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Examination of Energy Needs and Hormone Levels in Male Endurance Athletes

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by

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DEDICATION

I dedicate this to the Lord for granting me the patience, strength and tenacity to fulfill this goal for his honor. To God be the Glory:

Let nothing disturb you,

Let nothing frighten you,

All things are passing away:

God never changes.

Patience obtains all things,

Whoever has God lacks nothing;

God alone suffices.

~St. Teresa of Avila

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ABSTRACT

A plethora of literature examining the physiological consequences associated with deficits in energy availability (EA) for female athletes exists, however literature examining male athletes is sparse. **Purpose:** To determine the effects of high exercise energy expenditure (EEE) on Male Triad symptoms (EA with or without an eating disorder [ED], reproductive hormones Testosterone [T] and Luteinizing hormone [LH], and bone mineral density [BMD]) and other metabolic markers (Insulin, Leptin, Cortisol and Interleukin-6 [IL-6]) in endurance-trained male athletes. **Methods:** We utilized a cross-sectional design on 14 endurance trained male athletes (age: 26.4 $\pm$ 4.2 yrs.; weight: 70.6 $\pm$ 6.4 kg; and height: 179.5 $\pm$ 4.3 cm) whom were recruited from the local community. Two separate training weeks (low [LV] and high [HV] training volumes were collected including: dietary logs, exercise logs, BMD, and blood concentrations for 6 hormones (T, LH, Insulin, Leptin, Cortisol, and IL-6). Anthropometric measurements (height, weight, and body composition) were taken prior to data collection. **Results:** Overall, EA presented as 27.6 $\pm$ 12.1 kcal/kg FFM·d with 35% (n=5) of participants presenting with increased risk for ED. Examining Male Triad components: 1) 32.1% presented with LEA ($\leq$ 20 kcal/kg FFM·d) with or without ED, 2) Reproductive hormones T (1780.6 $\pm$ 1672.6 ng/dL) and LH (813.7 $\pm$ 314.2 pg/mL) were within normal limits compared to normative data, and 3) BMD was not compromised at 1.31 g/cm^2. Of those participants at risk for LEA ($\leq$ 20 kcal/kg FFM·d), 41.2% (n = 7) (HV: 50%, n = 4; LV: 33.3%, n = 3) demonstrated increased T levels (p = 0.20) while, 21.7% (n = 5) (HV:

18.2%, n = 2; LV: 25%, n = 3) presented with low Leptin levels ($p = 0.01$). Significant regressions revealed T levels from EA ($F(1, 24) = 4.8$, $p = 0.04$); RMR ($F(1, 23) = 16.2$, $p < 0.001$); EI ($F(1, 24) = 6.7$, $p = 0.02$), and DXA_BFP ($F(1, 24) = 51.9$, $p < 0.001$) and leptin levels from DXA_BFP ($F(1, 24) = 27.2$, $p < 0.001$). **Conclusion:** This study is the first to examine all 3 components of the Male Triad. We found 1 compromised component of the Triad (LEA with or without ED risk), however both reproductive hormones (T and LH) and BMD were not compromised. Resultant LEA demonstrated a significant negative relationship with Leptin. Relationships between body fat percent and the hormones T and Leptin demonstrated clinical uses for monitoring weight to assess hormonal profiles in males. More research investigating negative physiological consequences associated with the Male Triad, decreased EI and increased EEE is needed in the male population.

Key Words: Male Triad, Low Energy Availability, Bone Mineral Density, Testosterone, Luteinizing Hormone, Leptin.

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LIST OF SYMBOLS

$\pm$	Plus or Minus
$>$	Greater Than
$\geq$	Equal to or Greater Than
$<$	Less Than
$\leq$	Equal to or Less Than

LIST OF ABBREVIATIONS

ACSM ..... American College of Sports Medicine

BMD ..... Bone Mineral Density

BMI ..... Body Mass Index

DE ..... Disordered Eating

DXA ..... Dual-Energy X-Ray Absorptiometry

EA ..... Energy Availability

EB ..... Energy Balance

EEE ..... Exercise Energy Expenditure

ED ..... Eating Disorder

EDI-3 ..... Eating Disorder Inventory-3

EDI-3 SC ..... Eating Disorder Inventory-3 Symptom Checklist

EI ..... Energy Intake

ELISA ..... Enzyme-linked Immunosorbent Assay

FFM ..... Free Fat Mass

HDL ..... High-Density Lipoprotein

HR ..... Heart Rate

HV ..... High Volume

LDL ..... Low-Density Lipoprotein

LEA ..... Low Energy Availability

LH ..... Luteinizing Hormone

LV ..... Low Volume

IGF-I ..... Insulin-like Growth Factor-I

Il-6 ..... Interlukien-6

IOC ..... International Olympic Committee

PBF ..... Percent Body Fat

RED-S ..... Relative Energy Deficiency in Sports

RMR ..... Resting Metabolic Rate

SCD ..... Sudden Cardiac Death

SWA ..... BodyMedia SenseWear Armband

T ..... Testosterone

TDEE ..... Total Daily Energy Expenditure

TEE ..... Total Energy Expenditure

Triad ..... Female Athlete Triad

CHAPTER 1

INTRODUCTION

Energy Deficiencies in Male Endurance Athletes

Literature examining endurance athletes have demonstrated increased risks involving impaired physiological functions due to the high-energy expenditure demands of their sport participation.[1-5] Examples of impaired physiological functions include: metabolic rate, protein synthesis, cardiovascular, and overall bone health.[5-12] Low energy availability (LEA), also referred to as deficient energy availability, is defined as insufficient dietary energy intake to support the energy expenditure required for activities of daily life and the physical functioning of organs, muscles and other systems after the demands from the energy expenditure of exercise are met.[5-13] Over 30 years of literature has examined female athletes in relation to LEA. In 2014, the International Olympic Committee (IOC) issued a call for an encompassing *model* that addresses impaired physiological functions associated with deficient EA for all athletes; Relative Energy Deficiency in Sports (RED-S).[11] In this issuance, the IOC describes RED-S as impaired physiological functions driven by relative energy deficiency leading to compromised functions of metabolic rate, menstrual function, bone health, immunity, protein synthesis and cardiovascular health in *all* athletes.[11] While this appeal from the IOC demonstrates the need to examine populations other than women, including men and non-abled body athletes (e.g., Para-Olympic), RED-S does not have the empirical literature support for a

blanketed model encompassing females, males and non-abled body athletes at this point. Currently, literature examining deficient energy availability (EA) and its effect on physiological functions in male endurance athletes is limited.[6]

Hormonal Responses

Knowledge of the impact of deficient EA on male athletes and corresponding physiological systems is sparse. Established research on LEA in female athletes has shown negative health outcomes in various physiological systems including: cardiovascular, gastrointestinal, endocrine, reproductive, skeletal, renal and central nervous systems.[5-13] Specifically for women, the high-energy demands of endurance exercise leads to concerns regarding (a) energy availability (EA), (b) menstrual cycle function, and (c) optimal bone health; also known as the female athlete triad (Triad).[5-13] The Triad has highlighted key factors of impaired physiological functions in women including; low energy availability, menstrual cycle dysfunction, and decreased bone health.[5-13] Research has also highlighted LEA as a catalyst, which drives the Triad[6,12] and spurns the acute and long-term reduction of hormonal and metabolic functions in the female athlete.[6,11-13] Limited research has demonstrated deficient EA in males has negative effects and selected health risks associated with low gonadal and reproductive hormones.[14-16]

Upon examination of the research, a few trends have been established for male athletes. Most recently, in 2016, Koehler et. al.,[17] examined short-term EA reduction in exercising men. Energy availability for the males was reduced to 15 kcal/kg FFM·d resulting in the suppression of the hormones leptin and insulin. Additionally,

Friedl et. al.,[16] examined chronic energy deficits on the thyroid, gonada-, and somatrotrophic –pituitary axes on US Army Rangers during training camp in an extreme semi-starvation, multi-stressor environment. Gonadal and reproductive hormones (i.e. Testosterone (T), Thyroid, Sex Hormone Binding Globulin, and Insulin-like Growth Factor-I (IGF-I)) measured consistently low and were used as reliable and specific hormonal markers in assessing acute energy deficits in male U.S. Army Rangers while cholesterol and cortisol markers assessed a more chronic state of energy deficits in male U.S. Army Rangers.[16]

Bone Mineral Density

Research has also revealed a trend in compromised bone mineral density (BMD) in male endurance athletes.[18-20] Literature has shown 25%-63% of male cyclists (recreational and competitive road cyclists) were diagnosed with osteopenia within the spine and hip, while 9% of the competitive cyclists (~9 years of racing experience and training 7-22 hours/week) were diagnosed with osteoporosis at specific sites.[16,18,20] While 19% of runners demonstrated osteopenia within the spine and hip.[18] Dolan et. al.,[15] found similar hormonal outcomes in professional jockeys that Friedl et. al.,[16] found in US Army Rangers. More specifically, Dolan et. al.,[15] found that with hormonal decrements, jockeys congruently had reduced bone mass and an elevated rate of bone loss due to this disruption in hormone activity in response to chronic weight cycling. This congruent reduction of hormonal and BMD is akin with previous female literature suggesting a potential commonality to Triad-like symptoms in males. There is inadequate research on the negative physiological effects and health outcomes associated with male athletes

participating in high-energy expenditure activities amid decreased energy needs with or without disordered eating/eating disorders (DE/ED).

Disordered Eating/Eating Disorder Risk

Previous research has demonstrated women who train and compete in a LEA state have a 20%-48%[1,21] higher prevalence of negative health outcomes, including nutrient deficiency, chronic fatigue, and increased infections and illness.[6,7] While these outcomes are known in women, empirical deficient EA research is lacking in men relative to BMD, cardiovascular and reproductive systems.[21] In females, LEA results from an inadequate dietary intake which may be present with or without an eating disorder (ED)/disordered eating (DE).[5-13,21] Previous research has approximated only 8% of elite male athletes exhibit ED/DE.[11] However, Martinsen and colleagues[22] found the prevalence of DE in male athletes range from 10%-42%.

Mechanisms for LEA in women are reflective of either high exercise energy expenditure (EEE) or low energy intake (EI) with or without ED/DE's.[5-12] Mechanisms for deficient EA in male athletes have yet to be identified (ie. high EEE or EI with or without DE/ED's). However, research demonstrates possible mechanisms including, long periods of restricted EI or rapid weight loss have demonstrated associations to other negative effects on cognitive function, growth, and sport performance.[23] Hagmar and colleagues[24] observed another psychological mechanism with male leanness in Olympic sport athletes and found these athletes had significantly lower body fat proportions, lower free T, lower leptin levels, and an increase in IGF-I levels.[24] Some researchers believe due to the physiological characteristics specific to men, such as testosterone, males are

not as vulnerable to similar negative health outcomes associated with women and the Triad.[11,15] However, research has demonstrated males in various sporting activities have similar physiological results to the Triad in relation to decreases in reproductive hormones, BMD, and compromised dietary intake.[11,12]

Statement of Problem

Presently, limited research has identified males have similar mechanisms associated with reproductive health, bone health and EA; however it is unclear the extent of these outcomes.[14] Koehler et. al.[17] describes energy deficiency can lead to metabolic alterations, which are linked to long-term health outcomes and declares these outcomes need to be examined further to explore underlying mechanisms in relation to endocrine and metabolic responses in relation to energy deficits. The IOC's RED's model confirms the need to examine the physiological effects of energy deficiency in male athletes. De Souza et. al.,[7] and Mountjoy et. al.,[11] concur and have expressed that research examining energy deficiency in the male athlete is in its preliminary stages[7,11] and continued research is necessary for male athletes specifically.[11] De Souza et. al.,[7] also stresses the importance of examining physiological differences amongst males and females independently and suggests the need to establish independent clinical guidelines specific to males and females.

Manuscript 1: Specific Aims and Hypothesis

Aim 1.1: Examine the Male Triad symptoms (energy availability, reproductive function and bone mineral density) among endurance trained male athletes.

Hypothesis 1.1: Male endurance trained athletes will display at least one decreased component of either: EA, reproductive hormones (Testosterone [T] and Luteinizing Hormone [LH]) and/or bone mineral density.

Hypothesis 1.2: Male endurance athletes who display deficient EA will have corresponding decreased symptoms of the other two components: decreased reproductive hormone (T and LH) and bone mineral density.

Aim 2: Examine differences in EI, EA, EEE, and reproductive hormones (T and LH) between high-volume (HV) and low-volume training (LV) weeks.

Hypothesis 2.1: Male endurance trained athletes will display differences in EI, EA, EEE, and reproductive hormones (T and LH) between the 2 training weeks.

Hypothesis 2.2: Male endurance trained athletes will display a decrease in EI and EEE in the LV week

Hypothesis 2.3: Male endurance trained athletes will display a decrease in EA and reproductive hormones (T and LH) in the in the HV week.

Hypothesis 2.4: Male endurance trained athletes will display an increase in EEE in the HV week.

Aim 3: Examine dietary behaviors regarding macronutrient profiles in male endurance trained athletes.

Hypothesis 3.1: Male endurance trained athletes will display decreased carbohydrate (CHO) intake with increased protein (PRO) and fat intake in relation to the American College of Sports Medicine (ACSM) nutritional guidelines.

Aim 4: Examine pathogenic dietary behavior in endurance trained male athletes.

Hypothesis 4.1: Male endurance trained athletes will display increased risks of ED/DE.

Hypothesis 4.2: Male endurance trained athletes who display increased risks of DE/ED will also demonstrate decreased energy availability.

Manuscript 2: Specific Aims and Hypothesis

Aim 1: Examine the effect of EA on reproductive (T and LH) and metabolic hormones (insulin, leptin, cortisol, and interleukin-6 [IL-6]) in male endurance trained athletes.

Hypothesis 1.1: Endurance trained male athletes with deficient EA will present with increased metabolic hormone (cortisol and IL-6).

Hypothesis 1.2: Endurance trained male athletes with deficient EA will present with and decreases in metabolic (insulin and leptin) and reproductive (T and LH) hormones.

Hypothesis 1.3: Endurance trained male athletes without deficient EA will not demonstrate compromised metabolic markers.

Aim 2: Examine differences in metabolic (insulin, leptin, cortisol and IL-6) and reproductive (T and LH) hormones between high-volume (HV) and low-volume training (LV) weeks.

Hypothesis 2.1: Male endurance trained athletes will display differences in metabolic (insulin, leptin, cortisol and IL-6) and reproductive (T and LH) hormones between the 2 training weeks.

Hypothesis 2.2: Male endurance trained athletes will display a decrease in metabolic (insulin, leptin) and reproductive (T and LH) hormones in the HV training week.

Hypothesis 2.3: Male endurance trained athletes will display an increase in metabolic (cortisol and IL-6) hormones in the HV training week.

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CHAPTER 2

MALE TRIAD SYMPTOMS AMONGST ENDURANCE-TRAINED MALE ATHLETES[1]

[1] Erin M. Moore, Toni M. Torres-McGehee, Clemens Drenowatz, Thaddus C. Brodrick, Brittany T. Williams, David F. Stodden, Justin M. Goins. To be submitted to *MedSci Sports Exerc.*

ABSTRACT

Literature examining the physiological consequences associated deficits in energy availability (EA) for male athletes is sparse. **Purpose:** To examine Male Triad components (low energy availability [LEA] with or without an eating disorder [ED], reproductive hormones [testosterone (T), and Luteinizing hormone (LH)] and bone mineral density [BMD]) in endurance-trained male athletes. **Methods:** We utilized a cross-sectional design on 14 endurance trained male athletes (age: 26.4 $\pm$ 4.2 yrs.; weight: 70.6 $\pm$ 6.4 kg; and height: 179.5 $\pm$ 4.3 cm) whom were recruited from the local community. Two separate training weeks (low [LV] and high [HV] training volumes were collected including: dietary logs, exercise logs, BMD, and blood concentrations for 2 hormones (T and LH). Anthropometric measurements (height, weight, and body composition) were taken prior to data collection. **Results:** Overall, EA presented as 27.6 $\pm$ 12.1 kcal/kg FFM·d with 35% (n=5) of participants presenting with increased risk for ED. Examining Male Triad components: 1) 32.1% presented with LEA ($\leq$ 20 kcal/kg FFM·d), 2) Reproductive hormones T (1780.6 $\pm$ 1672.6ng/dL) and LH (813.7 $\pm$ 314.2 pg/mL) were within normal limits compared to normative data, and 3) BMD was not compromised at 1.31 g/cm^2. Macronutrient profile demonstrated 92.9% were under-consumed carbohydrates (CHO), 35.7% over-consume protein (PRO) and 42.9% over consumed fats compared to ACSM recommendations. **Conclusion:** Within this study, endurance-trained male athletes presented with one compromised component of the Triad (LEA with or without ED risk). Overall, both reproductive hormones (T and LH) and BMD were not compromised. Macronutrient profiles present similar to female literature, under-consuming CHO and over-consuming PRO and Fat. These results suggest similar mechanistic behaviors to previous literature related to Triad symptoms in female athletes.

Further examination into physiological mechanisms of Triad symptoms is needed in the male athlete population.

Key Words: Triad, Low energy availability, Bone mineral density, Testosterone, Luteinizing hormone.

Introduction

The Female Athlete Triad (Triad) established 3 components (1) low energy availability (LEA) with or without eating disorder, (2) hypothalamic reproductive dysfunction, and (3) compromised bone mineral density (BMD).[1,2] Energy availability markers for female athletes have been established at 45 kcal/kg FFM·d for energy balance,[2-10] and 30 kcal/kg FFM·d[4-7,10,11] for LEA. Previous literature found significant physiological changes, which occur at and below 30 kcal/kg FFM·d.[4-7,10,11] These physiological changes include: metabolic rate declines, bone reabsorption increases, and protein synthesis breakdown occurs at this level, and changes in reproductive hormones (i.e., LH, FSH, and estrodral).[2-7,10] These norms have not been established for the physically active male; therefore the Female Athlete Triad (Triad) Coalition and the International Olympic Committee (IOC) identified males as an under-researched population examining energy deficiency and its association to compromised physiological functions.[3]

More specifically, examination of Triad symptoms has not been examined in male endurance runners. However, literature suggest sports emphasizing leanness (e.g., endurance sports, etc.) and sports requiring weight control may be at increased risk for nutritional deficits, and in turn be at risk for compromised physiology functions (e.g., decreased BMD, decreased hormonal response (leptin, insulin, and Testosterone [T]).[12-19] The American College of Sports Medicine (ACSM)[20] has identified 3 categories describing the origins of energy deficiency in athletes; (1) lack of knowledge of energy needs based on demands of the energy expended, (2) clinical eating disorders and (3) intentional subclinical mismanagement of energy consumption to reduce body size and

fatness.[20] This could include disordered eating behaviors similar to various eating disorders such as fasting and purging.[20] Previously established research has examined and recognized the physiological differences in males and females, prompting De Souza et. al.,[3] in response to the IOC's REDs model, to stress the necessity to establish independent clinical guidelines in regards to energy deficiency and the physiological impacts specifically for males and females.

To date, only one study has examined reduced energy availability (EA) set at 15 kcal/kg FFM·d in 6 exercising men and found decreases in leptin and insulin hormones, but not for Testosterone (T).[17] However, Koehler et al. (2016)[17] did not examine BMD. A second study examined 4 triathletes' BMD and total T and revealed only 1 athlete's Z-score below -1.0 and 3 participants had low T levels (216 ng/dL to 242 ng/dL).[21]

Previous literature examining male military populations assessed hormonal responses to extreme training environments and found negative physiological effects.[14,18] More specifically, examination of energy deficits in soldiers demonstrated with both high exercise energy expenditures (EEE) and decreased dietary energy intake (EI), reproductive hormone T decreased with energy deficits in males.[17,18] Testosterone is considered a more robust hormone however, LH has proven critical during examination in female literature, due to its' precursory control over estrogen (females) and T (males). Additionally, male cyclists and jockeys have demonstrated trends of decreased hormonal outcomes in relation to reduced bone mass[22,23] and increased bone loss rates.[12,13]

Due to high EEE demands of endurance sports, male endurance athletes may be at increased risk of Male Triad symptoms. A review of literature found male athletes present similar symptoms to female athletes, with low caloric EI, lower reproductive

hormone levels (T), and decreased BMD.[19] Tenforde et al. (2017)[19] examined parallels between Triad symptoms in males and females and determined that for males, a better understanding of nutritional deficits including a definition of EA, confirmation whether disordered eating/eating disorder risk contributes to chronic energy deficits, and the relationship of EA to metabolic changes (specifically reproductive hormones and BMD) is needed.

Our study sought to examine Male Triad symptoms (energy availability [EA] with or without an eating disorder [ED], reproductive hormones [T an LH] and bone mineral density [BMD]) among endurance trained male athletes. We hypothesized endurance trained athletes would display at least one decreased component of the Male Triad. A secondary purpose was to examine differences in energy needs (EI, EA, and EEE) and hormonal changes (T and LH) across 2 separate training volume weeks (high volume [HV] training week and low volume [LV] recovery week). We hypothesized EI and EEE would be increased in the HV week and EA, T, and LH would be decreased in the HV week. Third, we aimed to examine dietary behaviors regarding macronutrient profiles and eating disorder (ED) behaviors. We hypothesized endurance trained athletes would display decreased carbohydrate (CHO) intake with increased protein (PRO) and fat intake in relation to the American College of Sports Medicine (ACSM) nutritional guidelines.[24]

Methods

Participants

Fourteen male participants (age: 26.4 ± 4.2 yrs.; weight: 70.6 ± 6.4 kg; and height: 179.5 ± 4.3 cm) were recruited from the local community. Specific inclusion criteria for participation included: participant is male, within a competitive season and actively training and racing >10 hours/week for at least 3 months,[7,14,25] has a body fat percentage ≤ 12%,[7,13,14,25,26] has maintained weight stability (± 3 kg in past 6 months),[17] has a VO_2max that is considered excellent to superior for age specific range (18-20 years: Excellent 51.0-55.9; Superior > 55.9 ml/kg/min, 20-29 years: Excellent 46.5-52.4; Superior > 52.4 ml/kg/min, 30-39 years: Excellent 45.0-49.4; Superior > 49.4 ml/kg/min), [17,27] and was required to be independent of any injury that would prevent them from full participation in a high-endurance sport (running, triathlon, or obstacle racing).

Specific exclusion criteria included any previous history of smoking, past or present diagnosis of clinical eating disorder, infectious disease within past 4 weeks, history of cardiovascular disease or orthopedic impairment that interferes with moderate to vigorous exercise, no history of thyroid or pituitary disease, use of medication, diabetes mellitus, known metabolic disease, and no long-term steroid use. Institutional Review Board was obtained prior to the start of the study and all participants provided consent prior to participation.

Instruments and Protocols

Basic Demographic Survey. Basic demographic information included: age; education level; ethnicity; exercise background; and pertinent medical history questions including known metabolic diseases, history of cardiovascular, thyroid, or pituitary diseases, and long-term steroid use were collected.

Anthropometric Measurements. Multiple anthropometric measurements were collected including height, weight, and body composition, which was measured according to ACSM standardized procedures.[20] Height was measured with a Stadiomometer (Shorr Productions, Maryland) to the nearest 0.1 cm and weight was measured wearing minimal clothes to the nearest .01 kg with a scale (Tanita SC-331S Body Composition Scale, Tanita Co., Tokyo, Japan). Body fat was assessed using Tanita scale (Tanita SC-331S Body Composition Scale, Tanita Co., Tokyo, Japan) for inclusion criterion and Dual-Energy X-Ray Absorptiometry (DXA) (GE Lunar Prodigy densitometer) for data analysis.

Dual-Energy X-Ray Absorptiometry (DXA). To measure bone mineral density (BMD) (g/cm^2) of the total body, we used the DXA (GE Lunar Prodigy densitometer)[2,20]. It is the gold standard for bone mineral density assessment.[20] Participants were instructed to: 1) not eat/overnight fast (12 hour fast), 2) refraining from vigorous exercise at least 15 hours prior to scan, 3) no caffeine and alcohol consumption during the preceding 24 hours, and 4) consume a normal evening meal the night before.[20] Scoring of BMD was in either "normal" or "low" categories. These categories were established from the T-scores and Z-scores. The T-score is recognized the subject's BMD score compared to the

average 30 year old adult.[28] The Z-score is the subject's BMD compared to the average score of subjects consisting of the same age, sex, weight, and ethnic or racial background.[28] Scoring for the Z-score is considered "normal" when the scores fell between -1.0 and +1.0, and were considered "low" if scores fall below -1 and -2.5.[28] Osteoporosis is a third category established with a Z-score below -2.5.[28]

Resting Metabolic Rate (RMR). Used to identify how many calories are necessary at rest. It was measured using indirect calorimetry (*Microlife MedGem*; HealtheTech, Golden, CO). The MedGem is a clinically-validated measurement device that assesses RMR.[29] While the MedGem is not the gold standard for measuring RMR; it is, however, clinically a very relevant tool.[29] Most accurate measurements are produced first thing in the morning, when the patient is rested and positioned in either a seated or semi-reclined position in a quiet room.[29] Measurements should be implemented when the patient has not eaten, exercised or drank any caffeine within the last four hours.

Total Daily Energy Expenditure (TDEE). Bodymedia SenseWear armbands continuously monitor TDEE and EEE. The armband is non-invasive, and participants wore it in all conditions (e.g., workouts, activities of daily life, sleeping) except to swim and shower.[29-36] The armband has been validated compared to indirect caliometry, double labeled water, and VO_{2max} metabolic cart. SenseWear© Software 8.0 was used to collect, save, and analyze data from the armband.[29-37]

Exercise Energy Expenditure (EEE). Two separate measurements were used to determine EEE; 1) Heart Rate Monitor (Garmin Forerunner 15) and 2) VO_{2max}-HR Regression. All results were reported as EEE in kcals.

1) **Heart Rate Monitor.** The participants wore a Garmin heart rate monitor (HRM) during exercise as a measure of EEE by calculating METs during exercise, derived from their HRM calculations. Various brands of HRM were found to accurately assess heart rates moderate activity ($r \geq 0.90$, SEE ≤ 5 beats/min).[38]
2) **VO_{2max}-HR Regression.** A VO_{2max} treadmill test using the method from Beashel and Taylor (1996)[39] targeted to endurance runners and the Parvo metabolic cart was administered as part of the inclusion criterion. Calculated VO_{2max} –HR regression slopes for each individual to match HR with EEE were used and reported in kcals.[25]

Dietary Intake. Participants recorded 2 separate weeks, 7-consecutive days per week of dietary intake. Portion sizes were explained, and take-home examples were given prior to food record distribution. Dietary records were analyzed for total kilocalories and macronutrient (carbohydrates [CHO], proteins [PRO], and fat) consumption using a dietary analysis software program (ESHA food processor 8.0, Salem, OR). Research has demonstrated that despite food intake restrictions, reported intake accuracy was superior using a 7-consecutive day weighted-diet record compared to a food-frequency questionnaire. Food records were used to examine EI, macronutrient intake and EA. Macronutrients were analyzed using ACSM standards; CHO 6-10g/ kg for endurance runners, PRO 1.2-2.0g/kg, and Fats calculated as 20-35% of total kcals consumed.[24]

Eating Disorder Inventory-3 (EDI-3) and Symptom Checklist (EDI-3 SC). The Eating Disorder Inventory-3 is a self-reported survey validated to identify subjects with disordered eating patterns.[40] The inventory includes 91 items, organized into 12 primary

scales, consisting of three eating-disorder specific scales and nine general psychological scales that are relevant but not specific to eating disorders.[40] The EDI-3 yields six composite scales, five general integrative psychological constructs (i.e. Ineffectiveness, Interpersonal Problems, Affective Problems, Over-control, and overall Psychological Maladjustment composite) and one eating disorder specific composite (Eating Disorders Risk Composite).[40] The EDI-3 is validated for age ranges of adolescent (13 years of age) through elderly (53 years of age) subjects.[40] Reliability for the EDI-3 composites are high.[40] Coefficient and median values for specific composites include; Eating disorder risk (r=.98, median=.95) and General Psychological Maladjustment (r=.97, median=.93).[40]

The EDI-3 SC is a screening tool designed specifically for Allied Health professionals to identify individuals at risk for eating disorders, providing information regarding the frequency of eating disorder risk behaviors or symptoms (i.e. binge eating, self-induced vomiting, exercise patterns, laxative use, diet pill use, and use of diuretics).[40].[40] EDI-3 Inventories (EDI-3 and EDI-3 SC) are copyright surveys from the Psychological Assessment Resources, Inc. and permission of use is granted with purchase of inventory.[40] To be determined "at risk" for ED, participants must be identified as "Typical Clinical" or "Elevated Clinical" for at least 1 EDI Composite score, and/or meet the criteria for risk of pathogenic behavior (e.g., restricting, excessive exercise, binge eating, vomiting, laxatives, diet pills, etc.).

Energy Availability (EA). Defined as the amount of dietary energy remaining after exercise, expressed as kcal/kg/free fat mass (EA = [EI – EEE] kcal/kg FFM·d).[2] Energy

availability was examined using EI and EEE over 2 separate weeks of 7-consecutive days. Low energy availability (LEA) was defined as ≤ 20 kcal/kg FFM·d.

Energy Balance (EB). Defined as the TDEE and dietary intake remaining at an equal level [dietary EI (kcals/day) = TDEE kcal/day)]. Energy balance was examined using dietary EI and TDEE over two 7-consecutive day weeks and will be defined as, 1) negative EB [EI < TDEE], 2) positive EB [EI > TDEE], or 3) balanced EB [EI = TDEE].[2-8,10,41]

Hormone Measures. All fasting blood samples were acquired with 24 hours of non-physical activity at the completion of each 7-consecutive day week. Blood samples were centrifuged, and plasma drawn out to assess T and LH using enzyme-linked immunosorbent assay (ELISA) Kits specific for each hormone. This study used enzyme-linked immunosorbent assay (ELISA) kits specific for T and LH. Research has shown ELISAs to report specific and highly sensitive procedures for identify various substances. Sensitivities of ELISAs are high, 1-10 ug/liter range with the correlation coefficient were reported between 0.95-0.99.[42] Establishment of cutoffs were identified as 1) low, 2) within normal limits, or 3) high based on previously established normative data specific for males (adult and age range specific) associated for T, while LH used the standard curve for the ELISA kit. The ranges include the following for reproductive hormones: T = 270-1070 ng/dL (average 679 ng/dL)[43] and LH = 140 pg/mL–10,000 pg/mL.

Training Conditions. Two separate training weeks were used to assess differences between energy needs and hormones.

1) **High Volume Training Week (HV).** A high-volume training week consists of at least 5 days of training and includes at least 10 hours of training with in a 7-consecutive day week.
2) **Low Volume Training Week (LV).** Low-volume training week, or a recovery week, was described as an unloading week for the participant. No specific requirements were established except participants were asked to work out a minimum of 2-3 days for the 7-consecutive day week.

Detailed Procedures

The data collection spanned across 2 separate weeks consisting of 1 day for an information and initial measurement session, and two 7-consecutive day weeks where 1 week was during the low volume/recovery week (LV), and 1 week was during the high volume-training week (HV).

Part I Recruitment:

Participants were recruited from local area running clubs and races. An information letter via email was used to give a brief overview of the study. All participants interested were individually corresponded with to set-up assigned dates and times for informational sessions and anthropometric measurements.

Part II Data Collection:

Informational Session: Participants attended an orientation session prior to the data collection sessions. This session consisted of a written and verbal overview of the study, participant expectations, instructions from the researchers describing various tools

used during data collection including: *ESHA FoodProdigy, BodyMeida SenseWear Armband, and Heart Rate Monitor*

Prior to Data Collection: Participants first completed a series of surveys, a brief interview, used to follow up on medical history, physical measurements (height, weight, and percent body fat), $V0_{2max}$ test, and resting metabolic rate (RMR). Blood Draws and DXA scans were scheduled for the 2 separate weeks of data collection. Blood draws consisted of 2 tubes (2.4 tsp) during each data collection. A total of 2 data collection sessions occurred, accumulating in 4 tubes (4.8 tsp) for the entire study.

Data Collection: Participants completed 2 separate 7-consecutive day weeks at 2 different levels of training volume (High/training and Low/recovery). All equipment (e.g., BodyMedia SenseWear Armband, HRM, and food log entry information) was provided on Day 1 and instructions were given both oral and written via email. Data collection HV week was a "normal" week with a minimum of 5 training days/week and participants were engaged in a competitive season. The procedures for each of the 2 weeks were identical; the only change was their volume load (HV and LV).

Training Weeks: Participants were instructed to not change their daily/weekly activities and physical activity/exercise, while recording their food and daily training for 7-consecutive days. They were instructed to wear the armband for 23 hours/day and wear a HR monitor only during exercise during training for the 7-consecutive days. At the end of the 7-consectuve day training week, participants came in for a fasting blood draw and DXA scan. All equipment was returned and the next week was scheduled.

Statistical Analysis

IBM SPSS statistical Software (version 24; SPSS Inc., Chicago, IL) and an *alpha* ≤ 0.05 was used for all analyses. Based upon power analysis a priori and based upon means of previous literature from Koehler et. al.,[17] and Loucks et. al.,[11] an effect size between 1.0 and 3.0 yielded a sample size of 6-10 subjects. Using the Wilcoxon signed rank test, 14 subjects allowed for full saturation. Descriptive statistics for all dependent variables were calculated. Frequencies and proportions with 95% confidence intervals were calculated for all categorical variables (Male Triad symptoms: at risk for LEA [with or without and ED], at risk for reproductive dysfunction, and at risk for Low BMD, pathogenic behaviors [e.g., restricting, excessive exercise, vomiting, etc.], and macronutrient assessment. Chi-square analysis were used to examine "at risk" for LEA, "at risk" for ED, and macronutrient profile compared to ACSM recommendations.

A 2 (week) X 7 (days) ANOVA and paired T-tests assessed differences between the two training weeks for EEE, EA, TDEE, EB, EI, and macronutrients. Paired T-tests were used to determine differences between the 2 training weeks and variables (e.g., Testosterone, LH, EEE, and EA). Pearson's correlation and regressions were used to examine relationships between EA and reproductive hormones.

Results

Eighteen participants began the study, 1 dropped out due to fear of needles and 3 were eliminated for lack of compliance with the required procedures of the study, yielding a total of 14 participants. Participants criterion demonstrated a VO_{2max} 62.3 $\pm$

6.9 ml/kg/min, FFM 65.7 ± 5.4 kg, and a DXA BFP 13.6 ± 3.5%. Ethnicity of participants demonstrated 78.6% Caucasian (n = 11), 14.3% African American (n = 2) and 7.1% Middle Eastern (n = 1). Education levels revealed 92.9% (n = 13) had some level of college and higher. Specifically, 28.6% (n = 4) attained some level of college, 21.4% (n = 3) attained a bachelor's degree, 35.7% (n = 5) attained a master's degree, and 7.1% (n = 1) attained a clinical doctorate, and 1 participant (7.1%) attained a GED. Overall distance was 49.2 ± 78 miles (HV: 63.4 ± 100.6 miles and LV: 34.9 ± 45.4 miles) with no significant difference between the 2 training weeks ($F_{(1,26)} = 0.93$, $p = 0.34$). Overall training time was 5.6 ± 4.3 hours per week (HV: 7.1 ± 5.3 hours and LV: 4.1 ± 2.5 hours) with no significant difference between the 2 training weeks ($F_{(1,26)} = 3.74$, $p = 0.06$).

Triad- Symptoms: Overall, zero participants met the criteria for all 3 Male Triad components (LEA, reproductive dysfunction, and low BMD), and no participates were at risk solely for reproductive dysfunction and low BMD. When examining LEA with or without ED risk using EEE from Garmin HRM, 32.1% (n = 9) of participants presented with LEA over the 2 training weeks (HV: 35.7%, n = 5; LV: 28.6%, n = 4). Twenty percent (n = 2) of participants presented having LEA with the risk of ED (HV: 20%, n = 1; LV: 20%, n = 1), while 38.9% (n = 7) of participants presented having LEA without the risk of ED (HV: 44.4%, n = 4; LV: 33.3%, n = 3). When examining LEA with or without ED risk using EEE from $V0_{2max}$-HR regression, 32.1% (n = 9) of participants presented with LEA over the 2 training weeks (HV: 35.7%, n=5; 28.6%, n = 4). Thirty percent (n = 3) of participants presented having LEA with the risk of ED (HV: 40%, n =

2; LV: 20%, n = 1), while 33.3% (n = 6) of participants presented having LEA without the risk of ED (HV: 33.3% n = 3; LV: 33.3% n = 3).

Energy Assessment: All means and standard deviations for energy needs assessment data (e.g., RMR, EI, EA etc.) can be found on Table 2.1. A 2 (training conditions: HV, LV) x 7 (days) ANOVA and paired T-tests were used for EA, EI, EEE, TDEE and EB. Overall EA_HRM was 29.7 ± 10.5 kcal/kg FFM·d (95% CI: 24.9, 34.5 kcal/kg FFM·d) and overall EA_VO_{2max} Regression was 30.2 kcal/kg FFM·d (95% CI: 25.6, 34.7 kcal/kg FFM·d). Paired t-tests demonstrated an average EA for both measurements: 1) EA_HRM: HV week: 26.9 $\pm$ 9.6 kcal/kg FFM·d and LV week: 29.5 $\pm$ 11.2 kcal/kg FFM·d ($t_{(13)} = -1.37$, $p = 0.15$); and 2) EA_VO_{2max} Regression: HV week: 25.2 $\pm$ 12.9 kcal/kg FFM·d and LV week: 29.9 $\pm$ 11.1 kcal/kg FFM·d ($t_{(13)} = -1.61$, $p = 0.13$). No significant differences were elicited between training weeks and EA. Regarding both training weeks, examination in the specific daily LEA account can be found in Table 2.1.

With or without an Eating Disorder. Overall, 35.7% (n = 5) of participants presented with at risk for composite scales EDI-3 (all data found in Table 2.2 and Table 2.3). Most participants did not demonstrate a specific risk for ED risk subscales and/or composite scale. Within the interpersonal problem composite (IPC), 21.3% (n = 3) scored as typical clinical, with the two subscales of 20% (n = 3) scoring typical clinical on the interpersonal insecurity (II) scale, and within the interpersonal alienation (IA) scale 21.4% (n = 3) scored typical clinical. The over control composite (OC) presented with 28.6% (n = 4) scoring typical clinical with the two subscales presenting 50.0% (n = 7) with typical clinical scores of and 7.1% (n = 1) elevated clinical scores for perfectionism scale and 21.4% (n = 3) typical clinical scores of Asceticism scale.

Maturity fears presented with the highest scores between all the sub-scales with 35.7% (n = 5) demonstrating typical clinical scores and 21.4% (n = 3) with elevated clinical scores. Two participants (14.3%) were identified as using restrictive behaviors while 42.9% (n = 6) of the participants used exercise to lose weight. Four participants (28.6%) used exercise to lose weight < 25% of the time while 14.3% (n = 2) used exercise for weight loss between 25%-50% of the time.

Energy Intake and Macronutrient Profile. One-way repeated-measures ANOVA (assumptions of sphericity were met due to only 2 weeks assessed) were calculated to compare difference across the 2 training weeks for EI and macronutrients (CHO, PRO, and Fats). Overall EI was 2658.9 ± 887.1 kcals (95% CI: 2314.9, 3002.9) and overall macronutrient intake demonstrated CHO: 4.9 ± 1.7g (95% CI: 4.2, 5.8), PRO: 1.7 ± 0.6 g (95% CI: 1.5, 1.9), and Fat: 32.3 ± 5% of total kcals (95% CI: 30, 34.6). No significant effect was found between the HV and LV training weeks for the following variables: EI: $F(1,13) = 2.04$; ($p = 0.18$), CHO: $F(1,13) = 0.15$; ($p = 0.70$), PRO: $F(1, 13) = 1.26$; ($p = 0.28$), and fat: $F(1,13) = 0.004$; ($p = 0.95$). Consumption of macronutrients demonstrated decreased CHO intake during both weeks, specifically 92.9% (n = 13) were below the recommendations in the HV week and 78.6% (n = 11) were below during the LV week. During the HV week, 35.7% (n = 5) were above the recommendations for protein, 21.4% (n = 3) were below recommendations for protein. Whereas, during the LV week, 28.6% (n = 4) over consumed protein and only 14.3% (n = 2) under consumed compared to the ACSM recommendations for protein. Participants (42.9%, n = 6) over-consumed fats during the HV week, while 35.7% (n = 5) over-consumed fat during the LV week. Chi-squares demonstrated that those presenting with LEA through Garmin HRM

demonstrated 36% (n = 9) under-consumed CHO, 33.3% (n = 2) under-consumed protein, and 20% (n = 2) over-consumed fats, while $V0_{2max}$-HR regression demonstrated, 36% (n = 9) under-consumed CHO, 66.7% (n = 4) under-consumed protein, and 30% (n = 3) over-consumed fats. Pearson's chi-square tests demonstrated no statistically significant association between overall EA_VO_{2max} Regression and CHO χ (2) = 1.6, p = 0.21, and overall EA_HRM and Fats χ (2) = 0.03, p = 0.87. However, overall PRO did demonstrate a significant association to EA_HRM χ (2) = 7.8, p = 0.02 (HV: χ (2) = 5.5, p = 0.06; LV: χ (2) = 2.4, p = 0.31) as well as the training weeks of Fat (HV: χ (2) = 4.3, p = 0.04; LV: χ (2) = 3.8, p = 0.05). When examining associations of EA_HRM to micronutrition's, only overall PRO (χ (2) = 7.1, p = 0.03) and Fat HV week (χ (2) = 4.3, p = 0.04) demonstrated statistically significant results.

Exercise Energy Expenditure: Two measurements were used to assess EEE. Due to missing data cells (determined by when participants exercised) an ANOVA was not used for EEE. A paired samples t-test was calculated to compare the mean of the 2 training weeks (HV and LV). The mean of HV week using Garmin HRM was 919.3 $\pm$ 538.2 kcals and the mean for the LV week using Garmin HRM was 696.5 $\pm$ 472.7 kcals. A significant difference between the 2 training weeks (HV vs. LV) was elicited ($t_{(13)}$ = 3.5; p = 0.004). The mean of HV week using VO_{2max}-HR regression was 1048.5 $\pm$ 805.6 kcals and the mean for the LV week was 682.3 $\pm$ 326.5 kcals. No significant difference between the 2 training weeks (HV vs. LV) was elicited ($t_{(13)}$ =1.7; p = 0.10).

Total Daily Energy Expenditure and Energy Balance: Participants TDEE for both weeks was 2993.0 $\pm$ 160.8 kcal, (HV: 3073.1 $\pm$ 180.5 kcals, LV: 2912.9 $\pm$ 149.4 kcals) which resulted in an overall negative EB of -139.3 $\pm$ 201.0 kcal, (HV: -289.4 $\pm$ 220.9

kcals and LV: 10.7 $\pm$ 205.6 kcals). There was no significant main effect found between the training weeks and TDEE: $F_{(1,11)} = 4.02$, ($p = 0.07$) or EB: no main effect $F_{(1, 11)} = 4.40$, ($p = 0.06$).

Hormones: A paired samples t-test was calculated to compare the means between the 2 training weeks (HV and LV) for T and LH. The mean for T was 1764.5 $\pm$ 1598.2 ng/dL (95% CI: 959.3, 1398.4) (T_HV: 1640.5 $\pm$ 1385.3 ng/dL and T_LV: 1888.3 $\pm$ 1831.1 ng/dL. The mean for LH was 795.6 $\pm$ 313.9 pg/mL (95% CI: 612.7, 794.9) (LH_HV: 385.7 $\pm$ 191.0 pg/mL and LH_LV: was 409.9 $\pm$ 119.9 pg/mL. No significant differences were found between the 2 training weeks and T ($t_{(12)} = -1.47$ $p = 0.17$) or LH ($t_{(12)} = -0.79$, $p = 0.44$). Neither T nor LH presented low compared to normative male value. A Pearson correlation coefficient was calculated for the relationship between T, LH and other variables. A strong positive correlation was found for both weeks; HV: ($r_{(13)} = 0.77$, $p = 0.002$) and LV: ($r_{(13)} = 0.84$, $p < 0.001$), indicating a significant linear relationship between the LH and BFP. A strong negative correlation was found for overall T to EI: ($r_{(26)} = -0.47$, $p = 0.02$), RMR: ($r_{(26)} = -0.64$, $p < 0.001$), and a strong positive correlation for overall T to DXA_BFP ($r_{(26)} = 0.83$, $p < 0.001$).

Testosterone (T): Two outliers were removed prior to regression analysis was ran. A simple linear regression was calculated to predict participant's T levels based on their EA. The regression equation was not significant ($F_{(1,23)} = 3.2$, $p = 0.89$) with an R^2 of 0.12. Energy availability is not a significant predictor of T levels. A simple linear regression was calculated to predict participants' T levels based on their RMR. A significant regression equation was found ($F_{(1,23)} = 16.23$, $p < 0.001$), with an R^2 of 0.4. Participants' predicted T level is equal to 3123.5 + -0.97(RMR) ng/dL when RMR is

measured in kcals. Participants' average T levels decreased -0.97 ng/dL for each kcal of RMR. A simple linear regression was calculated to predict participants' T levels based on their EI. A significant regression equation was found ($F_{(1, 24)} = 6.7$, $p = 0.02$), with an R^2 of 0.22. Participants' predicted T level is equal to 2591.9 + -0.44(EI) ng/dL when EI is measured in kcals. Participants' average T levels decreased -0.44 ng/dL for each kcal of EI. A simple linear regression was calculated to predict participants' T levels based on their DXA_BFP. A significant regression equation was found ($F_{(1, 24)} = 51.9$, $p < 0.001$), with an R^2 of 0.83. Participants' predicted T level is equal to -1273.5 + 197.2 (DXA_BFP) ng/dL when DXA_BF is measured in kg. Participants' average T levels increased by 197.2 ng/dL for each kg of DXA_BFP. A significant association was demonstrated between EA_VO_{2max} Regression and overall T (χ (2) = 4.4, $p = 0.04$) and T from the HV week (χ (2) = 5.8, $p = 0.02$). No other significant relationships were found.

Luteinizing Hormone (LH): Four outliers were removed prior to regression analysis. A simple linear regression was calculated to predict participant's LH levels based on their EA. The regression equation was not significant ($F_{(1,22)} = 1.6$, $p = 0.22$) with an R^2 of 0.07. EA is not a significant predictor of LH levels. A simple linear regression was calculated to predict participant's LH levels based on their DXA_BFP. The regression equation was not significant ($F_{(1,22)} = 2.08$, $p = 0.16$) with an R^2 of 0.09. The DXA_BFP is not a significant predictor of LH levels. No other significant relationships were found.

Bone Mineral Density: Total bone density was 1.3 ± 0.1 g/mc^2 with a Z-score of 1.3 ± 0.9 g/mc^2 suggesting overall, participants did not present with compromised bone health. Overall measurements from the DXA scan included a T-score of 1.1 ± 1.1 g/mc^2

and specific areas of the body including Legs 1.4 ± 0.1 g/mc^2, Pelvis 1.2 ± 0.2 g/mc^2, and Spine 1.2 ± 0.1 g/mc^2 indicating a healthy level of BMD.

Discussion:

In this study, we sought to identify and measure all 3 components of the Male Triad, LEA with or without an ED (set at 20 kcal/kg FFM·d), hypogonadotropic hypogonadism[19] (T and LH), and BMD across 2 separate training volume weeks (HV and LV). Our overall results supported our hypothesis that endurance trained male athletes would exhibit at least one component of the Male Triad. We found that 32.1% exhibited LEA (with or without an ED); however, our participants did not demonstrate decreased reproductive hormones (T and LH) or decreased BMD either with or without LEA.

Energy Availability

Within this study, 2 separate training weeks (HV and LV) were used to examine energy needs and demands specific to EA, EI including macronutrients (carbohydrates, proteins, and fats), and pathogenic eating behaviors, EEE, TDEE, and EB. To assess EA, we created a cutoff point for LEA at ≤ 20 kcal/kg FFM·d. Our results yielded an average EA of 25.2 kcal/kg FFM·d (HV week) and 29.9 kcal/kg FFM·d (LV week). These results demonstrated considerably lower overall EA compared to Reed et al. (2013)[44] study of free-living NCAA Division I female soccer players presenting with EA (LEA set at ≤ 30 kcal/kg FFM·d) at ~42 kcal/kg FFM·d in pre-season, ~32 kcal/kg FFM·d in mid-season, and ~43 kcal/kg FFM·d in post-season. While overall neither week demonstrated LEA using VO_{2max}-HR Regression EA, 71.4% (n = 10) of the participants

demonstrated LEA between 1 and 4 days during both HV and LV weeks. There currently is no research examining free-living EA in males to compare our results with.

The EEE seen in this study was congruent with previous studies in females. Melin et al.[19] reported an average of 879 kcal EEE, with participants who presented with LEA reported EEE of 1222 kcal in female elite endurance athletes. Reed et al.[18] reported an average of approximately 800 kcal during preseason and 600 kcal midseason in free living female collegiate soccer athletes. Conversely, the female recreational athletes represented in this study reported an average of 531 kcal EEE. Therefore, the participants in this study did not have to exercise at the same level as seen in those collegiate and elite athletes to demonstrate LEA. This may be due to the great decrease in EI mentioned before. As the participants in this study did not eat as much as seen in the previous studies, the participants did not require as high of a level of EEE to put them at risk for LEA.

However, EEE seen in this study was far lower compared to male participants in other studies. In Friedl et al.[10] study on army rangers, the estimated EEE was approximately 4000 kcal/day, whereas Dolan et al.[16] estimated EEE on race days for professional jockeys was 3952 kcal. The male participants in this study's EEE were only a fraction of that amount at an average of 687.8 kcal. This may be due to a few reasons, 1) the military operation studies required significant EEE demands, while our study examined free- living runners and 2) the other studies may be defining and calculating EEE in a different way than we did in our study.

Dietary EI did not reveal a statistical difference between the 2 training weeks, suggesting male endurance-trained athletes are not changing their EI in relation the

demands of the training volumes. This may be due to a lack of nutrition knowledge or may be related to pathogenic behaviors. Caloric EI (2658.9 ± 230.4 kcals/day) was lower compared to male and female mountain runners (3199 ± 701 kcal/day) pre-race day diet[45] and higher compared to female endurance runners during regular training (Low: 2140 ± 130 kcals) and intensified training (INT: 2318 ± 343 kcals).[46] Another study examining EI specifically in males was Malinauskas et al.[47] study, which examined game (home and away) days and a non-game day during a summer league college baseball season. The baseball players caloric intake for 3161 ± 709 kcals/day on non-game days, 2968 ± 26 calories/day for home game days, and 2679 ± 701 kcals/day for away game days compared to our EI of 2658.9 ± 230.4 kcals during active competitive racing season.

The macronutrient profile of our participants was similar to previous research within female athletes, specifically, the under consumption of CHO and over consumption of PRO and fats.[2,8,44] This is concerning as because within the female literature, LEA is the catalyst for negative physiological functions associated with the Triad.[1] Koehler et al.[17] (in males) and Loucks et al.[7] (in females) have both demonstrated LEA is elicited with or without exercise, which emphasizes the importance of proper nutritional practices throughout training. Within our participants, we did not notice a significant difference between the EA and the training weeks, but the HV training week did demonstrate a lower EA. This decrease in EA, was not due to a change in EI, but rather to a significant difference noted in EEE with the LV training week eliciting lower EEE kcals.

Low Energy Availability: With or Without an Eating Disorder

Overall, 35.7% presented with an increase eating disorder (ED) risk. This is elevated compared to the limited research on male athletes. Research has estimated 5% of elite male athletes present with anorexia nervosa or bulimia nervosa specifically[48] and another 1% of males represent "other specified feeding or eating disorders".[49] Norwegian researchers found 13.5% of both male and female athletes were diagnosed with a clinical and/or subclinical ED.[50] When compared to non-athletes (4.6%), this was twice as high for the athletes.[50] Sundgot-Borgen et. al.[50] also demonstrated endurance athletes presented with 9% diagnosed ED, which was higher than ball-game athletes with 5% diagnosed ED. There is limited research examining specific eating disorders prevalence in male athletes. Rates in males are currently unknown for anorexia nervosa, however an estimated 10:1 ratio has been demonstrated for females to males.[51]

The EDI-3 has specific composite and sub-scale scores which examine specific behavioral traits similar to those diagnosed with EDs, with "typical clinical" and "elevated clinical" scores indicating increased risk factors for EDs. Two specific psychological risks identified in the over-control composite, demonstrated 57.1% were classified as typical and elevated clinical for perfectionism and maturity fears. The over-control composite reflects the significant need to avoid disappointing others as well as be the best and contains sub-scales perfection (P) and asceticism (A). The sub-scale perfectionism is important to note, as this sub-scale is a distinguishing feature of EDs as well as for athletes in general, which is demonstrated in this study, with over 50% of our participant had typical or elevated scores.

Males also presented with pathogenic eating behaviors, similar to females. One pathogenic behavior, like female athletes, includes using exercise for weight control. With 28.6% using exercise to lose weight < 25% and another 14.3% using exercise to lose weight between 25%-50%. Research suggests 37% of male athletes (age 18-22) exercise 2 or more hours per day for the purposeful intention to burn calories.[52] Similar to the female literature, elite male athletes participating in sports that require leanness (endurance running) shows increases in ED risk.[49] However, to date the literature focused on ED is limited and there is not the ability to compare male athletes to other male control groups.[49]

Energy Balance

Endurance-trained male athletes demonstrated negative EB (-289.4 kcals) in the HV training week, which eliciting concern as this is indicative of poor fueling to meet the demands of EEE and TDEE. Previous research has demonstrated prolonged negative EB can lead to decreases in EA and decreased physiological processes.[6] The disparity amongst EB and EA is that an athlete can have LEA but maintain their EB. This is due to the suppression of various physiological processes due to the lack of EA.[6] Strubbs et. al.,[53] provided an example of the contrast between EB and EA. Eight lean men had a suppressed caloric energy intake and an increased exercise energy expenditure resulting in a constant energy availability of 30 kcal/kg FFM·d.[53] Strubbs et. al.,[53] found the negative energy balance decreased towards zero at a rate of 90kcal/day due to the decreased physiological processes, and estimated 3 weeks for participants to elicit an EB of zero while stilling remaining in a severely LEA state.[53]

Reproductive Hormones

We did not see a decrease in Testosterone (T) in either training week. This may be due in part to the large "normative" range of T. Both weeks demonstrated larger values than the "normal" range of 270-1070 ng/dL. With the average for both weeks high (HV: 1640.6 $\pm$ 1385.3 ng/dL and LV: 1888.3 $\pm$ 1831.1 ng/dL), this was not congruent with most of the literature examining endurance runners. Testosterone is considered a more robust hormone, with delays in response to external stimuli (i.e., decreased body fat[54], increased mileage).[55-57] One thought is the "volume threshold" proposed by De Souza and Miller,[58] that found decreases in T when participants trained at $\geq$100km/week, which our participants met in the HV week for distance but did not demonstrate decreases in T. As this study was observational in nature, EA (~25-29 kcal/kg FFM·d) and body fat percentages (~13.5%) may not have been low enough, or EEE (~670 kcals) or mileage (~49 miles; HV ~63 miles; LV: ~34) may not have been high enough, to elicit adverse reactions and decreases in T levels. Our results are congruent with Koehler et al.,[17] whom examined 6 cyclists and did not show a decrease in T when EA was acutely reduced to 15 kcal/kg FFM·d for 4 days. We were not able to calculate a prediction equation for T or LH using EA, however using RMR, EI and DXA_BFP demonstrated significant regressions equations to predict T levels in male endurance-trained athletes. This could be impactful for clinicians whom do not have access to specific blood testing.

Luteinizing hormone did not demonstrate a significant difference between the 2 weeks. Freidl et al.,[14] 2001 examined LH in US Army Rangers during Ranger school (8 weeks) and found soldiers presented with low LH levels during high training volume. This may have been due to the sudden and dramatic decrease in EI with simultaneous

dramatic increase in EEE resulting in an average body fat percentage under 5% during the 8 weeks. Our results found a positive correlation between LH and BFP, and previous literature may suggest a link with BFP and hormonal deficits[14-16,54]

Bone Mineral Density

No participants in this study demonstrated low BMD, however 29% (n=4) participants ranged from -0.4 to 0.9 including an African American participant with a BMD at -0.4, which was interesting due to African American BMD typically is more dense in comparison to Caucasians.[59] Also, 2 participants were well over a Z-score of 2; these were Obstacle course runners and therefore did large amounts of weight lifting along with endurance running. Limited research examining male endurance runners have demonstrated similar risks to female endurance runners.[60] However, it has been demonstrated that endurance runners have equal or higher BMD compared to inactive controls, but lower BMD compared to higher-impact sports. Many participants were active in a weight lifting regimen, which may be related to the adequate BMD levels and increased T levels.

Limitations and Future Research:

There were limitations identified in this study examining energy needs, hormones and BMD. First, EI collection was a self-reported measurement, however 7-day recall has been found to be more reliable and valid compared to food frequency questionnaires and 3-day recalls.[61] The use of mechanical equipment including the armband, HR monitor, as well as participant operator error may have had an influence during data

collection. Examination of EEE was calculated using HRM and Vo2max-HR regression. Double-labeled water would be a more accurate method to estimate EEE. The DXA scans were of total body and not segmental. Segmental scans should be used in the future to examine specific sites including spine and femur. Luteinizing Hormone is pulsatile in nature, and while we only measured it once as a fasting draw to give a gross overview of LH, a more accurate multi-draw technique to assess the pulsatile nature of LH is preferred. Future research should examine reproductive hormones more specifically in relation to LH to examine the pulsatile nature of the hormones response, and possibly examining sperm count to assess the output measures of LH and T in males.

Conclusion

This is the first study to examine all 3 components of the Male Triad (EA with or without an ED, hypogonadotropic hypogonadism [T and LH], and BMD) of endurance-trained male athletes in a free-living setting. Our study demonstrated the endurance-trained males presented with 1 compromised component of the Triad, LEA with or without ED was demonstrated. While reproductive hormones and BMD were not negatively affected, this study provides results that demonstrate the need for further examination of the Male Athlete Triad. Also, with the compromised macronutrient profile and our participants demonstrating pathogenic dietary behaviors, further research is needed to examine specific nutritional behaviors, EA with or without ED and the corresponding physiological health markers of male athletes. The mechanistic nature seen in the female population with EA set at 30 kcal/kg FFM·d needs to be assessed and

identified in the male population. Currently, there is no set cut-off point for LEA in the male population or a clear understanding of physiological consequences for males regarding Triad symptoms. More research examining male athletes and the Male Triad needs to be conducted.

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TABLES

Table 2.1: Energy need assessments and macronutrient intake for Endurance Trained Male Athletes (n=14). Values are presented in Mean ± Standard Deviation.

	ALL	
	M	SD
Energy Needs Assessment		
Resting Metabolic Rate (kcals)	1799.3	549.0
Energy Intake (kcals)	2658.9	230.4
Exercise Energy Expenditure (kcals)-GHR	636.7	127.1
Exercise Energy Expenditure (kcals)-VO_2	865.4	566.1
Energy Availability (kcal/kg FFM·d)-GHR*	28.2	10.4
Energy Availability (kcal/kg FFM·d)-VO_2*	27.6	12.0

Low Energy Availability Risk	HV%	N	LV%	N
LEA ≤ 2 days per week-GHR	42.8	6	35.7	5
LEA 3-4 days per week-GHR	21.4	3	21.4	3
LEA≤ 2 days per week-VO_2	35.7	5	50.0	7
LEA 3-4 days per week-VO_2	35.7	5	21.4	3
LEA ≤ 2 days per week-Arm	35.7	5	21.4	3
LEA 3-4 days per week-Arm	21.4	3	28.6	4
LEA 5-6 days per week-Arm	21.4	3	21.4	3
LEA 7 days per week-Arm	7.7	1	7.7	1

Macronutrients	HV Mean	HV SD	LV Mean	LV SD
CHO g	327.9	35.1	329.0	35.8
CHO g/kg	4.6	0.5	4.7	0.5
PRO g	122.9	12.0	119.8	11.5
PRO g/kg	1.7	0.2	1.7	0.1
Fat kcal	96.8	11.4	93.7	10.6
Fat (%)	31.6	1.7	31.5	1.4

*Note: LEA for the week was calculated by taking the average of the 7 days of EA. LEA = low energy availability; GHR = Garmin Heart Rate Monitor; VO_2=VO_{2max}-HR Regression; Arm=Armband. EEE- calculated by the average number of days of exercise /week across individuals

Table 2.2: Eating disorder characteristics among Endurance Trained Male Athletes (n=14). Data is presented in frequency (n) and percent (%).

	EDI Classification							
	Raw Score		Low Clinical		Typical Clinical		Elevated Clinical	
	Mean	SD	n	%	n	%	n	%
Eating Disorders Risk Scale								
Drive for Thinness (DT)	1.6	2.4	14	100	-	-	-	-
Bulimia (B)	0.9	1.9	13	92.9	**1**	**7.1**	-	-
Body Dissatisfaction (BD)	1.6	2.4	14	100	-	-	-	-
Eating Disorder Risk Composite (EDRC)	82.3	5.8	14	100	-	-	-	-
Psychological Scale								
Low Self-Esteem (LSE)	2.2	3.8	13	92.9	**1**	**7.1**	-	-
Personal Alienation (PA)	1.9	2.3	14	100	-	-	-	-
Interpersonal Insecurity (II)	4.9	4.4	11	78.6	**3**	**21.4**	-	-
Interpersonal Alienation (IA)	3.4	3.2	10	71.4	**4**	**28.6**	-	-
Interceptive Deficits (ID)	0.8	1.1	14	100	-	-	-	-
Emotional Dysregulation (ED)	0.6	0.8	14	100	-	-	-	-
Perfectionism (P)	10.1	4.3	6	42.9	**7**	**50**	**1**	**7.1**
Asceticism (A)	5.4	3.8	11	78.6	**3**	**21.4**	-	-
Maturity Fears (MF)	7.1	5.8	6	42.9	**5**	**35.7**	**3**	**21.4**
Composite								
Ineffectiveness Composite (IC)	63.1	9.6	13	92.9	**1**	**7.1**	-	-
Interpersonal Problems Composite (IPC)	76.3	11.5	11	78.6	**3**	**21.4**	-	-
Affective Problems Composite (APC)	66.6	2.1	14	100	-	-	-	-
Over control Composite (OC)	80.6	12.4	10	71.4	**4**	**28.6**	-	-
General Psychological Maladjustment (GPMC)	331.1	30.5	14	100	-	-	-	-

Table 2.3. Eating disorder pathogenic behaviors among endurance trained male athletes (n=14). Data is presented in frequency (n) and percent (%)

	All Data	
Exercise to Control Weight	**N**	**%**
0% of time	8	57.1
<25% of time	**4**	**28.6**
25%-50% of time	**2**	**14.3**
More than 75% of time	0	0
100% of time	0	0

Table 2.4: Hormone and bone mineral density values for Endurance Trained Male Athletes (n=14). Values are presented in Mean ± Standard Deviation.

Hormones	M	SD
Testosterone ng/dL	1780.6	1672.6
Luteinizing Hormone pg/mL	813.7	314.2
Bone Mineral Density	M	SD
Total Z-score	1.30	0.96
Total Score (g/cm^2)	1.31	0.01
Legs (g/cm^2)	1.40	0.12
Spine(g/cm^2)	1.17	0.13
Pelvis (g/cm^2)	1.22	0.16

*Hormones and BMD were measured after 24 hours of rest and as AM fasting

FIGURES

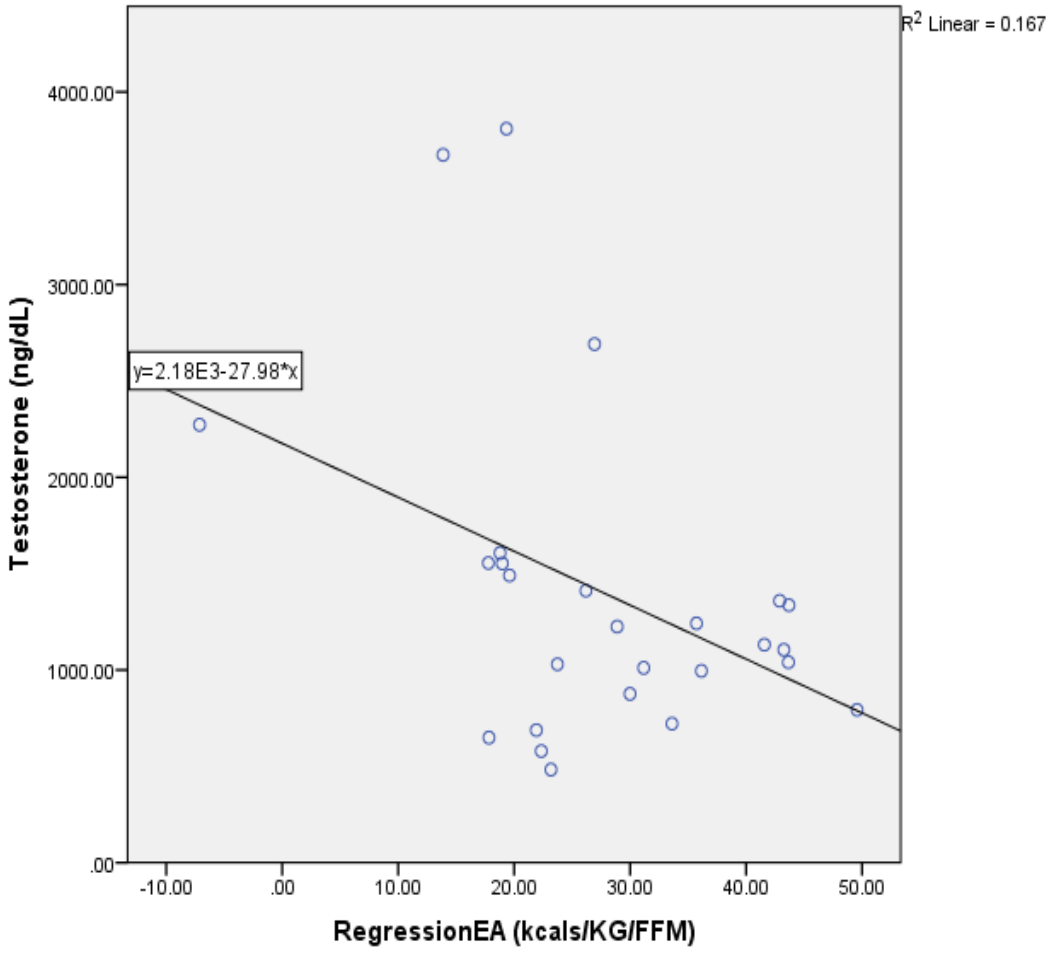

Figure 2.1: Testosterone levels as a function of $V0_{2max}$-HR Regression Energy Availability in endurance-trained male runners. Testosterone measured ng/dL and Regression EA measured in kcal/kg FFM·d , n= 26; p = 0.89.

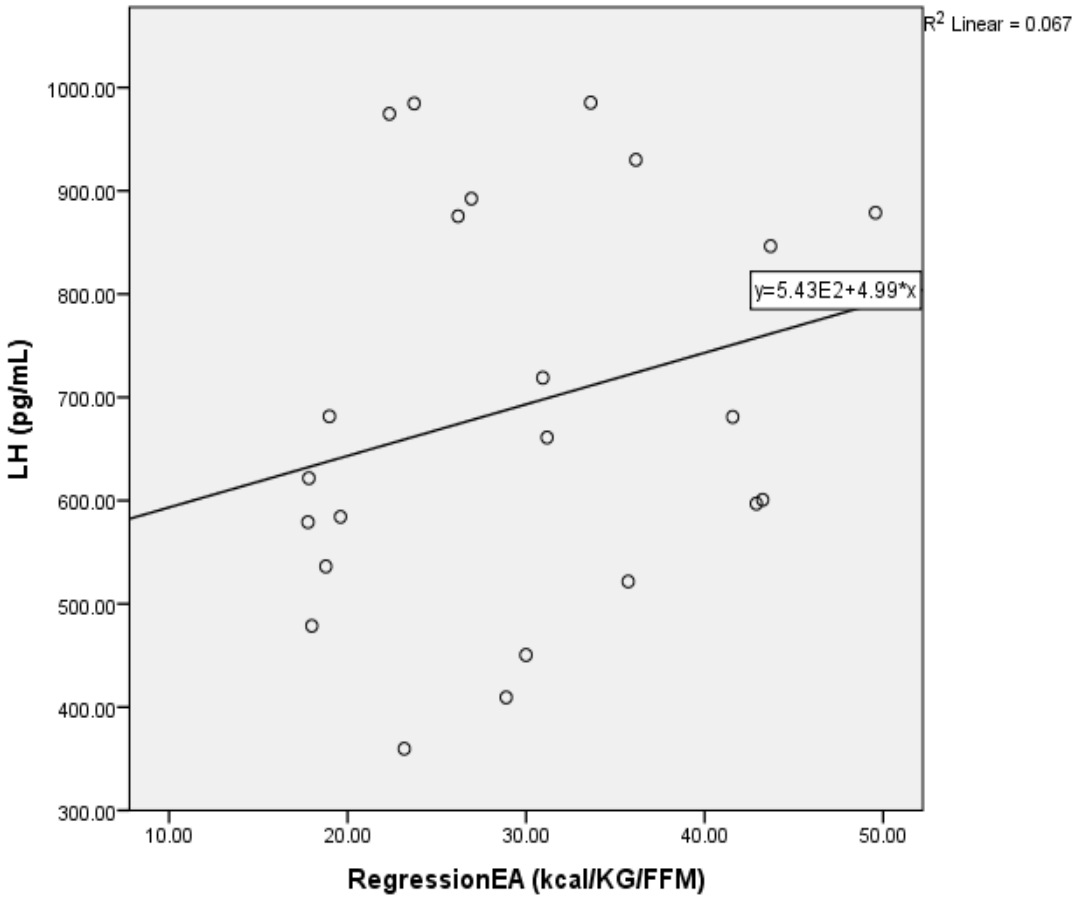

Figure 2.2: Luteinizing Hormone levels as a function of $V0_{2max}$-HR Regression Energy Availability in endurance-trained male runners. LH measured pg/mL and Regression EA measured in kcal/kg FFM·d , n= 24; p = 0.22.

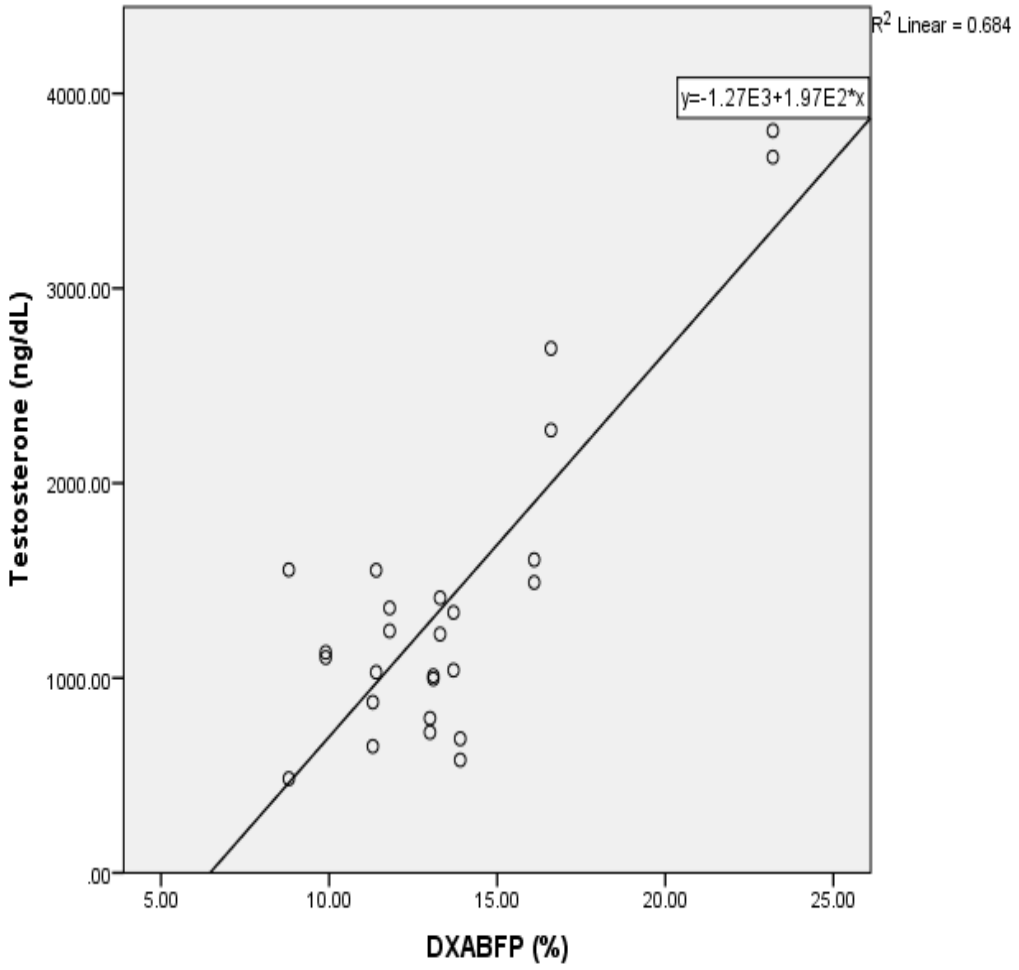

Figure 2.3: Testosterone levels as a function of Body Fat Percentage in endurance-trained male runners. Testosterone measured ng/dL and DXABFP measured in percentage (%); ($r_{(24)} = 0.83$, $p < 0.001$).

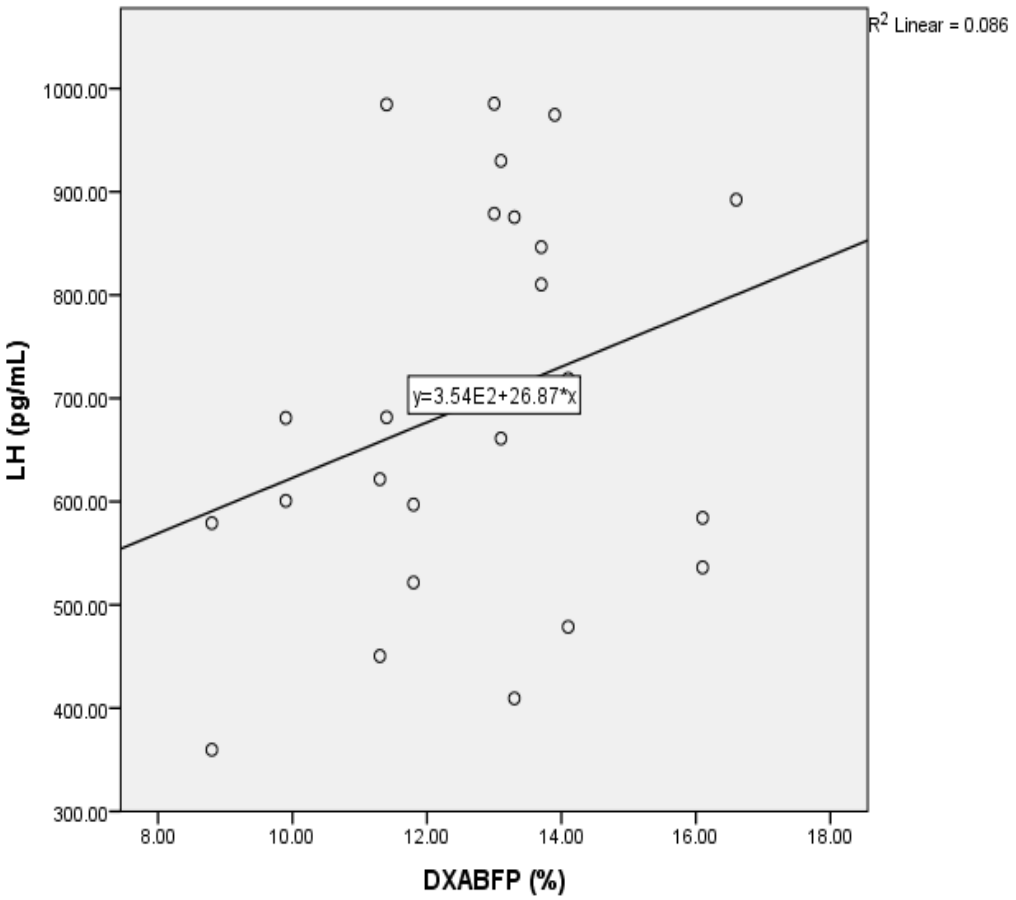

Figure 2.4: Luteinizing Hormone levels as a function of Body Fat Percentage in endurance-trained male runners. LH measured pg/mL and DXABFP measured in percentage (%), $p < 0.001$.

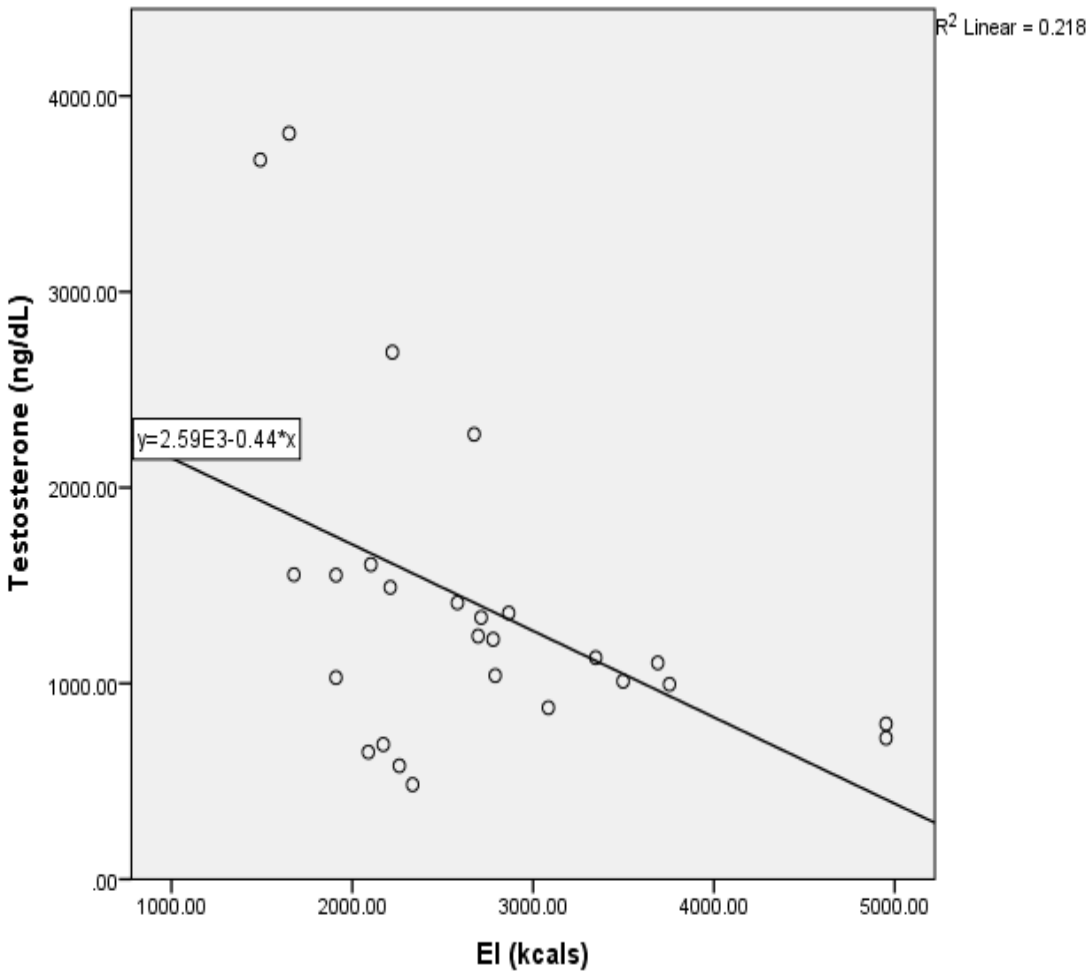

Figure 2.5: Testosterone levels as a function of Energy Intake in endurance-trained male runners. Testosterone measured ng/dL and EI measured in kcals, n = 26; ($r_{(24)} = -0.47$, $p = 0.02$). A significant regression equation was found ($F_{(1, 24)} = 6.7$, $p = 0.02$), with an R2 of 0.22.

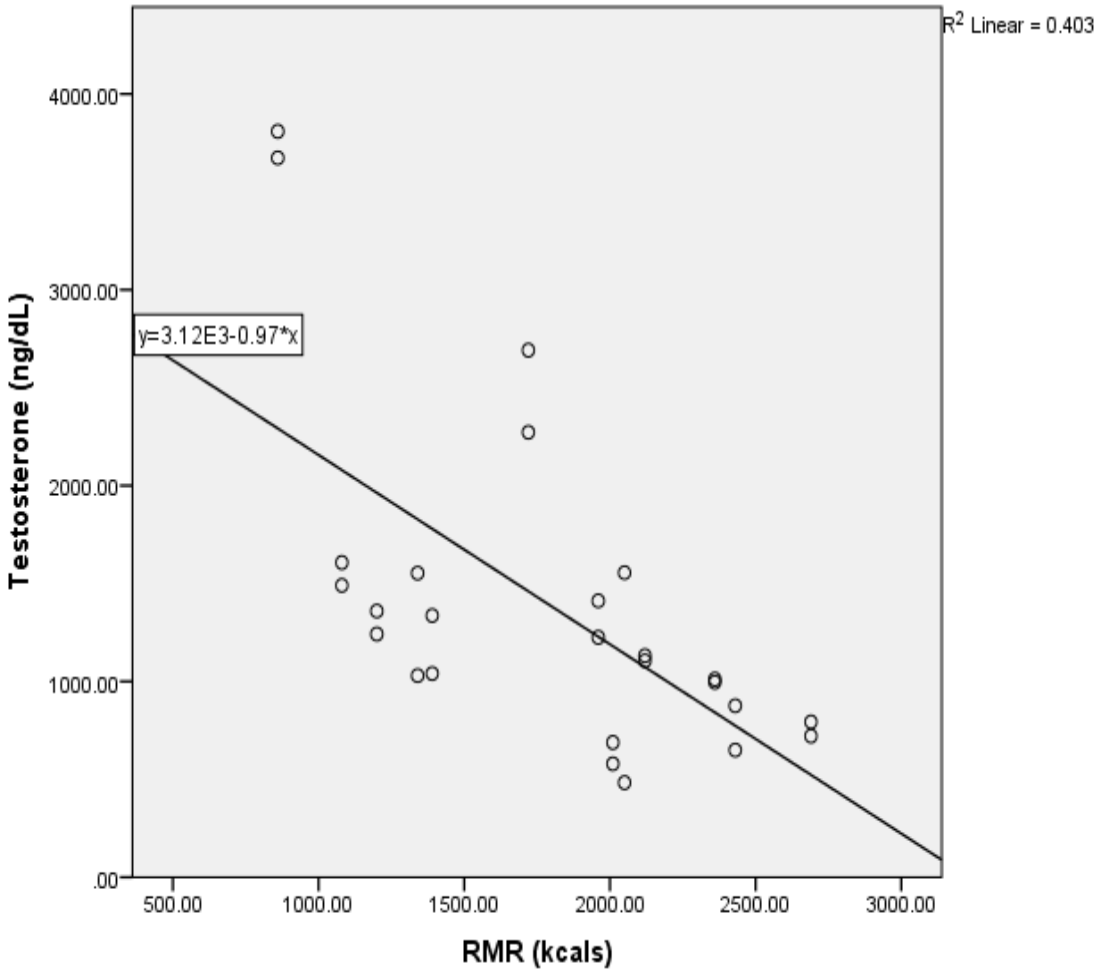

Figure 2.6: Testosterone levels as a function of Resting Metabolic Rate in endurance-trained male runners. Testosterone measured ng/dL and RMR measured in kcals; ($r_{(26)}$ = -0.64, $p < 0.001$). A significant regression equation was found ($F_{(1,23)} = 16.23$, $p < 0.001$), with an R^2 of 0.4.

CHAPTER 3

EXAMINATION OF ENERY AVAILABILITY ON HORMONAL PROFILE OF ENDURANCE TRAINED MALE ATHLETES[2]

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ABSTRACT

Examination of the negative physiological effects due to decreased energy availability (EA) in male athletes is not understood. **Purpose:** Examine the effect of EA on reproductive (Testosterone [T] and Luteinizing Hormone [LH]) and metabolic hormones (Insulin, Leptin, Cortisol, and Interleukin-6 [IL-6]) in male endurance-trained athletes. **Methods:** We utilized a cross-sectional design on 14 endurance trained male athletes (age: 26.4 $\pm$ 4.2 yrs.; weight: 70.6 $\pm$ 6.4 kg; and height: 179.5 $\pm$ 4.3 cm) who were recruited from the local community. Participants completed 2 separate training weeks (low [LV] and high [HV] training volumes) and each week included: 7-day dietary logs, 7 day-exercise logs, and one blood draw each week to determine concentrations for 6 hormones (T, LH, Insulin, Leptin, Cortisol, and IL-6). Anthropometric measurements (height, weight, and body composition) were taken prior to data collection. **Results:** Participants at risk for LEA ($\leq$ 20 kcal/kg FFM·d), demonstrated 41.2% (n = 7), HV: 50%, n = 4; LV: 33.3%, n = 3) had increased T levels ($p = 0.20$). Of those participants with LEA, 21.7% (n = 5) (HV: 18.2%, n = 2; LV: 25%, n = 3) presented with low Leptin levels ($p = 0.01$). Significant regressions revealed T levels from RMR ($F(1, 23) = 16.23$, $p < 0.001$); EI ($F(1,24) = 6.7$, $p = 0.02$), and DXA_BFP ($F(1, 24) = 51.9$, $p < 0.001$) and leptin levels from DXA_BFP ($F(1, 24) = 27.18$, $p < 0.001$). **Conclusion:** Overall, participants demonstrated LEA, which highlighted a significant negative relationship between LEA and Leptin. Relationships between BFP and T, BFP and Leptin both demonstrate more clinical uses for clinicians in monitoring males' weight and hormonal profiles for those without access to blood testing. These results suggest that BFP impacts

endurance-trained male athletes' hormonal profiles. Ultimately, insights on the near and long-term health outcomes for male athletes are needed.

Key Words: Energy Availability, Testosterone, Luteinizing Hormone, Insulin, Leptin, Interleukin-6.

As high-energy exercise in the elite sport realm has been popular (e.g., soccer, cycling, running, etc.); a new fad of sports such as ultramarathons, extreme challenge races and triathlons have developed to promote physical activity, increase community involvement in exercise, and used as a counter-measures to obesity. This increase participation in high-energy exercise has changed from purely elite sport participation to the recreational athletes. Endurance runners are unique athletes due to the high-energy expenditure physical demands within their sport. These increased demands escalate endurance athletes' (e.g. distance runners) risk of impaired physiological functions (e.g., decreased hormonal profile [Testosterone (T), leptin, insulin, increased cortisol, decreased BMD, and compromised macronutrient profile)[1-9] however it is unclear how male endurance athletes' impairments are compared to the well-established research of female endurance athletes.[1-9]

Energy Availability

Research examining energy availability (EA) and the resultant physiological effects of low EA (LEA) in male athletes is currently unknown. The only study to examine short-term EA reduction in exercising men demonstrated significant suppression of leptin and insulin hormones due to the reduction of EA to 15 kcal/kg FFM·d in 6 exercising men, which are similar results to Loucks et. al.,[10,11] research in exercising female athletes. Other male research has examined chronic energy deficits and its effect on various hormones;[12-19] however, EA was not calculated or examined specifically. Within the Female Triad, LEA has been established at ≤ 30 kcal/kg FFM·d [3-6,9,11] due to significant physiological changes, including metabolic rate declines, bone reabsorption increases, protein synthesis breakdown occurs, and reproductive hormones decrease (i.e.,

Luteinizing hormone [LH], Follicular stimulating hormone [FSH], and estrodral).[1-6,9] To date, there are no established LEA markers for the male athlete. Mechanisms responsible for energy deficiency in athletes include; 1) lack of knowledge of energy needs based on demands of the energy expended, (2) clinical eating disorders and (3) intentional subclinical mismanagement of energy consumption to reduce body size and fatness.[13] Due to physiological differences between males and females, De Souza et. al.,[2] stresses the necessity to establish independent clinical guidelines regarding LEA and the physiological impacts specifically for males.

Hormones

Military research has shown male soldiers during occupational missions are exposed to dietary restrictions and increases in EEE causing compromised EA.[14,18] Fridel et. al.,[14] examined 2 separate groups of US Army Rangers during their 8-week Ranger course. During these 8 weeks; 4 cycles of restricted energy intakes (EI) and re-feeding, incorporating specific EI deficits of 1,000-1,200 kcal/day occurred.[14] Due to deficits in EI and high EEE in the male US Army Rangers, gonadal and reproductive hormones (testosterone (T), thyroid, sex hormone-binding globulin, and insulin-like growth factor I (IGF-I)) were consistent markers to use when assessing acute energy deficits.[14] Cortisol and cholesterol were specific hormonal markers, which assessed the chronic state of energy deficits in the male US Army Rangers.[14] Congruently, Kryolainen et. al.[18] examined hormonal alterations during prolonged military field exercises in Finnish soldiers and the effect of various energy deficits prompted through different exercise intensity and consistent EI. Results revealed an average energy deficit (difference between EEE and EI) of 4,000 kcal/day in phase I of training (one week long)

demonstrated significant increases in cortisol (+32%) and growth hormone (+616%) where exhibited while insulin (-70%), total T (-27%) and free T (-26%) decreased.[18] Once energy deficits were reduced (<1,000 kcal/day in Phases II and III), all hormonal markers stabilized.[18] Understanding the mechanistic nature of hormonal alterations in relation to decreased EI, increased EEE, and compromised EA is needed. The roles of hormones (T, LH, Insulin, Leptin, Cortisol, IL-6) have demonstrated associations to various physiological functions including the hypothalamic-pituitary axes, which in part controls reproductive and metabolic processes. These physiological functions can be suppressed by 1) decreased EI, 2) increased EEE, or 3) a combination of both. Specific roles for each hormone are listed below.

Reproductive Hormones. Testosterone is a steroid hormone produced in the testes (specific for males) and adrenal cortex.[20] Testosterone stimulates development of male secondary sexual characteristics.[20] The mechanistic nature of decreased T is currently unknown, however research directs to one impression that involves dysfunctions in the hypothalamic-pituitary-testicular regulatory axis.[21] Another reproductive hormone includes LH. Luteinizing hormone in males stimulates the production of testosterone and in part, aids in the role of sperm production.[22] Previous literature suggests exercise intensity plays an important role in relation to LH outcomes (positive and negative).[23] Other results suggest a hypothalamic gonadotropin-releasing hormone deficiency may occur due to suppression from other hormones (e.g., cortisol) related to training.[24] Establishing mechanistic behaviors (decreased EA and EI and/or increased EEE) for decreases in reproductive hormones (T and LH) need to be determined.

Metabolic Hormones. Insulin is a peptide hormone and produced from Beta cells within the islets of Langerhans in the pancreas and contributes an important role in metabolism.[25] Insulin aids in the absorption of glucose into muscle, fat, and liver cells for energy.[25] Insulin also stimulates muscle and liver tissue to store excess glucose in the form of glycogen, in turn suppressing gluconeogenesis by the liver.[25] Two mechanisms have been suggested for decreased insulin including 1) adaptations of glucose uptake in runners and 2) a global effect on the hypothalamic-pituitary axes.[3,25]

Leptin, a metabolic hormone, is an adipoocytokine hormone that affects neurons in the hypothalamus responsible for the regulation of body mass loss and satiety regulating dietary energy intake and energy expenditure.[26] Circulating leptin levels are influenced by exercise and glucose uptake and through sympathetic and direct mechanism which induces increase of fatty acid disposal in metabolism.[26] Leptin stimulates appetite suppression and reduces the level of brain chemicals that stimulate appetite.[26] Leptin receptors have various actions due in large part to the numerous types of tissues that contain leptin receptors.[26] Most prominent tissues include the skeletal muscle and the liver.[26] Previous literature has demonstrated a parallel relationship of leptin and body weight suggesting an acute reduction of leptin is linked to an acute metabolic signal for energy conservation. [26,27] Research has also demonstrated key endocrine axes (reproductive, growth hormone, IGF-I, and thyroid axes) are associated to reduced leptin levels.[27] The synergistic effect of body weight and leptin and leptin's associations with other hormones establishes an endocrine effect that needs to be understood in relation to Male Triad symptoms.

A third hormone with associations and effect on other hormones include cortisol. Cortisol is the primary hormone related to stress (physical, psychological and physiological) and is a glucocorticoid hormone from the adrenal cortex.[28] Cortisol has various roles in the body including increasing glucose up-regulation into the bloodstream and brain, inhibits nonessential functions that would be detrimental in a "flight-or fight" state, and alters the immune system response.[28] Cortisol has demonstrated the ability to decrease functions of other systems (reproductive, digestive, and growth processes) and high cortisol levels over time have adverse effects on health. [28] With specific effects on the reproductive and metabolic processes through hypothalamic-pituitary axes due to changes in EI, EEE, and body composition, examination of compromised EA and its effect on the male endurance-trained athletes' hormonal profile needs to be assessed.

A pro-inflammatory hormone that has local and global effects is Interleukin-6. Interleukin-6 is a cytokine produced in the skeletal muscle and released systemically. It has many functions including induction of lipolysis, stimulation of cortisol production, and suppression of tumor necrosis factor alpha (TNF) production, another cytokine involved in systemic inflammation.[29,30] During exercise and low muscle glycogen levels, IL-6 is activated,[29] and Pedersen et al.,[29] demonstrated CHO supplementation during exercise blunted IL-6 release in muscles. These reproductive and metabolic hormones are all associated with physiological effects related to exercise intensity, high EEE, low EI, as well as actions on other hormones. With this involvement, understanding the endurance-trained male athletes' hormonal profiles during decreased EI and/or compromised EA needs to be examined further.

Currently, there is inadequate literature existing for male athletes who participate in high-energy expenditure activities with decreased energy needs. Understanding the physiological demands and consequences of energy needs in male athletes is critical for acute and long-term health and prevention of injuries and illness. Existing literature has established long periods of restricted energy intake or rapid weight loss in male athletes have an affiliation to negative hormonal effects.[12,14-19,31] Our study sought to examine the effect of EA on reproductive (T and LH) and metabolic hormones (insulin, leptin, cortisol, and interleukin-6 [IL-6]) in male endurance-trained athletes. We hypothesized endurance-trained athletes who displayed LEA would present with decreased reproductive (T and LH) and metabolic (Insulin and Leptin) hormones as well as increased metabolic hormones (cortisol and IL-6). A secondary purpose was to examine differences in reproductive (T and LH) and metabolic (Insulin, Leptin, Cortisol and IL-6) hormones between the 2 training (HV and LV) weeks. We hypothesized male endurance-trained athletes would display a difference between the 2 training weeks. Third, we aimed to examine secondary measures (EI, EEE, DXA_BFP, RMR, and mileage) and their relationships to the reproductive (T and LH) and metabolic hormones (Insulin, Leptin, Cortisol, IL-6). We hypothesized some of the secondary measures would demonstrate a relationship with the hormones (i.e., increased mileage would have a negative effect on the hormonal profile).

Methods

Participants

Fourteen male participants (age: 26.4 $\pm$ 4.2 yrs.; weight: 70.6 $\pm$ 6.4 kg; and height: 179.5 $\pm$ 4.3 cm) were recruited from the local community. This study was part of a larger study conducted and therefore shares similar methodology. Specific inclusion criteria for participation included: participant is male, within a competitive season who is actively training and racing >10 hours/week for at least 3 months,[6,14,32] has a body fat percentage $\leq$ 12%,[6,12,14,32,33] has maintained weight stability ($\pm$ 3 kg in past 6 months),[17] has a VO_2max that is considered excellent for age specific range (18 - 20 years: Excellent 51.0 - 55.9 ml/kg/min; 20 - 29 years: Excellent 46.5 - 52.4 ml/kg/min; 30 - 39 years: Excellent 45.0 - 49.4 ml/kg/min),[17,34] and was required to be independent of any injury that would prevent them from full participation in a high-endurance sport (running, triathlon, or obstacle racing).

Specific exclusion criteria included no previous history of smoking, past or present diagnosis of clinical eating disorder, infectious disease within past 4 weeks, history of cardiovascular disease or orthopedic impairment that interferes with moderate to vigorous exercise, no history of thyroid or pituitary disease, use of medication, diabetes mellitus, known metabolic disease, and no long-term steroid use. Institutional Review Board was obtained prior to the start of the study and all participants provided consent prior to participation.

Instruments and Protocols

Basic Demographic Survey. Basic demographic information including: age, education level, ethnicity, exercise background, and pertinent medical history questions including known metabolic diseases, history of cardiovascular, thyroid, or pituitary diseases, and long-term steroid use were collected.

Anthropometric Measurements. Multiple anthropometric measurements were collected including height, weight, and body composition, which were measured according to ACSM standardized procedures.[13] Height was measured with a Stadiomometer (Shorr Productions, Maryland) to the nearest 0.1 cm and weight was measured wearing minimal clothes to the nearest .01 kg with a scale (Tanita SC-331S Body Composition Scale, Tanita Co., Tokyo, Japan). Body fat was assessed using a Tanita scale (Tanita SC-331S Body Composition Scale, Tanita Co., Tokyo, Japan) for inclusion criterion and Dual-Energy X-Ray Absorptiometry (DXA) (GE Lunar Prodigy densitometer) for data analysis.

Dual-Energy X-Ray Absorptiometry (DXA). The gold standard for body fat percentage (BFP) measurement and bone mineral density assessment.[13] Participants were instructed to: 1) not eat/overnight fast (12 hour fast), 2) refraining from vigorous exercise at least 15 hours prior to scan, 3) no caffeine or alcohol consumption during the preceding 24 hours, and 4) consume a normal evening meal the night before.[13] Scoring of BFP was reported in percentage.

Resting Metabolic Rate (RMR). Used to identify how many calories are necessary at rest. It was measured using indirect calorimetry (*Microlife MedGem*;

HealtheTech, Golden, CO). The MedGem is a clinically-validated measurement device that assesses RMR.[35] While the MedGem is not the gold standard for measuring RMR; it is, however, a clinically a very relevant tool.[35] Most accurate measurements are produced first thing in the morning, when the patient is rested and positioned in either a seated or semi-reclined position in a quiet room.[35] Measurements should be implemented when the patient has not eaten, exercised or drank any caffeine within the last four hours.

Exercise Energy Expenditure (EEE). Two separate measurements were used to determine EEE; 1) VO_{2max}-HR Regression and 2) Heart Rate Monitor (Garmin Forerunner 15). All results were reported as EEE in kcals.

1) **VO_{2max}-HR Regression.** A VO_{2max} treadmill test using the method from Beashel and Taylor (1996)[36] targeted to endurance runners and the Parvo metabolic cart was administered as part of the inclusion criterion. Calculated VO_{2max} –HR regression slopes for each individual to match HR with EEE were used and reported in kcals.[32]
2) **Heart Rate Monitor.** The participants wore a Garmin heart rate monitor (HRM) during exercise as a measure of EEE by calculating METs during exercise, derived from their HRM calculations. Various brands of HRM were found to accurately assess heart rates moderate activity ($r \geq 0.90$, SEE ≤ 5 beats/min).[37]

Dietary Intake. Participants recorded 2 separate weeks, 7-consecutive days per week of dietary intake. Portion sizes were explained, and take-home examples were given prior to food record distribution. Dietary records were analyzed for total

kilocalories consumption using a dietary analysis software program (ESHA food processor 8.0, Salem, OR). Research has demonstrated that despite food intake restrictions, reported intake accuracy was superior using a 7-consecutive day weighted-diet record compared to a food-frequency questionnaire. Food records were used to examine EI and EA.

Energy Availability (EA). Defined as the amount of dietary energy remaining after exercise, expressed as kcal/kg/free fat mass (EA = [energy intake–EEE] kcal/kg FFM·d).[1] Energy availability was examined using EI and EEE over 2 separate weeks of 7-consecutive days. Low energy availability (LEA) was defined as $\leq$ 20 kcal/kg FFM·d.

Hormone Measures. All fasting blood samples were acquired with 24 hours of non-physical activity at the completion of each 7-consecutive day week. Blood samples were centrifuged, and plasma drawn out to assess 6 different hormones (Testosterone [T], Luteinizing Hormone [LH], Insulin, Leptin, Cortisol, and Interluekin-6 [IL-6]) using enzyme-linked immunosorbent assay (ELISA) Kits specific for each hormone. This study used ELISA kits specific for T, LH, Insulin, Leptin, Cortisol, and IL-6. Research has shown ELISAs to report specific and highly sensitive procedures for identify various substances. Sensitivities of EISAs are high, 1-10ug/liter range with the correlation coefficient were reported between 0.95-0.99.[38]

Establishment of cutoffs were identified as 1) low, 2) within normal limits, or 3) high based on previously established normative data specific for males (adult and age range specific) associated for T, Insulin, Leptin, Cortisol, and IL-6 while LH used the standard curve from the ELISA kit. Normative ranges for each hormone includes: T =

270 - 1070 ng/dL (average 679 ng/dL)[39], LH = 1.8-12.0 mlU/L,[39,40] fasting insulin = ≤ 5 ulU/mL (8 - 10 ulU/mL is also an accepted range),[41] leptin = 1.2 -9.5 ng/ml,[42] cortisol specific to the morning includes: 7 - 28 ug/dL, and IL-6 = < 1.8 pg/mL.[43]

Training Conditions. Two separate training weeks were used to assess differences between energy needs and hormones.

1) **High Volume Training Week (HV).** A high-volume training week consists of at least 5 days of training and includes at least 10 hours of training with in a 7-consecutive day week.
2) **Low Volume Training Week (LV).** Low-volume training week, or a recovery week, was described as an unloading week for the participant. No specific requirements were established except participants were asked to work out a minimum of 2-3 days for the 7-consecutive day week.

Detailed Procedures

The data collection spanned across 2 separate weeks consisting of 1 day for an information and initial measurement session, and two 7-consecutive day weeks where 1 week was during the high volume-training week (HV) and 1 week was during the low volume/recovery week (LV).

Part I Recruitment:

Participants were recruited from local area running clubs and races. An information letter via email was used to give a brief overview of the study. All

participants interested were individually corresponded with to schedule assigned dates and times for informational sessions and anthropometric measurements.

Part II Data Collection:

Informational Session: Participants attended an orientation session prior to the data collection sessions. This session consisted of written and verbal overview of the study, participant expectations, instructions from the researchers describing various tools used during data collection including: *ESHA FoodProdigy, BodyMeida SenseWear Armband, and Heart Rate Monitor*

Prior to Data Collection: Participants first completed a series of surveys, a brief interview, used to follow up on medical history, physical measurements (height, weight, and percent body fat), $V0_{2max}$ test, and resting metabolic rate (RMR). Blood Draws and DXA scans were scheduled for the 2 separate weeks of data collection. Blood draws consisted of 2 tubes (2.4 tsp) during each data collection. A total of 2 data collection sessions occurred, accumulating in 4 tubes (4.8 tsp) for the entire study.

Data Collection: Participants completed 2 separate 7-consecutive day weeks at 2 different levels of training volume (HV and LV). All equipment (e.g., BodyMedia SenseWear Armband, HRM, and food log entry information) was passed out and instructions were emailed. Data collection HV week was a "normal" week with a minimum of 5 training days/week and participants were engaged in a competitive season. The procedures for each of the 2 weeks were identical; the only change was their volume load (HV and LV).

Training Weeks: Participants were instructed to not change their daily/weekly activities and physical activity/exercise, while recording their food and daily training for 7-consecutive days. They were instructed to wear the armband for 23 hours/day and wear a HR monitor only during exercise during training for the 7-consecutive days. At the end of the 7-consectuve day training week, participants came in for a fasting blood draw and DXA scan. All equipment was returned and the next week was scheduled.

Statistical Analysis

IBM SPSS statistical Software (version 24; SPSS Inc., Chicago, IL) and an *alpha* ≤ 0.05 was used for all analyses. Based upon power analysis a priori and based upon means of previous literature from Loucks et. al.,[11] and Koehler et. al.,[17] an effect size between 1.0 and 3.0 would yield a sample size of 6-10 subjects. Using the Wilcoxon signed rank test, 14 subjects should allow for full saturation. Descriptive statistics for all dependent variables were calculated. Frequencies and proportions with 95% confidence intervals were calculated for all categorical variables (at risk for LEA, at risk for decreased hormonal profile (T, LH, Insulin, Leptin, Cortisol, IL-6). A chi-square analysis was used to examine "at risk" for LEA. A 2 (week) X 7 (days) ANOVA and paired T-tests assessed differences between the 2 training weeks and variables (e.g., EA, hormones, EEE, and EI). Pearson's correlation and regressions were used to examine relationships and predictive qualities between EA, secondary measures (DXA_BFP, RMR, EI, EEE, distance/mileage), and hormones (T, LH, Insulin, Leptin, Cortisol, and IL-6).

Results

Eighteen participants began the study, 3 were eliminated for lack of compliance with the required procedures of the study, and 1 dropped out due to fear of needles yielding a total of 14 participants. Participants' criterion demonstrated a VO_{2max} 62.3 $\pm$ 6.9 ml/kg/min, FFM 65.7 $\pm$ 5.4 kg, and a DXA_BFP 13.6 $\pm$ 3.5%. Ethnicity of participants demonstrated 7.1% Middle Eastern (n = 1), 14.3% African American (n = 2), and 78.6% Caucasian (n = 11). Education levels revealed 92.9% (n = 13) had some level of college and higher. More specifically, 7.1% attained a clinical doctorate (n = 1), 35.7% attained a master's degree (n = 5), 21.4% attained a bachelor's degree (n = 3), 28.6% attained some level of college (n = 4), and 1 participant attained a GED (7.1%).

Energy Assessment

Overall, when examining LEA using EEE from VO_{2max} - HR regression and Garmin HRM, 32.1% (n = 9) of participants presented with LEA over the 2 training weeks (HV: 35.7%, n = 5; 28.6%, n = 4). Using VO_{2max} - HR regression EA, participants that presented at risk for LEA, demonstrated 47.1% (n = 8) (HV: 62.5%, n = 5; LV: 33.3%, n = 3) had increased T levels (p = 0.04). Of those participants with LEA, 26.1% (n = 6) (HV: 27.3%, n = 3; LV: 25%, n = 3) presented with low Leptin levels (p = 0.14). Using Garmin HRM EA, participants that presented at risk for LEA, demonstrated 41.2% (n = 7) (HV: 50%, n = 4; LV: 33.3%, n = 3) had increased T levels (p = 0.20). Of those participants with LEA, 21.7% (n = 5) (HV: 18.2%, n = 2; LV: 25%, n = 3) presented with low Leptin levels (p = 0.01). No other specific hormones presented with compromised levels due to LEA.

Energy Availability: All means and standard deviations for energy needs assessment data (e.g., RMR, EI, EE, EA etc.) can be found on Table 3.1. A 2 (training conditions: HV, LV) x 7 (days) ANOVA and paired t-tests were used for examination of EA, EI, and EEE. Paired t-tests demonstrated an average EA for both measurements: 1) EA_VO_{2max} regression: 27.6 $\pm$ 12.1 kcal/kg FFM·d (HV: 25.2 $\pm$ 12.9 kcal/kg FFM·d and LV: 29.9 $\pm$ 11.1 kcal/kg FFM·d) ($t_{(13)}$ = -1.61, p = 0.13); and 2) EA_HRM: 28.2 $\pm$ 10.3 kcal/kg FFM·d (HV: 26.9 $\pm$ 9.6 kcal/kg FFM·d and LV: 29.5 $\pm$ 11.2 kcal/kg FFM·d) ($t_{(13)}$ = -1.37, p = 0.15). No significant differences were elicited between training weeks and EA. Regarding both training weeks, examination in the specific daily account of LEA per week can be found in Table 3.1.

Energy Needs. One-way repeated-measures ANOVA (assumptions of sphericity were met due to only 2 weeks assessed) were calculated to compare difference across the 2 training weeks for EI. No significant effect was found between the 2 training weeks for EI. Two measurements were used to assess EEE. Due to missing data cells (determined by when participants exercised) an ANOVA was not used for EEE. A paired samples t-test was calculated to compare the mean of the 2 training weeks (HV and LV). The mean of HV week using VO_{2max}-HR regression was 1048.5 $\pm$ 805.6 kcals and the mean for the LV week was 682.3 $\pm$ 326.5 kcals. No significant difference between the 2 training weeks (HV vs. LV) was elicited ($t_{(13)}$ = 1.7; p = 0.10). The mean of HV week using Garmin HRM was 919.3 $\pm$ 538.2 kcals and the mean for the LV week using Garmin HRM was 696.5 $\pm$ 472.7 kcals. A significant difference between the 2 training weeks (HV vs. LV) was elicited ($t_{(13)}$ = 3.5; p = 0.004).

Hormonal Profile

Reproductive Hormones:

Testosterone (T). A paired samples t-test was calculated to compare the means between the 2 training weeks (HV and LV) for T with a mean of 1764.5 $\pm$ 1598.2 ng/dL (T_HV: 1640.5 $\pm$ 1385.3 ng/dL and T_LV: 1888.3 $\pm$ 1831.1 ng/dL). No significant differences were found between the 2 training weeks and T ($t_{(13)} = -1.53$ $p = 0.15$). Testosterone presented high compared to normative male values (above 1070 ng/dL). A Pearson correlation coefficient was calculated for the relationship between T and other variables (i.e., DXA_BFP, RMR, EI, EEE, etc.). A strong negative correlation was found for overall T to RMR: ($r_{(24)} = -0.64$, $p < 0.001$), a weak negative correlation was found for overall T to EI: ($r_{(24)} = -0.47$, $p = 0.02$), as well as a strong positive correlation for overall T to DXA_BFP ($r_{(24)} = 0.83$, $p < 0.001$). Significant associations were demonstrated between EA_VO_{2max} Regression and overall T ($\chi(2) = 4.4$, $p = 0.04$) and HV_T ($\chi(2) = 5.8$, $p = 0.02$).

Two outliers were removed prior to regression analysis. A simple linear regression was calculated to predict participant's T levels based on their EA, RMR, EI, EEE, DXA_BFP, and distance. Energy availability via 1) $V0_{2max}$-HR regression was not statistically a significant predictor of T levels ($F_{(1,24)} = 3.2$, $p = 0.09$) with an R^2 of 0.12 or via 2) GarminHR ($F_{(1, 24)} = 3.8$; $p = 0.06$) with an R2 of 0.14. A significant regression equation was found for T and RMR: ($F_{(1, 23)} = 16.23$, $p < 0.001$), with an R^2 of 0.4. Participants' predicted T level is equal to 3123.5 ± -0.97 (RMR) ng/dL when RMR is measured in kcals. Participants' average T levels decreased -0.97 ng/dL for each kcal of

RMR. A significant regression equation was also found for T and EI: ($F_{(1, 24)}$ =6.7, p = 0.02), with an R^2 of 0.22. Participants' predicted T level is equal to 2591.96 ± -0.44 (EI) ng/dL when EI is measured in kcals. Participants' average T levels decreased -0.44 ng/dL for each kcal of EI. A significant regression equation was found for T and DXA_BFP: ($F_{(1,24)}$ = 51.9, p < 0.001), with an R^2 of 0.83. Participants' predicted T level is equal to -1273.5 ± 197.2 (DXA_BFP) ng/dL when DXA_BF is measured in kg. Participants' average T levels increased by 197.2 ng/dL for each kg of DXA_BFP. No other significant relationships were found for T (see Table 3.2).

Luteinizing Hormone (LH). A paired samples t-test was calculated to compare the means between the 2 training weeks (HV and LV) for LH. The mean for LH was 795.6 ± 313.9 pg/mL (LH_HV: 385.7 ± 191.0 pg/mL and LH_LV: was 409.9 ± 119.9 pg/mL). No significant differences were found between the 2 training weeks and LH ($t_{(12)}$ = -0.79, p = 0.44). Luteinizing hormone did not present low compared to normative male values. A Pearson correlation coefficient was calculated for the relationship between LH and other variables (i.e., DXA_BFP, RMR, EI, EEE, etc.). Non-significant weak correlations were found for all secondary measures (see Table 3.2). Secondary measures (DXA_BFP, RMR, EI, EEE) and LH are not related.

Four outliers were removed prior to regression analysis. A simple linear regression was calculated to predict participant's LH levels based on their EA, RMR, EI, EEE, and DXA_BFP. The regression equation was not significant for EA via 1) $V0_{2max}$-HR regression was ($F_{(1,22)}$ = 1.6, p = 0.22) with an R^2 of 0.07 and via 2) GarminHR ($F_{(1,22)}$ = 0.47, p = 0.50) with an R^2 of 0.02. EA is not a significant predictor of LH levels.

No other significant predictors were found when examining RMR, EI, EEE, and DXA_BFP.

Metabolic Hormones:

Insulin: A paired samples t-test was calculated to compare the means between the 2 training weeks (HV and LV) for Insulin with a mean of 7.7 $\pm$ 2.6 mU/L (IN_HV: 7.1 $\pm$ 1.1 mU/L and IN_LV: 7.3 $\pm$ 1.5 mU/L). No significant differences were found between the 2 training weeks and T ($t_{(13)} = -1.10$; $p = 0.29$). Insulin presented within normative male values (< 25 mU/L) for both HV and LV weeks. A Pearson correlation coefficient was calculated for the relationship between Insulin and other variables (i.e., BFP, RMR, EI, EEE, etc.) and no statistically significant relationships were found between Insulin and the other variables. One outlier was removed prior to regression analysis. A simple linear regression was calculated to predict participant's Insulin levels based on their EA, RMR, EI, and DXA_BFP. The regression equation was not significant for EA via 1) $V0_{2max}$-HR regression was ($F_{(1,25)} = 0.40$, $p = 0.53$) with an R^2 of 0.02 and via 2) Garmin_HR ($F_{(1,25)} = 0.29$, $p = 0.59$) with an R^2 of 0.01. Energy availability was not statistically a significant predictor of Insulin levels. No significant predictors were found for Insulin.

Leptin. A paired samples t-test was calculated to compare the means between the 2 training weeks (HV and LV) for Leptin with a mean of 0.75 $\pm$ 0.57 ng/dL (Lep_HV: 0.82 $\pm$ 0.59 ng/dL and Lep_LV: 0.68 $\pm$ 0.58 ng/dL). A significant difference was found between the 2 training weeks and Leptin ($t_{(13)} = 1.61$ $p < 0.001$). Within the HV week, 78.6% (n = 11) of participants presented with low levels compared to normative male

values (between 1.2-9.5 ng/dL), while 85.7% (n = 12) presented with low leptin levels in the LV week. A Pearson correlation coefficient was calculated for the relationship between Leptin and other variables (i.e., DXA_BFP, RMR, EI, EEE, etc.). A strong negative correlation was found for overall LH to DXA_BFP: ($r_{(24)} = -0.73$, $p < 0.001$). No other correlations were found. A significant association was demonstrated between Garmin HRM and overall Leptin ($\chi (2) = 6.4$, $p = 0.01$) as well as HV_Leptin week ($\chi (2) = 6.9$, $p = 0.01$).

Two outliers were removed prior to regression analysis. A simple linear regression was calculated to predict participant's leptin levels based on their EA, RMR, EI, and DXA_BFP. Energy availability was not statistically a significant predictor of leptin levels via 1) $V0_{2max}$-HR regression was ($F_{(1,24)} = 0.98$, $p = 0.33$) with an R^2 of 0.04 and via 2) Garmin_HR ($F_{(1,24)} = 0.92$, $p = 0.35$) with an R^2 of 0.04. A significant regression equation was found for Leptin and DXA_BFP: ($F_{(1,24)} = 27.18$, $p < 0.001$), with an R^2 of 0.51. Participants' predicted Leptin level is equal to -1.02 + 0.13(DXA_BFP) ng/mL when DXA_BF is measured in kg. Participants' average leptin levels decreased by -1.02 ng/dL for each kg of DXA_BFP. No other significant results were found for leptin.

Cortisol. A paired samples t-test was calculated to compare the means between the 2 training weeks (HV and LV) for cortisol with a mean of 12.9 $\pm$ 2.8 ug/dL (Cort_HV: 12.6 $\pm$ 2.8 ug/dL and Cort_LV: 13.1 $\pm$ 2.6 ug/dL). No significant differences were found between the 2 training weeks and T ($t_{(13)} = -0.91$ $p = 0.38$). Cortisol presented within the normative range for males between 7-28 ug/dL. A Pearson correlation coefficient was calculated for the relationship between Cortisol and other

variables (i.e., BFP, RMR, EI, EEE, etc.). A weak negative correlation was found for overall Cortisol to Regression EEE ($r_{(24)} = -0.46$, $p = 0.01$). A simple linear regression was calculated to predict participant's Cortisol levels based on their EA, RMR, EI, and DXA_BFP. Energy availability was not statistically a significant predictor of Cortisol levels via 1) $V0_{2max}$-HR regression was ($F_{(1,26)} = 1.1$, $p = 0.30$) with an R^2 of 0.20 and via 2) GarminHR ($F_{(1,26)} = 0.01$, $p = 0.92$) with an R^2 of 0.00. A significant regression equation was found for Cortisol and $V0_{2max}$-HR RegressionEEE: ($F_{(1, 26)} = 7.07$, $p = 0.01$), with an R^2 of 0.21. Participants' predicted Cortisol level is equal to 14.68 ± -0.002 (EEE) ug/dL when EEE is measured in kcals. Participants' average Cortisol levels decreased -0.002 ug/dL for each kcal of EEE. A significant regression equation was also found for Cortisol and DXA_BFP: ($F_{(1, 26)} = 12.0$, $p = 0.002$), with an R^2 of 0.32. Participants' predicted Cortisol level is equal to 19.12 +-.46 (DXA_BFP) ug/dL when DXA_BFP is measured in percentage. Participants' average Cortisol levels decreased -0.46 ug/dL for each percent of DXA_BFP. No other significant relationships/predictors were found for Cortisol.

Interluekin-6 (IL-6). A paired samples t-test was calculated to compare the means between the 2 training weeks (HV and LV) for IL-6 with a mean of 0.68 $\pm$ 0.60 pg/mL (IL-6_HV: 0.80 $\pm$ 0.80 pg/mL and IL-6_LV: 0.56 $\pm$ 0.39 pg/mL). No significant differences were found between the 2 training weeks and IL-6 ($t_{(13)} = 0.95$; $p = 0.36$). Interleukin-6 presented with most all the participants within the normal range (< 1.8 pg/mL) with 1 participant above the normative male value. A Pearson correlation coefficient was calculated for the relationship between IL-6 and other variables (i.e., BFP, RMR, EI, EEE, etc.). Weak correlations that were not significant were found for

DXA_BFP ($r_{(25)}$ = -0.4; p=0.84), RMR ($r_{(25)}$ = -.15; p = 0.46), EI: ($r_{(25)}$ = -.25; p = 0.22), V02max-HR Regression EEE ($r_{(25)}$ = .02; p = 0.94), Garmin_HRM EEE ($r_{(25)}$ = -0.22; p = 0.27), Distance ($r_{(25)}$ = -.23; p = 0.26). These secondary measures (DXA_BFP, RMR, EI, EEE, and distance) are not related to IL-6 levels.

One outlier was removed prior to regression analysis. A simple linear regression was calculated to predict participant's IL-6 levels based on their EA, RMR, EI, and DXA_BFP. Energy availability was not statistically a significant predictor of IL-6 levels via 1) $V0_{2max}$-HR regression was ($F_{(1,25)}$ = 0.94, p = 0.34) with an R^2 of 0.04 and via 2) GarminHR ($F_{(1,25)}$ = 0.09, p = 0.77) with an R^2 of 0.004. No significant regression equation was found for IL-6 with the other measures (DXA_BFP, RMR, EI, EEE, Distance).

Discussion

In this study we sought to find a relationship between EA and specific reproductive (T and LH) and metabolic hormones (Insulin, Leptin, Cortisol, and IL-6). Our overall results partially supported our hypothesis that LEA (≤ 20 kcal/kg FFM·d) would impact metabolic hormones, however, LEA only impacted Leptin. Overall, participants presenting with LEA, 21.7% (n = 7) also presented with low levels of Leptin. Our participants did demonstrate increased levels of testosterone (47.1%, n = 8) in relation to LEA. However, no other hormones were negatively impacted by LEA.

Energy Availability

Within this study, 2 separate training weeks (HV and LV) were used to examine energy needs and demands specific to EA and EEE. To assess EA, we created a cutoff point for LEA at ≤ 20 kcal/kg FFM·d. Our results yielded an average EA of 25.2 kcal/kg FFM·d (HV week) and 29.9 kcal/kg FFM·d (LV week). There currently is no research examining free-living EA in males to compare our results with. However, one study examining NCAA Division I female soccer players examining EA (LEA set at ≤ 30 kcal/kg FFM·d) demonstrated higher EA levels compared to our results (EA levels between 32 to 43 kcal/kg FFM·d for 3 measurements throughout the season). While overall neither week demonstrated LEA (≤ 20 kcal/kg FFM·d) using VO_{2max} - HR regression EA, 71.4% (n = 10) of the participants demonstrated LEA between 1 and 4 days during both HV and LV weeks (see Table 3.1).

Hormones

Overall, we did not see significant relationships between EA and the reproductive (LH) and metabolic (Insulin, Leptin, Cortisol, IL-6) hormones. However, secondary measures (DXA_BFP, RMR, EI, and EEE) did demonstrate significant relationships between: 1) T and RMR, 2) T and EI, 3) T and DXA_BFP, 4) Leptin and DXA_BFP and 5) Cortisol and EEE. We were able to calculate a prediction equation from RMR, EI and DXA_BFP that all demonstrated significant regression equations to predict T levels in male endurance-trained athletes. Examining linear regressions, DXA_BFP was a good predictor for Leptin levels while EEE was a good predictor for Cortisol levels. This could be impactful for clinicians whom do not have access to blood testing capabilities.

Reproductive Hormones

Testosterone. Increases in T level were found with both weeks of training for our participants. More specifically, both weeks demonstrated larger values than the "normal" range of 270-1070 ng/dL.[39] With the average for both weeks high (HV: 1640.6 $\pm$ 1385.3 ng/dL and LV: 1888.3 $\pm$ 1831.1 ng/dL), this was not congruent with most of the literature examining endurance runners. For example, previous research has demonstrated endurance training has a negative effect on testosterone levels in males.[21] Additionally, resting testosterone levels were measured lower in endurance-trained males compared to untrained males.[21] The mechanistic nature of this decrease is currently unknown. One impression is that the decreased testosterone levels are related to dysfunctions in the hypothalamic-pituitary-testicular regulatory axis, which is congruent with literature within the female population.[21] Presently, there is no existing literature that indicates any testosterone dysfunction is instigated by endurance training in males.[21]

De Souza and colleagues[44] established a "training volume-threshold" (~100 km/week), which demonstrated significant changes in the male reproductive function.[44] High volume of endurance running (> 104 km/wk) showed associations with changes in both sex hormones profiles (decreased testosterone), and quality of semen (decreased mobility and increases in immature cell numbers).[21,44] On average, our participants did meet this distance in the HV week but did not demonstrate decreases in T overall in either HV or LV week. Peripheral and central mechanisms have been proposed as reasons for decreased testosterone.[44] These proposed mechanisms for endurance runners include: peripheral mechanisms such as intrinsic failure of the tests to maintain steroid biosynthesis and altered hepatic clearance and metabolism of testosterone and central

mechanism include alterations in the hypothalamic and pituitary axes.[44] As this study was observational in nature, energy deficiency (EA ~25-29 kcal/kg FFM·d) and body fat percentages (~13.5%) may not have been low enough, or EEE (~670 kcals)/mileage (~49 miles; HV ~63 miles; LV: ~34) may not have been high enough, to elicit decreases in T levels.

Luteinizing Hormone. Luteinizing hormone did not demonstrate a significant difference between the 2 weeks. Previous literature is in discordance regarding LH. Research has shown with increases in exercise, testosterone decreases; however LH has not shown to be significantly changed during these increases of training loads.[22] Conversely, Kuoppasalm et. al. [23] demonstrated that with long term, high intensity running, roughly 30 minutes after long-term runs, plasma LH significantly dropped below baseline levels by 42% (moderate run) and 45% (intense run). Suggesting intensity of the exercise is important in regards to negative LH outcomes.[23]

Female literature has shown hypothalamic gonadotropin-releasing hormone is common in female athletes (hypothalamic amenorrhea).[24] Male literature examining male reproductive hormones (T and LH) have seen similar results as the female literature. MacConnie et. al.[24] examined highly trained male marathon runners (125 – 200 km/week) and found the runners had diminished frequency of spontaneous LH pulses and the amplitude of their LH pulses were decreased compared to healthy controls.[24] These results suggest highly trained male athletes, akin to the female athlete, have deficiency of hypothalamic gonadotropin-releasing hormones. Male studies examining military populations found similar results of decreased LH to distance runners. Freidl et al.,[14] and Kyrolainen et al.,[18] examined military soldiers during active training camps who

presented with low LH levels during high training volume. This may have been due to the sudden and dramatic increase in EEE and simultaneous dramatic decrease in EI. Our results found a strong negative correlation between LH and DXA_BFP, and previous literature may suggest a link with BFP and hormonal deficits[14-16,45]

Metabolic Hormones

Insulin: Our results yielded "normal" ranges for Insulin across both training weeks with no statistically significant relationship to EA or other measures. Our results are not congruent with previous literature that demonstrated decreases in insulin due to high EEE and low EI.[14,18] Koehler et. al.,[17] found decreases in insulin in relation to EA suppression (EA = 15 kcal/kg FFM·d) in male cyclists. Previous research has demonstrated that when insulin is low or absent, glucose is not utilized as an energy source, and the body will begin to utilize fat as an energy source, consequently, insulin aids in the prevention of fat utilization for energy.[25] Insulin aids in the regulation of amino acid uptake and has other anabolic effects throughout the body.[25] Proposed mechanistic reasons for decreased insulin include a global effect on the hypothalamic-pituitary axes[3] or due to adaptations of glucose uptake in runners.[25] It is suggested that in aerobically trained athletes, adaptations related to the increased blood flow (capillarization) causes augmented glucose disposal due to increased glucose transport proteins (GLUT 4) globally with less insulin required.[46] More research is needed to understand the mechanism of decreased insulin in a LEA state.[46]

Leptin. Overall, Leptin was the only hormone that demonstrated significant decreases compared to LEA and difference between the 2 training weeks ($t_{(13)} = 1.61$ $p <$

0.001). Our results found 78.6% (HV) and 85.7% (LV) of the participants presented with low leptin levels, which is congruent with Hagmar et. al.,[15] who examined 18 Olympic male athletes that participated in leanness sports and demonstrated low Leptin levels (1.04 ng/mL) as well as low DXA_BFP (11.7 $\pm$ 3.4%) compared to non-lean sports. Typically, body weight has an analogous relationship with leptin (i.e. weight loss reduces leptin while weight gain increases leptin levels).[26] It is hypothesized that the reduction of leptin is an acute metabolic signal of starvation and energy conservation.[26,27] This is suggested due to the reductions in leptin are associated with the suppression of key endocrine axes (reproductive, growth hormone, IGF-I, and thyroid axes).[27] During acute energy deficits, the rapid reduction of leptin is associated with changes in body composition.[47]

Cortisol. Our results demonstrated our endurance-trained male athletes cortisol levels were within normal limits (7-28 ug/dL) means between the 2 training weeks (HV and LV) for cortisol with a mean of 12.9 $\pm$ 2.8 ug/dL (Cort_HV: 12.6 $\pm$ 2.8 ug/dL and Cort_LV: 13.1 $\pm$ 2.6 ug/dL) with no differences between the 2 training weeks. However, we did find EEE and DXA_BFP was a good predictor of cortisol levels. Cortisol also has the ability to suppress various systems including the reproductive and digestive system, as well as other growth process.[28] Long-term triggering and overexposure to cortisol can disrupt bodily process and increase the risk to various health issues including, anxiety, depression, digestive issues, headaches, heart disease, sleep problems, and weight issues.[28] Hill et. al.,[28] demonstrated moderate to high intensity exercise invokes an increase in circulating cortisol levels, while in contrast; low intensity exercise (40% VO_2max) decreases circulating levels.[28] Cortisol levels may increase due to either an

increased need to catabolize other energy sources other than fat stores, or due to a reduction in clearance.[14]

Interleukin-6. Our results demonstrated no differences between the 2 training weeks with a mean of 0.68 ± 0.60 pg/mL (IL-6_HV: 0.80 ± 0.80 pg/mL and IL-6_LV: 0.56 ± 0.39 pg/mL) or an increase in IL-6 levels compared to normative data (≤ 1.8pg/mL). This is not congruent with previous literature that found increases in IL-6 levels after strenuous exercise as an inflammatory marker.[48,49] However, this was reported as an acute response, while our blood draws were done after 24 hours of rest from exercise on the 8th day of the study. Fischer[50] examined IL-6 responses to acute and training loads, and found that there was a training effect for the down regulation of IL-6. This low plasma levels of IL-6 can be characterized as a training adaptation.[50] This training effect, may explain our lower levels of IL-6. Research has also demonstrated glucose aids in decreased acute localized muscular release of IL-6.[51] This mechanism of decreased IL-6 due to training or glucose needs to be examined as well as the inverse relationship, in the event of decreased EI and potential pathogenic dietary behaviors, which decrease carbohydrate ingestion in male athletes.

Limitations and Future Directions

There were limitations identified in this study examining EA and hormones. First, an aspect of EA includes EI and EEE. Energy intake was a 7-day self-reported dietary log but is most valid and reliable measurement compared to other self-reported food intake measurements (3-day and food frequency questionnaires.)[52] Second, double-

labeled water would be a more valid and reliable examination of EEE was calculated using HRM and Vo2max-HR regression. Double-labeled water would be a more accurate method to estimate EEE. Additionally, data collection for the hormones was a one-time fasting blood draws on the morning on the 8th day. However, some measures would have benefited from different measurements. Specifically, LH is pulsatile in nature and would be better to examine LH multiple times. Testosterone would have been more expressive if measured daily compared to one draw. Another hormone, IL-6, has demonstrated more acute responses to exercise and may have given more information to inflammatory responses if measured within a few hours after exercise compared to after 24 hours of rest at the end of the week. Future studies should examine hormones to assess a more accurate assessment of LH, T and IL-6. Also, an intervention study should be implemented examining specific EA levels and the response of reproductive and metabolic hormones specific to set EA levels.

Conclusions

Overall, LEA demonstrated a relationship with Leptin. Other variables BFP, RMR, EI and EEE also demonstrated relationships with T, Leptin and Cortisol. The implications of these relationships, speaks to the physiological influence of EA, EI, EEE and body composition on hormones. This knowledge is impactful for general knowledge of clinicians regarding the hormonal profile of male athletes, especially those with decreased EA, EI, and BFP, and increased EEE. Male runners should monitor their EEE and EI to maintain appropriate levels of EA; which currently we have evidence to suggest

above 20 kcal/kg FFM·d due to altered hormonal response of Leptin. Valid and reliable predictive equations for hormones (T, Leptin, Cortisol) may become useful tools for clinicians whom do not have access to blood work.

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TABLES

Table 3.1: Energy need assessments for Endurance Trained Male Athletes (n=14). Values are presented in Mean ± Standard Deviation.

	ALL	
	M	SD
Energy Needs Assessment		
Resting Metabolic Rate (kcals)	1799.3	549.0
Energy Intake (kcals)	2658.9	230.4
Exercise Energy Expenditure (kcals)-GHR	636.7	127.1
Exercise Energy Expenditure (kcals)-VO_2	865.4	566.1
Energy Availability (kcal/kg FFM·d)-GHR*	28.2	10.4
Energy Availability (kcal/kg FFM·d)-VO_2*	27.6	12.0

Low Energy Availability Risk	HV%	N	LV%	N
LEA ≤ 2 days per week-GHR	42.8	6	35.7	5
LEA 3-4 days per week-GHR	21.4	3	21.4	3
LEA ≤ 2 days per week-VO_2	35.7	5	50.0	7
LEA 3-4 days per week-VO_2	35.7	5	21.4	3
LEA ≤ 2 days per week-Arm	35.7	5	21.4	3
LEA 3-4 days per week-Arm	21.4	3	28.6	4
LEA 5-6 days per week-Arm	21.4	3	21.4	3
LEA 7 days per week-Arm	7.7	1	7.7	1

*Note: LEA for the week was calculated by taking the average of the 7 days of EA. LEA = low energy availability; GHR = Garmin Heart Rate Monitor; VO_2=VO_{2max}-HR Regression; Arm=Armband. EEE- calculated by average number of days of exercise per week across individuals.

Table 3.2: Hormonal Profile for Endurance Trained Male Athletes (n=14). Data is presented in mean (M) and standard deviation (SD).

	Overall		HV Week		LV Week	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Reproductive						
Testosterone ng/dL	1764.5	1608.2	1640.6	1385.3	1888.3	1831.1
Luteinizing Hormone pg/mL	398.8	155.5	385.7	191.0	409.9	119.9
Metabolic						
Insulin mU/L	7.25	1.23	7.15	1.07	7.34	1.46
Leptin ng/dL	0.63	0.38	0.82	0.59	0.68	0.58
Cortisol ug/dL	12.92	2.78	12.63	2.75	13.11	2.61
Interleukin-6 pg/mL	0.68	0.60	0.80	0.80	0.56	0.39

*Note: Hormones were collected on the 8th day after 24 hours of rest.

FIGURES

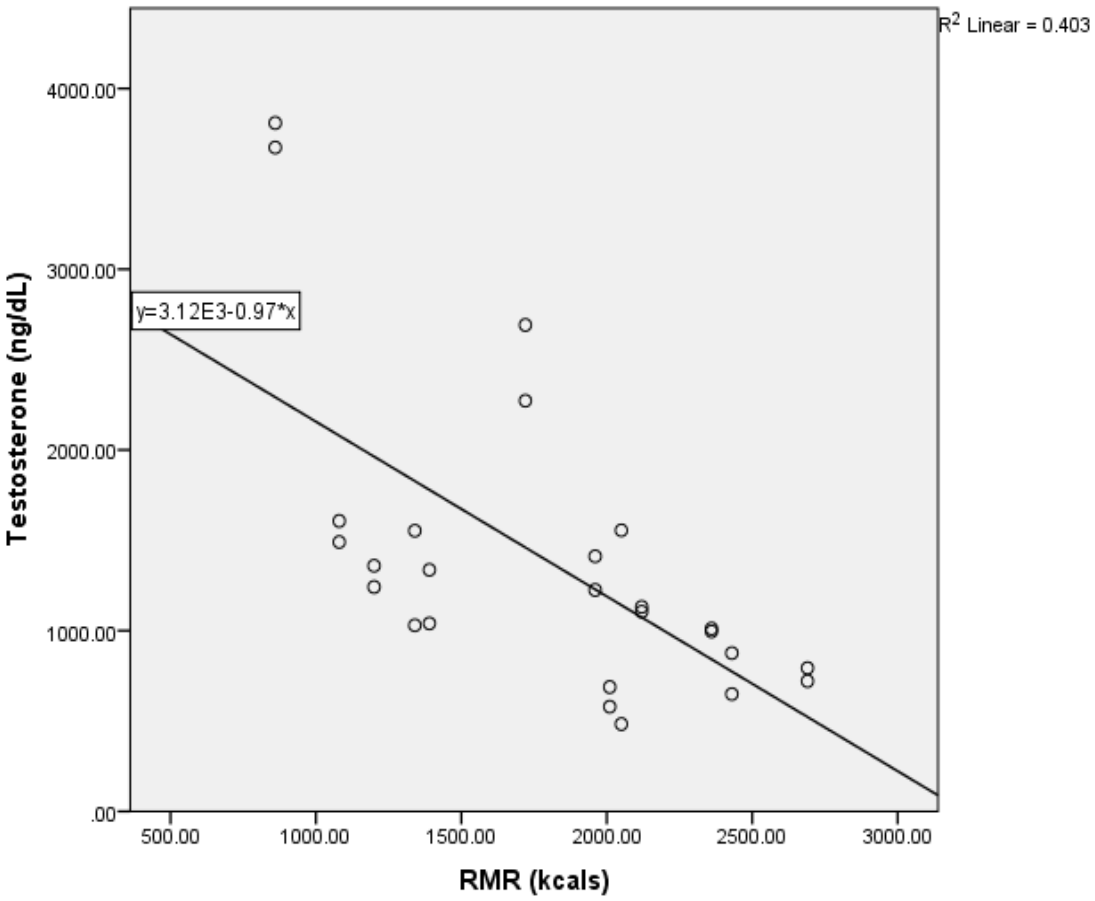

Figure 3.1: Testosterone levels as a function of Resting Metabolic Rate in endurance-trained male runners. Testosterone measured ng/dL and RMR measured in kcals; ($r_{(26)}$ = -0.64, $p < 0.001$). A significant regression equation was found ($F_{(1,23)} = 16.23$, $p < 0.001$), with an R^2 of 0.4.

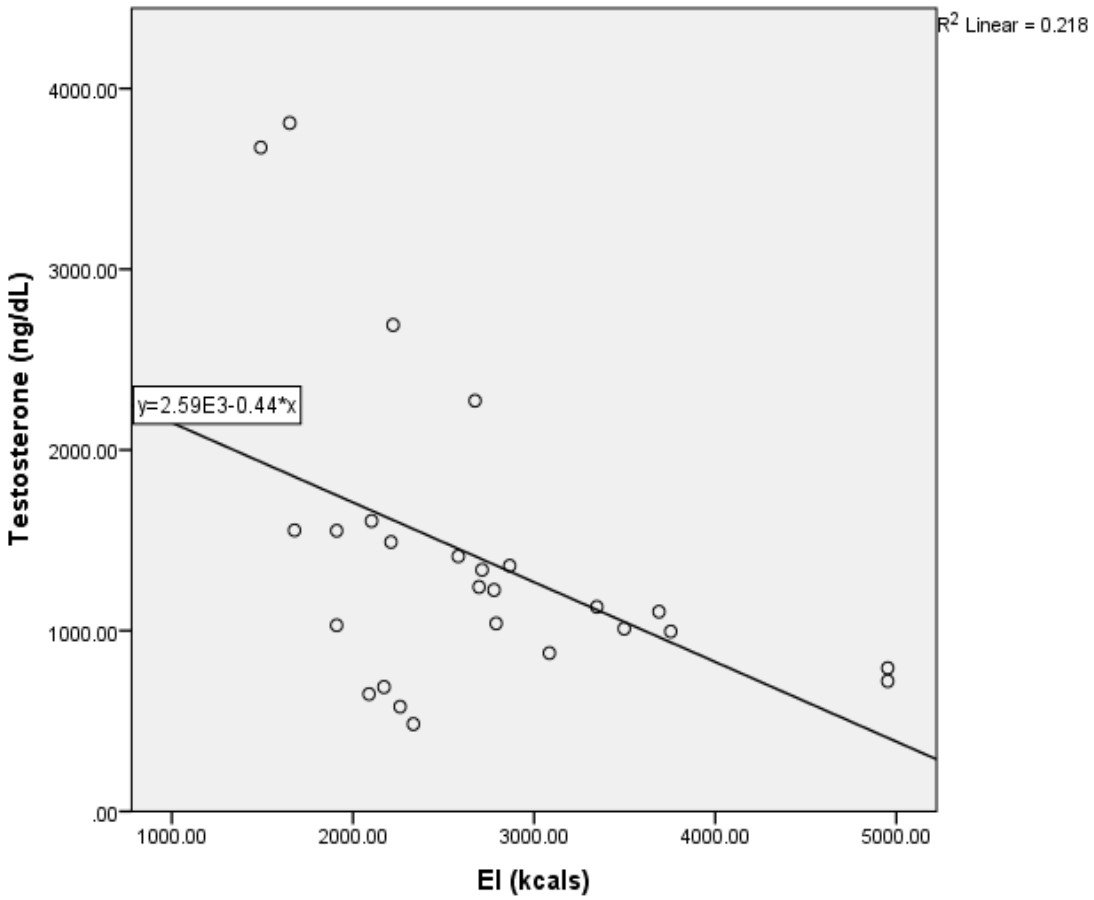

Figure 3.2: Testosterone levels as a function of Energy Intake in endurance-trained male runners. Testosterone measured ng/dL and EI measured in kcals, n = 26; ($r_{(24)} = -0.47$, $p = 0.02$). A significant regression equation was found ($F_{(1, 24)} = 6.7$, $p = 0.02$), with an R2 of 0.22.

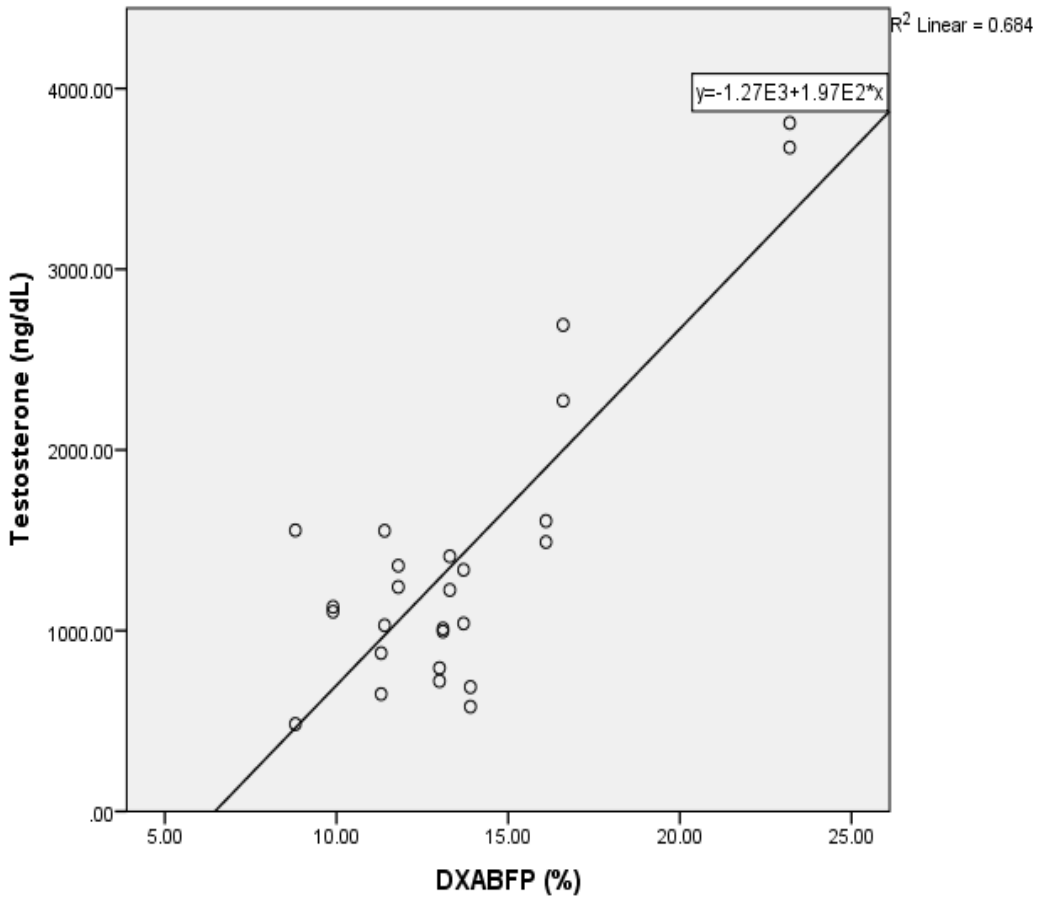

Figure 3.3: Testosterone levels as a function of Body fat percentage in endurance-trained male runners. Testosterone measured ng/dL and DXABFP measured in percentage (%); ($r_{(24)} = 0.83$, $p < 0.001$).

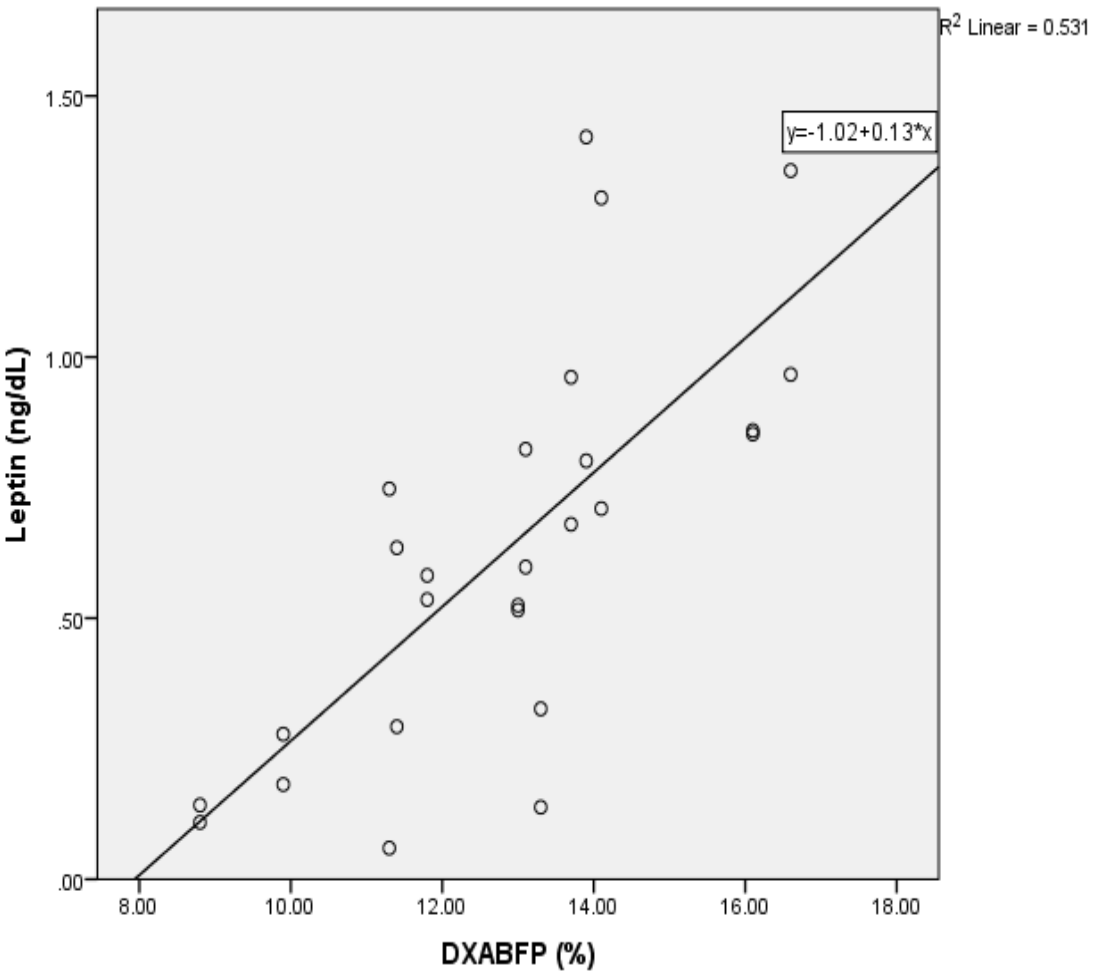

Figure 3.4: Leptin levels as a function of Body Fat Percentage in endurance-trained male runners. Leptin measured ng/dL and DXABFP measured in percentage (%); ($r_{(24)} = -0.73$, $p < 0.001$). A significant regression equation was ($F_{(1,24)} = 27.18$, $p < 0.001$), R^2 of 0.51.

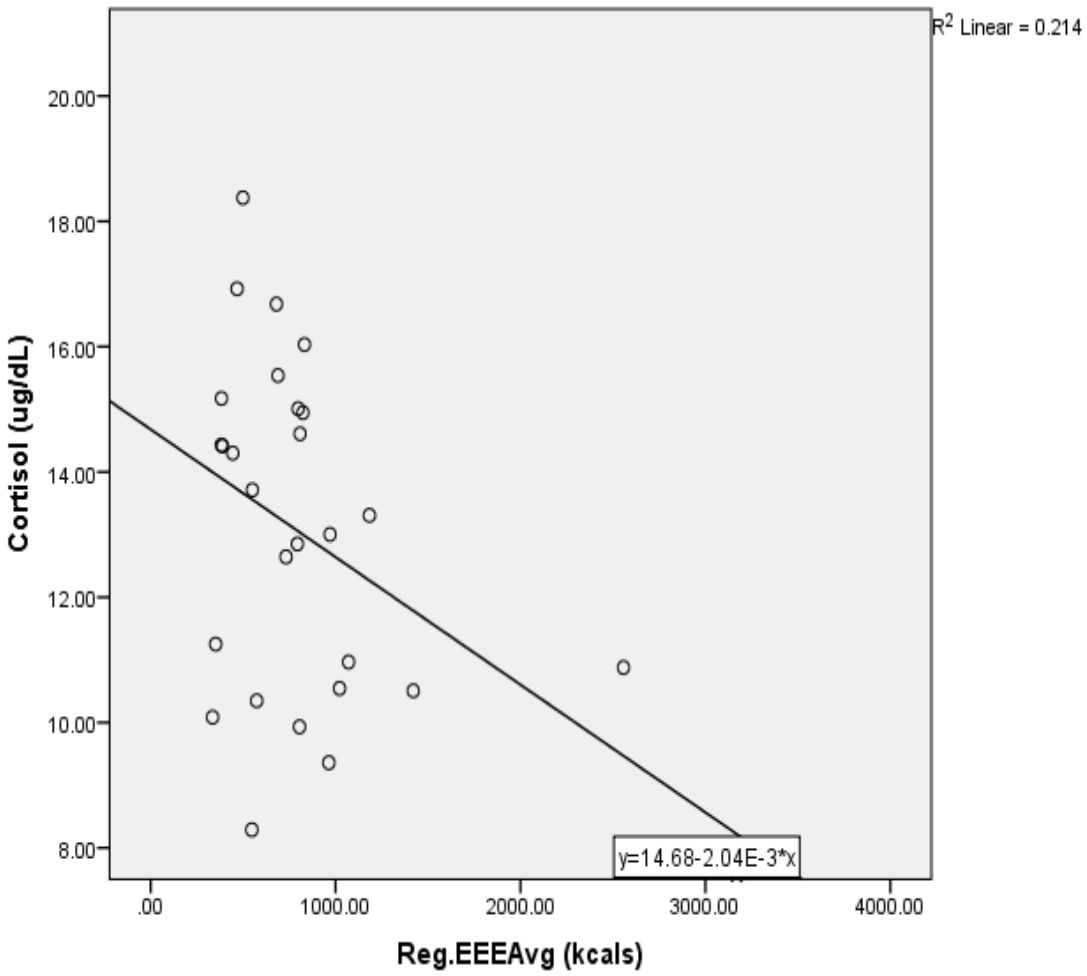

Figure 3.5: Cortisol levels as a function of V02max-HR Regression Exercise Energy Expenditure in endurance-trained male runners. Cortisol measured ug/dL and EEE measured in kcals; ($r_{(24)} = -0.46$, $p = 0.01$). A significant regression equation was found ($F_{(1, 26)} = 7.07$, $p = 0.01$), R^2 of 0.21.

CHAPTER 4

REVIEW OF LITERATURE

Endurance athletes an at-risk population

Endurance athletes are unique due to the physiological demands of their high-energy expenditure sport participation. These increased psychological demands increase endurance athletes' (e.g. distance runners) risk of impaired physiological functions.[1-9] Endurance athletes are exposed to an increased risk of injuries and illnesses compared to other sports. Specifically examining injuries in endurance runners; due to the repetitive impact forces, stress fractures are more common in distance runners than other endurance athletes.[10] The NCAA estimates the incidence of stress fractures range from 1% - 2.6% in all athletes compared to 15% in runners.[10] A 10 year retrospective review highlighted NCAA Division I college distance runners suffered most stress fractures of all male and female athletes.[10] Current research suggests differences in loading forces and the high-energy expenditure bone health of endurance runners is inferior to other sport athletes.

Endurance athletes are also exposed to increased risks of illness.[10] Hagmar et. al.,[11] confirmed more lean Olympic athletes reported being ill during a 3month period prior to competition compared to non-lean athletes (38.5% vs 21.6%, $P < 0.05$)[11] and had specifically higher frequencies of mild diseases including upper respiratory tract infections.[11,12] Another illness specific to endurance athletes includes potential adverse

cardiovascular effects.[13] Emerging data has shown long-term excessive endurance exercise (marathons, ultramarthons, ironman distance triathlons, and distant bicycle races) has associations with coronary artery calcification, diastolic dysfunction and artery wall stiffening, and sudden cardiac death (SCD).[13] While long-term excessive exercise may cause adverse cardiovascular remolding, further investigation is still needed at this time to understand mechanistic causes.[13] Rates for SCD are rare, 1 SCD/100,000 participants (.00001).[13] However, while the risk of SCD has not increased, the absolute rates for mortality of SCD has increased as annual participation in marathons have increased 20-fold.[13]

Most link negative body image issues to females only; however, literature has shown this is tenuous. Mellor et al.[14] confirmed an inverse relationship in adult men between self-esteem and body image. Unique results of this study reported men, when compared to women, place greater importance on their appearance and described high levels of body dissatisfaction.[14] Hagmar and colleagues[11,12] have examined male Olympic athletes and the differences between leanness and non-leanness sports. Male athletes in leanness sports reported lower BMI (22.7 ± 2.7 kg/m^2 vs 23.7 ± 2.3 kg/m^2 for non-lean athletes, $P < 0.05$) and greater variations in weight (5.3% vs 4.7%, $P < 0.05$) compared to non-lean athletes.[11] Lean athletes also reported more frequent attempts of weight loss ($P < 0.001$) as well as reporting longer training times and more pronounced training loads ($P < 0.001$).[11] Significant differences were discovered between male Olympic leanness and non-leanness athletes when examining body composition.[11,12] Leanness athletes exhibited a total body fat and total body fat percentage significantly

lower when compared to non-leanness athletes (leanness 9.2±3.5 kg, 11.7% ± 3.4; non-leanness 13.2 ± 5.3 kg, 16.4 ± 5.8%).[11,12]

Relative Energy Deficit in Sports (RED-S)

In 2014, the International Olympic Committee (IOC) issued a Consensus Statement, "Beyond the Female Athlete Triad: Relative Energy Deficiency in Sport (RED-S)".[7] This new paradigm addresses energy deficiency in sports for *all* types of athletes, including females, males, and non-able bodied (e.g. Para-Olympic athletes).[7] This syndrome of RED-S, designed to be all encompassing model, highlights physiological impairments to the athlete resulting from relative energy deficiency.[7] These impairments comprise various physiological systems including: cardiovascular health, bone health, menstrual function, immunity, protein synthesis, and metabolic rate.[7] While the IOC's RED-S is lacking empirical literature to illustrate RED-S as an encompassing model, it does provide awareness and the necessity to examine other populations (males and non-able bodied athletes).[7] Current literature examining men, have shown similar physiological decrements to the Female Athlete Triad (Triad), compromising of compromised reproductive hormones,[15-20] decreased bone mineral density,[10,21,22] and negative energy deficiency[11,12,18,23]. Currently the only research specifically examining low energy availability (LEA) in the male population was Koehler et. al.,[18] in 2016, after the issuance from the IOC.

Energy Balance vs. Energy Availability

Energy balance (EB), as defined in the field of dietetics, is the dietary energy intake (EI) minus the *total* energy expenditure (EB= EI-TEE).[5] Specifically, this is the amount of dietary energy, either auxiliary or used from the body's overall energy stores once all physiological systems have completed their energy expenditure demands for the day.[5] Energy balance is measured as an output from those systems, and categorized when EB=0 kcal/day.[5] Energy availability (EA) is defined as the dietary EI minus the energy expended in exercise (EA=EI-EEE).[5] Energy availability is the dietary energy residual after exercise training for all other physiological and metabolic processes.[5] Energy availability is an input to the physiological systems and categorized at a healthy status of EA=45 kcal/kg FFM·d. [5]

The disparity amongst EB and EA is that an athlete can have low energy availability (LEA) but maintain their EB. This is due to the suppression of various physiological processes due to the lack of EA.[5] Strubbs et. al.,[23] provided an example of the contrast between EB and EA. Eight lean men had a suppressed caloric energy intake and an increased exercise energy expenditure resulting in a constant energy availability of 30 kcal/kg FFM·d.[23] Strubbs et. al.,[23] found the negative energy balance decreased towards zero at a rate of 90kcal/day due to the decreased physiological processes, and estimated three weeks for participants to elicit an EB of zero while stilling remaining in a severely LEA state.[23] Due to EB being an output from of physiological systems it is does not provide the most reliable information in regards to energy requirements of athletes, therefore is not the most useful in managing energy.[5]

Energy Availability

Koehler et. al.,[18] is the first study to examine short-term energy availability reduction in exercising men. Similar to Loucks et. al.,[24,25] examination in females; Koehler et. al.,[18] reduced EA to 15 kcal/kg FFM·d in six exercising men. This reduction of EA resulted in a significant suppression of leptin and insulin hormones. Koehler et. al.,[18] found a decreasing trend in the hormone testosterone.[18] Other research has examined chronic energy deficits and its effect on various hormones and bone mineral density.[11,12,16-19,23,26] However, EA was not calculated or examined specifically. To date, there are no established LEA markers for the male athlete.

Energy availability markers for female athletes have been established at 45 kcal/kg FFM·d.[1-9] Literature has established this is due to significant physiological changes, which occur at and below 30 kcal/kg FFM·d.[3-6,9,25] These physiological changes include: metabolic rate declines, bone reabsorption increases, protein synthesis breakdown occurs at this level, and reproductive hormones (i.e.. LH, FSH, and estrodral) decrease.[1-6,9] In the female literature, LEA is the catalyst for Triad symptoms. The American College of Sports Medicine (ACSM)[26] has identified 3 categories describing the origins of energy deficiency in athletes; (1) lack of knowledge of energy needs based on demands of the energy expended, (2) clinical eating disorders and (3) intentional subclinical mismanagement of energy consumption to reduce body size and fatness.[26] This could include disordered eating behaviors similar to various eating disorders such as fasting and purging.[26] Previously established research has examined and recognized the physiological differences in males and females, prompting De Souza et. al.,[2] in response to the IOC's REDs model, to stress the necessity to establish independent clinical

guidelines in regards to energy deficiency and the physiological impacts specifically for males and females.

Female Athlete Triad

The Female Athlete Triad has a strong empirical literature foundation encompassing thirty years of literature on the female athlete and the effects of reduced EA. The Triad is defined, from the "2014 Female Athlete Triad Coalition Consensus Statement on Treatment and Return to Play of the Female Athlete Triad"[1], as a medical condition observed in physically active girls and women involving 3 components: (1) low energy availability with or without disorder eating, (2) menstrual dysfunction, and (3) low bone mineral density.[1] In 2007, the Triad conceptually changed from a triangle model (disordered eating, amenorrhea, and osteoporosis) into a 3 interrelated spectrum describing the Triad as a syndrome of LEA with or without DE/ED, functional hypothalamic amenorrhea, and compromised BMD.[8] This spectrum model allows for the early recognition of subclinical abnormalities and early intervention for at risk female athletes.[1] Health consequences associated with the Triad range a spectrum of systems within the body including; endocrine, gastrointestinal, renal, neuro-psychiatric, musculoskeletal and cardiovascular health. [1]

Triad-Like Symptoms in Males

Trends have been established in previous literature in males expressing Triad like symptoms. When EA is compromised, various gonadal and reproductive hormones are

suppressed,[15-20] BMD is reduced,[15,16,21,22] and limited research has described a prevalence of disorder eating ranging from 10%-42% in male athletes.[11,12,27]

Military research has shown male soldiers during occupational missions are exposed to dietary restrictions causing decreased EA. Fridel et. al.,[17] examined US Army Rangers during their 8-week Ranger Course. During these 8 weeks; 4 cycles of restricted energy intakes (EI) and refeeding, incorporating EI deficits of 1,000-1,200 kcal/day occurred.[17] Research has revealed that due to deficits in EA in males, gonadal and reproductive hormones (testosterone (T), thyroid, Sex hormone-binding globulin, and insulin-like growth factor I(IGF-I)) were reliable hormonal markers when assessing acute energy deficits.[17] Cortisol and cholesterol were specific hormonal markers, which assessed the chronic state of energy deficits in the male US Army Rangers.[17] Congruently, Kryolainen et. al.[19] examined hormonal alterations during prolonged military field exercises in Finnish soldiers and the effect of various energy deficits prompted through exercise intensity. Kryolainen et. al.[19] found during an average energy deficit of 4,000 kcal/day in phase I of training (one week long) significant increases in cortisol (+32%) and growth hormone (+616%) where exhibited while insulin (-70%), total T (-27%) and free T (-26%) decreased. Once energy deficits were reduced (<1,000kcal/day in Phases II and III), all hormonal markers stabilized.[19]

Research examining endurance sports (running, biking, and swimming) have been found to have lower BMD than athletes participating in other sports and even lower than inactive peers. Hetland et. al.,[28] demonstrated lumbar spine BMD was negatively correlated to average weekly mileage for distant runners. Dolan et. al.,[15,16] demonstrated professional jockeys had reduced bone mass and an elevated rate of bone loss due to a

disruption in hormone activity in response to chronic weight cycling. Research has revealed 25%-63% of male cyclists were diagnosed with osteopenia, while 9% were diagnosed with osteoporosis.[21,22]

Previous research has shown women are more at risk for eating disorders (ED)/disorder eating (DE) compared to males, however literature has shown males especially in lean sports have increased risks of similar energy restriction behavior and similar associated negative physiological responses. Martensen et. al.,[27] found prevalence in male athletes to range from 10% to 42%, for DE behaviors. In male Olympic athletes, Hagmar and colleagues[12] observed participants who were categorized within "lean sports" had significantly lower body fat proportions, lower free T, leptin levels, and an increase in IGF-I. [12]

Eating Disorders/Disorder Eating:

Currently research examining ED risks in male athletes are inconclusive. It is evident ED risks are higher in females than males; however, the rate of males are becoming more prevalent in literature. Rates range from 10%-42% for disordered eating prevalence.[27] Similar to female literature, there are increased ED risks associated with specific sports such as weight class sports (e.g. wrestling, rowing, Judo).[11,12,27] Rosendahl et. al.,[29] examined Elite Sports School, an elite high school for athletes, and found boys in leanness sports had a twofold higher frequency of DE compared to non-leanness athletes (risk ratio = 2.11, 95% CI 1.11-4.04).[29] While female literature has examined a larger variety of female athletes and sports, there is limited research examining males across a variety of sports.[29]

Assessment measurements (instrumentation and questionnaires) used to assess the risk of disordered eating in males needs to be more congruent to address males at risk.[29] While dieting is linked to mostly the female population, male athletes were most frequently dieting to enhance performance.[29] One third of the male athletes were dieting because a coach or teacher pressured them.[29] Within the leanness male athletes, 13% were dieting while 6% were using pathogenic weight control measures compared to 11% dieting and 5% pathogenic weight control measures in the non-leanness sports.[29] Disordered-eating symptoms among adult elite athletes have previously been under reported.[29] However, predictors associated with symptoms of DE among both female and male athletes were found to be body mass index (BMI) and gender ($p<0.001$ and $p=0.012$).[29]

The DSM-5 characterizes feeding and eating disorders as a consistent disruption in eating-related behavior which results in altered or compromised intake of food that has significant impairment on the physical and mental health of the eater.[30] The DSM-5 addresses diagnostic criteria for six specific feeding and eating disorders; pica, rumination, avoidant/restrictive food intake disorder, anorexia nervosa, bulimia nervosa, and binge eating disorder. [30]

Pica

Pica is defined as the persistent eating of nonnutritive, nonfood substances over a minimum period of one month, which includes substances that are inappropriate to the development level of the individual and not culturally or socially normative practices.[30]

Pica can occur within the context of another mental disorder (e.g. autism spectrum disorder, schizophrenia, intellectual disability) and other medical conditions such as pregnancy, which would warrant additional clinical attention.[30]

Rumination

Rumination is characterized as the repeated regurgitation, re-chewed, re-swallowed, or spit out, of food over a one-month period. [30] Regurgitation is not associated with gastrointestinal medical conditions such as gastroesophagela reflux and pyloric stenosis.[30] Rumination could occur concurrently but not exclusively with anorexia nervosa, bulimia nervosa, binge-eating disorder, or avoidant/restrictive food intake disorders.[30] Rumination disorder can present with concurrent symptoms with another mental disorder (e.g. intellectual disability, another neurodevelopmental disorder, and generalized anxiety disorder), which may warrant additional clinical attention.[30]

Avoidant/Restrictive Food Intake Disorders

Avoidant/restrictive food intake disorders is described as an eating or feeding disturbance based on the sensory characteristics of food; concern about adverse consequences of eating manifested by persistent failure to meet appropriate nutritional and/or energy needs associated with one or more of the following: (1) significant weight loss, (2) significant nutritional deficiency, (3) dependence on enteral feeding or oral nutritional supplements, (4) marked interference with psychosocial functioning.[30] The

disturbance in energy intake is not explained by lack of available food or associated culturally sanctioned practices.[30] Avoidant/restrictive food intake disorders can concurrently or non-exclusively occur with anorexia nervosa or bulimia nervosa.[30] However, with avoidant restrictive food intake disorder, there is no associated concurrent medical condition or mental disorder.[30]

Anorexia Nervosa

Anorexia Nervosa is the restriction of energy intake relative to requirements leading to a significantly low body weight in context of age, sex, developmental trajectory and physical health.[30] Significantly low body weight is determined as a weight less than minimally normal. Anorexia nervosa is associated intense fear of gaining weight or becoming fat or a persistent behavior that interferes with weight gain despite having low body weight.[30] The disturbance alters body weight or shape and a persistent lack of recognition of the seriousness of the low body weight.[30] There are 2 subtypes: restricting and binge-eating/purging type categorized with Anorexia Nervosa.[30] Restricting type is defined for an individual, over the course of 3 consecutive months, has not participated in binge eating or purging behaviors (e.g., vomiting, laxatives, enemas, and diuretics).[30] Binge-eating/purging behaviors are described as an individual engaging in recurrent episodes of binge eating or purging behaviors (e.g., vomiting, laxatives, enemas, and diuretics) over the course of 3 consecutive months.[30]

Bulimia Nervosa

Bulimia Nervosa is characterized as recurrent episodes of binge eating, eating in a discrete period of time (e/g within any 2 hour period), larger than normal amounts of food in that most individuals would eat in a similar time frame.[30] This is also accompanied by a lack of control during the eating episode.[30] Inappropriate compensatory behaviors to prevent weight gain are accompanied with the recurrent binging eating.[30] These behaviors include self-induced vomiting, misuse of laxatives, diuretics, and other medications, fasting, or excessive exercise.[30] Occurrence of the binge eating and inappropriate compensatory behaviors occur concurrently on average at least once per week for 3 consecutive months.[30] The eating/purging disturbances do not exclusively occur during episodes of anorexia nervosa, and self-evaluation is unduly influenced by body shape and weight.[30]

Binge-Eating Disorder

Binge-Eating Disorder is defined as recurrent episodes of binge eating characterized by 2 components; eating a larger amount of food in a discrete time period, which is more than most people would eat in a similar time period under similar circumstances and a lack of control over eating during the episode.[30] Binge episodes are linked with 1 or more of the following behaviors: eating more rapidly than normal, eating until uncomfortably full, eating large amounts of food without physical hunger, eating alone due to embarrassment over the amount of food one's consumed, and negative feelings of disgust, depression or guilt after consuming food.[30] Binge eating is classified

as a minimum of 1 binge per week for 3 consecutive months.[30] Binge eaters are distressed regarding behavior, and the behavior is not concurrently associated with bulimia nervosa or anorexia nervosa.[30]

Prevalence of Eating Disorders in Males

Prevalence of eating disorders (ED) in male athletes has been lacking in the literature when compared to female athlete literature. Previous research has demonstrated 73.6% of all elite athletes meet at least one criterion for diagnosing an eating disorder.[31] Roughly one million males present with an ED, however researchers believe this is a gross underestimation due to under-reporting and males being less likely report EDs or seek diagnosis/treatment.[32] Research has estimated 5% of elite male athletes present with anorexia nervosa or bulimia nervosa[32] and another 1% of males represent "other specified feeding or eating disorders.[33] Norwegian researchers found 13.5% of both male and female athletes were diagnosed with a clinical and/or subclinical ED.[34] When compared to non-athletes (4.6%), this was twice as high for the athletes.[34] Sundgot-Borgen et. al.[34] also demonstrated endurance athletes presented with 9% diagnosed ED, which was higher than ball-game athletes with 5% diagnosed ED.

There is limited research examining specific eating disorders prevalence in male athletes. Rates in males are currently unknown for anorexia nervosa, however an estimated 10:1 ratio has been demonstrated for females to males.[30] Male rates for bulimia nervosa is currently unknown; however female rates are estimated between 1%-1.5%.[30] Binge eating rates for males are estimated at 0.8%.[30] Rates are presently unknown across

ethnicity and race.[30] Binge eating and anorexia nervosa are the two most common eating disorders in athletes.[31] The National Institute of Mental Health reports roughly 5%-15% of males present with anorexia or bulimia.[35] Other ED rates are unknown; Pica is usually seen in children and rumination is often seen in infants between the ages of 3-13 months of age.[30]

Males also present with pathogenic eating behaviors, similar to females. Research suggests 37% of male athletes (age 18-22) exercise 2 or more hours per day for the purposeful intention to burn calories.[36] Males demonstrated engagement in restrictive diets at least twice in 1 calendar year.[36] This may be due to a distorted self-image where males observe that their BMI is larger than their actual BMI.[37] Research presents inconclusive motives for pathogenic behaviors for males currently. Roughly half of male research presents with concurrent EDs and body dissatisfaction or with EDs without body dissatisfaction.[38] Similar to the female literature, elite male athletes participating in sports that require leanness shows increases in ED risk.[33] However, to date the literature focused on ED is limited and there is not the ability to compare male athletes to other male control groups.[33]

Bone Mineral Density

Literature suggests endurance athletes have decreased BMD compared to other athletes. Scofield and Hecht[39] examined endurance runners, and found runners had consistently lower BMD when compared with sprinters, gymnasts and ball sports athletes.[39] When endurance runners were examined with other runners (sprinters), cortical density was inversely related to competitive distance, with the lowest cortical density

elicited with long distance running.[40] Taafee and colleagues[41] examined distance runners and confirmed a reduction in BMD over time compared to cross-sectional studies.[41] Other research examining other endurance sports has revealed increased risks of osteopenia, and osteoporosis in cyclists and swimmers.[12,20,22,39] Dolan et. al.,[15,16] examined professional horse jockeys and found not only reduced bone mass but an elevation in the rate of bone loss. Endurance running is a weight-bearing sport that requires high metabolic demands; energy availability may be a critical role in long-term bone health.[40]

Hormones

Existing literature has established long periods of restricted energy intake or rapid weight loss in male athletes have affiliation to negative hormonal effects.[11,12,15-19,23] Many researchers believe T levels create a protective mechanism that shields men from similar components of the Triad found in women. Previous research has demonstrated various negative hormone effects when energy deficits occur.[15-17,19] Gonadal and reproductive hormones; T, sex hormone-binding globulin, thyroid hormones, and IGF-I, cholesterol, and cortisol have been reliable markers in assessing energy deficits in males.[11,12,15-19,23] Koehler et. al.,[18] is the only existing literature to date to examine short-term energy availability reduction in exercising men. Energy availability was reduced to 15 kcal/kg FFM·d resulting in the suppressions of leptin and insulin.[18] A trend was found in the decrease in T.[18] Reasons for this incongruence to the previous literature could be due to the 4 days of energy restriction or to the limited size of the participants (n=6).[18]

Reproductive Hormones

Testosterone

Testosterone (T) is a steroid hormone produced in the testes (specific for males) and adrenal cortex.[42] Testosterone stimulates development of male secondary sexual characteristics.[42] Research has demonstrated endurance training has a negative effect on testosterone levels in males.[43] Resting testosterone levels were measured lower in endurance-trained males compared to untrained males.[43] The mechanistic nature of this decrease is currently unknown. Research directs to one impression, that the decreased testosterone levels are related to dysfunctions in the hypothalamic-pituitary-testicular regulatory axis, which is congruent with literature within the female population.[43] Presently, there is no existing literature that indicates any testosterone dysfunction is instigated by endurance training in males.[43] De Souza and colleagues[44] established a "training volume-threshold" (~100km/week), which has significant changes in the male reproductive function.[44] High volume of endurance running (>104 km/wk) showed associations with changes in both sex hormones profiles (decreased testosterone), and quality of semen (decreased mobility and increases in immature cell numbers).[43,44] Peripheral and central mechanisms have been proposed as reasons for decreased testosterone.[44] These proposed mechanisms for endurance runners include: peripheral mechanisms such as intrinsic failure of the tests to maintain steroid biosynthesis and altered hepatic clearance and metabolism of testosterone and central mechanism include alterations in the hypothalamic and pituitary axes.[44]

Luteinizing Hormone

Luteinizing Hormone (LH) in males stimulates the production of testosterone and in part, aids in the role of sperm production.[45] Research has shown that with increases in exercise testosterone decreases; however LH has not shown to be significantly changed during these increases of training loads.[45] Conversely, Kuoppasalm et. al. [46] showed that with long term and high intensity running, roughly 30 minutes after long-term runs, plasma LH significantly dropped below baseline levels by 42% (moderate run) and 45% (intense run). Suggesting intensity of the exercise is important in regards to negative outcomes.[46] Female literature has shown hypothalamic gonadotropin-releasing hormone is common in female athletes (hypothalamic amenorrhea).[47] MacConnie et. al.[47] examined highly trained male marathon runners (125-200km/week mileage) and found the runners had diminished frequency of spontaneous LH pulses and the amplitude of their LH pulses were decreased compared to healthy controls.[47] These results suggest highly trained male athletes, akin to the female athlete, have deficiency of hypothalamic gonadotropin-releasing hormone. It is hypothesized that due to repetitive elevations of other hormones, which, suppress gonadotropin-releasing hormone, are related to daily training.[47]

Metabolism

Insulin

Insulin is a peptide hormone and produced from Beta cells within the islets of Langerhans in the pancreas and contributes an important role in metabolism.[48] Insulin aids in the absorption of glucose into muscle, fat, and liver cells for energy.[48] Insulin also

stimulates muscle and liver tissue to store excess glucose in the form of glycogen, in turn suppressing gluconeogenesis by the liver.[48] When insulin is low or absent, glucose is not utilized as an energy source, and the body will begin to utilize fat as an energy source, consequently, insulin aids in the prevention of fat utilization for energy.[48] Insulin aids in the regulation of amino acid uptake and has other anabolic effects throughout the body.[48] Proposed mechanistic reasons for decreased insulin include a global effect on the hypothalamic-pituitary axes[3] or due to adaptations of glucose uptake in runners.[48] It is suggested that in aerobically trained athletes, adaptations related to the increased blood flow (capillarization) causes augmented glucose disposal due to increased glucose transport proteins (GLUT 4) globally with less insulin required.[49] More research is needed to understand the mechanism of decreased insulin in a LEA state.[49]

Insulin-Like Growth Factor I

Insulin-like Growth Factor-I (IGF-I) is a polypeptide derived from the liver that plays an important role in the intervention of metabolic and anabolic cellular responses during altered energy states.[50] Changes in body composition, negative energy balance and protein-energy malnutrition all effect IGF-I.[50] Other processes IGF-I contributes vital roles for includes stimulating protein synthesis and maintaining muscle mass.[50] Previous research found IFG-I correlated significantly with relative body mass loss and fat-free mass loss.[51] Nindl et. al.,[51] examined the IGF-I system and found all components were directionally associated with the decrease of energy deficits and losses of body mass. [51] However, more research is needed prior to using IFG-I system as a predicative ability as a biomarker.[51]

Metabolic

Leptin

Leptin is an adipoocytokine hormone that affects neurons in the hypothalamus responsible for the regulation of body mass loss and satiety regulating dietary energy intake and energy expenditure.[52] Through the sympathetic and direct induced increase of fatty acid disposal in metabolism.[52] Leptin stimulates appetite suppression and reduces the level of brain chemicals that stimulate appetite.[52] Leptin receptors have various actions due in large part to the numerous types of tissues that contain leptin receptors.[52] Most prominent tissues include the skeletal muscle and the liver.[52] Typically, body weight has an analogous relationship with leptin (i.e. weight loss reduces leptin while weight gain increases leptin levels).[52] It is hypothesized that the reduction of leptin is an acute metabolic signal of starvation and energy conservation.[52,53] This is suggested due to the reductions in leptin are associated with the suppression of key endocrine axes (reproductive, growth hormone, IGF-I, and thyroid axes).[53] During acute energy deficits, the rapid reduction of leptin is associated with changes in body composition.[54]

Cortisol

Cortisol is the primary hormone related to stress (physical, psychological and physiological) and is a glucocorticoid hormone from the adrenal cortex.[55] Cortisol has various roles in the body including increasing glucose up regulation into the bloodstream and brain, inhibits nonessential functions that would be detrimental in a "flight-or fight" state, and alters the immune system response.[55] Cortisol also has the ability to suppress various systems including the reproductive and digestive system, as well as other growth

process.[55] Long-term triggering and overexposure to cortisol can disrupt bodily process and increase the risk to various health issues including, anxiety, depression, digestive issues, headaches, heart disease, sleep problems, and weight issues.[55] Hill et. al.,[55] demonstrated moderate to high intensity exercise invokes an increase in circulating cortisol levels, while in contrast; low intensity exercise (40% VO_2max) decreases circulating levels.[55] Cortisol levels may increase due to either an increased need to catabolize other energy sources other than fat stores, or due to a reduction in clearance.[17]

Cholesterol

Cholesterol is a fat-like substance which can be found within the body and consumed food.[56] Cholesterol is needed to make hormones and with the aid of digestion of food. [56] Cholesterol travels though the bloodstream as a lipoprotein, which are lipids surrounded by proteins.[56] There are 2 types of lipoproteins; low-density lipoprotein (LDL) and high-density lipoprotein (HDL). [56] High levels of LDL lead to buildup of cholesterol (plaque) in your arteries increasing risk of heart disease.[56] High levels of HDL are desirable due to HDL's ability to carry cholesterol from various parts of the body back to the liver.[56] Having a balance of healthy levels of LDL and HDL is important for overall health.[56] It has been hypothesized that the increased cholesterol is due to the mobilization of cholesterol from fat deposits at higher rates during fat mobilization.[57] Hypercholesterolemic response has been established during late phase starvation.[17,57]

Conclusion

As high energy exercise in the elite sport realm has been popular (e.g., soccer, cycling, running, etc.); non-traditional sports such as dance, horse racing, and jujitsu along with a new fad of sports such as Crossfit©, ultramarathons, extreme challenge races and triathlons has developed. The increase participation in high-energy exercise has changed from purely elite sport participation to the recreational athlete. There is inadequate research on the negative health outcomes and physiological effects associated with male athletes participating in high-energy expenditure activities with decreased energy needs with or without an ED/DE. Limited research has demonstrated males have similar mechanisms associated with the Triad; however, it is unclear the extent of these outcomes. Understanding the physiological demands and consequences of energy needs in male athletes is critical for their energy balance, injury and illness prevention, and counteracting long-term negative health consequences. Ultimately, insights on the near and long-term health outcomes for male athletes need to be provided.

CHAPTER 5

METHODS

Research Design and Setting

This study will implement a cross-sectional design within the Southeastern region of the United States. Independent variables include recreational male endurance athletes (distance runners n=16). Dependent variables include energy availability, energy expenditure, dietary intake, bone mineral density (BMD), reproductive (Testosterone (T) and Luteinizing hormone (LH) and metabolic (Insulin and Insulin-like growth hormone-I (IFG-I) Leptin, Cholesterol, and Cortisol) hormone profile. Anthropometric measurements including age, height, weight, and sport participation history will be surveyed.

Participants

A convenience sample of endurance trained male athletes (18-30 years) will be recruited from local recreational clubs and organizations (n=~16). Participants will be required to be independent of any injury that would prevent them from full participation in a high-endurance sport (running) prior to participation in the study. Specific inclusion criteria for participation includes: participant is male, within a competitive season and actively training and racing >10 hours/week for at least 3 months,[6,17,58] has a body fat percentage ≤ 12%,[6,16,17,20,58] has maintained weight stability (± 3 kg in past 6 months),[18]

and has a VO_2max that is considered excellent for age specific range (18-20 years: Excellent 51.0-55.9; Superior >55.9 ml/kg/min, 20-29 years: Excellent 46.5-52.4; Superior >52.4 ml/kg/min, 30-39 years: Excellent 45.0-49.4; Superior >49.4 ml/kg/min).[18,59] Specific exclusion criteria includes no history of smoking, past or present diagnosis of clinical eating disorder, infectious disease within past 4 weeks, history of cardiovascular disease or orthopedic impairment that interferes with moderate to vigorous exercise, no history of thyroid or pituitary disease, use of medication, diabetes mellitus, known metabolic disease, and no long term steroid use. Internal Review Board approval will be obtained prior to the start of the study and all participants will sign consent forms prior to participation.

Instrumentation

Basic Demographic Survey: Basic demographic information will be collected including the age, education level, ethnicity, exercise background, and pertinent medical history questions including known metabolic diseases, history of cardiovascular, thyroid, or pituitary disease, and long-term steroid use.

Anthropometric Measurements: Multiple anthropometric measurements will be collected including height, weight, and body composition, which will be measured according to ACSM standardized procedures.[26] Height will be measured with a Stadiomometer (Shorr Productions, Maryland) to the nearest 0.1 cm. Weight will be measured wearing minimal clothes to the nearest .01 kg with a scale (Tanita, 331S, Tokyo, Japan). Body fat will be assessed using Tanita scale (Tanita SC-331S Body Composition Scale, Tanita Co., Tokyo, Japan), and Dual-Energy X-Ray Absorptiometry

(DXA) (GE Lunar Prodigy densitometer). All skin folds will be measured in accordance with ACSM standardized procedures including the following sites; thigh, triceps, suprailiac, chest, supscapular, mid-axillary and abdomen.[26] The Tanita scale, a bioelectrical impedance method (BIA), and skinfolds are reliable and valid measurements to obtain percent body fat.[60] While DXA is the gold standard, BIA and skinfold measurements were found to be reliable (R=0.957-0.987) with standard errors ranging from 0.9-1.5% fat.[60]

Dual-Energy X-Ray Absorptiometry (DXA): Will be used to measure bone mineral density (BMD) (g/cm^2) of the lumbar spine (L1-L4) total left hip, left femoral neck, and total body will be measured using DXA (GE Lunar Prodigy densitometer)[1,26] DXA is considered a safe method for estimating body composition and bone mineral density. It is the gold standard for bone mineral density assessment.[26] Radiation exposure is less for DXA than other x-rays. The average skin dose is 1 to 3 mrad per DXA scan, which is comparable to a typical weekly exposure (3.5mrad) of environmental background radiation.[26] Participants will undergo the DXA scan after an overnight fast (12 hour fast), refraining from vigorous exercise at least 15 hours prior to scan, no caffeine and alcohol during the preceding 24 hours, and consuming a normal evening meal the night before.[26] Scoring of BMD will be in either "normal" or "low" categories. These categories will be established form the T-score and Z-score. The T-score is recognized the subject's BMD score compared to the average 30 year old adult.[61] Your Z-score is the subject's BMD compared to the average score of subjects consisting of the same age, sex, weight, and ethnic or racial background.[61] Scoring for the T-score is considered "normal" when the scores fall between -1 and +1, and will be considered

"low" if scores fall below -1 and -2.5.[61] Osteoporosis is a third category established with a T-score below -2.5.[61]

Energy Availability (EA): Defined as the amount of dietary energy remaining after exercise, expressed as kcal/kg lean body mass (EA = (energy intake–EE) kcal/kg FFM·d).[1] EA will be examined using dietary intake and energy expenditure over 7-days. Two 7-consectutive day online food logs (FoodProdigy) will examine dietary intake and two 7-consectutive day exercise activity log daily examine energy availability (see Total Energy Expenditure below). Dietary records will be analyzed for kilocalories, carbohydrate, protein, and fat composition using a dietary analysis software program (ESHA food processor 8.0, Salem, OR).

Energy Balance (EB): Defined as the total energy expenditure and dietary intake remaining at an equal level [dietary intake (kcals/day) = Total Daily Energy Expenditure (TDEE, kcal/day)]. Energy balance will be examined using dietary intake and TDEE over two 7-consectuvie days. Negative energy balance will be defined as TDEE (kcals/day) > dietary intake (kcals/day).[1-7,9,62]

Resting Metabolic Rate (RMR): Will be used to identify how many calories are necessary at rest. It will be measured using indirect calorimetry (*Microlife MedGem*; HealtheTech, Golden, CO). The MedGem is a clinically-validated measurement device that assesses RMR.[63] While the MedGem is not the gold standard for measuring RMR; it is, however, clinically a very relevant tool.[63] Most accurate measurements are produced first thing in the morning, when the patient is rested and positioned in either a seated or semi-reclined position in a quiet room.[63] Measurements should be implemented when the

patient has not eaten, exercised or drank any caffeine within the last four hours. Procedures for the MedGem include, placing a plastic nose clip comfortably on their nose, and to breathe nasally and the volume of air (inspired and expired) will be measured by an ultrasonic sensor. The measurement is conducted with the patient holding the calorimeter resting on their chest and time for completion.[63]

Total Daily Energy Expenditure (TDEE): A 7-consecutive day daily training (exercise) log will be used to calculate TDEE. Variables such as duration, intensity of activity, weight, age, and gender from the Ainsworth compendium of physical activity will be used.[64] Exercise energy expenditure (EEE) will be calculated using SenseWear Armbands. *SenseWear* armbands contain accelerometers that continuously monitor the individual wearing the device for TEE and EEE. The armband has been validated compared to indirect caliometry, double labeled water, and VO2 max metabolic cart. SenseWear© Software 8.0 will be used to collect, save, and analyze data from the armband.[63-71]

Dietary Intake: Participants will record their dietary intake for two separate weeks, 7-consecutive days per week. Two 7-consecutive day food records will examine energy intake, macronutrient intake and energy availability. Portion sizes will be explained, and take-home examples given prior to food record distribution. Dietary records will be analyzed for total kilocalories and macronutrients (carbohydrates, proteins, and fat) consumption using a dietary analysis software program (ESHA food processor 8.0, Salem, OR). Research has demonstrated that despite food intake restrictions, reported intake accuracy was superior using a 7-consecutive day weighted-diet record compared to a food-frequency questionnaire.

A 7-consectuive day food record was chosen over a 3-day food record or a food-frequency questionnaire.[72] This study examined the validity of the 7-consecutive day food record and confirmed the 7-consecutive day food record has greater ability to detail food intake.[72] The 7-consecutive day food diary includes color photographs of seventeen foods, presenting with three different portion sizes for each food.[72] Participants can choose with photograph representation their individual portion size or can gauge whether they consumed more or less than the picture depicts.[72] Participants can also describe food intake and portion sizes in other measurements when appropriate including weights and household units.[72] Detailed instructions on food descriptions and quantifications are given prior to food record distribution and during the course of the study. [72]

Exercise Energy Expenditure (EEE): A 7-consecutive day daily training log will be recorded. Exercise energy expenditure will be measured two separate ways. The first measurement will be calculated using the Ainsworth equation {EEE= duration(minutes) x ((METs X 3.5 x weight (kg))/200).[64] The second form of measurement will be the Bodymedia SenseWear Armbands (Bodymedia Inc, Pittsburgh, PA).

Bodymedia SenseWear Armbands: The Bodymedia SenseWear armbands continuously monitor total daily energy expenditure and EEE during physical activities. The armband is non-invasive and participants can wear it in all conditions (e.g workouts, activities of daily life, sleeping) except to swim and shower.[63,65-71] Exercise energy expenditure will be examined using the data from the armbands over 14-days (two 7-consecutive day weeks). The armband has been proven to be clinically reliable in several studies in providing an accurate estimate of energy expenditure when compared to indirect calorimetry during exercise periods.[63,65-71] In a comparison of energy expenditure

measuring devices, the BodyMedica SenseWear Armband (SWA) , the CSA, and the TriTrac-R3D, the SWA estimated the best total energy expenditure at most speeds.[63] Drenowatz et al.[73] assessed the validity of the SWA. Results demonstrated a celling effect around ten METs causing an underestimation of energy expenditure at high intensities (65, 75 and 85% of VO_{2max}).[73,74] To aid in this underestimation of EEE, participants will wear a heart rate monitor during exercise.[58,75]

Heart Rate Monitor: The participants will wear a POLAR heart rate monitor during exercise as a secondary measure of EEE by calculating METs during exercise, derived from their heart rate and the corresponding percent of VO_{2max}. Various brands of heart rate monitors, including POLOAR, were found to accurately assess heart rates during rest and moderate activity ($r \geq 0.90$, SEE $\leq$ 5 beats/min).[75] Accuracy did decrease at the highest speed however the HR monitor will be used as a backup to the SenseWear Armbands to aid in calculating exercise energy expenditure.[58,75]

VO2max: A VO_{2max} treadmill test using the Bruce Protocol[76] will be administered as part of the inclusion criterion. The Bruce Protocol is a submaximal exercise test used to estimate VO_{2max}.[76] However, procedurally, the predictive equation from ACSM associated with the Bruce protocol will not be used, as direct measurement using a Cosmed K4b2 unit will be administered to measure VO_{2max}. Koutlianos, Dimitros, Metaxas, Deligiannis, and Kouidi[77] examined the indirect estimation of VO2max in national and international athletes by the ACSM's equation and found the Bruce protocol does not accurately equate in participants 18-37 years of age.[77] For this reason the Cosmed K4b2 unit will be used as it provides good reliability and repeatability for

measurements of VO2 ($r^2 = 0.84$), and VCO2 ($r^2 = 0.92$) when compared to the metabolic cart measurements.[78]

EDI-3

The Eating Disorder Inventory-3 (EDI-3) is a self-reported survey validated to identify subjects with disordered eating patterns.[79] The inventory includes 91 items, organized into 12 primary scales, consisting of three eating-disorder specific scales and nine general psychological scales that are relevant but not specific to eating disorders.[79] The EDI-3 yields six composite scales, five general integrative psychological constructs (i.e. Ineffectiveness, Interpersonal Problems, Affective Problems, Over-control, and overall Psychological Maladjustment composite) and one eating disorder specific composite (Eating Disorders Risk Composite).[79]

The EDI-3 is validated for age ranges of adolescent (13 years of age) through elderly (53 years of age) subjects.[79] Reliability for the EDI-3 composites are high.[79] Coefficient and median values for specific composites include; Eating disorder risk (r=.98, median=.95) and General Psychological Maladjustment (r=.97, median=.93).[79] The EDI-3 Symptom Checklist (EDI-3 SC) provides information regarding the frequency of eating disorder risk behaviors or symptoms (i.e. binge eating, self-induced vomiting, exercise patterns, laxative use, diet pill use, and use of diuretics).[79] The EDI-3 SC is a screening tool designed specifically for Allied Health professionals to identify individuals at risk for eating disorders.[79] EDI-3 Inventories (EDI-3 and EDI-3 SC) are copyright surveys from the Psychological Assessment Resources, Inc. and permission of use is granted with purchase of inventory.[79]

Hormone Measures

All fasting blood samples will be acquired during the two separate 7-consecutive day data collection. Blood samples will be centrifuged, and plasma drawn out to assess the following hormones: Testosterone, IGF-I, LH, Cortisol, Cholesterol, and Leptin using enzyme-linked immunosorbent assay (ELISA) Kits specific for each hormone. Establishment of cutoffs will be identified as low, within normal, or high based on previously established normative data specific for males (adult and age range specific) associated to each of the 7 hormones. The ranges include the following for reproductive hormones: T=270-1070 ng/dL (average 679 ng/dL)[80] and LH= 1.8-12.0 mlU/mL.[80,81] Normative ranges for the dietary hormone leptin is 1.2 -9.5 ng/ml.[82] Metabolism hormone markers are: for a fasting insulin = ≤ 5 ulU/mL (8-10 ulU/mL is also an accepted range)[83] and IGF-I is age specific with ages 16-24 ranging from 182-780 ng/mL and ages 25-39 ranging from 114-492 ng/mL.[84] Steroidal hormones have multiple components to them. As we will be doing a fasting morning blood draw, cortisol specific to the morning includes 7-28 ug/dL, however afternoon values decrease from 2-18 ug/dL.[85] Hormonal markers for cholesterol include a total cholesterol range below 200 mg/dL, however "high" would be above 240 mg/dL.[86] Specific ranges for LDL consist of values below 100 mg/dL while a "high" LDL is considered above 159 mg/dL.[86] Ranges for HDL consist of values above 60 mg/dL and "low" values are considered below 40 mg/dL.[86]

ELISA Kits

This study will be using enzyme-linked immunosorbent assay (ELISA) kits specific for each of the previous mentioned hormones. These include the hormones Testosterone, Leptin, Luteinizing hormone, Insulin, IGF-I, cortisol and Cholesterol. Research has shown enzyme-immunoassay (EIAs), an alias for ELISA, to report specific and highly sensitive procedures for identify various substances. Sensitivities of EIAs are high, 1-10ug/liter range with the correlation coefficient were reported between 0.95-0.99.[87]

Detailed Procedures

All participants will sign consent forms and grant permission before data collection. The data collection will span across 2 separate weeks consisting of one day for an information and initial measurement session, and two 7-consecutive day weeks where one week will be during a low volume-training week (LV), and one week will be during a high volume-training week (HV).

Part I Recruitment:

Participants will be recruited from local area running clubs. An information letter via email will be used to give a brief overview of the study. All participants interested will be asked to fill out a short information and availability sheet, so researchers can individually correspond with participants and set up assigned dates and times for informational session and anthropometric measurements.

Part II Data Collection:

Informational Session: Participants will attend an orientation session prior to the data collection sessions begin. This session will consist of written and verbal overview of the study, participant expectation, instructions from the researchers describing various tools during data collection including, *ESHA FoodProdigy, BodyMeida SenseWear Armband, and POLAR Heart Rate Monitor.* The ESHA FoodProdigy website is where participants will record their daily food diaries and activity record. The BodyMedia SenseWear Armband wear and care instructions will be dispensed to the participants. All instructions will also be sent home for participants as a reference during the data collection time. Contact information of the researcher will be provided for any questions that may present after instructional session.

*Prior to Data Collection: P*articipants will begin by completing a series of surveys, a brief interview, used to follow up on medical history, physical measurements (height, weight, and percent body fat) and resting metabolic rate (RMR). This process will take roughly sixty minutes to complete this portion of the study. Blood Draws and DXA scans will be scheduled for the two separate weeks of data collection.

Data Collection: Participants will complete two separate 7-consecutive day weeks at two levels of training volume (High and Low). All equipment, BodyMedia SenseWear Armband, heart rate monitor and food log entry information will be passed out and instructions will be emailed out. The research and participant will discuss and plan out a low-volume and high-volume training week to use during data collection. Data collection weeks should be a "normal" week with a minimum of 5 training days/week

and participants must be engaged in a completive season. The procedures for each of the 2 weeks will be identical; the only change will be their volume load (LV and HV).

Training Weeks: Participants will be instructed to not change their daily/weekly activities and physical activity/exercise, while recording their food and daily training for 7-consecutive days. They will wear the armband for 23 hours/day and wear a HR monitor only during exercise and record HR during training for the 7-consecutive days. At the end of the 7-consectuve day training week, participants will come in for a fasting blood draw and DXA scan. All equipment (Armband and HR monitor) will be returned and the next week will be scheduled.

Statistical Analysis

SPSS statistical software (Version 23; SPSS Inc, Chicago, IL) and *alpha* ≤ 0.05 will be used for all analyses. Based upon power analysis a priori and based upon means of previous literature from Koehler et. al.,[18] and Loucks et. al.,[25] an effect size between 1.0 and 3.0 would yield a sample size of 6-10 subjects. Using the Wilcoxon signed rank test, 16 subjects should allow for full saturation. A regression model will be used to examine predicative components for energy availability cut offs. Basic descriptive statistics will be used to examine demographic information (e.g height, weight, BMI, TDEE, EEE, etc.). Energy availability will be calculated using the equation {EA = (EI-EEE)/FFM}[1,3-6,9,24,25] and exercise energy expenditure will be calculated from 3 methods; 1) BodyMedia SenseWear Armband algorithm, 2) using the recorded HR as a percentage of VO_{2max} to calculate METs, and 3) as well as {EEE= duration(minutes) x ((METs X 3.5 x weight (kg))/200).[64] Risk for low energy availability (LEA) will be defined as ≤ 20

kcal/kg FFM·d. Frequencies and proportions with 95% confidence intervals will be calculated for categorical variables along with means and standard deviations for continuous variables. Chi-square analyses will be used to examine the proportion of participants classified as "at risk" of LEA, low BMD, risk for reproductive dysfunction, and risk for disordered eating/eating disorders and/or pathogenic behaviors (e.g., restrictive eating, binge eating, purging, laxatives, diet pills or diuretics to control weight).

TABLES

<table>
<tr><td colspan="5">Table 4.1 Study procedures and protocol for Examination of Energy Needs and Hormone Levels in Endurance Trained Male Athletes</td></tr>
<tr><td>Informational Session</td><td colspan="2">Data Collections</td><td colspan="2">Concluding Session</td></tr>
<tr><td>Prior to study</td><td>High Volume</td><td>Low Volume</td><td>High Volume</td><td>Low Volume</td></tr>
<tr><td>-Explain procedures

-Consent forms signed

-VO2 Max test

-Instructions for data collection session

-Pass out equipment of study:
1. Armband
2. HR monitor</td><td>* Day 1 only: Basic survey; Anthropometric measurements and RMR

-Record online 7-day food record

-Recording daily training log

-Wear HR monitor only during exercise

-Wear the armband for 23 hours/day</td><td>* Day 1 only: Basic survey; Anthropometric measurements and RMR

-Record online 7-day food record

-Recording daily training log

-Wear HR monitor only during exercise

-Wear the armband for 23 hours/day</td><td>-Participants will report for Scheduled:
1.Fasting morning blood draw
2. DXA scan*

-Participants will return all equipment of the study:
1. Armband
2. HR monitor</td><td>-Participants will report for Scheduled:
1.Fasting morning blood draw
2. DXA scan*

-Participants will return all equipment of the study:
1. Armband
2. HR monitor</td></tr>
<tr><td>-Schedule:
1. DXA scan (1 scan)
2. Fasting Blood Draws [2 draws; 1 (HV week) and 1 (LV week)]</td><td colspan="2">* ‘Day One’ will occur only once for this study, however will depend on which week (High or Low volume) will be the first week scheduled for data collection for each individual participant.
Data collection weeks should be a “normal” week (minimum of 5 training days/week) and participants must be engaged in a competitive season</td><td colspan="2">*One DXA scan will occur for this study over the course of the two separate weeks. However, it can be scheduled in either the High or Low Volume week.</td></tr>
</table>

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APPENDIX A – INFORMED CONSENT

Title of the Study: *Examination of Energy Needs and Hormone Levles in Male Enduranced Trained Athletes.*

Investigators: Erin M. Moore, MS, ATC; Toni M. Torres-McGehee, PhD, ATC; David F. Stodden, PhD, Justin M. Goins, PhD, ATC; Clemens Drenowatz, PhD

The purpose of this study is to determine the effects of high exercise energy expenditure (EEE) on energy availability (EA), bone mineral density (BMD) and 7 metabolic markers including: Testosterone (T), Luteinizing hormone (LH), Insulin, Insulin-like Growth factor-I (IGF-I), Cortisol, and Cholesterol. The study is being conducted to fulfill partial requirements for a Doctorate degree. You are being invited to participate in this study because you are between the ages of 18 and 30yrs, endurance trained, and are in good general health. The study will take place at the Exercise Science Laboratories at the University of South Carolina's Arnold School of Public Health. If you agree to participate, you will be participating with approximately 15 other people.

STUDY PARTICIPATION

After signing the informed consent form, potential participants will complete a Health and Injury History Questionnaire. Any potential participant with a health or safety

concern will be required to have his questionnaire reviewed by the physician overseeing this study (Dr. Murphy) to determine inclusion or exclusion into the study.

Prior to acceptance into the study, participants will complete a treadmill test to measure VO2max and have their body fat percentage measured using 7-site skinfold and Tanitia scale (BIA). If you agree to participate in the study the following will occur:

Part One

You will be asked to complete a series of surveys, a brief interview (follow up on medical history), and physical measurements (height, weight, and body composition using skin fold measurements and Tanita scale) and resting metabolic rate. It will take roughly 60 minutes to complete this portion of the study. All measurements will be taken by one of the researchers. You will be asked to wear shorts and a tee shirt to this meeting. All measurements will be taken in private (no other participants in the room). Some of the questions are about health behaviors and you may be somewhat uncomfortable answering. You do not have to answer any questions that you feel uncomfortable with. Questions asked include:

- Age, education, type of exercise involvement
- Drug use (e.g., anabolic steroids, alcohol, etc.),
- Medical history

Part Two

Dual Energy X-ray Absorptiometry (DXA) Scan.

DXA is a method used to measure bone mineral density and body composition. The machine uses x-rays that yield precise, high quality images of the body. You will meet with one of the research investigators to schedule one DXA scan at your convenience. You will be asked to wear loose, comfortable clothing, and avoid clothing with belts, zippers, or buttons made of metal to the appointment. During the scan, you will be asked to lie still on a padded table, with an x-ray generator below the table, and the imaging device above your body. The DXA machine will send a thin, invisible beam of low-dose x-rays with two distinct energy peaks through the body. The exposure levels from the DXA X-ray unit are very low. While the radiation used for the DXA scan has no observable radiological or biological effect, there is always a risk associated with radiation exposure, even very low exposure. If you want more information on the exposure levels, please contact the lead research on this study. This procedure will take about 10-20 minutes. The DXA machine, based on the standards of the DXA manufacturer, will automatically generate the bone mineral density measurement. The scans will not be read by a radiologist and are performed for research purposes only. The investigators will not go over individual results with you. Appointments for the scan will be at one of the designated venues for DXA scans.

Blood Work

As part of this study, two one-time blood draw sessions are required. You will have roughly 60 ml of blood taken from you twice. The blood will be taken from the arm.

The total amount of blood taken for the whole study will be about 120ml. There are associated risk of bruising, tenderness, fainting, and infection associated with blood draw. OSHA guidelines will be followed to minimizing risk.

Dietary Assessment & Exercise Assessment

In addition, you will be asked to complete a food record and a daily training log for 2 separate consecutive weeks. You will be asked to record all food and drinks consumed every day during the14 days (2 separate- 7 consecutive days) (roughly 10-15 minutes per day). In addition, you will be required to log all physical activities and exercise (including any races) for 14 days (2 separate-7 consecutive days) (about 5-10 minutes per day). Finally, you will be asked to wear an armband throughout the entire 14 days (2 separate-7 consecutive days). The armband should be worn at all times, including during sleep, but should not be worn in the water (e.g., shower, swimming, etc.).

During the course of this study, you will be asked to maintain normal levels of physical activity and dietary food intake.

CONDITIONS

Training Weeks

The research and you will examine your schedule and determine the two weeks that will be used for data collection. One week will be a low training volume (LV) week and the second will be a high training volume week (HV).

The aim of the study is to observe the participant during his normal training sessions. The aim is not to induce any type of intervention or change within their eating or training regimens.

INSTRUMENTS AND PROTOCOLS

Treadmill VO2max Test

Used to measure VO2max, this treadmill test progressively increases in difficulty and respiratory variables are measured throughout. Participants with a VO2max between 41-60 ml/kg/min for males will be considered endurance trained and qualify to participate in the study.

Blood Measures

To obtain the blood we will draw blood from a vein in your arm at the end of each data collection week a total of 2 times. For each blood draw, we will take 7 tubes (1.2 tsp). Approximately 1 blood draw will be done during one data collection session for a total of 8.4 tbsp ($1/6^{th}$ of what you would donate to the American Red Cross).

Heart Rate

To ensure participants remain at safe limits during the VO2max test and to determine exercise energy expenditure (EEE) during training runs, the participant will be given a Polar Heart Rate monitor and watch to use during exercise activities.

Diet and Activity Logs

You will be asked to complete an online form to track your diet and activity for 2 separate 7-consectuive day sessions. You will be given a login and password for your personal account.

SECURITY OF PERSONAL INFORMATION

Participation will be confidential. To assure your confidentiality, all information pertaining to this study and participation will be stored in a secure and locked cabinet. Additionally, a code number will be assigned to each participant at the beginning of the project. This number will be used on project records rather than your name, and no one other than the researchers will be able to link your information with your name. Data from all participants will be analyzed together. All blood samples will be discarded after analysis. All data will be stored on a secure, password protected computer and only researchers will have access to the data. The results of this study may be reported in professional journals or presented at meetings; however, you will not be identified.

RISKS

As with any exercise, there is a small risk of a cardiac event due to an undetected cardiac condition. Heart rate will be monitored during the VO2max test and the participant will be able to tract their heart rate during exercise throughout the stud. All investigators are American Red Cross AED, CPR Professional Rescuer and First Aid certified.

Other risks of this study are general risks associated with exercise, such as muscle or tendon strains. These risks are no different from what the participant is already doing in his own training sessions.

Risks of drawing blood include temporary discomfort from the needle stick, bruising, tenderness and infection. Fainting could also occur. OSHA guidelines will be followed to minimizing these risks. Investigators will abide by all OSHA guidelines and will be able to act if there is an emergency or other medical issue during the study.

PARTICIPANT SAFETY

In the event that you experience a sudden stop in your heart, during the VO2max test, there is access to an Automated External Defribillator (AED), room 316 of the Public Health Research Center and/or inside the cardio deck of the Solomon Blatt Physical Education Center. All investigators are trained to use an AED and in CPR. If you experience any medical issues such as a cardiac event as a participant in this study, the investigators will provide appropriate immediate care and you will be referred for additional medical treatment if necessary. However, in the event you suffer from a research related injury, there will be no financial compensation from the investigators or the University of South Carolina to assist with medical fees.

The investigators will terminate your participation in the study, without your consent, if you suffer any symptoms or conditions that the investigators believe make it inadvisable for you to continue in the study. In the case of a trial being terminated due to adverse

symptoms an incident report will be completed and given to the physician overseeing the study. In the case the participant is able to continue with the study, the participant cannot continue until the physician has reviewed the incident report and deems it appropriate for the participant to continue with the study. In the case of a more severe incident (for example, cardiac event) the participant will not be allowed to continue in the study.

BENEFITS

Participating in this study will increase your awareness of personal training and fueling behaviors. Each participant will receive a personalized fueling protocol that includes caloric needs during exercise. The primary investigator will also be available to discuss the individual reports and answer any specific questions the participant has regarding fluid and electrolyte needs during exercise.

The information from this study will be helpful for competitive athletes as well as recreational athletes and athletic trainers regarding energy availability and hormonal responses to high-energy expenditure activities in male athletes.

VOLUNTARINESS

Participation is voluntary. You may decide not to participate at all, or to stop participating at any time, for any reason without negative consequences. Participants wishing to withdraw voluntarily from the study should notify the primary investigator (Dawn Emerson) by phone or email. Your participation, non-participation and/or withdrawal will

not affect your grades or your relationship with your professors, college(s), or the University of South Carolina.

QUESTIONS

If you have any questions, please feel free to ask at any time. You should contact Erin M. Moore at (cell) (603) 860-6916 or (email) erinmm@email.sc.edu or Dr. Toni Torress-McGehee (email) Torresmc@mailbox.sc.edu if you have any questions or if you believe that you have suffered a research related injury.

If you have any questions about your rights as a research subject contact, Lisa Marie Johnson, IRB Manager, Office of Research Compliance, University of South Carolina, 901 Sumter Street, Byrnes 515, Columbia, SC 29208, Phone: (803) 777-7095 or LisaJ@mailbox.sc.edu. The Office of Research Compliance is an administrative office that supports the USC Institutional Review Board. The Institutional Review Board (IRB) consists of representatives from a variety of scientific disciplines, non-scientists, and community members for the primary purpose of protecting the rights and welfare of human subjects enrolled in research studies.

SIGNATURES

I have read the contents of this Consent Form. The purpose of this study, procedures to be followed, risks and benefits have been explained to me. I have been allowed to ask questions, and my questions have been answered to my satisfaction. I have been told whom to contact if I have additional questions. I agree to participate in this study,

knowing that I may withdraw at any time. I have been given a copy of this Consent Form to keep for my reference.

_____

Date

_____ _____

Signature of Participant Name (Please Print)

I have explained and defined in detail the research procedure in which the participant has agreed to participate and have offered him a copy of this informed consent form.

_____

Date

_____ _____

Signature of Investigator Name (Please Print)

University of South Carolina

Athletic Training Program

Blatt PE Center

APPENDIX B – MEDICAL HISTORY

Medical History Review-Interview Questions

Are you currently, or have you in the past year, followed a particular "diet"?	YES	NO
How many meals (i.g., breakfast, lunch, dinner) do you eat each day?		
How many snacks do you have each day?		
Are there certain food groups that you refuse to eat (meat, breads, etc.)		
Do you ever limit food intake to control weight?	YES	NO
If yes, do you (circle below): a. Decrease the amount of food you eat during the day b. Skip meals c. Limit carbohydrates intake d. Limit fat intake e. Cut out snack items f. Other _____		
Do you ever feel out of control when eating or feel that you cannot stop eating?	YES	NO

Do you take vitamin supplements? If yes, what type: _____ How often (daily, a few times a week? _____	YES	No
Do you take nutritional supplements? If yes, what type: _____ How often (daily, a few times a week? _____	YES	NO
What do you currently weight?	lbs	

Are you happy with this weight? If not, what would you like to weight? _____lbs	YES	NO
What was the most you've weighed in the past year?	lbs	
What was the least you've weighted in the past year?	lbs	
Do you gain or lose weight regularly to meet the demands of your performance?	YES	NO
Has anyone recommended that you change your weight or eating habits? If yes, specify (instructor, director, etc.): _____	YES	NO
Has anyone ever set a target weight for you or subjected you to routine weigh ins? If yes, specify (instructor, director, etc.): _____	YES	NO
Have you ever had to lose weight using any of the following methods? a. Vomiting b. Laxatives c. Diuretics d. Diet Pills e. Exercise	YES	No
Do you regularly exercise outside of your normal practice schedule? If yes, describe your activities: _____	YES	NO
Have you ever been diagnosed with an eating disorder?	YES	NO
Do you think that you might have an eating disorder?	YES	NO
Have you ever been treated for a stress fracture? If yes, how many have you had? _____ What body part(s) were involved? _____ When did the injury occur? _____ How was the diagnosis made (x-ray, bone scan, MRI, CT)?	YES	NO