Pathogenesis and Symptomology of the Exercise-Hypogonadal Male Condition

Dissertation

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Abstract

Men that engage in high volumes of long distance running have been previously shown to demonstrate low testosterone concentrations. However, both the cause and the consequences of the condition remain undetermined. The purpose of this study was to identify the pathogenesis of the condition as well as to determine whether symptoms that are typically seen in other populations with reduced testosterone are present. 9 men (Age: 36.3 ± 9.2 years; Height: 180.0 ± 8.8 cm; Weight: 77.2 ± 6.8 kg) performing an average of 81 ± 14 km per week of running for the past 12 months (EHMC) were compared to 8 men who served as control (CONT) subjects (Age: 30.8 ± 6.3 years; Height: 176.9 ± 5.2 ; Weight: 77.3 ± 10.7 kg) performing no regular exercise for the past 12 months. Blood samples were taken every 15 minutes beginning at 08:00 and continuing until 12:00, for a total of 17 blood draws. Blood was analyzed for testosterone (T), luteinizing hormone (LH), follicle stimulating hormone (FSH) and cortisol (C). Subjects underwent a dual x-ray absorptiometry (DEXA) scan to assess bone density and body composition. Subjects also completed the Aging Male Symptoms (AMS) questionnaire and a Food Frequency Questionnaire (FFQ). As expected, T concentrations were significantly ($P \leq 0.05$) reduced in the EHMC group compared with CONT at all time points. There were no differences in LH.

The EHMC group demonstrated significantly ($P \leq 0.05$) higher AMS scores (EHMC: 26.0 ± 7.1 vs CONT: 21.7 ± 5.5). There were no differences in body composition or bone density. There were no differences in energy intake (EHMC: 2710.6 ± 800.1 vs CONT: 2742.8 ± 969.0 kcal), but there was a significantly ($P \leq 0.05$) higher contribution from carbohydrate in the EHMC group (EHMC: 48.6 ± 3.8 vs CONT: 36.5 ± 9.0 %). This study was the first to document that reduced T concentrations resulting from high volumes of long distance running leads to the demonstration of hypogonadal symptoms. This study also revealed that despite running an average of over 80 km per week at an estimated energy cost of 901 kcal/day, the EHMC group do not consume any more calories than their weight matched sedentary controls. This suggests that the long distance runners are likely in a substantial caloric deficit and failing to consume adequate calories in the diet to support the high levels of activity may be contributing to the condition. Furthermore, examining individual differences showed that some men may be protected against the reduced T response, while others may suffer particularly severe symptoms. Individual differences also showed that in some cases LH concentrations can be severely dampened, possibly due to an exhaustion of the GnRH neuron. In conclusion, this study revealed that long distance runners with reduced testosterone concentrations do exhibit symptoms consistent with the condition of hypogonadism and thus may warrant treatment. However, the symptoms are mostly minor and a carte blanche pharmaceutical intervention is not advised at present.

Dedication

My Ph.D. is dedicated to my family. To my parents, Paul and Vanessa who gave me the opportunity and love I needed to pursue my dream. To my sister, Claire, for showing me that a Hooper can go to University. To my wife and children, Stephanie, Jace, Landon and Alexa. You were the inspiration for graduate school and the reason that I made it through.

I love you all.

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Awards

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Fields of Study

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Table of Contents

Abstract	ii
Dedication	iv
Acknowledgements	v
Vita	vi
List of Tables	xvi
List of Figures	xviii
Chapter 1: Introduction	1
Chapter 2: Review of Literature	6
Introduction	6
Background.....	7
Testosterone Synthesis.....	8
Testosterone Secretion	10
Testosterone Action	13

Factors that Influence Testosterone Concentration.....	18
Chronic Response of Testosterone to Exercise	20
Basal Testosterone Responses to Aerobic Exercise.....	22
Basal Testosterone Responses to Resistance Exercise	24
Acute Response of Testosterone to Exercise	24
Acute Testosterone Response to Aerobic Exercise	25
Acute Testosterone Response to Resistance Exercise.....	27
Summary	34
Chapter 3: Methods.....	36
Chapter 4: Results.....	42
Chapter 5: Discussion	49
References	57
Appendix A: Institutional Review Board Approval.....	64
Appendix B: Consent Form.....	65

List of Tables

Table 1. Participant characteristics. EHMC = Exercise Hypogonadal Male Condition, CONT = Control. No differences were found between the two groups.	37
Table 2. Luteinizing Hormone concentrations and pulse characteristics of the CONT and EHMC groups. AUC = Area Under the Curve, LH = Luteinizing Hormone, EHMC = Exercise Hypogonadal Male Condition, CONT = Control. No significant differences were discovered between groups.....	43
Table 3. Dietary composition as measured by a food frequency questionnaire. EHMC = Exercise Hypogonadal Male Condition, CONT = Control. * = Significantly ($P \leq 0.05$) different between groups.	45
Table 4. Total body measures of bone densitometry as measured by Dual-energy X-ray Absorptiometry (DEXA). BMD = Bone Mineral Density, BMC = Bone Mineral Content, EHMC = Exercise Hypogonadal Male Condition, CONT = Control. No significant differences were observed.....	46

Table 5. Total body measures of body composition as measured by Dual-energy X-ray Absorptiometry (DEXA). EHMC = Exercise Hypogonadal Male Condition, CONT = Control. No significant differences were observed.	47
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List of Figures

Figure 1. Testosterone synthesis. Cholesterol is the precursor to all steroid hormones. After conversion to pregnenolone, several pathway permutations are possible, but all lead to the conversion to androstenedione. The pathway via DHEA is approximately 4 fold more common in humans, and is highlighted by thicker arrows. The final step in the synthesis is the conversion of androstenedione to testosterone in the testis and prostate in men, and the ovary and mammary gland in women. Following its' production, testosterone can then be converted to estradiol. 7

Figure 2. Testosterone Secretion. The kisspeptin neuron begins the testosterone secretion process by mobilizing the peptide Kiss 1. Upon interacting the Kiss 1 receptor, the GnRH neuron receives the signal to secrete GnRH down the hypothalamic-hypophyseal portal vein (Hh-PV) from the hypothalamus to the anterior pituitary where it meets its receptor on the gonadotrophs. Luteinizing hormone (LH) is then sent out in to the systemic circulation, where it can meet its receptor, either in the testes (T) or prostate (P) in men, or the ovaries (O) or mammary gland (MG) in women. Finally, when testosterone concentrations are adequate, the AR is targeted on the kisspeptin neuron and a negative feedback system is activated that prevents further testosterone secretion. 12

Figure 3. Genomic and nongenomic androgen action. Left side: 1. Hydrophobic testosterone readily diffuses across the cell membrane. 2. The androgen receptor is inactive before testosterone binds to due its association with heat shock proteins. 3. Testosterone binding occurs after heat shock protein dissociation and results in a conformational change of the complex. The receptor also dimerizes facilitating future binding to the androgen response element (ARE) 4. The complex trans-locates to the cell nucleus. 5. With the help of transcription factors, the complex binds to the ARE on the DNA sequence near the target gene. 6. Following an increase in transcription, there is an increase in the product of the gene..... 15

Figure 4. Testosterone concentrations in control (CONT) (gray) and Exercise-Hypogonadal Male Condition (EHMC) (black) groups. A repeated measures ANOVA revealed a significant ($p \leq 0.05$) main effect for group. 42

Figure 5. Differences in AMS questionnaire score between the EHMC and CONT group. EHMC = Exercise Hypogonadal Male Condition, CONT = Control. * = Significantly ($P \leq 0.05$) different between groups. 44

Figure 6. Total luteinizing hormone area under the curve for individual subjects in the EHMC group. 48

Chapter 1: Introduction

Testosterone is a hormone that is essential for the development of the male phenotype (8) and also plays a key role protein synthesis (32). Despite the well-known benefits of aerobic exercise, high volumes of aerobic activity, such as the levels conducted by men who train for ultramarathons, has been consistently shown to be associated with reduced testosterone concentrations (27, 28, 51, 52, 81, 82). Upon its discovery, the parallel was drawn to the already previously established condition in women, characterized by amenorrhea that resulted from heavy distance running (16). Just as high levels of physical activity seemed to be linked to menstrual dysfunction in women, it appeared men were also suffering from an endocrinological response where sex hormone secretion was being impaired as a result of the endurance activity, possibly due to dysfunction not only in the gonads, but with a hypothalamic and/or pituitary source, where the signal for sex hormone secretion originates.

After several studies utilized a similar cross-sectional design where long-distance runners were compared to inactive controls, the cause and even the presence of the condition at all remained equivocal. Studies reported either no difference in

testosterone but a difference in luteinizing hormone (51); changes in testosterone but not luteinizing hormone (27, 28); or, changes in both testosterone and luteinizing hormone (52). Studies also suggested that high concentrations of cortisol could be playing a role (27), as it had been previously found that hydrocortisone injections can inhibit testosterone secretion without changes in luteinizing hormone (13). The equivocal findings appear to be due to differences in the characteristics of the experimental subjects, mainly that variations in running volumes and length of time performing such volumes may have contributed to the discrepancies. More recent literature has suggested that this so called "Exercise-Hypogonadal Male Condition" is limited to those who have been persistently involved in chronic endurance training for an extended period of time (23). This hypothesis has been supported by studies that have taken single blood samples of aerobic athletes around elite competitions, such as the Ironman World Championships (35), the Western States Ultramarathon (43) and Susitna 100 Ultramarathon (39), and found even lower testosterone concentrations than the prior studies that have been mentioned discovered.

Although the identification of this condition came from examining aerobic athletes, this condition appears to impact other populations as well, particularly those exposed to high levels of physical activity coupled with a caloric deficit. For example, competitive wrestlers, throughout the course of a season, appear to

suffer reductions in their total testosterone concentrations as their body mass concomitantly reduces (63). Even more acutely, military populations under the stress of 8 days of energy restriction and high energy expenditure demonstrated a 50% reduction in testosterone concentration (3).

As this condition appears to be observed more frequently and across more populations, the need for more research in this area is apparent due to the potential long term anabolic or androgenic consequences that could result from men who are exposed to reduced testosterone concentrations for prolonged periods of time (26). Theoretically, these men could suffer from the same side effects as those with androgen deficiency, such as infertility, low bone density, loss of muscle mass and sexual dysfunction (4). However, at the same time, it has been acknowledged that this condition could exist either as an altered 'set point' for the body, that perhaps just requires less testosterone in these individuals, or that it could even have protective effects on cardiovascular health (26).

It is particularly surprising that the consequences of this reduced concentration of testosterone have not been more thoroughly assessed considering that a fundamental concept in the diagnosis of hypogonadism is the presence of symptomology. In fact, testosterone concentration itself is not a criteria for

diagnosing the condition (4). Rather, the condition of hypogonadism is determined by the use of a simple survey, such as the Aging Male Symptoms questionnaire (15). However, any assessment of such symptoms has been absent from the studies that have been previously described.

Another notable absence from the literature regarding the EHMC is the impact of nutrition, which has been well studied in the female equivalent condition, characterized by low estrogen and menstrual dysfunction, commonly known as the 'female athlete triad' (see (17) for review). In fact, a recent study clearly demonstrated that the simple intervention of a daily nutritional supplement containing 360 kcal comprised of 54g carbohydrate and 20g protein was able to reverse exercise-related menstrual dysfunction in 6 months in 8 female athletes (11). Also, as previously mentioned, 8 days of energy restriction coupled with high energy expenditure reduced testosterone concentrations in military men by 50% (3). Taken together, these studies clearly demonstrate that energy deficit could be playing a role, and that nutritional supplementation could potentially be a simple remedy to the condition in men without the need for pharmaceutical intervention, much like it has been in women that suffer menstrual dysfunction as a result of energy deficit.

The purposes of this study are threefold; Firstly, to identify whether luteinizing hormone and cortisol are playing a role in the condition. It is hypothesized that luteinizing hormone concentrations will be elevated. Secondly, to discover whether there is a presence of symptoms typically associated with low testosterone in these EHMC populations. It is hypothesized that there will the EHMC populations will suffer from symptoms. Thirdly, to identify whether nutrition could be playing a role in the development of the condition. Is it hypothesized that a caloric deficit may be contributing to the condition.

Chapter 2: Review of Literature

Introduction

Androgens are essential for the expression of the male phenotype (8) and play a key role in the greater lean tissue and higher levels of strength seen in men over women. Regardless of sex, exploiting the testosterone hormone has become an important component of exercise physiology. Whether a person is suffering from a muscle wasting disease or an elite athlete, most populations will see a benefit of improvements in strength and increases in lean tissue with increased exposure to testosterone. From the use of injections of the hormone itself or synthetic derivatives known as anabolic steroids to designing resistance training programs specifically to increase the circulation of the hormone, testosterone has a diverse range of applications to exercise physiology. The purpose of this review is to provide a synthesis of the research pertaining to the applications of testosterone physiology to exercise, with an emphasis on new findings which provide new interpretations of old studies and place some misconceptions into perspective.

Background

In order to fully understand the practical applications of testosterone to exercise, it is important to first cover the fundamental concepts of testosterone synthesis, secretion and action

Figure 1. Testosterone synthesis. Cholesterol is the precursor to all steroid hormones. After conversion to pregnenolone, several pathway permutations are possible, but all lead to the conversion to androstenedione. The pathway via DHEA is approximately 4 fold more common in humans, and is highlighted by thicker arrows. The final step in the synthesis is the conversion of androstenedione to testosterone in the testis and prostate in men, and the ovary and mammary gland in women. Following its' production, testosterone can then be converted to estradiol.

Testosterone synthesis

Each step of the synthesis of testosterone is shown in Figure 1. The precursor to all steroid hormones is cholesterol, whose synthesis is complex and highly energetically expensive. The following text describes each step, and the corresponding enzyme is shown in parentheses:

Cholesterol to Pregnenolone (CYP11A)

The initial step in all steroid hormone synthesis involves the conversion of cholesterol to pregnenolone, which is also the rate limiting step. The reaction requires three molecules of oxygen and three molecules of NADPH which are utilized for three sequential oxidation reactions. Each of the three steps is catalyzed by CYP11A to convert cholesterol to pregnenolone by removing a 6 carbon side chain, isocaproaldehyde. The enzyme, CYP11A is expressed in the adrenal cortex, ovary, testis and placenta.

Δ^5 -3 β -hydroxysteroids to Δ^4 -3-ketosteroids (3 β HSD1) and pregnenolone/progesterone to DHEA/androstenedione (CYP17)

Pregnenolone can be converted to one of two substrates. The first pathway involves the conversion of pregnenolone to dehydroepiandrosterone (DHEA) via an intermediate, 17 α -hydroxypregnenolone by the enzyme CYP17. All three of these molecules are known as Δ^5 -3 β -hydroxysteroids. The second pathway for

pregnenalone, which can also be used by the other Δ^5 -3 β -hydroxysteroids is then converted to a respective Δ^4 -3-ketosteroid by the enzyme 3 β HSD1. This involves two reactions in sequence. The first reaction involves the reduction of NAD⁺ to NADH. The reduced NADH then activates the isomerization of the Δ^5 -3-ketosteroid to the respective Δ^4 -3-ketosteroid. As shown in Figure 1, pregnenolone can be converted to progesterone; 17 α -hydroxypregnenolone to 17 α -hydroxyprogesterone; and DHEA to androstenedione.

Each Δ^4 -3-ketosteroid can be converted to androstenedione using the same CYP17 enzyme as is used to convert each Δ^5 -3 β -hydroxysteroid to DHEA. CYP17 catalyzes two oxidase reactions, including a hydroxylation and a cleavage of C17-C20, with each step requiring NADPH and O₂. Of these several possible permutations, the predominant pathway is shown in Figure 1 by the thicker arrows, which occurs at approximately a 4 fold greater rate (36). Both CYP17 and 3 β HSD are expressed in the adrenal cortex as well as the Leydig cells and the ovaries.

Androstenedione to testosterone (17HSD3 and 17HSD5)

Using predominantly NADPH as a cofactor, 17HSD3 converts androstenedione, a weak androgen, to testosterone, a highly potent androgen (60). As 17HSD3 is only expressed in the testis it is unable to explain the presence of testosterone in

women. A different form of the 17β -HSD enzyme known as type 5 has been found to be expressed in the ovary (49) as well as the mammary gland (61), and thus is believed to be responsible for the presence of testosterone (47) in women, albeit in much lower concentrations.

Testosterone to estradiol (CYP19)

Using the microsomal electron transfer system and 3 molecules of O_2 and NADPH, CYP19 (or often called aromatase) converts testosterone to estradiol in the Leydig cells of the testicles in men or the ovaries in women.

Testosterone Secretion

The system for the release and control of the testosterone hormone is known as the Hypothalamic-Pituitary-Gonadal Axis (HPGA) (Figure 2). A recent advancement in the understanding of the HPGA was the discovery of kisspeptins, produced by the KISS1 gene, and their role in the regulation and secretion of gonadotropin releasing hormone (GnRH). In fact, KISS1 is the initial signal for GnRH secretion, and is now universally recognized as the major central regulator of the HPGA (67). Kisspeptin neurons are located in the brain in two areas, the arcuate nucleus (ARC) and the anteroventral periventricular nucleus (AVPV). The kisspeptin receptors (KISS1R) are located on GnRH neurons in the preoptic area of the anterior hypothalamus, which stimulates

GnRH release into the hypothalamic-hypophyseal portal vein, connecting the hypothalamus to the anterior pituitary. At the anterior pituitary, the GnRH receptor (GnRHR) receives the signal to secrete luteinizing hormone (LH) into the circulation, which is done in a pulsatile manner. LH is then the signal for testosterone secretion, which as described above can occur in several tissues, predominantly the testis in men and the ovaries in women. When appropriate concentrations are reached, androgens as well as estrogens (which are synthesized from testosterone) provide negative feedback to the KISS1R to stop further secretion (21). On this note, the discovery that kisspeptins are androgen sensitive is therefore able to answer the question of how the negative feedback mechanism for testosterone secretion occurs when GnRH neurons do not express androgen receptors (71).

Figure 2. Testosterone Secretion. The kisspeptin neuron, located in the arcuate nucleus (ARC) and anteroventral periventricular nucleus (AVPV) regions of the brain, begins the testosterone secretion process by mobilizing the peptide Kiss 1. Upon interacting the Kiss 1 receptor, the GnRH neuron receives the signal to secrete GnRH down the hypothalamic-hypophyseal portal vein (Hh-PV) from the hypothalamus to the anterior pituitary where it meets its receptor on the gonadotrophs. Luteinizing hormone (LH) is then sent out in to the systemic circulation, where it can meet its receptor, either in the testes (T) or prostate (P) in men, or the ovaries (O) or mammary gland (MG) in women. Finally, when testosterone concentrations are adequate, the AR is targeted on the kisspeptin neuron and a negative feedback system is activated that prevents further testosterone secretion.

Testosterone Action

Once testosterone is in circulation it can be loosely bound to albumin (~20-30%), tightly bound to sex hormone binding globulin (SHBG) (~50-70%), bound to other proteins (~4%) or unbound, known as 'free' (~1-3%) (18). Traditionally, free testosterone has been thought of as the only form of testosterone that is biologically available, often referred to as the "free hormone hypothesis" (56). Due to the hydrophobic nature of testosterone, the binding proteins were believed to act as a mode of transport for the hormone which would not readily dissolve in the blood. It was also suggested that binding proteins were used as a way to keep testosterone in an inactive state and thus serve as a means of regulating the amount of active hormone available for diffusion.

The idea of binding proteins inactivating circulating hormones centers on the classical action of testosterone (Figure 3, left side), where the bound complex is unable to cross the cell membrane. In contrast, free testosterone is hydrophobic and can readily be diffused through the membrane in to the cell cytoplasm where it can reach the androgen receptor (AR) (5). Before testosterone reaches the AR, the AR is associated with a large complex of chaperones, including heat shock proteins (HSPs) which keep the AR inactive yet still ready for binding (62). The AR binds both dihydrotestosterone (DHT) and testosterone with high affinity,

although testosterone binds with a twofold lower affinity as well as a fivefold higher dissociation rate than DHT (22). Upon binding, HSPs are then dissociated, accompanied by a conformational change of the complex. This change is associated with an increased affinity for the androgen response element on the DNA sequence. Once the complex has been translocated to the nucleus, the receptor then dimerizes and binds to DNA sequences known as the androgen response element, where it can now influence transcription. This occurs by an interaction between the AR and many different classes of transcription factors, including general, sequence specific, co-activators, co-repressors and chromatin factors. The end result of this process is a promotion of the expression of target genes (8). Therefore, the effects of testosterone are not seen until the protein products that form as a result of the increased gene expression are created. Such effects on protein synthesis are not seen for at least half an hour (57) and up to hours or days (59). This process has also been referred to as the 'slow action' of testosterone due to this amount of time that is required as a result of the genomic nature.

Figure 3. Genomic and nongenomic androgen action. Left side: 1. Hydrophobic testosterone readily diffuses across the cell membrane. 2. The androgen receptor is inactive before testosterone binds to due its association with heat shock proteins. 3. Testosterone binding occurs after heat shock protein dissociation and results in a conformational change of the complex. The receptor also dimerizes facilitating future binding to the androgen response element (ARE) 4. The complex translocates to the cell nucleus. 5. With the help of transcription factors, the complex binds to the ARE on the DNA sequence near the target gene. 6. Following an increase in transcription, there is an increase in the product of the gene.

The rate at which the effects of testosterone are seen is an important difference in the two mechanisms of response illustrated in Figure 3. Where the genomic response requires an interaction with nuclear DNA before changes can be observed, another mechanism of testosterone action (Figure 3, right side) can exert its effects via signal transduction systems that can show a measureable biological response within seconds (59). Evidence of these non-genomic responses has been presented in all classes of steroids across many laboratories (59). Such research into the non-genomic response has unveiled a complex network of signaling cascades beyond the scope of this review. For a detailed review of these effects see references (57, 59). However, the main concept (highlighted in Figure 3) is that these networks appear to lead to activation of the Akt/mTOR and/or mitogen-activation protein kinase (MAPK) pathways, critical pathways for hypertrophy.

Interestingly, although the non-genomic role of testosterone might be seen as an alternative to the free hormone hypothesis, a seminal paper by Mendel (1989) for the proposal of the free hormone hypothesis actually predicted that it may not hold for all hormones and all tissues, and particularly questioned its validity for steroid hormones [11]. However, it is not until much more recently that alternatives to the free hormone hypothesis have been studied. As previously mentioned, the reason for bound testosterone initially being considered inactive

is due to the attached protein preventing the complex from crossing the membrane and blocking any interaction with the intracellular receptor. Therefore, these non-genomic signaling cascades in response to testosterone center on the potential role for the bound hormone through a putative membrane receptor.

In a pivotal paper with regards to the non-genomic role of androgens, Estrada et al. (19) demonstrated that testosterone that was conjugated with bovine serum albumin (and therefore unable to pass the cell membrane) led to an increase in ERK-1/2 phosphorylation that was comparable to that of free testosterone in skeletal muscles. Further experiments identified that such an increase was not inhibited by an androgen receptor antagonist, clearly indicating an alternate androgen receptor present in the cell membrane. Also, ERK-1/2 phosphorylation was blocked by a G-Protein antagonist (pertussis toxin), suggesting that this pathway is mediated by a G-protein mechanism. Estrada (2003) went on to state that calcium was likely the second messenger responsible for signal transduction, although at the time was unable to provide a mechanism as to how this signal went on to stimulate the ras/mek/erk pathway, but did elude to the possibility that the process was mediated by calmodulin or protein kinase C. Although the study of the non-genomic role of testosterone is still in its infancy, there is substantial evidence for its existence and should now begin to be

considered in studies that pertain to the role of testosterone in exercise physiology.

Factors that Influence Testosterone Concentration

Before a simple testosterone concentration can be interpreted, it is essential to first consider the context that the sample was taken in. There are a multitude of factors that can impact a single testosterone concentration, including feeding, time of day and exercise. With an established context for a testosterone concentration, one can begin to utilize the information in a manner that could contribute to the optimization of resistance training adaptations.

Feeding Influences on Testosterone Concentration

Studies have demonstrated an acute effect of diet on testosterone concentration. For example, fat rich meals (78), carbohydrate (33) and mixed meals (42) all reduce testosterone in the post prandial phase (the time period immediately following food intake) to below that of fasting. In fact, the latter study clearly demonstrated the caution that must be taken when interpreting a simple circulating testosterone concentration. Following the typical rise in testosterone seen in response to a resistance training protocol, Kraemer et al. (42) demonstrated that when consuming a mixed macronutrient supplement

(56% carbohydrate, 16% protein and 28% fat) testosterone concentrations drop to below that of a fasted state. This reduction in circulating testosterone might have lead one to speculate that food intake can negatively affect the contribution of testosterone to resistance training adaptations. However, this drop in testosterone coincided with an increase in androgen receptor content in the muscle, suggesting that perhaps the testosterone had moved from the circulation to the muscle where it can exert its positive effects on protein metabolism. What is certain, however, is that measuring testosterone concentration in the fed state could lead to mistakes in interpreting a testosterone value.

Time of Day Influences Testosterone Concentration

In addition to feeding, time of day can affect the circulating testosterone concentration, with a peak in the early morning, and a substantial nadir in the evening (23). When considering these factors, it is essential that for a basal concentration to be determined, the sample should be taken between 7-10am, following a normal night's sleep and in a fasted and rested state (4).

Exercise Impacts Testosterone Concentration

Once the factors such as feeding and time of day have been taken in to consideration, testosterone concentrations can begin to be measured around exercise and interpreted. Interestingly, testosterone concentrations can change

and return to baseline quickly in both aerobic and resistance exercise but to varying degrees. Also, prolonged periods of regular exercise can alter basal concentrations of testosterone, with aerobic and resistance exercise resulting in differential responses. For this reason, the acute and chronic changes in testosterone concentration will be discussed separately, as too will aerobic and resistance exercise.

Chronic Response of Testosterone to Exercise

Changes in basal concentrations of testosterone can have a drastic impact on the body. Despite flaws in the design of early studies evaluating the effectiveness of androgens leading to even the American College of Sports Medicine declaring them ineffective from 1976-1984 (53, 54), the positive effects of supraphysiological concentrations of testosterone on lean mass and strength are no longer questioned (34). It has also been demonstrated that after suppression of basal testosterone concentration, that lean mass and strength increase in a dose response manner over the course of 16 weeks in young men, with exogenous testosterone doses ranging from 25mg-600mg per week. These doses altered basal concentrations on a range from below normal (for the 25mg per week group) to nearly 4 fold above normal (for the 600mg per week group), clearly demonstrating the benefits of testosterone supplementation to populations across an entire spectrum of basal concentrations. Thus, it is not merely

supraphysiological concentrations that are required to yield benefits from testosterone supplementation (6).

In addition to the clear support for benefits of increasing basal testosterone concentrations, the drawbacks of the removal of testosterone are equally powerful in the opposite direction. Following the use of a gonadotropin releasing hormone (GnRH) analogue (which blocks testosterone secretion), strength and lean mass gains were attenuated in young men when compared to placebo following 8 weeks of strength training (44). The processes by which these strength and lean mass attenuations occur has been described more recently by the same author group, where it appears that when testosterone is blocked, satellite cells are unable to differentiate to myonuclei which prevents the muscle from being able to grow (45).

Although testosterone concentrations were artificially reduced in the aforementioned study, these findings may be of concern for individuals who acquire hypogonadism. Such populations include individuals with a disease of the pituitary which can alter testosterone secretion as well as older populations due to the fact that cross sectional studies have demonstrated testosterone concentrations decrease with normal aging (30). In addition, low testosterone concentrations are associated with decreased lean mass in otherwise healthy

hypogonadal men when compared with controls (37). In addition, a wide range of symptoms have been reported in association with hypogonadism, such as infertility, reduced libido, reduced muscle mass and strength, loss of body hair and breast discomfort to name a few (4).

Clearly, changes in basal testosterone concentrations can have a powerful impact on the health of the individual, and long term exercise has been shown to alter testosterone. However, the chronic responses to aerobic and resistance exercise are entirely different.

Basal Testosterone Responses to Aerobic Exercise

Despite the clear benefits of aerobic exercise on cardiovascular health, one adaptation to such training, albeit at very high volumes, is a reduction in testosterone known as the Exercise Hypogonadal Male Condition (EHMC), named by Hackney et al. (26). Although it was clearly identified in the 1980s that men who perform high volumes of intense aerobic exercise show reduced testosterone concentrations (27, 51), to this day many fundamental questions regarding the condition remain unanswered (23), such as whether the reduced testosterone is a result of the inability of the testes to secrete testosterone (81), or whether it is a reduction in luteinizing hormone which signals testosterone secretion (52).

Another critical question that remains unanswered with regards to EHMC is the presence of any symptomology, which other than in one case study (9), is not reported in studies that pertain to the condition (24, 25, 27, 28, 51, 52, 81, 82). This is an essential missing element of this area of study as it ultimately determines whether this reduced testosterone concentration is something that needs to be treated, or is simply an altered set-point that is a consequence of heavy aerobic training. Hypogonadism is not a condition that is determined by a blood hormone concentration, but one that is diagnosed based on the presence of negative symptoms associated with reduced testosterone, such as sexual dysfunction, infertility, reductions in muscle mass and strength as well as less specific symptoms such as reduced energy, depression, sleep disturbance and lack of concentration (4). These symptoms can easily be identified with the use of questionnaires, such as the Aging Male Symptoms (AMS) questionnaire (31), as well as others, although the AMS questionnaire appears to be the most widely used (4). Only once these symptoms have been identified could there be any cause for treatment (4). As mentioned, low testosterone has been associated with a litany of substantial side effects that could potentially impact this population as well, although at this point the presence of hypogonadal symptomology in this group is unknown.

Basal Testosterone Responses to Resistance Exercise

Where long term aerobic exercise can lead to reductions in testosterone, basal concentrations of testosterone have been found to demonstrate significant increases after just 5 weeks of resistance training (73). Beyond initial increases in previously untrained individuals, testosterone concentrations have been shown even in elite weightlifters to continue to increase over the course of two years of training (29). However, other studies have failed to show any chronic increases, leading to the speculation that perhaps varied strength training programs using higher volumes may be needed to alter resting concentrations of testosterone (41). Although, of course, when increases were found they did not match the types of concentrations seen with pharmaceutical intervention (6), increases in basal testosterone concentrations are associated with increases in lean mass and strength and it is reasonable to suggest that such adaptations from resistance training would also be positive for human performance. Although the exact role of this adaptation is unknown, it has been speculated it may lead to optimized strength development (29).

Acute Response of Testosterone to Exercise

Where the effects of chronic changes in testosterone concentration have been well documented, such as the negative effects of hypogonadism and the positive effects of increases in testosterone, the impact of acute testosterone responses to exercise are poorly understood, despite being identified in aerobic exercise in

1973 (74) and resistance exercise shortly after in 1976 (20). The main questions that remain in this area pertain to the physiological role of these responses and their consequences.

Acute Testosterone Response to Aerobic Exercise

As mentioned, it was first documented in 1973 that serum androgens acutely rise following maximal swimming and rowing (74), although these observations were made following a normal training session in which the activities were not tightly controlled. However, these results have been replicated in a laboratory setting. For example, acute testosterone increases were demonstrated at intensities of 75- and 100% $\dot{V}O_2$ Maximum during a graded maximal treadmill test (41). This acute increase in testosterone has been much more thoroughly studied following resistance training, however, and the implications of this response has been well debated in the literature and will be described later.

Although this acute rise in testosterone has been demonstrated following aerobic exercise, acute reductions in testosterone have also been shown (39, 43). However, in these cases, testosterone was measured before and after the completion of an ultra-endurance event. For example, Kraemer et al. (39) measured testosterone concentration before and after a 160km race across Alaska, whereby competitors completed the race either cycling or running. The

results clearly showed a substantial reduction in testosterone following the race. In a similar study, Kupchak et al (43) measured resting testosterone in 12 men prior to running the 100 mile Western States Ultramarathon race. Blood samples were taken immediately following, but also at 1 and 2 days following the race. This study again demonstrated an acute reduction in testosterone, but this study also noted that these concentrations did not return to baseline levels until the final time point 2 days following the race.

The reason for this acute drop in testosterone following ultra-endurance activity is unknown. It has been suggested that the reduction is a result of a dampening of luteinizing hormone (39), however, this particular hormone is highly pulsatile and would require serial blood draws over several hours to adequately assess, which has not taken place following these events. It has also been suggested that the elevated cortisol concentrations following these highly physically stressful events could be playing a role, particularly as cortisol has been previously linked with a suppression of testosterone secretion (13). Although it is clear that the hypothalamic-pituitary-gonadal axis is suppressed following extreme physiological stress, the exact mechanism and the consequences of the response remain to be determined.

Acute Testosterone Response to Resistance Exercise

Soon after the increase in testosterone was demonstrated following aerobic exercise, the response was also identified following resistance exercise (20). In this study, elevations were seen in college aged males, but not in college aged females or high school aged males (20), suggesting that higher basal concentrations of testosterone play a role in this response. Later, pioneering work in the field of resistance training endocrinology was undertaken by Kraemer et al. (40) to characterize the types of resistance training exercise that led to the greatest acute increases in growth factors, including testosterone. This study demonstrated that higher volumes (5 vs. 10 repetitions) and shorter rest periods (3 minutes vs 1 minute) resulted in greater acute testosterone responses (40).

Immediately following such a revelation, the obvious question was posed: does this response play a role in chronic adaptations to resistance training? The initial answer, as was stated by Kraemer et al. (40), is that whether any subsequent adaptations are related to the acute temporal increases remains unknown.

Incredibly, despite hundreds of studies regarding this topic having been published since this finding in 1990, the answer essentially remains the same. However, 25 years of research has led to a greater understanding of the processes that accompany the response as well as certain contexts when the acute rise in testosterone, termed acute testosterone (AT), may be relevant.

After the discovery of AT, future research began to investigate the presence of the androgen receptor (AR) in the recruited muscle. This was an essential area of discovery as we know that the hormone itself does not have an effect on protein synthesis until it has interacted with its receptor. Several studies together characterized the changes in expression of the muscle androgen receptor, which appears to stabilize immediately following an AT-inducing workout (72), then down regulate at the 1 hour time point (64), before showing up regulation (72) for at least 3 hours. When mapped along with the AT response, it appears that after the stimulation of circulating testosterone, the presence of testosterone in the blood begins to dissipate and the expression of the receptor in the muscle increases, suggesting that the hormone has moved from the circulation to the muscle, where it can interact with its receptor and induce its protein synthesis response. Interestingly, each of these aforementioned studies were conducted with their participants in the fasted state, which is known to be a state in which net protein balance remains negative (7). Only one study has measured the AR response in the fed state, which in contradiction to prior research that found a down regulation of the AR at the 1 hour time point in the fasted state (64), found a significant increase in AR content at the same time point following consumption of a mixed meal (56% carbohydrate, 16% protein, 28% fat). Although speculative, there is a clear link between increased protein synthesis in the fed state which also shows an up regulation in muscle AR content.

Another possible means of testosterone playing a role in resistance training adaptation is via the process of intracrinology. In this process first put forward by Labrie (46), testosterone in skeletal muscle may act in an intracrine manner, where precursors to testosterone such as DHEA, progesterone and androstenedione may be converted to testosterone at the muscle site itself where it would act upon, rather than at the testis and sent in to circulation in the classic endocrine action. The significance of the role of intracrinology is clearly evidenced by the fact that when testicular synthesis is removed, circulating testosterone concentration drops 95-97%, but function only reduces to approximately 40% (50). These processes have also been supported by the presence of 17 β HSD3 and 3 β HSD in human skeletal muscle, which perform the aforementioned peripheral conversions of testosterone precursors to testosterone and led to the study of their responses following resistance training (77). Although this study found no evidence of intracrinology in the AT response, this particular study was undertaken in the fasted state. As has been previously shown, protein synthesis is much higher in the fed state, and the process of intracrinology may have required a supply of energy as well as amino acids to fully function, and therefore this study may have failed to activate intracrinology processes.

When research has suggested an important role for AT, it has been previously dubbed “the hormone hypothesis” (68). What is often misunderstood in the literature, however, is the context for which the role of AT has been considered relevant. Although it is sometimes insinuated that proponents of the so called “hormone hypothesis” have suggested that AT is *necessary* for hypertrophy, this certainly does not seem to be the case, particularly in untrained individuals. Early resistance training research clearly documented significant hypertrophy from high load, long rest strength training protocols that are not typically associated with high hormonal responses (14, 75). Even light load training at just 30% 1RM has been shown to result in significant hypertrophy following 10 weeks of resistance exercise 3 times per week in a previously untrained population (58), although other studies have failed to find significant increases in hypertrophy following light load resistance training in beginners (10). The context for which AT has been suggested to be beneficial for hypertrophic adaptations in resistance training is in trained populations, where such a response may optimize adaptations (1). However, there is a dearth of studies comparing resistance training programs with and without AT in trained populations (68).

The lack of research in trained populations has not prevented some authors from continuing to entirely discount the role of AT in resistance training programming. For example, in a creative study design, participants trained elbow flexors 3-4

times per week for 15 weeks, but each elbow flexor was trained individually on different days, with one day using exclusively elbow flexor exercises to keep circulating hormone concentrations low, and the other elbow flexor in conjunction with leg exercises designed to stimulate AT (80). Despite the presence of AT for one of the elbow flexors, there were no differences in strength or hypertrophy between the elbow flexors. Although initially these results may appear to discount the role of AT, it is important to consider the mechanism by which testosterone induces its favorable effects on protein synthesis. As previously mentioned, recruited musculature shows an up regulation in AR content for upwards of 3 hours following resistance training, and it is the interaction of testosterone with its receptor that induces the beneficial response. Although this study increased circulating testosterone, it did so with the use of the lower body musculature, which would not have increased the AR content in the elbow flexors. As a result, the increased circulating testosterone would not have a concomitant increase in receptors to bind to in the elbow flexors, and therefore would not be able to have any effect.

Other studies have attempted to discount the role of AT by comparing the effects of resistance training programs that do and do not stimulate AT and balance their effects on muscle protein synthesis (MPS). In a between-subjects design, an identical resistance training program was conducted on men (who would

demonstrate an AT response) and women (who were unable to produce an AT response) and their respective post exercise MPS rates were compared (79). After the failure to uncover any differences in MPS between the two sexes, the authors concluded that the AT response must not be relevant in stimulating protein synthesis. However, it is important to consider the context for which MPS can be used. Of course, resistance training is beneficial due to its positive effects on net protein balance, where synthesis exceeds degradation and hypertrophy can occur. In a within-subjects design, particularly if the stimulus is identical, it is fair to assume that protein degradation would be similar, such as in the studies that have compared protein ingestion protocols on MPS and developed protein recommendations. However, protein degradation can be drastically different when comparing populations, as was demonstrated by a cross-sectional study comparing young and old subjects, as well as men and women, which showed that older women have the highest MPS rates, but they also have the highest degradation rates (70). Clearly, changes in MPS alone, particularly when comparing across sexes do not adequately predict changes in protein balance as it does not take in to account protein degradation. As a result, this study is unable to adequately discount a positive effect of the AT response on long-term changes in protein balance.

What is clear regarding AT response is that it is very consistent and responsive to subtle changes in program design, such as an increase in rest period (40). The AT response also involves the conversion of cholesterol, a highly energetically expensive molecule (69), which would be extremely wasteful for the body to send in to circulation for no reason. These observations at least indirectly point to the body considering the AT response to be important following the high volume, moderate load, short rest protocols that induce the response. What remains unclear is what the purpose of this response is. One novel suggestion at this point is that the role of AT could be in order to stimulate protein synthesis through the recently identified non-genomic mechanism. Surprisingly, support for such a mechanism has actually come from the authors of the previously described study that failed to find differences in MPS (79). As previously described, measurement of MPS alone fails to adequately predict changes in protein balance as it fails to measure protein breakdown. It has also been noted that changes in protein synthesis measured following resistance training do not always occur in parallel with chronic up regulation of causative myogenic signals (12) and is not necessarily predictive of long term hypertrophic response to regular resistance training (76). However, the authors measured factors related to the non-genomic role of testosterone and found increased phosphorylation of Akt and mTOR in men, as well greater AT response and increased AR content, showing a clear link between the AT response and an increase in non-genomic testosterone responses (58). Other research by the same author group has demonstrated an

increase in phosphorylation of p70S6K in resistance training programs that utilize 80% 1RM that is associated with the AT response compared with 30% 1RM (58). Thus, these findings are clearly helping to provide a link between the AT response and the non-genomic mechanism of testosterone which may lead to increased protein synthesis.

Summary

The study of testosterone in exercise physiology is very complex. Many studies have been performed within the context of the free hormone hypothesis which suggests that free testosterone is the only form that is biologically available. However, non-genomic responses to testosterone have been clearly identified in-vitro and must begin to be considered as a possible mechanism of testosterone in exercise physiology. Also, a circulating concentration only provides limited information about the physiological processes that are occurring. For example, only testosterone that is interacting with its receptor will lead to a physiological response. In addition, many factors impact testosterone concentrations, for example, it is a pulsatile hormone that also shows diurnal variation, which provides more challenges in establishing a 'baseline' concentration on which to compare to.

Other factors such as food also appear to impact testosterone concentration, meaning that nutrition must be standardized. It has also been shown that food significantly reduces testosterone below a baseline value, in conjunction with an upregulation of the AR, suggesting that when circulating testosterone returns to baseline it may not mean the end of the response, but could signify the beginning of the process as it is taken up by the receptor.

In terms of the exercise responses, resistance and aerobic exercise have independent effects on testosterone. The increase following resistance training may or may not be contributing to hypertrophy, however the response is both significant and consistent. The reason for this response is currently unknown, although it would seem illogical for an energetically expensive molecule to be sent out into circulation in conjunction with its receptor being upregulating in skeletal muscle without it being part of a critical response. Heavy aerobic exercise, however, appears to lead to a chronic reduction in testosterone and the long-term consequences of such a response remain undetermined. Clearly, the applications of testosterone physiology to exercise continue to grow, and there is still much more to learn in order to discover how this powerful hormone can be exploited and correctly applied in the context of exercise physiology.

Chapter 3: Methods

Experimental Approach

A between-groups design which compared long-distance runners to sedentary controls was used. The comparisons that were made included blood hormones (described in detail below) as well as symptoms of hypogonadism, including both physiological (such as blood markers and bone density) and psychological factors (measured by questionnaires). A dietary analysis was also performed to see if food intake was contributing to any differences.

Participants

9 men who had ran an average of 81 ± 14 km per week for the past 12 months were compared to 8 men who had not ran regularly for the past 12 months. Participant characteristics are provided in Table 1. Caloric expenditure from running was estimated assuming a 10:00 min/mile average pace with caloric expenditure data obtained from Ainsworth et al. (2). Height was measured using a stadiometer (Seca, Hamburg, Germany). Weight was measured using a calibrated scale (OHAUS Corp., Florham Park, NJ). All subjects were fully informed of the protocol design and associated risks of this

investigation before signing an informed consent approved by The Ohio State University Institutional Review Board for use of human subjects.

	EHMC	CONT
N	9	8
Age (yrs)	36.3 ± 9.2	30.8 ± 6.3
Height (cm)	180.0 ± 8.8	176.9 ± 5.2
Weight (kg)	77.2 ± 6.8	77.3 ± 10.7
Weekly km ran past 12 months	81 ± 14	0 ± 0
Estimated calorie expenditure from running	901 ± 141	0 ± 0

Table 1. Participant characteristics. EHMC = Exercise Hypogonadal Male Condition, CONT = Control, km = kilometers. No differences were found between the two groups.

Procedures

Participants arrived at the laboratory at 07:30 following an overnight fast.

Participants were encouraged to drink at least a cup of water the night before, as well as the morning of testing to ensure adequate hydration. Hydration was verified with a urine sample that was measured for urine specific gravity (USG) with a refractometer (Reichert, Depew, NY) and testing would not begin if USG

was greater than 1.025. Subjects first underwent a dual x-ray absorptiometry (DEXA) scan. Following the DEXA, an indwelling catheter was inserted by a trained phlebotomist into an antecubital vein which allowed for serial blood draws from the same site. 6ml of blood was drawn into a serum vacutainer every 15 minutes beginning at 08:00 and lasting until 12:00 for a total of 17 time points. During the blood draws participants completed a food frequency questionnaire (FFQ) and the aging male symptoms questionnaire (AMS).

Blood variables

Comparisons included testosterone (T) concentrations, as well gonadotropin concentrations (luteinizing hormone (LH) and follicle stimulating hormone (FSH)) to determine whether the possible low testosterone was of a primary or secondary nature. In a primary condition, participants would show normal concentrations of LH and FSH, but testosterone would remain low due to a low functioning testis. In a secondary condition, gonadotropin secretion would be compromised, leading to LH and FSH concentrations being low, and thus LH fails to send an adequate signal to stimulate T secretion. If gonadotropin function is disrupted, FSH could also be compromised which could negatively impact spermatogenesis. Cortisol (C) was also measured as it has been shown to inhibit T secretion and could be another contributing factor to reduced T. LH was

measured at all 17 time points, T was measured every 60 minutes and FSH and C were measured for a single time point at 08:00.

Questionnaires

Comparisons were also made regarding symptoms of hypogonadism using the Aging Male Symptoms (AMS) questionnaire, which has been previously described (15). One small modification was made to the questionnaire, where participants were asked if the symptoms pertained to them during the past month, rather than at that specific time, so as to ensure the responses reflected more of a chronic condition, rather than any acute factors that could have impacted them within the previous 24 hours. Subjects completed the AMS two times, on separate days, with an average score used for analysis. A food frequency questionnaire was also completed and analyzed with Nutritionist Pro software (Axxya, Redmond, WA).

DEXA

Body composition and bone density was measured by dual energy x-ray absorptiometry (DEXA) (iDexa, GE Lunar, model: LU44535, Madison, WI). All scans were performed and analyzed by the same technician using Encore v14.1.

Blood Processing and Biochemical Analysis

Blood was collected in serum vacutainers, which were centrifuged at 1500xg at 4°C for 15 minutes. Serum was then aliquoted and stored at -80°C until it was later analyzed. Samples were thawed once only and analyzed in duplicate.

Luteinizing hormone was measured at all 17 time points. Testosterone was measured at 5 time points (8:00, 9:00, 10:00, 11:00 and 12:00). Cortisol and follicle stimulating hormone were measured only at the 8:00 time point.

Testosterone, luteinizing hormone, cortisol and follicle stimulating hormone were all measured using an ELISA (CALBiotech, Spring Valley, CA) with sensitivities of 0.8 nmol·L⁻¹, 3.1 mIU·L⁻¹, 11 nmol·L⁻¹ and 5 mIU·mL⁻¹ respectively. The luteinizing hormone ELISA analyses were conducted on a total of 9 plates, with an inter-assay coefficient of variance (CV) of 7.7%, and intra-assay CV of 11.86 ± 2.8%. The testosterone ELISA analyses were conducted on 3 plates, with an inter-assay CV of 3.0% and an intra-assay CV of 9.3 ± 1.5%. Cortisol and FSH analyses were ran on single plates, with intra-assay CVs of 6.47 and 10.45% respectively. All sample plates were measured on a Versamax tunable microplate reader (Molecular Devices, Sunnyvale, CA) at a wavelength of 450nm.

Statistical Analysis

For the testosterone concentrations, a 2 way ANOVA with repeated measures was used to compare the control (CONT) group to the exercise-hypogonadal

male condition group (EHMC) across the 5 time points measured during the protocol. For luteinizing hormone concentrations, area under the curve was taken for each subject across the 17 time points measured during the protocol and the two groups were compared using an independent t-test. Further, a pulsatile analysis was conducted as previously described by Reame et al. (65). In brief, a pulse was considered an increase in hormone concentration that was 3 times greater than the intra-assay CV calculated for the corresponding plate. The frequency of pulses, as well as the amplitude of each pulse was calculated for each subject. For pulse frequency and amplitude as well as all other comparisons, independent t-tests were used to compare the differences between the two groups. For the comparison of AMS scores, one CONT group subject was greater than two standard deviations from the mean and was removed as an outlier. For all other analyses, independent t-tests were used with a Bonferroni correction factor to control for alpha inflation. Statistical significance in this investigation was set at $p \leq 0.05$.

Chapter 4: Results

Hormone Analyses:

There was a significant main effect between groups for testosterone concentration. EHMC demonstrated significantly lower testosterone concentrations than the CONT at each time point (see Figure 4).

Figure 4. Mean $\pm$ SD testosterone concentration in CONT (n=8) (gray) and EHMC (n=9) (black) groups. CONT = control, EHMC = Exercise-Hypogonadal Male Condition, * = significantly ($p \leq 0.05$) higher than EHMC at corresponding time point.

Group	AUC LH (mU·mL ⁻¹ ·4h ⁻¹)	LH Pulse Frequency (No.·4h ⁻¹)	LH Pulse Amplitude LH (U·L ⁻¹)
EHMC	57.16 ± 31.12	2.0 ± 0.87	2.00 ± 1.07
CONT	42.27 ± 10.01	2.0 ± 1.07	2.23 ± 0.99

Table 2. Mean ± SD Luteinizing Hormone concentration and pulse characteristics of the CONT (n=8) and EHMC groups (n=9). AUC = Area Under the Curve, LH = Luteinizing Hormone, EHMC = Exercise Hypogonadal Male Condition, CONT = Control. No significant differences were discovered between groups.

There were no significant differences in mean luteinizing hormone concentrations or in pulse characteristics (see Table 2). There were no significant differences between groups for cortisol (EHMC: 242.92 ± 84.53 nmol·L⁻¹ vs. CONT: 287.21 ± 144.67 nmol·L⁻¹) or follicle stimulating hormone concentrations (EHMC: 3.52 ± 2.22 IU·L⁻¹ vs. CONT: 3.08 ± 0.99 IU·L⁻¹).

Aging Male Symptoms Questionnaire:

The EHMC group demonstrated significantly ($p \leq 0.05$) higher AMS scores than the CONT group (see Figure 5).

Figure 5. Mean $\pm$ SD AMS questionnaire score for the EHMC (n=9) and CONT (n=8) group. EHMC = Exercise Hypogonadal Male Condition, CONT = Control. * = Significantly ($P \leq 0.05$) different between groups.

Nutrition:

Analysis of the Food Frequency Questionnaire (FFQ) demonstrated significantly higher carbohydrate content in the EHMC group, but no other differences (see Table 3).

	EHMC (n=9) (mean $\pm$ SD)	CONT (n=8) (mean $\pm$ SD)
Calories (Kcal)	2623.1 $\pm$ 796.1	2742.8 $\pm$ 969.0
Carbohydrate (%)	48.7 $\pm$ 4.1*	36.5 $\pm$ 9.1
Protein (%)	14.5 $\pm$ 2.0	17.6 $\pm$ 3.2
Fat (%)	33.1 $\pm$ 4.2	42.1 $\pm$ 1.0

Table 3. Dietary composition as measured by a food frequency questionnaire. EHMC = Exercise Hypogonadal Male Condition, CONT = Control. * = Significantly ($P \leq 0.05$) different between groups.

DEXA

The results from the DEXA scan showed no differences in bone density or body composition (Tables 4-5).

Bone Density

Total Body

	EHMC (mean $\pm$ SD)	CONT (mean $\pm$ SD)
Total BMD (g/cm ²)	1.354 $\pm$ 0.142	1.332 $\pm$ 0.173
Total BMC (g)	3333 $\pm$ 513	3216 $\pm$ 526
Total Area (cm ²)	2455 $\pm$ 175	2410 $\pm$ 154
Total T-Score	1.5 $\pm$ 1.4	1.3 $\pm$ 1.7
Total Z-Score	1.6 $\pm$ 1.4	1.4 $\pm$ 1.4

Table 4. Total body measures of bone densitometry as measured by Dual-energy X-ray Absorptiometry (DEXA). BMD = Bone Mineral Density, BMC = Bone Mineral Content, EHMC = Exercise Hypogonadal Male Condition, CONT = Control. No significant differences were observed.

Body Composition

	EHMC (mean $\pm$ SD)	CONT (mean $\pm$ SD)
Total Bone Mass (kg)	3.3 $\pm$ 0.5	3.2 $\pm$ 0.5
Total Fat Mass (kg)	13.5 $\pm$ 3.7	15.3 $\pm$ 6.1
Total Lean Mass (kg)	60.4 $\pm$ 5.0	58.5 $\pm$ 7.8
Total Tissue Mass (kg)	73.9 $\pm$ 6.3	73.8 $\pm$ 10.3
Total Fat-Free Mass (kg)	63.6 $\pm$ 5.1	61.7 $\pm$ 8.3
Total Mass (kg)	77.2 $\pm$ 6.5	77.1 $\pm$ 10.7

Table 5. Total body measures of body composition as measured by Dual-energy X-ray Absorptiometry (DEXA). EHMC = Exercise Hypogonadal Male Condition, CONT = Control. No significant differences were observed.

Individual Differences

Figure 6. Total luteinizing hormone area under the curve for individual subjects in the EHMC group.

Chapter 5: Discussion

The primary finding of this study is that men who demonstrate low testosterone concentrations that result from high volumes of long-distance running present with similar symptoms to men with androgen deficiency (see Figure 5). Although many studies have identified this curious hormone value, both in long-distance aerobic athletes (27, 28, 35, 39, 43, 51, 52, 81, 82), as well as other populations, such as competitive wrestlers (63) and military (3), this new finding is critical as hypogonadism is not determined from a blood value, but by the presence of symptomology (4). As a result, it appears that this condition may warrant some form of treatment.

However, although these long-distance runners did score significantly higher on the Aging Male Symptoms questionnaire (AMS), the severity of the symptoms for most, but not all (described later), was apparently minor. For example, the assessments of blood hormone markers, such as Follicle Stimulating Hormone which plays a role in spermatogenesis, as well as the DEXA which assessed muscle mass and bone density, failed to show any differences between the long-distance runners and the controls (see Tables 4-5). Therefore, at present, assessments of the symptoms in men that demonstrate the EHMC do not appear

to suffer the same level of severity that has been shown in women, who have been reported to suffer from low bone density (66).

Another important new discovery in this area is that nutrition could be playing a role in the development of the EHMC. Despite running an average of over 80 miles per week at an estimated energy cost of 901 kcal/day, the EHMC group do not consume any more calories than their weight matched sedentary controls, suggesting that the long distance runners are likely in a substantial caloric deficit. A long term caloric deficit has been shown to play a significant role in the female athlete triad (17), and has been shown acutely to reduce testosterone concentrations in military male populations (3). In women who suffer from menstrual dysfunction, a 360 kcal nutritional supplement was found to reverse the condition when taken daily for 6 months (11). Although speculative, a reduction in the caloric deficit from simply increasing caloric intake could be a successful intervention for men that demonstrate this condition.

Many of the early studies in this area focused on other hormones that could be disrupted and leading to the reduced testosterone concentration, such as luteinizing hormone and cortisol. With regards to luteinizing hormone, this study failed to show any significant differences in its concentrations or pulse characteristics, although examining individual differences may be able to suggest

luteinizing hormone is playing a role (described later). In terms of cortisol, however, prior research demonstrated that hydrocortisone injections can inhibit testosterone secretion without impacting luteinizing hormone (13). In this study, subjects were instructed to rest for 48 hours prior to blood analysis, which led to cortisol concentrations being equal between the EHMC and control groups. Despite no difference in cortisol concentrations, the EHMC group still demonstrated reduced testosterone, suggesting therefore that cortisol is not playing a major role in the condition. However, cortisol is a pulsatile hormone, and this lack of difference must be viewed with caution as cortisol was only measured at one single time point.

With regards to bone density, although hypogonadal populations have been previously shown to exhibit low bone density, such as young men with a congenital disorder (48), or older populations whose testosterone has declined with age (38), this study failed to demonstrate any differences in bone density (Table 4). There are several possible explanations for this finding. Firstly, it is possible that the impact levels that these men are exposed to during high mileage running, which is a known stimulator of bone mass (reference), is able to negate the deleterious effects that low testosterone appears to have on bone mass in the other populations previously described. It is also possible that despite the EHMC subjects studied in this population having reduced

testosterone, it was still not as low as those with a congenital disorder, who have demonstrated testosterone concentrations of less than 1 nmol·L⁻¹ (48).

Therefore, it is possible that these men were still above the threshold of testosterone that is associated with low bone density. Also, these men were much younger than the older populations shown to have low bone density with low testosterone, who were over 75 years old (38). Perhaps there is an amount of time that a person has to be exposed to reduced testosterone before bone density begins to suffer, which these much younger men have not reached. It remains to be seen as these EHMC men age whether they will show a greater prevalence of osteoporosis.

The DEXA scan also revealed no differences in body composition, despite similar caloric intake and a drastic difference in caloric expenditure. Although the subjects were weight matched, it may have been expected that the control subjects would have a higher percentage of body fat, with more a higher contribution of fat free mass in the runners. However, similar results have been demonstrated in women, where reduced energy availability resulting from low caloric intake and high energy expenditure was not associated with any differences in body weight or fat free mass as measured by DEXA (55). This particular study was able to explain the curious finding by measuring resting metabolic rate, and finding a significant reduction in the energy deficient women

when compared with control (55). It appears that much like their female counterparts, these energy deficient men may have undergone energy sparing adaptations in order to compensate for their training and diet.

Individual differences

One potential subject (Outlier) was excluded from the study because despite running over 70 km per week regularly for over 10 years, he did not suffer from reduced testosterone (Outlier: $25.2 \text{ nmol}\cdot\text{L}^{-1}$ vs. EHMC: $9.16 \pm 2.28 \text{ nmol}\cdot\text{L}^{-1}$). Further analysis revealed that his caloric intake was considerably higher than the control group and the EHMC group (Outlier : 3499 vs. CONT: 2742 vs. EHMC: 2623 kcal), but his higher testosterone cannot fully attributed to the higher caloric intake as 2 other subjects from the EHMC group had similarly high caloric intakes but still exhibited low testosterone. However, this one individual does clearly demonstrate that not all athletes performing very high volumes of aerobic exercise will show reduced testosterone.

On the other hand, Subject 5 showed a testosterone concentration of more than 2 standard deviations below the mean of the EHMC group (Subject 5: $4.47 \text{ nmol}\cdot\text{L}^{-1}$ vs. EHMC: $9.16 \pm 2.28 \text{ nmol}\cdot\text{L}^{-1}$). In conjunction with the extremely low testosterone value, Subject 5 suffered from particularly high symptoms of

hypogonadism. His AMS score was well above the CONT as well as the rest of the EHMC group (Subject 5: 34.5 vs. EHMC: 27 vs. CONT: 20). Subject 5 also had very low bone mineral density (Subject 5: 1.136 vs. EHMC: 1.354 ± 0.142 vs. CONT: 1.332 ± 0.173 g/cm²). Surprisingly, Subject 5 was the newest to long-distance running in the EHMC, having only been participating in high mileage running for 2 years. Therefore, Subject 5 demonstrates that although most appear to only suffer minor symptoms of hypogonadism, some can suffer severe symptoms and these can manifest within just 2 years of regular long-distance running.

Although luteinizing hormone concentrations were not different between groups (see Table 2), a closer look at the individual differences may reveal a possible paradigm for the large variations seen in past studies regarding this hormone (Figure 6). It appears that in the majority of the subjects (7 of 9) luteinizing hormone is elevated. However, 2 subjects are clearly bringing down the average substantially. These two subjects, Subject 1 and 5, also show the highest AMS scores, and the lowest bone densities in the group and Subject 5, as mentioned, had the lowest testosterone. Although speculative, it may be that as testosterone concentrations fall, the negative feedback mechanism for the reduction of GnRH and subsequently luteinizing hormone is not initiated and therefore luteinizing hormone concentrations begin to rise. If the testis continues to fail to respond to

the elevated luteinizing hormone concentrations, it is possible that eventually the GnRH neuron could become exhausted and luteinizing hormone concentrations drop substantially. In this scenario, hypogonadism would become both a primary and a secondary condition, and could explain why these two subjects demonstrated both low luteinizing hormone as well as far more substantial symptoms of hypogonadism. Furthermore, Figure 6 might suggest that Subject 9, with very high luteinizing hormone may soon suffer a GnRH neuron exhaustion too.

In conclusion, not all aerobic athletes appear to demonstrate low testosterone. Although many aerobic athletes do suffer from symptoms similar to androgen deficiency when their testosterone is reduced, the symptoms appear to be relatively minor in most cases, suggesting that a carte blanche pharmaceutical intervention in all cases of exercise-related low testosterone would be excessive. However, just as some athletes can tolerate high volumes of running with no change in testosterone, others suffer substantial decreases and do go on to suffer significant symptoms such as low bone density. Surprisingly, despite running over 80 km per week, the EHMC group did not consume any more calories than the sedentary, weight matched controls, suggesting that nutrition could be playing a role in the condition. This is further evidenced by other populations that are also exposed to energy deficits exhibiting reduced

testosterone concentrations. Thus, the condition may not only apply to ultra-endurance athletes, but in fact, may be more widespread.

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Appendix A: Institutional Review Board Approval

Biomedical Sciences Institutional Review Board

Office of Responsible Research Practices
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Phone (614) 688-8457
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April 9, 2015

Protocol Number: 2014H0481
Protocol Title: PATHOGENESIS AND SYMPTOMOLOGY OF THE EXERCISE-HYPOGONADAL MALE CONDITION, of the Exercise-Hypogonadal Male Condition, William Kraemer, David Hooper, Human Sciences Administration
Type of Review: Initial Review
IRB Staff Contact: Matthew Carter
614-247-1357
Carter.1003@osu.edu

Dear Dr. Kraemer,

The Biomedical Sciences IRB APPROVED the above referenced research.

Date of IRB Approval: April 9, 2015
Date of IRB Approval Expiration: April 9, 2016

If applicable, informed consent (and HIPAA research authorization) must be obtained from subjects or their legally authorized representatives and documented prior to research involvement. The IRB-approved consent form and process must be used. Changes in the research (e.g., recruitment procedure, advertisements, enrollment numbers, etc.) or informed consent process must be approved by the IRB before they are implemented (except where necessary to eliminate apparent immediate hazards to subjects).

This approval is valid for one year from the date of IRB review when approval is granted or modifications are required. The approval will no longer be in effect on the date listed above as the IRB expiration date. A Continuing Review application must be approved within this interval to avoid expiration of IRB approval and cessation of all research activities. A final report must be provided to the IRB and all records relating to the research (including signed consent forms) must be retained and available for audit for at least 3 years after the research has ended.

It is the responsibility of all investigators and research staff to promptly report to the IRB any serious, unexpected and related adverse events and potential unanticipated problems involving risks to subjects or others.

This approval is issued under The Ohio State University's OHRP Federally Assurances #00006378. All forms and procedures can be found on the ORRP website - www.orrp.osu.edu. Please feel free to contact the IRB staff contact listed above with any questions or concerns.

Karla Zadnik, OD, PhD, Chair
Biomedical Sciences Institutional Review Board

hs-017-04 Approval New CR
Version 01/06/15

Appendix B: Consent Form

CONSENT
Biomedical/Cancer

IRB Protocol Number: 2014H0481
IRB Approval date: 04/08/2015
Version: 1

The Ohio State University Consent to Participate in Research

Study Title: Pathogenesis & Symptomology of the Exercise-Hypogonadal Male Condition

Principal Investigator: William J. Kraemer, Ph.D.

- **This is a consent form for research participation.** It contains important information about this study and what to expect if you decide to participate. Please consider the information carefully. Feel free to discuss the study with your friends and family and to ask questions before making your decision whether or not to participate.
- **Your participation is voluntary.** You may refuse to participate in this study. If you decide to take part in the study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your usual benefits. Your decision will not affect your future relationship with The Ohio State University. If you are a student or employee at Ohio State, your decision will not affect your grades or employment status.
- **You may or may not benefit as a result of participating in this study.** Also, as explained below, your participation may result in unintended or harmful effects for you that may be minor or may be serious depending on the nature of the research.
- **You will be provided with any new information that develops during the study that may affect your decision whether or not to continue to participate.** If you decide to participate, you will be asked to sign this form and will receive a copy of the form. You are being asked to consider participating in this study for the reasons explained below.

1. Why is this study being done?

It is well known that men that perform very high amounts of aerobic exercise (also known as 'cardio', or 'cardiovascular exercise') tend to show a reduced amount of testosterone (a hormone that essentially makes a man different from a woman) in their blood. This finding has been termed as the Exercise-Hypogonadal Male Condition (EHMC), which simply means that the high amount of exercise that a person is performing seems to be related to their low testosterone. Unfortunately, very little is known about the ultimate cause of the low testosterone, and surprisingly, it is currently unknown if there are even any negative effects that result from the low testosterone.

35 You are being asked to participate in the study as either an individual who performs very
36 high volumes of aerobic exercise that has resulted in a change in your blood testosterone,
37 or as a healthy control to compare that particular population to.
38

39 **2. How many people will take part in this study?**

40 We are looking to recruit up to 15 EHMC individuals and 15 matched controls.
41

42 **3. What will happen if I take part in this study?**

43 The study will involve two separate assessments that can be performed on the same day,
44 with schedules permitting.
45

46 **DEXA**

47 The first assessment is called a dual energy x-ray absorptiometry (DEXA) scan, which
48 will measure the amount of fat and muscle in your body as well as your bone density.
49 Before this procedure, you will need to refrain from eating the night before the scan and
50 have had plenty of water to drink. To ensure you are well hydrated, we will encourage
51 you to drink two cups of water the night before the assessment, and two cups of water the
52 morning of. In order to make sure you have drank enough water, we will request a urine
53 sample which we can check immediately. If you have not drank enough water, we will
54 simply ask you to drink more water and test again until you pass the urine test.
55

56 **Blood Draws**

57 Blood draws will be performed in after 12 hours without eating, which includes sleep
58 time. Therefore, we will ask you to not eat any breakfast before reporting to the lab. As
59 the hormones vary throughout the day, many blood draws are required for several hours.
60 This means that blood draws will begin at 08:00 hours and continue every fifteen minutes
61 until 12:00 hours, for a total of 17 draws. The blood draws will be performed with the use
62 of something called an indwelling catheter, which is a sheath that covers the needle and
63 enables us to only prick your skin once. The catheter remains in the vein throughout the
64 17 draws, but you will likely forget that it is in there as you cannot feel it. For each draw,
65 only 5ml of blood will be taken, leading to a total of 85ml, which is considerably less
66 than would be taken when a person donates blood.
67

68 The samples that you provide will later be analyzed for only the following hormones:

Analyte	Purpose of Measurement
Testosterone	Assesses the ability of the testicles to make testosterone
Luteinizing hormone (LH)	LH is the signal that tells the body to make testosterone.
Follicle Stimulating Hormone (FSH)	FSH will be a measure of hypogonadism (low testosterone) as it is responsible for making sperm.
Prolactin	If the amount of prolactin is either too small or too large it can result in changes in your testosterone amount.
Cortisol	Increases in cortisol can prevent your testicles from being able to release testosterone

69
70 Questionnaires
71 During the blood draws, you will be asked to complete a questionnaire that will ask you
72 questions that pertain to whether you experience symptoms typically associated with
73 hypogonadism (low testosterone). You will also be asked to complete a food frequency
74 questionnaire, which is a form about which foods you typically eat and how often you eat
75 them.

76
77 Following participation, you will be provided snacks and water.
78

79 **4. How long will I be in the study?**
80

81 The total time commitment for the study will be no more than 6 hours. If the DEXA scan
82 can be scheduled before the blood draws, these visits can be performed on the same day.
83
84

85 **5. Can I stop being in the study?**
86

87 You may leave the study at any time. If you decide to stop participating in the study,
88 there will be no penalty to you, and you will not lose any benefits to which you are
89 otherwise entitled. Your decision will not affect your future relationship with The Ohio
90 State University.
91
92

93 **6. What risks, side effects or discomforts can I expect from being in the study?**
94

95 There are slight risks associated with the DEXA scan as it utilizes X-Ray. However, the
96 amount of X-Ray exposure is extremely low, in fact it is approximately 1/30th the amount
97 of X-Ray exposure in a typical chest X-Ray. The scan will also be performed by a
98 licensed General Machine X-Ray Operator. There are also slight risks of discomfort and
99 bruising associated with blood draws. This will be minimized, however, by the use of an
100 indwelling catheter to prevent multiple sticks, and a trained phlebotomist will perform the
101 draws.
102

103 **7. What benefits can I expect from being in the study?**
104

105 By participating in the study you will receive information about the amount of muscle and
106 fat in your body as well as your bone density. You will also receive data on your
107 hormonal profile.
108

109 **8. What other choices do I have if I do not take part in the study?**
110

111 You may choose not to participate without penalty or loss of benefits to which you are
112 otherwise entitled.

113 **9. Will my study-related information be kept confidential?**

114

115 Efforts will be made to keep your study-related information confidential. However, there
116 may be circumstances where this information must be released. For example, personal
117 information regarding your participation in this study may be disclosed if required by state
118 law.

119

120 Also, your records may be reviewed by the following groups (as applicable to the
121 research):

122

- 123 • Office for Human Research Protections or other federal, state, or international
124 regulatory agencies;
- 125 • U.S. Food and Drug Administration;
- 126 • The Ohio State University Institutional Review Board or Office of Responsible
127 Research Practices;
- 128 • The sponsor supporting the study, their agents or study monitors; and
- 129 • Your insurance company (if charges are billed to insurance).

130

131 If this study is related to your medical care, your study-related information may be placed
132 in your permanent hospital, clinic, or physician's office records. Authorized Ohio State
133 University staff not involved in the study may be aware that you are participating in a
134 research study and have access to your information.

135

136 A description of this clinical trial will be available on <http://www.ClinicalTrials.gov>, as
137 required by U.S. law. This website will not include information that can identify you. At
138 most, the website will include a summary of the results. You can search the website at
139 any time.

140

141 You may also be asked to sign a separate Health Insurance Portability and Accountability
142 Act (HIPAA) research authorization form if the study involves the use of your protected
143 health information.

144

145 **10. What are the costs of taking part in this study?**

146

147 If you need to park on campus, you will be required to pay for parking.

148

149 **11. Will I be paid for taking part in this study?**

150

151 You will be paid \$50 for participating in the study.

152

153 **12. What happens if I am injured because I took part in this study?**

154

155 If you suffer an injury from participating in this study, you should notify the researcher or
156 study doctor immediately, who will determine if you should obtain medical treatment at
157 The Ohio State University Wexner Medical Center.

The cost for this treatment will be billed to you or your medical or hospital insurance. The Ohio State University has no funds set aside for the payment of health care expenses for this study.

13. What are my rights if I take part in this study?

If you choose to participate in the study, you may discontinue participation at any time without penalty or loss of benefits. By signing this form, you do not give up any personal legal rights you may have as a participant in this study.

You will be provided with any new information that develops during the course of the research that may affect your decision whether or not to continue participation in the study.

You may refuse to participate in this study without penalty or loss of benefits to which you are otherwise entitled.

An Institutional Review Board responsible for human subjects research at The Ohio State University reviewed this research project and found it to be acceptable, according to applicable state and federal regulations and University policies designed to protect the rights and welfare of participants in research.

14. Who can answer my questions about the study?

For questions, concerns, or complaints about the study you may contact David Hooper at hooper.131@osu.edu, or the principal investigator, Dr. William J. Kraemer at krnemer.44@osu.edu or 614-688-2354 (office telephone).

For questions about your rights as a participant in this study or to discuss other study-related concerns or complaints with someone who is not part of the research team, you may contact Ms. Sandra Meadows in the Office of Responsible Research Practices at 1-800-678-6251.

If you are injured as a result of participating in this study or for questions about a study-related injury, you may contact David Hooper at hooper.131@osu.edu.

Signing the consent form

I have read (or someone has read to me) this form and I am aware that I am being asked to participate in a research study. I have had the opportunity to ask questions and have had them answered to my satisfaction. I voluntarily agree to participate in this study.

I am not giving up any legal rights by signing this form. I will be given a copy of this form.

_____ Printed name of subject	_____ Signature of subject
	_____ Date and time
	AM/PM
_____ Printed name of person authorized to consent for subject (when applicable)	_____ Signature of person authorized to consent for subject (when applicable)
	_____ Date and time
	AM/PM
_____ Relationship to the subject	

Investigator/Research Staff

I have explained the research to the participant or his/her representative before requesting the signature(s) above. There are no blanks in this document. A copy of this form has been given to the participant or his/her representative.

_____ Printed name of person obtaining consent	_____ Signature of person obtaining consent
	_____ Date and time
	AM/PM

Witness(es) - May be left blank if not required by the IRB

_____ Printed name of witness	_____ Signature of witness
	_____ Date and time
	AM/PM
_____ Printed name of witness	_____ Signature of witness
	_____ Date and time
	AM/PM