and post-exercise. Diet analysis showed that the high and low carbohydrate groups consumed close to the prescribed percentage of carbohydrate (58.5 +/- 4.9% and 31.9 +/- 2.5%) and without a significant difference in total calorie intake. Resting serum cortisol increased significantly from baseline only within the low carbohydrate group. This study is consistent with other literature in carbohydrates and cortisol, but in opposition of our findings. While our data investigating serum cortisol in
response to carbohydrate did not find robust significant findings, there were some interesting trends including the potential quadratic relationship in cortisol and carbohydrate g/kg. This data continues to challenge us to consider the complexity of cortisol control and what is best for the body in terms of carbohydrate consumption and exercise adaptations. As well, habitual diet and potential adaptation to chronic patterns, like keto-adapting, should be considered, even in acute interventions, as these may be examples of a low carbohydrate intake that has normal, or even low, cortisol values.

**Protein Intake and Serum Cortisol**

Protein turnover is increased in athletes and endurance athletes may have as much as a 2-fold increase in protein turnover compared to sedentary individuals.\textsuperscript{110,111} Similarly, the literature has suggested a protein intake for athletes that is higher than the RDA.\textsuperscript{86} While the literature has demonstrated that appropriate protein intake is an important part of a balanced diet, it has received comparatively less focus among endurance athletes compared to carbohydrates and fat, and especially in women.

The findings within our study demonstrated a wide, and seemingly random, dispersion of serum cortisol values in relation to protein intake in grams/kilogram. Indeed, protein as a predictor of serum cortisol lacked a significant relationship, and did not appear to demonstrate any trend. The lack of a significant relationship or trend in our data reinforces a great deal of the current literature investigating dietary protein and cortisol.

The literature described earlier demonstrated no significant changes in cortisol following short term protein-manipulated diet and exercise interventions in endurance athletes.\textsuperscript{87,88} However, these are relatively small studies completed in men. The literature surrounding the stress response to differing dietary protein content is thin and requires more attention among larger populations. It would also be interesting for future studies to
attempt to control for habitual energy balance to continue to tease out the relationship with low energy availability. It is also noticeable in our female runner sample that protein was adequate (> 1 g/kg) in all but one participant.

**Fat Intake and Serum Cortisol**

As a dietary macronutrient for endurance exercise, fat has been approached with caution due to the belief that high fat diet may be involved in immunosuppression and greater oxidative stress.\(^{112}\) Additionally, some females, particularly those exercising for weight loss, will restrict fat in an attempt to reduce caloric intake.\(^2,7\) However, fat is essential to a balanced diet and aids in absorption of essential vitamins and minerals.\(^96\) Dietary fat recommendations are typically given as a percentage of total calories. Endurance athletes should generally consume 25-30% of total calories from fat.\(^22\)

Given the different format for these recommendations, our assessment of dietary fat was in total grams, as opposed to grams/kilogram. The analysis of serum cortisol in relation to dietary fat did not produce any statistically significant correlations. The scatterplot of cortisol and dietary fat demonstrated a large dispersion of the data, very similar to what was seen with protein. We used linear and quadratic lines of best fit to attempt to discern any trend in the data. While the quadratic was a more appropriate fit compared to the linear model, neither demonstrated a discernable pattern in the data.

While our data did not demonstrate a statistically significant prediction for dietary fat to estimate serum cortisol, the existing literature does provide support for the current sports nutrition recommendations. A 4 week study by Venkatraman et al\(^92\), mentioned previously, demonstrated that endurance athletes consuming a diet of approximately 30% fat could exercise longer and maintained more stable pre- to post-exercise serum cortisol values compared to lower and higher fat groups.\(^92\) However, a shorter study with a dietary intervention of either a low, or high fat diet failed to find the same hormone and
performance benefits with the higher fat intake. Opposing results in these studies may demonstrate the importance of longer studies to allow time for physiological adaptations to dietary interventions.

Overall, analysis of dietary macronutrients in relation to serum cortisol values did not demonstrate significant patterns. The lack of findings may be partially due to the fact that our inclusion for the study was relatively low in terms of training volume, including women running at least 15 miles for the previous 6 weeks. We know that lack of regular menstruation can be indicative of hormone imbalances linked to inadequate energy intake.53,7

A study by De Souza et al46 provides some insight into hormone status based on regular or irregular menstruation. Her study included three groups: eumenorrheic sedentary women (n = 6), eumenorrheic female runners (n = 9) and amenorrheic female runners (n = 9). The groups were physically similar except in that the amenorrheic runners consistently weighed less, indicating the potential for inadequate energy to support activity level. All of the participants had maintained their current menstrual status and activity level for at least 12 months prior, and were without thyroid or other medical conditions. Collection of fasted, early morning serum cortisol indicated that, on average, the amenorrheic runners had significantly higher values than both eumenorrheic groups.46 Although many of our female runners appeared to be fueling inadequately in carbohydrate and calories we did not observe any significant link to cortisol, and menstruation status was not included in our current analysis. However, given that nutrition and training regimens can take time before producing hormonal responses, follow-up with this group of runners deserves further exploration.
Serum Cortisol, Visceral Adipose Tissue and Energy Availability

The accumulation of VAT has been linked to several chronic disease states.\textsuperscript{51} Metabolic Syndrome, which is linked to heart disease, type II diabetes and kidney disease\textsuperscript{51}, provides a prime example of the connection between greater VAT and poor health.\textsuperscript{54} In addition to accumulation of VAT, a corresponding increase in serum cortisol may be more harmful than an increase in body size alone.\textsuperscript{54} Literature including both overweight\textsuperscript{16,45,58} and normal weight participants\textsuperscript{57} has demonstrated an increase in morning serum cortisol when more VAT is present. Despite the fact that VAT can cause increases in cortisol even in normal weight individuals\textsuperscript{57}, and is linked to many chronic health conditions, the relationship of VAT and cortisol has not received attention among an active population.

Our analysis of serum cortisol in correlation to VAT considered these variables in both a linear and quadratic model. While both models demonstrated significance, the linear model was the better fit given that one data point contributed to the fit of the quadratic model. The scatterplot of this data shows that serum cortisol crosses the y-axis at 19.57. This is relatively high considering our normal range for morning serum cortisol (10-20\,\mu g/dL\textsuperscript{33}). Ironically, our scatterplot also demonstrated that serum cortisol increases as VAT decreases. This trend is the opposite of what we would expect to see and begs the question: are the women with no measurable VAT failing to fuel adequately, leading to higher serum cortisol?

While the linear model of VAT as a predictor for serum cortisol was the better fit for this data, our quadratic model also demonstrated significance. We may be somewhat limited in our view of the data considering that our population displayed very low, and often zero, VAT. The single data point marking the greatest VAT also had a cortisol that was trending back up, allowing for the fit of our quadratic model. It would be interesting
to complete a similar analysis in a population with more VAT to see if serum cortisol would trend back upward as VAT increased beyond the limits of our data.

Our findings that the female runners without VAT had greater serum cortisol may indicate that they were fueling inadequately, thereby causing more physiological stress, indicated by higher serum cortisol. The study by De Souza et al., mentioned previously, supports this theory by demonstrating that a group of amenorrheic female runners had consistently, and significantly, lower body weight and higher fasted serum cortisol values compared to female runners with normal menstrual status. However, other literature has examined similar groups of female runners and failed to show the same pattern of elevated cortisol. Given our surprising finding that overall serum cortisol increased as overall VAT decreased, as well as the conflicting literature, this topic deserves a more thorough analysis in an active population over a wide range of VAT and different dietary styles.

Additionally it is important to consider an individual’s adaptation to habitual diet and training regimen. An athlete who has altered macronutrient intake so as to keto-adapt muscle would be expected to have a lower stress response, including cortisol, to a low carbohydrate diet in comparison to a carbohydrate trained athlete. If it goes unconsidered, this factor significantly confounds research on diet and training. Future research should likely consider the fuel preference of the trained muscle according to diet.

Limitations

One of the limitations of this study included potential inaccuracies in self-reported data. Despite the fact that participants were instructed in the proper procedure for tracking diet and activity, the 3-day diet histories and the Bouchard physical activity grids have the potential for human error. However, the same three researchers reviewed self-
reported data with participants to ensure as much accuracy as possible. Still, three days of dietary data does not allow for insight into whether participants were carbohydrate or fat adapted, which could impact the physiological (cortisol) response to dietary macronutrient composition. Additionally, dividing participants into categories to assess activity level, energy availability and carbohydrate adequacy in correlation to serum cortisol can limit data interpretation. Dividing any data into groups defines two categories as different (i.e.: “adequate” or “inadequate”), when the difference in two specific values could be as little as 0.1. Of specific note, this study population of female runners had very low levels of measurable visceral adipose tissue, significantly limiting the range of data available. An alternative to our insignificant findings is that the study subjects had great variability in the consumption of their macronutrients; hence a larger number of subjects might be more illuminating. Continued research on visceral adiposity in physically active populations will be interesting to consider.

The CoreScan algorithm for the iDXA machine is relatively new technology that has not been examined in a more active population. While more active individuals typically have very little visceral adipose tissue, CoreScan could identify patterns in the amount and distribution of fat depending on type and frequency of exercise or diet composition. Future research should attempt to compile normative data that considers the activity level of the population.

**Conclusion**

This study examined the association of carbohydrate, protein and fat intake with serum cortisol levels in recreational female runners. Additionally, it used relatively new technology, the GE CoreScan, to assess visceral adipose tissue for a correlation with serum cortisol. Macronutrient intake and serum cortisol levels in female runners is of concern because of the propensity of this population to under-fuel in order to lose
weight, potentially leading to inadequate recovery and the physiological inability to regulate cortisol within a normal range. Higher serum cortisol values also coincide with higher visceral adipose tissue, a contributing factor to many chronic diseases. Exercise is a generally healthy activity, but can produce unhealthy physical consequences when athletes fail to fuel adequately. The fact that runners often carry less weight does not mean that they are impervious to the accumulation of visceral adipose tissue and the coinciding negative health consequences. Future research should likely consider the interaction of diet, cortisol and visceral adipose tissue in an active population with a greater range of visceral adipose tissue.
References Cited


Appendix A: GE CoreScan VAT Printout
The Android region is that of the abdomen, and often the body type with increased fat in this area is described as "apple shaped." The Gynoid region is that around the hips and thighs and often the body type with increased fat in this area is described as "pear shaped." Understanding where fat is stored on the body is recognized as an important predictor of the potential health risks of obesity.

CoreScan estimates the VAT (Visceral Adipose Tissue) content within the android region. VAT is a specific type of fat that is associated with several types of metabolic diseases such as obesity, metabolic syndrome, and type 2 diabetes. CoreScan results have been validated for adults between ages 18-90, and with a BMI in the range of 18.5-40.
Appendix B: An Example Paper

Visceral Adipose Tissue and Serum Cortisol in Recreational Female Runners
Visceral Adipose Tissue and Serum Cortisol

The current literature has demonstrated the link between certain health conditions, such as Cushings Syndrome, and visceral adipose tissue.\textsuperscript{1,2} Similarly, an increase in accumulation of visceral adipose tissue (VAT) has been documented in health conditions, particularly Metabolic Syndrome.\textsuperscript{3} While cortisol and VAT are known to lead to poor health outcomes, it was not until relatively recently that the two factors were connected in literature involving normal weight subjects.\textsuperscript{4,5} Given the link to many chronic disease states, the presence of VAT and its link to serum cortisol deserves further attention in a population of individuals at normal weights.

Cortisol

Cortisol is a glucocorticoid secreted by the adrenal cortex, whose major functions include development, and regulation of the stress response and the immune system.\textsuperscript{6} In a healthy state, cortisol follows a circadian rhythm, similar to the sleep-wake rhythm, with higher values in the morning and relatively lower values in the evening.\textsuperscript{7,8} This circadian rhythm naturally promotes a healthy pattern of sleep and waking.\textsuperscript{9}

Fluctuations in cortisol occur in response to environmental or physiological stressors. In response to a bout of exercise in a healthy individual, cortisol increases and may remain elevated for hours after exercise is completed.\textsuperscript{10,11} Following stimulation of the hypothalamic pituitary adrenal (HPA) axis, cortisol should trend back toward pre-stress levels. This is accomplished through a negative feedback loop.\textsuperscript{12} In normal conditions, accumulation of cortisol following stress acts to reduce the release of the glucocorticoid and reduce circulating levels in the body.\textsuperscript{12}

Over time, unusual, or extreme, environmental and physiological conditions can cause shifts in the normal circadian rhythm of cortisol in an individual. For instance, cortisol values can remain chronically elevated in athletes exposed to increased
physiological stress as a result of overtraining.\textsuperscript{13–15} However, the fluctuation in cortisol following a period of exercise in healthy individuals, under normal conditions, makes it difficult to determine the long-term implications of elevated cortisol levels as a result of increased stress. Physiological stress has the potential to cause chronically elevated or chronically depressed cortisol.\textsuperscript{10,16} These variations may correspond to the presence or absence of VAT as they do in certain disease states.

As mentioned earlier, abnormally elevated cortisol values can be present in certain disease states where the normal rhythm of the HPA axis is disrupted. Polycystic Ovarian Syndrome (PCOS) and Cushing’s syndrome are two conditions that often present with elevated levels of cortisol.\textsuperscript{17} While Cushing’s syndrome provides an extreme example of the effect of hypersecretion of cortisol, it is useful in demonstrating some of the negative ramifications of prolonged elevation of cortisol. Assessment of body composition in women with glucocorticoid excess and Cushing’s syndrome has demonstrated significantly greater fat mass and lower lean body mass compared to healthy women.\textsuperscript{2} These conditions, involving endocrine malfunction and chronically elevated levels of circulating cortisol are related to a significant increase in mortality due to comorbidities including diabetes and cardiovascular disease.\textsuperscript{1} Cortisol is an integral hormone in the regulation of stress and normal body function; however, variations in normal secretion can lead to adverse health consequences.

While the mechanism of pathophysiology has yet to be clearly elucidated, chronically elevated cortisol is implicated in a greater distribution of fat to the visceral depot. In addition to elevated cortisol secretion, both PCOS and Cushing’s syndrome are commonly associated with obesity and an increase in VAT.\textsuperscript{18} Research has demonstrated that premenopausal women with Cushing’s syndrome experienced a significant decrease in serum cortisol and VAT following treatment as determined by a
computed tomography (CT) scan.\textsuperscript{19} The link between serum cortisol and VAT is clearly present in certain disease states; however, it is more difficult to evaluate this relationship in apparently healthier individuals.

Obesity and the subsequent presence of an inflammatory state is a known contributor to many diseases including diabetes, hypertension and cardiovascular disease. Cortisol rhythm and changes in adipose tissue distribution may play a role in contributing to these chronic diseases. Data in obese, premenopausal females, demonstrates an association between urinary and serum cortisol, and central adiposity indicated by higher waist-to-hip ratio (WHR).\textsuperscript{20} A prolonged elevation of cortisol could be detrimental to long-term health. There is considerable evidence from clinical, to cellular and molecular studies that elevated cortisol, particularly when combined with secondary inhibition of sex steroids and growth hormone secretions, causes accumulation of fat in visceral adipose tissues as well as metabolic abnormalities.\textsuperscript{21} Similar to PCOS, female endurance athletes may experience menstrual disturbances and dyslipidemia\textsuperscript{22} that are likely related to a heightened cortisol response to diet and training. Exploration of cortisol relative to VAT may demonstrate important correlations to these maladaptations and long-term health consequences in normal weight individuals. A deeper look at VAT will allow us to outline the associated health conditions and why they are of concern.

**Visceral Adipose Tissue (VAT)**

Adipose tissue is a metabolically active organ containing mature adipocytes, preadipocytes, vascular, neural and immune cells and is responsible for modulating many of these obesity-related risk factors.\textsuperscript{23} Specifically, regional differences in fat distribution are important in determining the risk of developing these obesity-related diseases, with an increased focus on an android (central) fat pattern.

Abdominal adipose tissue can be divided into two types of adipose tissue by a
membranous layer of fascia. Visceral adipose tissue is contained behind this layer of fascia, closer to vital organs. In particular, visceral adipose tissue is metabolically active and appears to be important for the pathogenesis of insulin resistance, dyslipidemia, glucose intolerance, hypertension and cardiovascular risk. These conditions, related to accumulation of VAT, are linked to several disease states where overweight and obesity is common. It remains to be determined if relatively higher serum cortisol and VAT is associated with the same disease conditions in a more active population.

The metabolic syndrome is a condition that demonstrates the link between VAT and chronic health issues. Metabolic syndrome is a constellation of risk factors for cardiovascular disease, type II diabetes mellitus, stroke and kidney disease. Symptoms of metabolic syndrome include insulin resistance, dyslipidemia, central obesity, hypertension, glucose intolerance and increased atherosclerotic disease. Central adiposity as a risk factor for the syndrome demonstrates the potential health risks associated with the presence of VAT.

It is widely accepted that overweight and obesity increase the risk of long-term health conditions. Additionally, chronically elevated circulating cortisol and the accumulation of VAT are each detrimental to health and may also lead to chronic health conditions. However, the link between elevated cortisol and an increase VAT, in particular, may contribute more to the development of these chronic health concerns than an increase in weight alone. Therrien et al found that obese subjects with an accumulation of visceral adipose tissue demonstrated increased awakening cortisol levels. Furthermore, visceral fat distribution in both lean and obese subjects has been associated with an increase in cortisol secretion following physical and psychological stressors. To date, no research has attempted to identify patterns between cortisol and visceral adipose tissue in a more active population. It remains to be seen if
interactions between awakening serum cortisol are correlated with fat distribution patterns similar to those seen in sedentary, overweight individuals.

Methods

This study is a retrospective analysis of a primary study on adult female runners, and thus the methods and data collection are limited to the previously executed protocol. The original study was set up to identify evidence of the female athlete triad in recreational female runners (IRB approved protocol 2009H0177). The study team recruited 125 women, ages 18 and older, who were running at least 15 miles per week for the immediate 6 weeks, and had no pre-existing thyroid, adrenal or bone issues.

A standard written three-day food record was used to collect dietary intake data from each of the female runners. Following submission of the food record, one of three researchers reviewed the dietary data with the participant to fill in missing information and ensure the highest possible accuracy level. After collection, data was entered into the professional grade ESHA Food Processor SQL version 10.2 and triple checked by different researchers to determine dietary intake. The data were compiled and variables, including total kilocalories and grams of carbohydrate, protein and fat, were exported to an excel spreadsheet.

Energy expenditure and duration of usual exercise were estimated using the Bouchard physical activity tool. Exercise energy expenditure (EEE) was determined using the self-reported Bouchard physical activity grid, where a level 6 or higher is considered exercise. The average frequency of 6 or higher was multiplied by 15 minutes to estimate usual duration of exercise, in minutes, to be used in determining adequacy of carbohydrate intake.

Collection of morning serum cortisol was completed in a fasted state, after an overnight rest, to reflect the serum cortisol values. Serum cortisol collection was
completed between 7am and 9am, with an occasional appointment at 9:30 or 10am. Data collection times were coordinated with the normal waking schedule of individual participants so that serum cortisol values would reflect typical early morning values for each subject. Normal serum cortisol concentrations are higher in the early morning, ranging from 10 to 20 μg/dL (275 to 555 nmol/L).\textsuperscript{7,9}

The GE Lunar iDXA was used in this study for body composition and partitioning of the VAT became possible with the additional CoreScan software. A two-compartment body composition was estimated by the GE lunar iDXA densitometer to determine fat mass and fat free mass (FFM) for participants.\textsuperscript{35} The iDXA is a relatively easy, low radiation means of measuring body composition, and is recognized as one of the more accurate measurements of fat and fat free mass.\textsuperscript{35} The iDXA protocol required height and weight measurements, where heights were taken using a stadiometer (nearest cm) and weights were taken via a Healthometer scale (nearest tenth of a pound). Use of the iDXA is central to this analysis and body composition variables. The lean body mass (including bone) was used in the energy availability calculation.

For the current project, total body scans were analyzed using the GE EnCore version 15 software to determine the amount of visceral adipose tissue.\textsuperscript{36} CoreScan provides VAT measurements in both mass (lbs) and volume (in\textsuperscript{3}). Studies validating the CoreScan algorithm used volumetric CT and iDXA VAT; therefore, volumetric measurements of VAT were chosen for use in this study. VAT volume and body fat measurements obtained from the iDXA scan were compiled and entered into the excel spreadsheet containing all other data.
Results

Serum Cortisol, Visceral Adipose Tissue and Energy Availability

The initial analysis of visceral adipose tissue as a predictor for serum cortisol indicated a significant relationship in both the linear ($p = 0.024$) and quadratic ($p = 0.045$) model. Given the lower $p$-value for the linear model, as well as the apparent influence of a single data point for the quadratic model, the linear model is more significant. The scatterplot of this data demonstrates the low VAT measured within our study population, where several women had no measurable VAT. Indeed, in the linear model, the increase per unit of VAT is very small (0.19) for changing serum cortisol. The scatter plot also shows that serum cortisol crosses the $y$-axis at 19.57 (near the high cusp of normal) and increases as VAT decreases. Given that this is the opposite of what we would expect to see, additional analysis included EA in the model.

When we included EA in the model as a categorical variable (3 groups) using an ANOVA, the model to see if VAT can significantly predict cortisol looks significant ($r^2 = 0.073$ and $p = 0.026$). At the risk of giving those categories too much weight where there are many values close to the cut-offs, we also chose to look at EA as a continuous variable in the same model.

When EA was modeled as a continuous variable in a linear model with VAT to predict cortisol, it was not statistically significant ($p = 0.778$). Additionally, we assessed EA as a quadratic variable in the model to examine VAT volume controlled for EA as a predictor for serum cortisol. However, when EA was analyzed as a quadratic variable in the overall model, the model $p$-value was improved to indicate a potential trend ($p=0.059$), but as a quadratic variable EA (modeled with VAT) was still not a significant predictor for serum cortisol ($p = 0.123$ and 0.129).
Discussion

Serum Cortisol, Visceral Adipose Tissue and Energy Availability

The accumulation of VAT has been linked to several chronic disease states\(^2\). Metabolic Syndrome, which is linked to heart disease, type II diabetes and kidney disease, provides a prime example of the connection between greater VAT and poor health\(^{26}\). In addition to accumulation of VAT, a corresponding increase in serum cortisol may be more harmful than an increase in body size alone\(^{21}\). Literature including both overweight\(^1,24\) and normal weight subjects\(^{26}\) has demonstrated an increase in morning serum cortisol when more VAT is present. Despite the fact that VAT can cause increases in cortisol even in normal weight individuals\(^{26}\), and is linked to many chronic health conditions, the relationship of VAT and cortisol has not received attention among an active population.

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