

**Male Endurance Athlete Tetrad**

**DISSERTATION**

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By

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## Abstract

In 2011 greater than 518,000 marathon finishing times were recorded in the USA, nearly 60% were male. It is commonly accepted that regular weight-bearing physical activity can promote bone health throughout the lifespan. However, available literature suggests male runners may have a decreased bone mineral density (BMD), specifically in the lumbar spine and radius. Researchers speculate a decrease in BMD may be the result of several factors including inadequate diet, low testosterone, and/or genetic predisposition. Decreased testosterone levels are present in males engaged in prolonged endurance activities such as running. Energy availability (EA) is the amount of dietary energy remaining for other body functions after exercise training. Low EA may be the underpinning factor for decreases BMD, and is the outcome of inadequate dietary intake and/or increasing exercise energy expenditure. **PURPOSE:** To explore the relationship between EA, testosterone level, blood lipids and BMD in male athletes. **METHODS:** Dual-Energy X-Ray Absorptiometry (DXA) bone density scans were completed for total body, non-dominant hip, lumbar spine, non-dominant forearm, and body composition. Additionally, blood samples, three-day food and activity records, and three-day accelerometry data were collected and analyzed. **RESULTS:** A total of 41 subjects completed the study. Bone density results revealed that 5 of 41 subjects had bone insufficiency (BMD Z-score  $\leq -1$ ) in the femoral neck, 13 in the lumbar spine (L1-L4) and 18 in the ultra-distal radius. Ten of 41 subjects presented with low testosterone and

24 subjects had a suboptimal blood lipid profile. Energy availability results revealed that mean EA was 32.1 kcals / kg FFM and 19 of 40 subjects consumed less than 30 kcals / kg FFM. **CONCLUSION:** The detriments of low bone density can be severe and irreversible and male endurance runners are practicing habits that may support low BMD. Consequently, it is important to gain more insight into the nutrition attitudes and behaviors of male distance runners that possibly elicit negative consequences to help future prevention and education efforts.

## **Dedication**

This document is dedicated to my wife, Kristen, for her continued love, support and encouragement.

To my family who has always been a source of pride and inspiration.

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## Publications

1. CK Spees, **JM Scott** and CA Taylor. Differences in the Amounts and Types of Physical Activity by Adult Obesity. American Journal Health Behavior. 2012 January;36(1): 56-65.
2. **JM Scott**, CK Spees, CA Taylor and R Wexler. Racial Differences in Barriers to Hypertension Control in a Family Practice Setting. Journal of the Primary Care and Community Health. 2010 October;1(3): 200-205.

3. **JM Scott**, L McDougle, KP Schwarian, and CA Taylor. Differences in the Dietary Intake Habits by Diabetes Status for African American Adults. *Ethnicity and Disease*. 2010 Spring;20(2):99-105.
  
4. KD Evans, **JM Scott**, CA Taylor, ME Geraghty and CD Ashcraft. Quantitative ultrasonography of calcaneal bone mass and its relationship to calcium consumption among impoverished hispanic women. *Journal of Diagnostic Medical Sonography*. 2009;25(3):127.

### **Fields of Study**

Major Field: Health and Rehabilitation Sciences

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## **Chapter 1: Introduction and Background**

The surgeon General stated that 20% of males will develop osteoporosis during their lifetime (1). At further risk are male distance runners who have demonstrated shifting bone densities, and yet, little research has occurred in this area. Over the last 20 years, however, substantial research has been conducted on female runners to investigate the female athlete triad (FAT), energy availability, bone mineral density, and menstrual status (2-10). Definitions and research have focused primarily on females, and there is recent interest to determine if males sustain a similar occurrence of symptoms.

Ultimately, we wanted to evaluate trained male runners to see if similar characteristics to the FAT exist. To date, no study has explored the link between energy availability and testosterone, or energy availability and bone mineral density (BMD) and cardiovascular health in male athletes (11). There is speculation that a male equivalent exists. Thus, the four components we were interested in studying were BMD, energy availability, testosterone level, and cardiovascular health in a cohort of trained male runners. We have coined the term Male Endurance Athlete Tetrad (MEAT) to describe the model, see Figure 1.

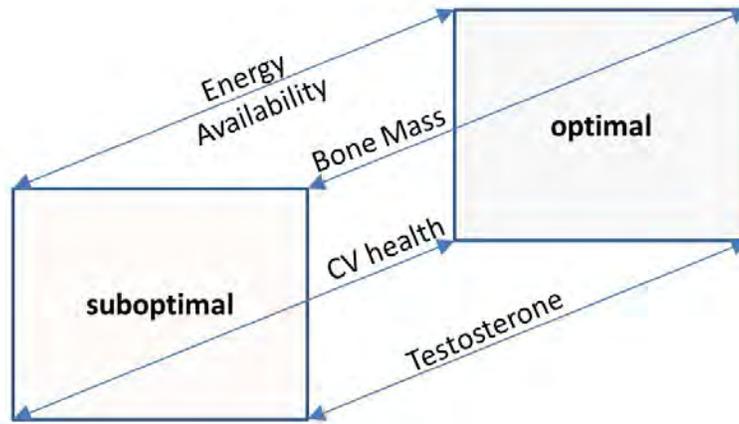


Figure 1: Visual Representation of the Male Endurance Athlete Tetrad

### **Background on Female Athlete Triad**

Historically, FAT was first described by Yeager et al in 1992 when the American College of Sports Medicine (ACSM) assembled a panel of experts to address this increasing area of concern within sports medicine (2). At that time, the panel defined the FAT as the sum of three disorders including: disordered eating, amenorrhea, and osteoporosis (2). The panel also noted that elite athletes may be at greatest risk, especially those in endurance and appearance sports such as gymnastics or dance (2).

In 1997, the ACSM released a formal position paper on FAT. Hallmark characteristics of each disorder (disordered eating, amenorrhea, and osteoporosis) of the triad were outlined (3). Disordered eating referred to a variety of harmful behaviors used to lose weight or achieve a lean appearance (3). Amenorrhea was defined based on the age, menarche, and pubertal development. “Primary amenorrhea was marked by absence

of menstruation by 16 years of age and absence of secondary sex characteristics and secondary amenorrhea was the absence of three or more consecutive menstrual cycles after menarche” (3). The statement adopted the World Health Organization’s definition of osteoporosis as a bone mineral density (BMD) greater than 2.5 standard deviations below the mean of young adults (T-score) (3). Similarly, the position paper also recognized osteopenia as bone mineral density (BMD) between 1 and 2.5 standard deviations below the mean of young adults, and normal BMD as no more than 1 standard deviation below the mean of young adults (3). Otis and colleagues (3) theorized that the pressure placed on young women to achieve or maintain unrealistically low body weight was the underpinning factor leading to the development of FAT. Consequently, adolescents and women training in sports in which low body weight is emphasized for athletic activity or appearance are considered at greatest risk. Otis et al (3) also recommended that individuals with one component of the triad should be screened for the other two components. This advice still carries today.

The current position on FAT was released in 2007 by ACSM with a few modifications (4). The current FAT paradigm including the inter-related spectrums of energy availability, menstrual status and bone mineral density (BMD) are depicted in Figure 2 where the arrows are used to show the relationship between components and are intentional from the research literature (4, 11, 12). Each component of the triad falls along a continuum from health to disease (4). Specifically, BMD refers to a spectrum of bone health ranging from optimal bone health to osteoporosis (4). ACSM defines osteoporosis as an Age-Matched Z-score less than or equal to 2 standard deviations below the mean (gender and ethnicity matched) (4). Low bone mass, formerly

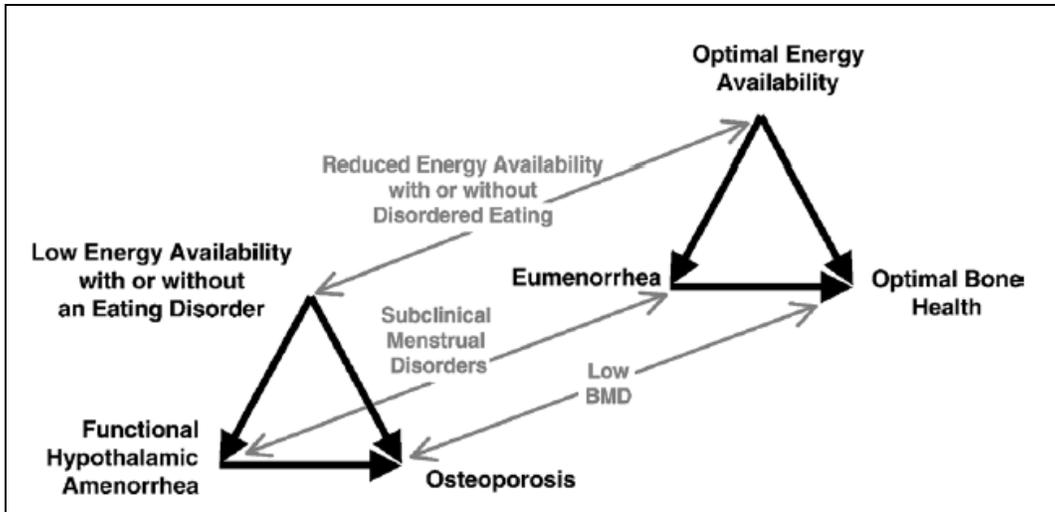


Figure 2: Graphic representation of the Female Athlete Triad and corresponding components. Adapted from Nattiv et al 2007 (4).

osteopenia, is defined as an Age-Matched Z-score between -1.0 and -2.0 (4). Normal bone mass is now defined as an Age-Matched Z-score greater than -1.0 (4). Because the Z-score compares the BMD of an individual to age, ethnicity and gender-matched controls, these same guidelines can be considered for male athletes.

The nutritional paradigm of the triad has significantly shifted from disordered eating to energy availability. Energy availability (EA), defined as dietary energy intake minus exercise energy expenditure normalized to fat free mass (FFM), can also fall along a continuum from optimal EA to low EA (4). Adequate EA is defined as greater than or equal to 30 kcals/kg FFM per day, whereas low EA is defined as less than 30 kcals/kg FFM per day; however, up to 45 kcals/kg FFM may be required for optimal health. Nattiv et al (4) recognize that low EA may be the result of intentional (disordered eating)

or unintentional behaviors (lack of knowledge). In general, low energy availability is caused by restricting dietary energy intake (intentional or unintentional), increasing exercise energy expenditure or a combination of both (4, 11). Although there is little research on energy availability or balance in male runners the same definition (calculation) of energy availability can be adopted. Due to limited research, it is less clear if the adequate and optimal values for EA are consistent for males

Menstrual function now encompasses a spectrum from eumenorrhea to amenorrhea. Eumenorrhea is defined as menstrual cycles that occur on a regular basis, every 28 days (plus or minus 7 days) (4). Oligomenorrhea is defined as menstrual cycles with longer than 35 day intervals. Primary amenorrhea is a delay in menarche beyond 15 years of age and secondary amenorrhea is the absence of menstrual cycles lasting greater than 90 days after menarche (4). The hypothalamic-pituitary-ovarian axis in females is analogous to the hypothalamic-pituitary-testicular axis in males; however, it is more difficult to measure the effects of testosterone. Unlike estrogen, testosterone levels are not dependent on timing of a monthly cycle, thus are used as the marker of the testicular axis.

Low energy availability appears to be the driving factor behind FAT. When energy availability becomes too low, physiological mechanisms reduce the amount of energy used for reproduction, growth and cellular maintenance (11, 13). As a result, estrogen levels are reduced and consequently decreases in bone density occur. Nattiv et al (4) recognize those at the greatest risk for low energy availability are individuals who restrict dietary energy intake, who exercise for prolonged periods, who are vegetarian and who limit the variety of food they eat.

In recent years, there has been much debate about the inclusion of a fourth component to suggest a tetrad. In 2005, Rickenlund et al (14) concluded that athletic amenorrhea was associated with endothelial dysfunction and an unfavorable lipid profile in a group of female runners. Similarly, Hoch et al (12) suggested cardiovascular dysfunction may exist within young athletic females with triad symptoms because menopause is known to accelerate cardiovascular risk. Normal estrogen levels can reduce low-density lipoprotein cholesterol, increase high-density lipoprotein cholesterol, and as a result women with reduced estrogen are at an increased risk for cardiovascular and endothelial dysfunction (12, 15). Interestingly, endothelial function was improved as women regained regular menses (15). Perregaux et al (16) also demonstrated that menopause was associated with reduced endothelium-dependent dilation, a risk factor for cardiovascular disease. Ultimately, Lanser and colleagues (17) recommended including cardiovascular dysfunction as part of the triad thereby creating a tetrad.

#### **Available research on male runners**

Limited BMD and energy availability research is available on male runners. Available literature suggests male runners may have a decreased bone mineral density (BMD), specifically in the lumbar spine and radius (18-22). Decreased BMD may be the result of low testosterone levels, inadequate diet and/or genetic components (23, 24). Additional studies found decreased testosterone levels in males engaged in prolonged endurance activities such as running (25-28). These studies regarding energy availability and cardiovascular risk issues suggest a similar male athlete triad (or tetrad) condition similar to the female athlete triad.

Energy availability, defined as the amount of dietary energy or Calories remaining after exercise training needed to sustain other metabolic processes, may be the underpinning factor (28, 29). To date, no study has explored the link between energy availability and testosterone, or energy availability and BMD and cardiovascular health in male athletes (11). Speculation exists that a male equivalent exists; however no formal study has been reported.

### **Male Endurance Athlete Tetrad and Assessment**

The Male Endurance Athlete Tetrad (MEAT) represents the interrelationships between energy availability, bone density, testosterone level, and cardiovascular health. To evaluate the presence of MEAT within a cohort of trained male runners, we examined four components: bone health (density), energy availability, testosterone levels, and cardiovascular health (lipid panels). BMD can be assessed using Dual-Energy X-ray Absorptiometry (DXA). Energy availability can be estimated using a written three-day food record coupled with an accelerometer and/or tracking activity for three days using the Bouchard grid. Both testosterone and blood lipids can be quantified with a fasting morning blood draw.

### **Bone**

#### *Bone Physiology*

Bone is a dynamic tissue responsible for structural adaptations in response to loading, protecting vital organs and maintaining mineral homeostasis. Bone can be classified as either cortical (compact) or trabecular (spongy). Bones can also be classified based on shape into one of four broad categories: long bones, short bones, flat bones, or irregular bones. Long bones consist of a shaft and two ends, and have one end

longer than the other. In contrast, short bones are nearly equal in both length and diameter. Flat bones are typically found in the skull and sternum and are composed of two layers of compact bone with a layer of spongy bone in the middle. Lastly, irregular bones consist of any bone that does not fit into any of the preceding categories such as vertebrae. Mature bones are composed of cylindrical units known as osteons or Haversian systems. Bone is innervated and has a vascular system within the Haversian canal whereby nutrients can be delivered (30, 31). Osteogenesis, including exercise-dependent changes, likely depends on this complex network.

Bone has a mineralized extracellular matrix allowing it to support fleshy structures, protect vital organs, and house bone marrow (31, 32). The main structural components of bone are type I and type V collagen, which make up nearly 90% of the bone matrix. The matrix also contains other components such as ground substance in the form of glycosaminoglycans, glycoproteins, and sialoproteins. Collagen and the ground substance are mineralized to form bone. Lacunae are spaces found within the bone matrix that contain osteocytes (bone cells) (31). Osteocytes are responsible for maintaining the viability of the bone tissue. Canaliculi are also found throughout the bone matrix and connect adjacent lacunae. Bone also contains three other unique cells: osteoprogenitor cells, osteoblasts, and osteoclasts (31, 33). Osteoprogenitor cells are precursors to osteoblasts. Osteoblasts are located at the surface of bone tissue and are responsible for the synthesis of the bone matrix, while osteoclasts are found within the lacunae and are responsible for the breakdown and resorption of bone tissue (30, 31, 33). The balance of the osteoblast to the osteoclast activity controls the balance of bone remodeling (30). The activation of additional osteoblasts or the inhibition of osteoclasts

results in net osteogenesis to improve bone density. Weight-bearing exercise (under well-fueled circumstances) may activate osteoblasts while estrogen is known to inhibit osteoclasts providing a framework for the bone changes associated with the triad (34, 35)

#### *Bone Mineral Density*

Bone mineral density (BMD) is the measure of the amount of minerals within a specific volume and is proposed to be a surrogate measure of the strength of bone. Loss of minerals over time, such as calcium, causes a decrease in bone mass resulting in weaker and more porous bone. The male skeleton reaches peak mass at approximately age 20 and remains fairly stable across young adulthood if all factors support stability (36). Adolescence is the most crucial time for acquiring bone mass and increasing BMD (36). In males, total body BMD remains stable until approximately age 50. The male skeleton should be stable between 20 and 50 years of age.

#### *Impact of Exercise on Bone System and Mechanostat Theory*

Dr. Julius Wolff (1892) is often credited with the development of the foundational framework used to explain how bone changes (or adapts) over time in response to mechanical stresses. The framework proposed by Wolff became known as Wolff's Law. Translated from German, Wolff's Law states "Every change in the form and function of bone or of their function alone is followed by certain definite changes in their internal architecture, and equally definite alteration in their external conformation, in accordance with mathematical laws" (37).

Nearly 100 years later, in 1987 Dr. Harold Frost proposed and developed a paradigm to explain the growth, modeling and remodeling of bone (38). The basis for much of Frost's work followed the basic premise of Wolff's Law whereby bone responds

to stress. Frost used the term “mechanostat” to describe a proposed sensing mechanism within the bone that can be influenced by outside factors to change bone mass and shape. In his theory, Frost proposed that bone has different set-points whereby remodeling can be “turned on” or “turned off” based on the amount of stress the bone encounters. A list of terms commonly used by Frost are presented below to help further explain the Mechanostat Theory (38):

- MU: mechanical usage, including any strain(s) applied to the skeleton
- MES: minimal effective strain, measured in units of microstrain or  $\mu\text{E}$
- r: the amount of strain below which the remodeling (breakdown) process is activated
- m: the amount of strain above which the modeling (building) process is activated
- p: the amount of strain above which “plastic” changes in the bone occur or the bone microdamage: threshold above which woven bone is formed instead of normal lamellar bone

MU to the skeleton produces strain on the bone and depends on the architecture and arrangement of the bone. Under ideal conditions, the MU will match the properties of the bone and strain will be maintained between 100-1000  $\mu\text{E}$ . If MU is low, as in bed rest or in a microgravity environment, the mechanostat will turn “on,” bone remodeling occurs and is accompanied by a subsequent decrease in bone loss. Alternatively, when MU exceeds the bone design, typically above 1000  $\mu\text{E}$ , the mechanostat will turn “on” bone modeling in an effort to strengthen the bone. Consistent strains above 3000  $\mu\text{E}$ , will result in rapid production of weak, woven bone and decreases in strong, lamellar bone in

order to compensate for the high strain. The fracture threshold for bone is estimated at approximately 25,000  $\mu\text{E}$ .

In a later paper, Frost attempted to explain the low bone density observed in distance runners (39). Frost theorized that an increase in muscular endurance does not depend on and is not accompanied by an increase in bone strength (39). Therefore, if bone strains stayed below the threshold needed to stimulate bone growth, no matter how much prolonged running is completed subsequent increases in bone density would not occur (39).

Only two studies have measured strain in vivo with human subjects (40, 41). Lanyon et al (40) placed a strain gauge on the anteromedial portion of the tibial midshaft on a single subject. Strains were measured while the subject walked on either the treadmill or floor, and un-weighted or carrying various amounts of weights (up to 71 kg). The greatest observed strain in the study was approximately 800  $\mu\text{E}$ , below the 1000  $\mu\text{E}$  threshold proposed by Frost whereby bone modeling is “turned on” in an effort to strengthen the bone (40). As expected, strains produced during running were two to three times higher than strains recorded during walking (41).

A second study by Burr et al placed two strain gauges, one at the midshaft of the tibia and the other 2 cm below the midshaft, on two subjects and observed changes in strain during four activities (walking, walking with a 17 kg pack, running, and zigzagging) (41). Results indicated that strains produced during vigorous activity, including uphill zigzagging and sprinting on a level surface, were highest and exceeded the 1000  $\mu\text{E}$  threshold but were still modest at less than 2000  $\mu\text{E}$ . Strains observed in both studies were well below the 25,000  $\mu\text{E}$  fracture threshold and even below the 3000

$\mu\text{E}$  threshold which has previously been proposed as the threshold whereby bone remodeling becomes weaker (38). The Lanyon study strains failed to meet the threshold of 1000 microstrains necessary to stimulate osteogenesis while the Burr study of more intense activities did meet the theoretical mechanostat requirements.

Although the mechanostat theory offers an explanation of bone change in microstrains and thresholds, others use the measure of ground reaction forces (GRF) to describe the potential osteogenic impact on bone. GRF is the equal and opposite force exerted by the ground on the body while doing various activities, and can be expressed as numeric values relative to body weight. Strain gauge force plates are commonly used to quantify the amount of GRF produced during an activity (42). While GRF of various activities are undoubtedly attenuated or amplified by foot wear and athlete mechanics, mean values for various activities are expressed in the literature for some activities.

A study by Fehling et al compared the differences in BMD between impact loading sports (volleyball and gymnastics) to active loading (swimming) and controls (43). After controlling for height and weight, the volleyball group had 5-19% higher BMD at most measured sites (total body, lumbar spine and proximal femur) compared to controls and swimmers. The volleyball group also had higher BMD in the lower extremities compared with the gymnasts; however, the gymnasts had 11% higher BMD in the upper extremities compared to the volleyball group. No significant differences were found between the swimmers and controls.

A second study by Robinson et al compared the bone mass of gymnasts to runners and controls (44). Lumbar spine and total BMD was significantly lower in runners compared to both gymnasts and controls. Runners also had lowest BMD at the femoral

neck. Overall runners had lower BMD at all measured sites compared to gymnasts despite a similar prevalence of amenorrhea. The researchers speculate that the intensive weight-bearing nature of gymnastics provides protective increases in BMD compared to runners.

Greater GRFs produced during activity have been well-related to improved osteogenic outcomes. Previous studies in children have found that a minimum GRF of 3.5 times body weight (per leg) is needed to produce beneficial osteogenic effects (45-47). In children, activities such as jumping are able to create GRFs in excess of 8 times body weight (45-47). Previous studies have revealed that GRFs during running rarely exceed 3 times body weight and did not differ between those with a previous history of lower-extremity stress fractures and those with no history (48-55). Additionally, the GRF in the lumbar spine during running is less than two times body weight (56), whereas weight lifters may experience loads of up to 36 times body weight during heavy lifting (57). Although running is weight bearing, running appears to fall below the minimum threshold needed to have osteogenic benefits. Therefore, as one moves vertically up the body, running GRFs and subsequent loading on the bones are predicted to decrease.

#### *Assessment of BMD*

##### Dual-Energy X-ray Absorptiometry (DXA)

X-ray technology has long been used to diagnosis bone disease. Dual-Energy X-ray Absorptiometry (DXA) was developed during the 1980's and became widely available for clinical use during the late 1990's. Improvements in DXA technology were motivated by the clinical use of the DXA to diagnosis osteoporosis (58). DXA machines are now used to measure bone mineral density (BMD) for the total body, anteroposterior

(AP) or lumbar vertebrae (L1-L4 or L2-L4), total femur and corresponding regions (femoral neck, upper neck, trochanter and Ward's area), and total radius and corresponding regions (ultradistal-UD and 33% sites). The DXA has also demonstrated a wide application in the ability to estimate body composition (fat mass versus lean tissue mass) (59).

DXA technology utilizes a dual-energy filtered x-ray beam along with specialized computer software analysis algorithms to estimate fat mass, lean tissue mass and bone mineral content (58). When x-rays are passed through a tissue, they are attenuated or weakened based on the tissue electron density. The more electron dense the tissue, the more the energy is attenuated. DXA uses two low level x-ray beams at two different energy levels to predict bone mineral content, fat mass and lean tissue mass. Proprietary algorithms developed by different manufactures rely on previously established constants to determine and differentiate between tissue types (60, 61). Figure 3 is a visual representation of the fundamental principles of how DXA functions.

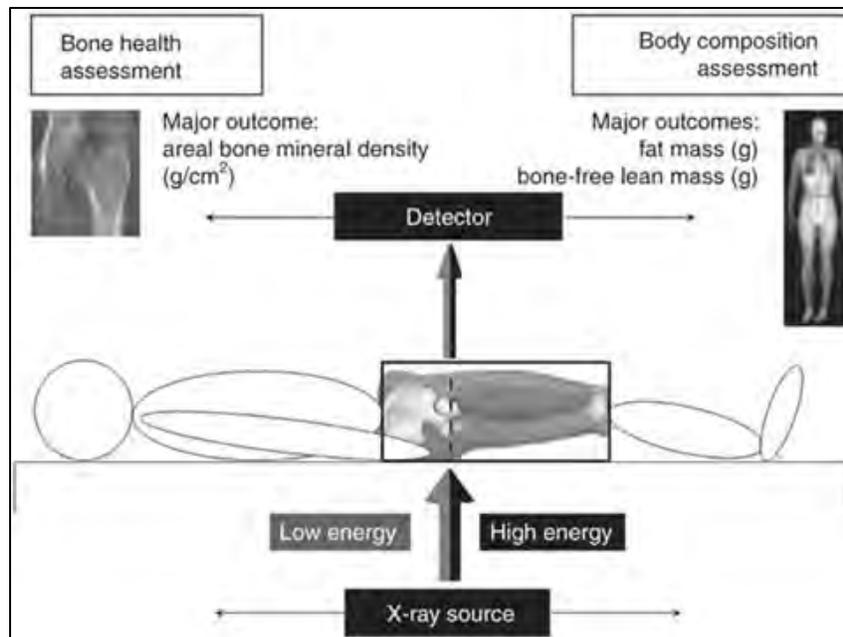


Figure 3: Fundamental principles of how DXA functions and estimates bone mineral content, fat mass and lean mass. Adapted from Toombs et al 2012 (62).

DXA is recognized as the accepted standard when determining BMD (63).

Previous research has found excellent in vivo precision for BMD measurements for the total body (CV 0.63%), lumbar spine (CV 0.41%), and hip (CV 0.53%) when using the GE Lunar iDXA (64, 65). Additionally, excellent in vivo precision has been established for the Lunar iDXA for measurements of body composition including lean tissue mass (CV 0.50%), total fat mass (CV 0.82%), and total bone mineral content (CV 0.60%) (65).

The position of the International Society of Clinical Densitometry (ISCD) recommends the screening for osteoporosis of men, without risk factors, begin at age 70. However, the ISCD suggests screening should be completed earlier if known risk factors including dementia, poor health, recent falls, prolonged immobilization, smoking, alcohol abuse, low body weight, vitamin D deficiency, hypogonadism, history of fragility

fracture in a first degree relative, or steroid use for more than three months have been identified (66, 67). When testing males under the age of 50, the ICSD recommends using age-matched Z-scores (Z-scores) when comparing BMD measurements. The Z-score is an age, gender, and ethnicity-adjusted value corresponding to the number of standard deviations above or below average BMD and is based on normative values from reference database (66). Using Z scores for comparison of runners to age-matched controls is consistent with ISCD recommendations as well as the current guidelines for the already developed female athlete triad.

ACSM cites that athletes in weight-bearing sports tend have a 5–15% higher BMD compared to non-athletes (43, 44, 68). Consequently, ACSM suggests if an athlete presents with a BMD Z-score  $\leq -1.0$ , further investigation is necessary to help determine what has contributed to the low BMD, even in the absence of a prior fracture. In females, ACSM goes further to define normal BMD as a BMD Z-score  $> -1$ , low BMD as BMD Z-score between  $-1$  and  $-2$ , and osteoporosis is classified as having a BMD Z-score  $< -2$  (4). Since the ACSM classification of bone health takes into consideration the unique characteristics of the athlete, the use of ACSM's classifications for bone health will be used.

#### Quantitative Computed Tomography (QCT)

Not all researchers believe BMD is the best measure of bone strength (69-71). Similar to DXA, QCT measures attenuation of radiation as it passes from the source, through the object of interest, and into the detector. QCT is mainly used in research to measure volumetric density ( $\text{mg}/\text{cm}^3$ ) of both cortical and trabecular bone. Also, QCT is capable of producing three-dimensional images of bone and can distinguish between

cortical and trabecular bone content. Bone geometry can be determined using QCT, which is used to predict bone strength. Some research suggests that bone geometry is the most accurate way to predict bone strength (69-71). Recent literature examining athletes demonstrates a change in shape of the bone where three dimensional volume and density of the bone would improve bone strength (72-74). This same literature makes the case that DXA technology is not sensitive to the shape and three dimensional volume changes, thus underestimates the potential strength of bone in athletes.

QCT also has many limitations to consider. Previous research indicates that QCT has poor precision (up to 4%) and accuracy errors up to 15% (75, 76). It also provides a greater radiation dose compared to DXA. A single spine assessment using QCT may provide a radiation dose up to 200 millirem (mrem) compared to less than 1 mrem using DXA. A third limitation of QCT is its inability to determine total body composition. Since QCT is site specific, it is unable to predict total body composition. Lastly, no standardized procedures exist for completing QCT studies (69-71). The use of DXA to estimate BMD as a surrogate for bone strength is still considered a valid measure due to the limitations of QCT.

### **Energy Availability (balance of physical activity and nutrition)**

Energy availability (EA) is commonly defined as dietary energy intake, calories consumed or energy intake (EI), minus exercise energy expenditure or calories expended during exercise (EEE), normalized to fat free mass (FFM) (4).

$$EA = (EI - EEE) / FFM, \text{ with a unit of kcal/kg FFM}$$

Energy availability represents the amount of energy (calories) available for the body to use for physiological functions, including cellular maintenance and growth, reproduction

and thermogenesis, after exercise training. When energy availability is too low in females, generally less than 30 kcals/kg FFM, some body functions (such as reproduction) are impaired in an effort to promote survival (77). Energy availability can be reduced as a result of increasing exercise energy expenditure, by reducing energy intake, or a combination. Regardless of whether energy availability is reduced intentionally or unintentionally, similar impairments on health result.

Loucks and Thuman demonstrated that luteinizing hormone pulse frequency significantly decreases, in females, when EA falls below 30 kcals / kg FFM ( $P < 0.05$ ) (78). The inhibition of the pulsatile release of LH causes a suppression of ovarian steroidogenesis. Loucks and Thuman further theorize that the brain relies on a constant supply of glucose to maintain homeostasis and if the supply of glucose to the brain is not sufficient, subsequent decreases in the levels of sex hormones will result. In a second study, Ihle and Loucks (79) found significant ( $p < 0.05$ ) decreases in biomarkers related to bone formation (breakdown and remodeling) when EA fell below 30 kcals / kg FFM. Emerging literature on female athletes suggests that a minimum of 45 kcals / kg FFM is needed in order to maintain optimal skeletal health (77, 136).

High mileage training results in increased EEE and potential to increase bone resorption (breakdown) if sufficient calories are not consumed (79). Evidence has established a link between negative calorie balance, reduced bone formation and low BMD in females (79). Research also supports that prolonged aerobic exercise suppresses *ad lib* food intake in men and females (80-83). Therefore, the coupling of high mileage training with a suppressed appetite may increase the risk for low EA and subsequent low BMD.

Westerterp et al. (80) studied the long-term effects of physical activity on energy balance in a group of 16 male participants under free living conditions. Participants were sedentary, had a BMI between 19.4-26.4, and participated in less than one hour of physical activity per week. Over the course of the 44-week study, participants completed an aerobic endurance training regimen that concluded with running a half marathon (13.1 miles). EI was measured by a 7-day written food record at baseline, weeks 8, 20 and 40. Similarly, energy expenditure was measured using the gold standard doubly labeled water (DLW) over the same time period. Weeks 20 - 40 represented the greatest increase in endurance training, with sessions four days per week, lasting between 30 and 90 minutes. Interestingly, energy expenditure increased nearly 30% from baseline to week 20 while EI decreased by 5% over this same time period. Thus, on average, subjects were expending nearly 800 more calories daily than they were consuming (energy expenditure = 3486 calories vs EI = 2698 calories). These results support that there appears to be no compensatory increase in caloric intake when caloric expenditure is increased through aerobic exercise.

A second study by Stubbs et al (82) explored the relationship between increases in energy expenditure through physical activity on appetite and EI under free living conditions. A total of six men with a mean age of 31 years and BMI of 23.3 were included in the study. Subjects were studied three times over a 9 day period which corresponded to each treatment condition: no exercise (control), moderate exercise (subjects were assigned to two 40 minute exercise sessions per day), and high level of exercises (subjects were assigned to three 40 minute exercise sessions per day). The order of the three treatments was randomized and separated by at least one week. On

days 1-2 of the study, subjects were provided a diet that balanced energy expenditure and EI. Over the next 7 days subjects completed one of three treatment conditions and concurrently kept a weighed and written food record. Subjects were not given any additional instructions as to how or what to eat. Changes in subjective appetite, hunger, and satiety were assessed each hour during waking hours throughout days 1-9. Over the duration of the study period no significant increases in caloric intake were reported, regardless of treatment condition. However, energy expenditure increased 30% from baseline to the high levels of exercise condition and 25% from the moderate exercise condition to the high levels of exercise condition. Under the high levels of exercise condition, subjects expended on average 1240 more calories than they consumed despite no increase in subjective appetite, hunger and satiety. Thus, lean men appear able to tolerate negative energy balance, created by exercise, over 7 days without subsequent increases in EI.

Unfortunately, none of the aforementioned studies in males measured changes in BMD or biomarkers for bone remodeling. Consequently, there is still no clear evidence in males if changes in bone remodeling occur as a result of low EA or if another potential mechanism exists. However, the evidence appears to support the notion that no strong biological drive to match caloric intake with caloric expenditure exists, when increased caloric expenditure is the result of increasing aerobic activity.

#### *Physical Activity*

It is commonly accepted that regular weight-bearing physical activity can promote bone health throughout the lifespan (84). However, prolonged endurance activities have been associated with low BMD (18-22, 39) and premature bone loss in women (85, 86).

Historically, low BMD in athletes has been a hallmark characteristic of FAT (87).

Emerging literature suggests male distance runners may have a trend towards decreased BMD, specifically in the lumbar spine and radius (18-22). Researchers speculate a decreased BMD in the lumbar spine may be related to several factors including inadequate dietary intake, low sex hormones, and/or a genetic predisposition.

Bilanin et al (20) measured bone density in 13 male long distance runners with a mean age of 28.7 yrs, compared to 11 male non-runners mean age of 26.8. BMD was measured at the lumbar spine and mid-tibia using dual photon absorptiometry, and at the mid-radius using single photon absorptiometry. Runners, trained on average 57.2 miles per week (92.2 km), had significantly lower bone density in the lumbar spine compared to non-runners ( $p < 0.05$ ). Interestingly, tibial and radial bone mineral density did not differ between the groups. Ultimately, Bilanin et al suggested that long distance running may lead to decreased vertebral BMD. A potential explanation for decreased BMD, is an unfavorable change in hormone levels (increased cortisol and decreased testosterone) as a result of prolonged endurance training.

A second study by Hetland and colleagues (18) investigated the impact of running on bone mass in men. A total of 120 healthy, physically active men 19-56 years of age, including runners and non-runners, were studied. Bone mineral content was measured in the lumbar spine, total body, and proximal femur using DXA, and in the forearm using single photon absorptiometry. Biomarkers of bone turnover were also assessed. Results revealed a significant negative correlation between lumbar bone mineral content and weekly running mileage ( $r = -0.37$ ;  $p < 0.0001$ ). Biomarkers of bone resorption were 20% higher in those running greater than 60 miles (100 km) per week compared to

controls. A significant correlation ( $r=0.27$ ,  $p<0.01$ ) was also found between bone resorption and weekly distance ran. Levels of sex hormone status (testosterone) were within expected ranges and did not differ based on running mileage. Hetland et al concluded that males participating in long distance training had reduced bone mass and increased bone turnover compared to controls, suggesting accelerated bone loss. The underlying mechanism was not established in this study, but the researchers speculated that sex hormones do not play a key role.

Hind et al compared BMD between male and female endurance runners with a reference population (19). A total of 44 men and 65 women with a mean age of 25 participated in the study. On average, the runners had trained for at least 3 years with a mean weekly training distance of 60 miles (96 km) for men and 51 miles (82 km) for women. BMD was measured at the lumbar spine and hip using DXA. Mean lumbar spine, total hip, and femoral neck T-scores were not different between genders. Interestingly, the mean lumbar spine T-scores for both men and women runners was -0.8. More than one third of the male runners presented with a low lumbar spine BMD (T-score  $<-1.0$ ) ( $n=16$ ). Multiple regression analyses revealed weekly running distance and training years were the best predictors of lumbar spine T-scores ( $r^2=.400$ ,  $p<0.01$ ). The authors conclude that male runners face the same bone threat at the spine, as female runners and suggest further research on male runners.

A cross-sectional study completed by Fredericson et al (22) compared the bone density of 20-30 year old male elite athletes from running ( $n=15$ ) and soccer ( $n=15$ ), to sedentary controls ( $n=15$ ). All distance runners had been training at least 70 miles (112 km) per week over the past year. DXA measurements for the lumbar spine, right hip,

right leg, and total body were completed. After adjusting for age, weight, and percent body fat, soccer players had significantly higher right hip and spine BMD values compared with runners ( $p = .012$ ;  $p = .009$ ). Also soccer players had significantly higher total body ( $p=0.008$ ), spine ( $p=0.041$ ), right hip ( $p<0.001$ ), right leg ( $p=0.019$ ) and calcaneal ( $p<0.001$ ) BMD values compared with controls. No differences were found between runners and controls. Similar to previously published reports, 40% of runners ( $n=6$ ) had low BMD in the lumbar spine, T-score  $< -1$ . The authors suggest that the activities soccer players are exposed to on a regular basis including sprinting, jumping, accelerating, decelerating as well as transverse and torsional loads may be contributing factors to observed increases in BMD compared to runners and controls.

Recently, Ackerman et al (21) published a cross-sectional study which investigated the endocrine determinants of BMD in male collegiate athletes. A total of 13 long-distance runners, six wrestlers, and seven golfers were included in the study. DXA measurements of the total body, postero-anterior spine, hip, and radius were completed along with blood variables including total and free estradiol and total and free testosterone. Mean postero-anterior spine and radius Z-scores were  $-0.8$  and  $-0.9$ , respectively for runners. More than 50% of runners ( $n=7$ ) had a Z-score  $< -1$  at the postero-anterior spine. Significant correlations were found between total estradiol at the postero-anterior spine ( $r=0.41$ ,  $p=0.038$ ) and radius ( $r=0.58$ ,  $p=0.002$ ) and free estradiol at the postero-anterior spine ( $r=0.49$ ,  $p=0.012$ ) and radius ( $r=0.61$ ,  $p<0.001$ ). Free testosterone levels only revealed a significant correlation at the radius ( $r=0.47$ ,  $p=0.014$ ). Ideal body weight and lean body mass were positively correlated with BMD while  $VO_2$  max was a negative determinant of BMD at the spine. Ackerman and colleagues

suggested that estradiol levels are more important determinants of BMD in collegiate male athletes than testosterone.

### **Bouchard grid**

The Bouchard grid can be used to estimate caloric expenditure through physical activity as well as total daily energy expenditure in adults and children (88). The Bouchard grid has high reproducibility ( $r = 0.96$ ) for mean energy expenditure over 3 days (88). The grid divides a 24-hour day into fifteen minute intervals (96 intervals for every day) and the subjects report their average intensity during each time interval on a categorical scale from 1 to 9. The scale represents activities corresponding to a metabolic equivalent of task (MET) intensity level of 1 MET-7.8 METs. Examples of activities at every intensity level are provided to the subjects to facilitate accurate reporting. An estimated total daily energy expenditure can be computed using an average METs value for each category along with body weight in kg. The sum of caloric values for activities greater than or equal to six (6) on the Bouchard Grid, representing moderate and vigorous activity, can be used to determine energy expenditure through exercise. Previous research has demonstrated a strong positive linear relationship between tri-axial accelerometry data and the Bouchard grid for total energy expenditure ( $r = 0.86$ ) (89).

### **Accelerometry**

An accelerometer is a small motion sensor used to estimate energy expenditure from exercise. The accelerometer is fitted to an elastic belt and secured around the waist on the anterior axillary line at the iliac crest. Accelerometers have a dynamic range of  $\pm 6$  g with frequency content between 0.25 and 2.5 Hz, sampled 60 times per second. The accelerometer must be initialized prior to use in order to provide output from each

individual directional axis using manufacturing specific software. Accelerometers are worn on the hip due to the proximity of the hip to the body's center of mass (CoM). Movement of the CoM is considered to be representative of whole body movement because the CoM is sensitive to the position of all body segments (90) .

Previous research has validated the use of Actigraph accelerometers for estimating energy expenditure compared to DLW (91, 92). In a study by Rothney et al (91), 22 subjects with a mean age of 41 years and BMI of 29.3 were included. Study participants wore an accelerometer for 14 days under free living conditions to estimate mean energy expenditure while concurrently measuring energy expenditure with DLW. No significant differences in energy expenditure were found between methods, DLW mean energy expenditure =  $2108 \pm 358$  MET minutes per day, accelerometer mean energy expenditure =  $2192 \pm 288$  MET minutes per day,  $P > 0.05$  (91).

A second study by John et al (93) found no difference in activity counts at any measured running or walking speed using any of the four available Actigraph accelerometers. A total of 10 men with a mean age of 23 years completed the study. Subjects wore one of four accelerometers while walking and running at 10 different speeds. Subjects walked or ran at each speed for 3 minutes. Participants first completed the walking stages continuously at 3, 5, and 7 k per hour. Participants then ran at 8, 10, 12, 14, 16, 18, and 20 km per hour. A two minute break was given between walking and running stages. Participants were given the option to take a three to five minute recovery break between running stages if they chose to do so. Speed during walking and running was monitored at the beginning of each stage using a tachometer. Data from the last two minutes of each stage were averaged and used to represent one minute activity counts.

No significant differences were found between any of the accelerometers at any of the reported speeds. The Actigraph Accelerometer appears valid and reliable for application in studies of physical activity.

## **Nutrition**

### *Nutritional factors and bone*

In addition to energy balance, there are a number of nutrients important to bone mass besides the aforementioned energy balance. Protein and sodium have been shown to influence bone mass (94, 95). Calcium and Vitamin D are well known to be essential to bone formation. Other less well known nutrients related to bone health include vitamin K, vitamin A and many of the minerals. Of these nutrients, calcium and vitamin D are typically considered for bone research evaluation.

Calcium is the most abundant mineral in the human body making up approximately 2% of one's total body weight. The mineral component of bone is primarily calcium phosphate in the form of hydroxyapatite crystals. Calcium is needed for proper skeletal growth and development contributing to peak bone mass amongst several other vital physiological functions (30). Dairy and fortified grains are the best sources of dietary calcium; however, supplemental calcium is available in various forms. It is notable that estrogen is also shown to influence the absorption fraction of calcium.

Vitamin D is more similar to a hormone than a vitamin and is responsible for maintaining serum calcium within the normal physiological range. When the skin is exposed to ultraviolet radiation from the sun, 7-dehydrocholesterol in the skin is converted to vitamin D<sub>3</sub> or cholecalciferol. Vitamin D can also be ingested in the diet through sources such as fatty fish, fortified dairy (D<sub>3</sub>), or supplementation (D<sub>2</sub> or D<sub>3</sub>).

Once vitamin D is present in the blood stream, it is transported to the liver and kidneys where it is finally converted to its most biologically active metabolite 1, 25-dihydroxyvitamin D<sub>3</sub>, 1,25 (OH)<sub>2</sub>D. However, 1,25 (OH)<sub>2</sub>D only exists in very small quantities in the serum. Vitamin D is more commonly found as 25-hydroxyvitamin D (96, 97). Vitamin D can impact calcium metabolism at several critical junctions including calcium transport, renal calcium reabsorption, intestinal calcium absorption, and mobilizing calcium from bone (92, 96).

#### *Three-day written food record*

Various techniques are available for measuring dietary intake including food records, food frequency questionnaires, and 24- hour dietary recalls. Except for controlling food intake in a clinical research setting, there is no truly accurate measure of current dietary intake. A diet record is considered the most accurate and feasible method for research in free-living subjects (98). A three-day written food record was previously demonstrated to accurately estimate energy intake (99). Self-reported, written three-day food records, involve recording all foods and beverages consumed for three (3) separate 24 hour time periods. Previous studies have found that periods of longer than three or four days reduce compliance which in turn reduces accuracy (100).

There are a number of food record protocols and training perspectives that might enhance the validity and reliability of food records. For instance, estimated household measures using common household symbols can be used such as one deck of playing cards is equal to approximately three ounces of cooked meat or a golf ball is approximately 2 tablespoons in volume (101). Upon completion of the food records, data should be screened by a trained researcher to improve accuracy of reporting or to clarify

incomplete data similar to the multi-pass method used in dietary interviews (102).

Finally, completed food records should be entered into professional grade food processing software for analysis of caloric intake and any other nutrients of choice. The Food Processor SQL as marketed by ESHA is considered a research quality software appropriate for analyzing macro and micronutrient intakes (103).

### **Hormonal influence on bone**

#### *Hypothalamic-pituitary axis*

The hypothalamic-pituitary axes are a complex network of feedback mechanisms which control the release of hormones throughout the body. The hypothalamus signals the pituitary which interacts through stimulating hormones with the adrenal and thyroid glands, as well as the testicles and liver as the organs which produce the metabolic hormones that influence bone. Figure 4 (below) represents the control of the hypothalamic pituitary axes on osteogenesis.

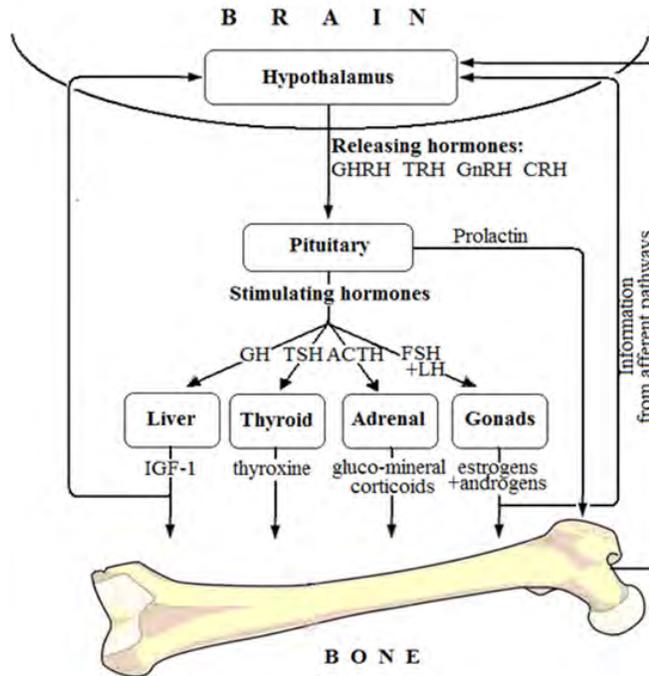


Figure 4: Visual depiction of the relationship between the hypothalamic-pituitary-axes and bone formation. Adapted from: <http://epigeneticscomesofage.com/wordpress/?p=6>

### *Total Testosterone versus Free Testosterone*

Testosterone ( $17\beta$ -hydroxy-4androst-3-one) is a steroid hormone derived from a metabolic pathway that begins with cholesterol. Serum total testosterone (TT) is the sum of unbound and protein-bound testosterone circulating throughout the body (104). Free testosterone (FT) represents the proportion of the total that is not bound to a protein and thus is available to target tissues for biological action including various bone cells (28, 104). FT represents approximately 0.5-3% of circulating testosterone (104) and has a shorter half-life compared to TT (105). Thus, the measure of total testosterone allows for a better clinical picture of the hormone available to the body.

Previous studies have demonstrated a decrease in testosterone in males engaged in prolonged endurance activities (24, 25, 27, 28). Hackney et al. compared the resting reproductive hormonal profiles of untrained (n = 11) and endurance-trained (n = 11) males (27). Total testosterone, free testosterone, estradiol, luteinizing hormone, prolactin, and cortisol were measured in the morning following an 8 hour fast and subsequently collected every 60 minutes for 4 hours. The endurance-trained group reported participating in 68.5 minutes of endurance activity per day on average, while the untrained group was sedentary. Neither group reported any history of hypothalamic-pituitary-testicular disorders. The overall 4 hour mean total testosterone and free testosterone levels were significantly ( $p < 0.05$ ) lower in the trained group compared untrained group, but all mean values were within normal ranges. The luteinizing hormone pulse frequency and amplitude did not differ between groups. Prolactin and cortisol levels were within normal limits and also did not differ between groups. The results suggest normal hypothalamic-pituitary function in the trained subjects. Prolactin and cortisol did not appear to explain the lowered testosterone levels in the study. However, chronic endurance training seemed to lower total and free testosterone in males possibly by an unknown mechanism.

Wheeler and colleagues (24) studied the effects of endurance training on serum total testosterone, sex hormone binding globulin, luteinizing hormone, follicle stimulating hormone, prolactin, and cortisol in 15 previously sedentary males. Over 6 month duration of the study, men increased weekly running mileage to an average of 35 miles (56 km) per week. Total testosterone levels decreased significantly compared to baseline ( $P < 0.05$ ), but still remained within the normal range. Levels of prolactin and cortisol also

decreased albeit not significantly, while luteinizing hormone and follicle stimulation hormone did not change. Overall, the authors concluded, these data confirm previous findings of reduction in serum testosterone and prolactin levels with increased endurance exercise. Similar to Hackney, decreases in testosterone were observed in male distance runners. They also proposed the decreases in testosterone were not related to changes in luteinizing hormone pulsatile release or increases in cortisol levels.

A study exploring the hormonal reproductive profile of endurance athletes was completed by Maimoun et al (28). The study compared levels of total testosterone, free testosterone, luteinizing hormone, estradiol and cortisol between cyclists (n=11), triathletes (n=14), swimmers (n=13) and controls (n=10). Serum samples were collected in the morning following an overnight fast. Overall, cyclists and triathletes had significantly lower levels of total testosterone compared to controls ( $p < 0.01$ ,  $p < 0.05$ , respectively). Cyclists also had significantly lower levels of total testosterone compared to swimmers ( $p < 0.05$ ). Levels of total testosterone were within the normal range for all groups. Other blood parameters did not differ between groups and were within the normal ranges. The underlying mechanism(s) whereby endurance training alters the hypothalamus feedback system, specifically reducing total testosterone, remains open to speculation.

In 2005 Hackney et al (106) coined the term “exercise-hypogonadal male condition” to explain the persistently and significantly reduced levels of free and total testosterone observed in chronically trained men. Hackney and colleagues (106) identified five common characteristics to men presenting with exercise-hypogonadal male condition:

1. Reduced resting basal testosterone levels, approximately 50-75% that of normal, healthy, aged-matched sedentary men;
2. Low testosterone levels do not appear to be the result of acute stress from exercise;
3. A potential alternation in the regulatory axis, thereby creating a new lower set-point for circulating testosterone;
4. A history of early involvement in organized sport and exercise which has persisted into daily exposure of regular physical activity;
5. Endurance training is most commonly reported including distance running, cycling or the triathlon.

Like others, the authors suggest more research be completed on male athletes to fully understand the mechanisms and consequences related to prolonged hypogonadism.

Hypogonadism has previously been identified as an accepted risk factor for secondary osteoporosis in men (107-110). Prior studies show testosterone deficiency in 50–66% of elderly men with hip fractures (111, 112) and 20% in men with vertebral fractures (109, 110, 113). The Baltimore Longitudinal Study of Aging measured total testosterone levels over a 30-year period in 890 men. Hypogonadism was defined as a total testosterone level <325 ng/dL, the prevalence was 12%, 19%, 28%, and 49% in men ages 50s, 60s, 70s, and 80s, respectively (114). Additionally, Snyder et al showed that 3 years of testosterone replacement increased spine BMD by up to 6% in men with a baseline testosterone below 300 ng/dL (115).

In 1989, Stěpán demonstrated androgen suppression led to loss of lean body mass, bone mineral density and an increase in bone turnover in a group of castrated men (116).

Human androgen receptors have also been detected in both osteoblasts and osteoclasts suggesting that bone is a target tissue for androgens (117, 118). Androgens and their receptors appear to play a major role in the acquisition of trabecular bone in men (119). While it is clear that androgens play a role in bone health, the exact role that testosterone plays in bone health and bone remodeling remains unclear (107).

## **Cardiovascular Health**

### *Lipid profile*

The National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) provides current guidelines for an optimal lipid profile (120), see Table 1.

<b>Category</b>	<b>Optimal</b>	<b>Low</b>	<b>High</b>
Total Cholesterol	<200 mg/dL		≥200 mg/dL
HDL Cholesterol	≥40 mg/dL	<40 mg/dL	
LDL Cholesterol	<100 mg/dL		≥100 mg/dL
Triglycerides	<150 mg/dL		≥150 mg/dL

Table 1: National Cholesterol Education Program Adult Treatment Panel guidelines for an optimal lipid profile (120).

Engaging in regular aerobic physical activity is commonly suggested as a means to favorably influence the lipid profile by lowering triglycerides and increasing HDL cholesterol (77, 120, 121). However, limited data are available on the blood lipid profile

of distance runners. Tomaszewski et al have reported the serum lipid profiles of 55 lean (BMI <25 kg/m<sup>2</sup>) and 12 non-lean runners (BMI 25-29.9 kg/m<sup>2</sup>) male distance runners participating in the 2000 Calissia Ultramarathon with surprising results (122). All runners reported regularly engaging in physical activity, 25-60 miles per week (40-100 km) for at least two years. Fasting serum samples were collected from peripheral blood and stored at -70°C until the biochemical analysis. Table 2 provides a summary of the blood lipid results based on lean versus non lean, data are presented as mean ± SDs. Interestingly, LDL cholesterol and triglycerides do not fall within the optimal category as described by the NCEP ATP III guidelines (120).

<b>Lipid Fraction (mg/dL) mean ± SD</b>	<b>Lean Runners</b>	<b>Non-Lean Runners</b>
<b>Total Cholesterol</b>	193.5 ± 34.8	224.5 ± 46.4
<b>LDL Cholesterol</b>	112.2 ± 21.1	170.3 ± 42.6
<b>HDL Cholesterol</b>	46.4 ± 11.6	34.8 ± 11.6
<b>Triglycerides</b>	168.3 ± 88.6	186.1 ± 106.3

Table 2: Summary of blood lipid values comparing lean runners to non-lean runners. Results are presented as means ± SD's. Adapted from Tomaszewski et al (122).

Similarly, in another study of fifteen nationally competitive male distance runners, the LDL, triglyceride and HDL levels fell outside the NCEP ATP III guidelines (123). In a cohort of 108 male runners (22 elite vs 86 recreational) mean LDL was 123

mg/dL regardless of level of training (124). High LDL cholesterol is a major risk factor for coronary heart disease (120). Furthermore, it is estimated that for every 30 mg/dL increase in LDL, above 40 mg/dL, is related to approximately a 30% increase in relative risk for developing coronary heart disease (120).

One possible explanation for patterns of observed dyslipidemia in male runners may be related to dietary intake. In general, runners are encouraged to consume a diet high in carbohydrates (125). However, diets high in carbohydrate have been linked to patterns of dyslipidemia, specifically a decrease in HDL cholesterol and an increase in triglyceride levels (126). It may be important to the overall health of the athlete to further evaluate the relationship between the quantity, and quality of carbohydrate in the habitual diet with cholesterol profiles.

### **Innovation and Value**

To date, no study has looked at all components of the male endurance athlete tetrad (BMD, serum testosterone, energy availability and cardiovascular health) in a cohort of trained male runners (Figure 3). Our primary aim is to look for evidence of the male endurance athlete tetrad. Our central hypothesis is suboptimal levels of the male endurance athlete tetrad components exist in a cohort of trained male runners.

H1. Male runners will have low bone mass, Z-score  $\leq -1$ , in at least one of the four measured sites: total body; lumbar spine; non-dominant hip or non-dominant forearm.

H2. Male runners will have low energy availability,  $< 30$  kcals / kg FMM

H3. Male runners will have low serum testosterone values, total testosterone  $\leq 300$  mg/dL and/or free testosterone  $\leq 9$  pg/m.

H4. Male runners will have a suboptimal fasted blood lipid profiles defined as above the optimal range in at least one of the following categories: total cholesterol; low-density lipoprotein; triglycerides; and/or below the optimal range for high-density lipoprotein.

Taken together, these efforts will provide data on the prevalence of MEAT, and perhaps justify future studies aimed at studying male runners. In addition, our paradigm shift paper, translating this previously female issue to also include males, will serve as the original manuscript to inform sports medicine professionals of the potential skeletal risks posed to males participating in long-term, high mileage training.

## **Chapter 2: Bone Insufficiency in the Trabecular Sites of Trained Male Distance Runners**

**Abstract:** It is commonly accepted that regular weight-bearing physical activity can promote bone health throughout the lifespan. However, available literature suggests male runners may have a decreased bone mineral density (BMD), specifically in the lumbar spine and radius. Researchers speculate a decrease in BMD may be the result of several factors including inadequate diet, low testosterone, and/or genetic predisposition. Decreased testosterone levels have previously been identified in males engaged in prolonged endurance activities such as running. Energy availability the amount of dietary energy remaining for other body functions after exercise training. Low energy availability may be the underpinning factor for decreases in BMD, and is the outcome from inadequate dietary intake and/or increasing exercise energy expenditure.

**PURPOSE:** To explore the bone health of trained male distance runners. **METHODS:** Dual-Energy X-Ray Absorptiometry (DXA) bone density scans were completed for total body, non-dominant hip, lumbar spine, non-dominant forearm, and body composition. Additionally, blood samples and three-day food and activity records were collected and analyzed. **RESULTS:** A total of 41 subjects completed the study. Bone density results revealed that 5 of the 41 subjects had bone insufficiency (BMD Z-score  $\leq -1$ ) in the femoral neck, 12 in the lumbar spine (L1-L4) and 18 in the ultra-distal radius.

**CONCLUSION:** The detriments of low bone density can be severe and irreversible and male endurance runners are practicing habits that may support low BMD. Consequently, it is important to gain more insight into the nutrition attitudes and behaviors of male distance runners that possibly elicit negative consequences to help future prevention and education efforts.

**Key Words:** DXA, Bone Mineral Density, Male Runners, Exercise

### **Introduction**

Increasingly, male distance runners are being recognized as potentially at risk for bone density concerns. Over the last 20 years, however, substantial research has been conducted on female runners as part of the female athlete triad (FAT) (2-10).

Unfortunately, little research has been completed on male distance runners despite the Surgeon General stating that 20% of males will develop osteoporosis during their lifetime (1). Available literature suggests male runners may have a decreased bone mineral density (BMD), specifically in the lumbar spine and radius (18-22). Researchers speculate a decrease in BMD may be the result of low testosterone levels, inadequate diet and or genetic components (23, 24); despite the fact that experts continue to recommend regular weight-bearing physical activity as a means to promote bone health throughout the lifespan (84).

Of the, greater than 518,000 marathon finishing times recorded in the USA in 2011, nearly 60% were male (127). With the number of males participating in endurance events, such as the marathon, on the rise a formal evaluation of bone density in trained male distance runners deserves more focused attention. Thus, the purpose of our study was to measure BMD of the total body, lumbar spine, non-dominant hip, and non-

dominant forearm in order to determine if a cohort of trained male distance runners have low bone mass. Low BMD is defined as Z-score  $\leq$  -1.

Null Hypothesis:

Male runners will not have low BMD at any of the four measured sites (total body, non-dominant hip, lumbar spine, or non-dominant forearm).

Research Hypothesis:

Male runners will have low BMD Z-scores, in at least one of the four measured sites (total body, non-dominant hip, lumbar spine, and/or non-dominant forearm).

## **Methods**

### *Subjects*

All runners were recruited from Ohio between October 2011 and June 2012. Interested participants completed an electronic survey to determine eligibility prior to enrollment. The eligibility criteria included: Caucasian, male, between 20-50 years of age, run on average at least 64 km (40 miles) per week for a minimum of at least two years, and run a minimum of 4 days per week. Individuals were excluded if they currently used any tobacco products (cigarettes, smokeless tobacco, cigars, pipes, etc.), any prescription medications to lower cholesterol, and/or any prescription medications known to influence bone mass including glucocorticoids. In addition, individuals were excluded if they were previously told by a healthcare provider they had low bone mass, osteopenia, osteoporosis, chronic kidney disease (elevated creatinine or decreased GFR), diabetes (type 1 or type 2), a thyroid disorder (hypothyroidism or hyperthyroidism); are currently taking; Subjects meeting eligibility criteria were sent a formal email inviting them to participate in the study.

Subjects were recruited from local running specialty stores, training groups, road races, ResearchMatch.org, and word of mouth. A total of 56 subjects qualified for the study based on the prescreening questionnaire, 41 chose to enroll in the study, and complete data were collected on 40 subjects. One subject did not have complete accelerometry data. The protocol was approved by the Biomedical Medical Sciences Institutional Review Board at The Ohio State University; all subjects provided written informed consent before study participation.

### *Protocol*

Study participants attended two outpatient visits. During the first visit subjects signed the informed consent, were instructed on how to complete the 3-day food and activity records, were fit for an accelerometer, and had basic anthropometric measurements (height and mass) taken by the same trained researcher. The second visit was usually scheduled within one week of the first visit and was scheduled between 7:00 am – 9:00 am.

During the second visit, iDXA, phlebotomy, and review of diet records were completed. BMD was measured by dual energy x-ray absorptiometry (iDXA) (GE Lunar iDXA, Madison, WI, USA) at the total body, lumbar spine (L1-L4), non-dominant hip, and forearm according to standardized procedures. DXA is recognized as the accepted standard when determining BMD (63, 64). An operational definition of non-dominant was defined as the self-reported answer to the following questions, “If you were going to kick a ball with power would you use your left or right leg. Are you left or right-handed?” The choice to measure the non-dominant hip and forearm was made to examine the sites with the lowest potential for mechanical loading while respecting

subject time and radiation. BMD is commonly used as a surrogate measure of bone health (128). All scans were completed by the same researcher. Machine calibration procedures were completed every day before measuring a subject. Throughout the duration of the study the coefficient of variation (CV) was 0.19%. Previous research has found excellent in vivo precision for BMD measurements for the total body (CV 0.63%), lumbar spine (CV 0.41%), and hip (CV 0.53%) when using the GE Lunar iDXA (64, 65). Additionally, excellent in vivo precision has been established for the Lunar iDXA for measurements of body composition including lean tissue mass (CV 0.50%), total fat mass (CV 0.82%), and total bone mineral content (CV 0.60%) (65). Upon completion of the scans, data were analyzed according to the manufacturer's analytic software algorithms and results shared with the subject. DXA data were exported from the database into an Excel spreadsheet and imported to IBM SPSS (version 19.1, Chicago, IL) for statistical analysis.

Prior to the blood draw, hydration status was assessed via a small urine sample using a urine refractometer, to avoid drawing dehydrated blood (artificially elevated serum values). A refractometer is a device used to measure urine specific gravity and has previously been used in athletes as a reliable instrument for measuring hydration status (129). Subjects presenting with a urine specific gravity of  $>1.020$  g/ml were considered dehydrated and had the blood draw delayed until adequate hydration was obtained. All blood samples were collected between 7:00 and 9:00 am after a minimum of a 9 hour fast. All serum samples were processed according to University Hospital specifications and were delivered to the University Hospital for analysis. Blood variables measured by the hospital included: total cholesterol, triglycerides, HDL, osmolality, cortisol, free T4,

total testosterone, thyroid stimulating hormone, hemoglobin, hematocrit, free testosterone and 25-OH vitamin D.

Energy availability (EA) was defined as dietary energy intake, Calories consumed (EI), minus calories expended during exercise, exercise energy expenditure (EEE), normalized to fat free mass (FFM) (4).

$$EA = EI - EEE / (FFM), \text{ with a unit of kcals/ kg FFM}$$

EI was estimated using a three-day written food record. Three day written food records have been previously demonstrated to accurately estimate energy intake (99), and involve recording all foods and beverages consumed for three (3) separate 24 hour time periods, in our case two typical weekdays and one typical weekend day. Use of common household symbols were used to help define portion sizes such as one deck of playing cards is equal to approximately three ounces of cooked meat or a golf ball is approximately 2 tablespoons in volume to improve validity and reliability. The records were screened by a trained researcher/dietitian to improve accuracy of reporting or to clarify incomplete data. Finally, completed food records were analyzed using a professional grade food processing software (ESHA, The Food Processor SQL) for analysis of mean three day calorie, calcium and vitamin D intake.

EEE was estimated using an Actigraph triaxial accelerometer (GT3X+, Actigraph, Pensacola, FL), ActiLife software (version 5.10. Actigraph, Pensacola, FL) in addition to monitor firmware (v. 2.4.0.). Subjects wore the accelerometer on their anterior superior iliac spine of the dominant hip during waking hours for the same three days as the food records were completed. Subjects were instructed to only take off the accelerometer when bathing or swimming. Data were collected at a sampling frequency of 60 Hz and

downloaded as 10 second epochs. In order to be considered complete data, subjects must have worn accelerometers for at least 10 hours per day for each of the three days that data were collected. EE was estimated using the vector magnitude equation which estimates caloric expenditure through exercise only.

### *Statistical Analysis*

All DXA results were compared to the manufacture's reference databases (GE Lunar Reference Population and NHANES III) using Age-Matched Z-scores which control for age, gender and ethnicity. Data were interpreted using the Z-score based on the classification of BMD by the American College of Sports Medicine (ACSM) (4). ACSM defines normal BMD as a Z-Score  $> -1$ , low BMD Z-score between  $-1$  and  $-2$  and osteoporosis as a Z-score  $\leq -2$ . Data are presented as mean  $\pm$  SD for anthropometric, demographic, blood values and Z-scores for each measured site. The number of individuals falling into each category (normal, low BMD and osteoporosis) is also presented. Statistical analysis was performed using IBM SPSS Statistics (version 19.1). Univariate regression models were constructed to investigate correlates of BMD and Pearson coefficients were reported. Statistical significance was set *a priori* at  $p < 0.05$ . Data are presented as mean  $\pm$  SD.

### **Results**

The demographic characteristics of the study participants are found in Table 5. Z-scores for the total body, lumbar spine, non-dominant hip and non-dominant forearm are reported in Table 4. Nearly 1 in 3 men (31.7%) presented with inadequate BMD in the lumbar spine, while almost half (43.9%) presented with inadequate BMD in the ultra-distal radius.

<b>Body Site</b>	<b>Mean Z-score ± SD</b>	<b>Low BMD Z<sub>≤</sub>-1.0 to -2</b>	<b>Osteoporotic Z<sub>≤</sub>-2.0</b>	<b>Frequency of suboptimal BMD (n=41)</b>
<b>Total body</b>	1.22 (1.0)	0/41	0/41	0/41 (0%)
<b>Femoral neck</b>	0.35 (0.96)	5/41	0/41	5/41 (12.2%)
<b>Lumbar spine</b>	-0.22 (1.13)	11/41	2/41	13/41 (31.7%)
<b>Ultra-distal radius</b>	-0.62 (1.18)	13/41	5/41	18/41 (43.9%)

Table 3: Mean BMD Z-scores and the number of subjects presenting with low BMD or osteoporosis for each of the four measured sites.

*Factors associated with BMD*

Few variables displayed a strong correlation with Z-score values. Significant correlations between total body Z-score and height, weight, lean body mass, mean energy availability and serum vitamin D were present. Only height, weight, lean body mass and mean energy availability remained significant correlates for femoral neck BMD. No significant correlations were found between lumbar spine and any of the variables of interest. Similar results were found for the ultra-distal radius despite the lumbar spine and ultra-distal radius having the lowest Z-scores. Pearson correlations for select variables are presented in Table 4

Variable	Mean (±SD)	Total Body Z- score		Femoral Neck Z-score		Lumbar Spine Z-score		Ultra-distal radius Z- score	
		R	P	R	P	R	P	R	P
<b>Height (cm)</b>	178.7 (6.8)	<b>0.407</b>	<b>0.009</b>	<b>0.384</b>	<b>0.014</b>	0.260	0.105	0.042	0.797
<b>Weight (kg)</b>	74.2 (10.4)	<b>0.534</b>	<b>&lt;0.001</b>	<b>0.366</b>	<b>0.020</b>	0.183	0.259	0.160	0.324
<b>BMI (kg/m<sup>2</sup>)</b>	23.1 (2.2)	<b>0.464</b>	<b>0.003</b>	0.224	0.165	0.080	0.622	0.203	0.209
<b>Age (years)</b>	34.6 (8.8)	0.150	0.356	-0.097	0.550	-0.008	0.960	0.302	0.058
<b>Body Fat (%)</b>	16.3 (4.7)	0.078	0.632	-0.099	0.544	-0.094	0.565	-0.093	0.570
<b>Mean energy intake (kcal)</b>	2813.8 (615.6)	-0.085	0.600	0.014	0.932	-0.072	0.657	-0.040	0.806
<b>Mean Exercise Energy Expenditure (kcal)</b>	860.9 (298.9)	0.304	0.060	<b>0.339</b>	<b>0.035</b>	0.232	0.155	0.162	0.324
<b>Fat free mass (kg)</b>	61.9 (7.6)	<b>0.511</b>	<b>0.001</b>	<b>0.409</b>	<b>0.009</b>	0.225	0.163	0.187	0.248
<b>Mean energy availability (kcal/kg FFM)</b>	32.1 (10.3)	<b>-0.426</b>	<b>0.007</b>	-0.302	0.062	-0.263	0.106	-0.199	0.225
<b>Serum Vit D (ng/dL)</b>	34.6 (12.8)	<b>0.317</b>	<b>0.046</b>	0.200	0.217	0.213	0.188	0.166	0.306
<b>Total testosterone (ng/dL)</b>	475.4 (151.2)	0.045	0.781	0.059	0.718	-0.023	0.888	0.032	0.845
<b>Free testosterone (pg/mL)</b>	11.5 (3.7)	0.153	0.345	0.143	0.378	-0.037	0.821	0.021	0.899
<b>Cortisol (µg/dL)</b>	14.9 (4.4)	-0.156	0.335	-0.178	0.273	0.039	0.809	-0.247	0.124

Table 4. Pearson correlations for select variables and BMD. p values < 0.05 are bolded.

## **Discussion**

Previous research has established that female runners with menstrual irregularity are at increased risk for suboptimal BMD; however, whether a similar risk for male runners is present is still unclear. In our study, runners had normal total body and normal hip BMD, lower lumbar spine and ultra-distal radius BMD. This may be the result of decreased loading at the lumbar spine and radius. This site-specific difference is consistent with previous research on both male and female runners. Previous studies suggest that greater BMD at the hip is due to a greater magnitude of loading at the hip compared to other measured sites during running. Additionally, since the hip is comprised of mainly cortical bone (130), compared to trabecular bone in the lumbar spine (131) and ultra-distal radius (132), negative influences at the hip may take a longer time or higher mileage to manifest.

We tested for correlations between BMD and several plausible factors in runners. Moderate associations between BMD and anthropometric variables (height, weight, BMI and lean body mass) were significant. We also examined the relationship between various hormones and BMD. Serum Vitamin D significantly predicted BMD, albeit weak, at the total body but no other measured site. No other hormones were correlated with bone mass (testosterone, free testosterone or cortisol). Previous research on male runners has demonstrated a decrease in testosterone levels with increases in training volume; however, a clear relationship between testosterone levels and BMD has yet to be established. A recent study by Ackerman et al (21) suggests a stronger relationship between estrogen levels and BMD in male runners, but no other studies have confirmed

this finding. To date, the scientific literature does not support a strong relationship between sex hormones and low BMD in male runners, yet the possibility of a relationship should not be dismissed.

Evidence has established a link between negative calorie balance, reduced bone formation and low BMD (79). High mileage training results in increased energy expenditure and potential to increase bone turnover if sufficient calories are not consumed. We found a weak correlation between mean EA and BMD for the total body. Research also supports that prolonged exercise suppresses *ad lib* food intake in males (80-83). Therefore the coupling of high mileage training with a suppressed appetite may increase the risk for energy deficiency and help to explain the low BMD found in runners.

The purpose of this study was to describe the BMD of multiple skeletal sites in a group of trained male runners. Since DXA was used as the primary measure of BMD, structural parameters such bone size and volumetric density were not assessed but would be useful in future studies. By selecting only Caucasian runners, we are limited in the explanation of our findings to only a single ethnic group, however future studies should aim to include other ethnicities to determine if similar patterns of BMD exist. Currently, the GE and NHANES database only includes complete reference data on Caucasians. Due to the cross sectional nature of the study, causation cannot be inferred from the results.

We conclude that trained male runners may be at a similar risk for decreased BMD as female runners; however, the exact mechanism whereby this occurs is still unclear. It is important to realize the relationship between form and function, especially

within the skeleton. Future studies should look at the impact of resistance training on non-loaded sites (spine and forearm) in trained male runners to see if improvements in BMD can be made. Future studies should also evaluate the relationship between calorie balance and BMD in males to determine if a specific threshold exists whereby BMD is decreased.

## **Chapter 3: Bone Redistribution While Training for a Single Marathon: A Case Report**

### **Abstract**

A 31 year old Caucasian male presented for baseline Dual Energy X-ray Absorptiometry (DXA) measurements to assess baseline body composition prior to initiating a 16-week marathon training program. His motivation for undertaking the marathon training was to lose weight. The athlete presented with no reported medical conditions or any long-term medication use that would negatively influence bone density. The athlete completed the training regime and the marathon. One-week post-marathon the athlete had a follow-up DXA to assess changes in body composition. Over the 16 week training cycle, the athlete demonstrated a significant decrease in bone density in his lumbar spine and a significant increase in his legs, while total body bone mass was relatively unchanged. This case study demonstrates bone-shifting activity to protect the lower extremities.

### **Background**

Previous cross-sectional studies have linked prolonged endurance running with decreases in BMD, specifically at the lumbar spine and radius (18-22, 39). In 2011 more than 518,000 marathon finishing times were recorded in the USA, nearly 60% were male (127). The average finishing time for males was 4:16:34 (127). It is commonly accepted

that regular weight bearing physical activity can promote bone health throughout the lifespan (84). However, the effects of training for a single marathon and subsequent skeletal adaptations have not been examined. The following case demonstrates changes in bone density distribution during a 16-week marathon training program.

### **CASE REPORT**

A 31 year old Caucasian male presented for baseline Dual Energy X-ray Absorptiometry (DXA) measurements to assess baseline body composition and bone health prior to beginning a 16-week marathon training program. The athlete had not previously trained for a marathon and presented with no reported medical conditions or any long-term medication use that would negatively influence bone density.

Bone mineral density (BMD) and body composition measurements were completed using the GE Lunar iDXA (GE Healthcare, Madison, WI, USA). DXA scans for the total body, lumbar spine, non-dominant hip, and non-dominant forearm were completed according to standardized procedures and analyzed with enCORE™ version 13.5 software. Initial BMD Z-scores were all within normal ranges ( $\geq -1$  standard deviations). Body fat was estimated at 19.6% (using a 2-compartment model).

Upon successfully completing the Chicago Marathon in less than four hours, the athlete returned one week post-marathon for a follow-up visit to assess changes in body composition (17 weeks post-initial body composition). Over the 16 week training cycle the athlete had dropped his overall body mass by 2.14 kg (4.71 pounds) and his body fat dropped to 17.5% (decrease of 2.1%). Of the 2.14 kg decrease in body mass the athlete lost 2.02 (4.44 pounds) kg of body fat. Lean body mass remained relatively unchanged, within 0.12 kg (0.26 pounds) between measurements.

Interestingly, at the end of the 16 week training cycle, the athlete showed a significant decrease in BMD for the lumbar spine (L1-L4) ( $p < 0.05$ , -2.5%) and a significant increase in BMD for the legs ( $p < 0.05$ , +4.1%). Using the regions of interest software, several other non-weight bearing sites in his body revealed a trend towards a decrease in BMD, specifically the ribs (-3.8%), arms (-3.0%) and head (-2.3%).

## **Discussion**

BMD is commonly used as a surrogate measure of bone health (128). DXA is recognized as the standard method to determine BMD (63). Previous research has found excellent in vivo precision for BMD measurements for the total body (CV 0.63%), lumbar spine (CV 0.41%), and hip (CV 0.53%) when using the GE Lunar iDXA (64, 65). Additionally, excellent in vivo precision has been established for the Lunar iDXA for measurements of body composition including lean tissue mass (CV 0.50%), total fat mass (CV 0.82%), and total bone mineral content (CV 0.60%) (65).

The concept of the “steal phenomenon” was first described by Smith et al (133) in 1984 while studying the effects of exercise on bone density in women 35-65 years of age. The “steal phenomenon” refers to the redistribution of bone from non-loaded sites to loaded sites in an effort to protect the loaded sites (134). Smith et al (133) found that the bone density in the forearm of an aerobic exercise group decreased at a greater rate than that of the control group over the first year. The exercise group participated in 45 minutes of aerobic exercise (predominately running and dancing) 3 days per week for 50 weeks per year. The primary focus of the first year of training was to increase aerobic capacity; consequently little attention was given to strengthening the upper body. Other researchers have noted similar redistribution of bone (21, 134, 135). Karlsson et al

observed this phenomenon in a group of professional ballet dancers, BMD was lower in the head of male dancers and in the arms of female dancers, yet higher BMD were observed in the lower extremities of female dancers and in the hips of male dancers (135). Heinonen et al (134) observed that individuals participating in a sport, such as running, where the upper limbs were not intensely loaded had lower BMD in the distal radius compared to weight lifters and squash players. Previous research supports the redistribution of bone to reflect the stress of the particular exercise.

The idea of a “steal phenomenon” appears to be consistent in our case report as the majority of non-loaded sites (spine, ribs, arms, and head) demonstrated a decrease in BMD while the legs, a loaded site during running, demonstrated an increase in BMD. Without collecting any nutrition information or serological markers, it is hard to determine whether this redistribution of bone was reflective of factors such as poor nutrition, a hormonal imbalance, or simply an adaptation to training.

Current research in male runners also supports the “steal phenomenon” as researchers have identified the lumbar spine and distal radius as the site with the greatest levels of bone insufficiency (18, 20, 21). To explain the low bone density observed in distance runners, Frost theorized that an increase in muscular endurance does not depend on and is not accompanied by an increase in bone strength (39). If bone strains stay below the threshold needed to stimulate bone growth, then regardless of the amount of prolonged running, subsequent increases in bone density would not occur (39).

Overall, future studies should investigate the short and long-term relationship between various physical activities and redistribution of bone. Nutrient intake, hormonal balance, and energy availability should also be assessed as potential contributors to this

redistribution. For instance, it is not known if the runner in this case consumed an adequate diet to support bone maintenance. If bone nutrients in the training diet were low, his changes may be different than if his diet were more supportive of bone health. Researchers in the field have identified patterns and differences in bone density that seem to coincide with the demands of the particular sport. To improve overall skeletal health and decrease risk for fractures, it may be important to include recommendations for bone health to personalize workout routines that cater to inadequate skeletal sites. Long-term follow-up studies would assist in identifying consequences and fracture risk associated with bone redistribution.

## **Chapter 4: Article Male Endurance Athlete Tetrad: A Paradigm Shift**

### **Historical Perspective**

Since the early 1990's a substantial body of research has been conducted on female long-distance runners and bone health as a component of the female athlete triad (FAT) (2-10). FAT refers to an interrelated spectrum of conditions encompassing energy availability, bone mineral density (BMD), and menstrual status. The literature reports one or two components of the triad in men, such as low lumbar or radial bone mass or low levels of testosterone, but evaluation for all three components simultaneously (energy availability, testosterone, and bone mass) does not exist. Additionally, recent evidence in females suggests that a fourth component of cardiovascular dysfunction exists as part of the model. We propose a similar tetralogy condition in males exists, and have coined the term Male Endurance Athlete Tetrad (MEAT) for male endurance athlete tetrad. For men, MEAT includes a spectrum of energy availability, testosterone levels, bone mass and cardiovascular health.

Energy availability (EA) is commonly defined as dietary energy intake (Calories consumed) minus exercise energy expenditure (calories expended during exercise) normalized to fat free mass (FFM) (4). EA represents the amount of energy (Calories) available for the body to use for physiological functions after exercise training. These physiological functions include cellular maintenance and growth, reproduction, and

thermogenesis. In the FAT model, energy availability (EA) is the driving factor influencing menstrual status and BMD (4). In females, when energy availability becomes falls below 30 kcals / kg FFM, health is impaired in an effort to promote survival (78). Energy availability can be reduced as a result of increasing exercise energy expenditure and/or by reducing energy intake (4, 29). Loucks suggest that marathon runners may reduce energy availability for one of three reasons: 1). initially- to change body size and composition in an effort to improve performance; 2). compulsively- consistent with patterns of disordered eating; or 3). unintentionally due to a lack of a biological drive to balance energy intake with activity-induced energy expenditure (29) Regardless of whether energy availability is reduced intentionally or unintentionally, similar impairments on health result (4). Consequently, energy availability should aim for at least 45 kcals / kg FFM to maintain optimal skeletal health (77, 136)

It is commonly accepted that regular weight bearing physical activity can promote bone health throughout the lifespan (84). Running, however, may not produce high enough ground reaction forces to produce osteogenic benefits (137). Prolonged endurance activities, including running, have been associated with low BMD (18-22) and premature bone loss in both men and women (85, 86). Current literature suggests male distance runners may have a decreased BMD, specifically in the lumbar spine and radius (18-21). Similarly, in older men the three most common sites for osteoporotic fracture include the hip, vertebrae, and distal radius all of which are sites comprised mainly of trabecular bone (138). The observed decrease in BMD in male runners may be the result of several factors including increased levels of stress hormones (139), lower testosterone

levels (24), lower estrogen levels (21) and/or increased inflammatory mediators that affect bone turnover (140).

Researchers are still unclear as to the exact role that testosterone plays in bone health and bone remodeling (107). Hypogonadism has previously been identified as a risk factor for osteoporosis. Androgen and androgen receptors appear to play a major role in the acquisition of trabecular bone in men (119). Previous studies have demonstrated decreased testosterone in males engaged in endurance activities, while also presenting with decreased lumbar spine BMD, a site composed mainly of trabecular bone (131)

The last component of MEAT includes cardiovascular health. Regular aerobic physical activity is commonly suggested as a means to address dyslipidemia, primarily to lower triglycerides and increase HDL cholesterol (120, 121). However, limited data are available on the blood lipid profile of distance runners. Fasting serum lipid profiles of 67 male distance runners participating in the 2000 Calissia Ultramarathon were analyzed by Tomaszewski et al (122). While the mean lipid profile of all runners was surprisingly high, runners were further divided into lean ( $\text{BMI} < 25 \text{ kg/m}^2$ )  $n = 55$  or non-lean runners ( $\text{BMI} 25\text{-}29.9 \text{ kg/m}^2$ )  $n = 12$  (Table 6).

Regardless of category, mean values for LDL cholesterol and triglycerides do not fall within the optimal category as described by the NCEP ATP III guidelines (120). Similarly, in a study of fifteen nationally competitive male distance runners, LDL, triglyceride, and HDL levels fell outside the NCEP ATP III guidelines (123). In a cohort of 108 male runners (22 elite vs 86 recreational), irrespective of level of training mean

LDL was 123 mg/dl (124). High LDL cholesterol is a major risk factor coronary heart disease (120).

Lipid Fraction (mg/dL)	Lean Runners	Non-Lean Runners
Total Cholesterol	193.5 ± 34.8	224.5± 46.4
LDL Cholesterol	112.2 ± 21.1	170.3 ± 42.6
HDL Cholesterol	46.4 ± 11.6	34.8 ± 11.6
Triglycerides	168.3 ± 88.6	186.1 ± 106.3

Table 5: Summary of blood lipid values comparing lean runners to non-lean runners. Results are presented as means ± SD's. Adapted from Tomaszewski et al (122).

Furthermore, it is suggested for every 30 mg/dl increase in LDL, above 40 mg/dl, there is approximately a 30% increase in relative risk for developing coronary heart disease (120). For a male with a marginal LDL value of 115, this would translate to a 75% increased risk.

We propose the Male Endurance Athlete Tetrad (MEAT) as a model to explain the relationship(s) between energy availability, bone density, testosterone level and cardiovascular health observed in trained male runners (Figure 5). With the number of males participating in the marathon exceeding 300,000 recorded finishing times in 2011, a formal evaluation of the consequences of high mileage training deserves evaluation.

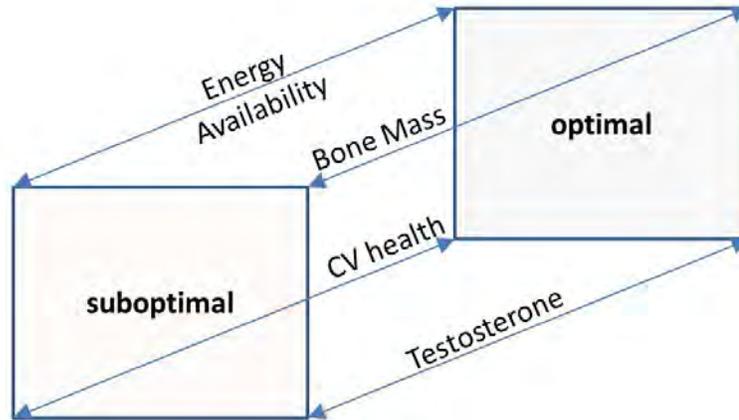


Figure 5: Visual representation of the Male Endurance Athlete Tetrad.

### **Assessing the Components of MEAT**

The evaluation of the tetrad will look similar to the female trilogy. Methods of assessment as well as cut points for each component of MEAT (BMD, EA, Testosterone level and Cardiovascular Health) are proposed in the following sections. When a male endurance athlete screens positive for one aspect of the tetrad, awareness of the potential interrelationship of the four axes would help the health care professional consider further evaluation of the other axes.

#### *Bone Mineral Density*

Dual energy X-Ray Absorptiometry (DXA) technology can be used to assess BMD. DXA is recognized as the accepted standard for determining BMD (63, 64). Previous research has found excellent in vivo precision (< 1%) for BMD measurements for the total body, lumbar spine, and hip when using the GE Lunar iDXA (64, 65).

Additionally, excellent in vivo precision has been established for the Lunar iDXA for measurements of body composition including lean tissue mass (CV 0.5%), total fat mass (CV 0.82%), and total bone mineral content (CV 0.6%) (65). The lumbar spine, hip and forearm, provide a measure of trabecular bone, which is most susceptible to fractures, while the total body scan provides a measure of cortical bone as well as body composition (fat mass vs lean mass). The adult human skeleton is made up of approximately 80% cortical bone and 20% trabecular bone (31). In theory, measurement of the non-dominant hip and forearm will provide information regarding the site in the body with the least amount of mechanical loading. An operational definition, used by our lab, of non-dominant hip is the opposite response to the following question, “If you were going to kick a ball would you use your left or right leg?” Similarly, an operational definition, used by our lab, of non-dominant forearm is defined as the opposite answer to the following question, “Are you left or right-handed?” Including all four skeletal scans will help to evaluate for bone sufficiency and also to provide a measure for body composition.

Results should be compared to normative data using an age-matched Z-score to classify BMD. Aged-matched Z-scores should be used as they control for age, gender, and ethnicity. The use of Z-scores, as opposed to T-scores, for BMD are based on the recommendations of the American College of Sports Medicine (ACSM) as well as International Society for Clinical Densitometry. For females, a normal BMD is defined as Z-score  $> -1$ , a low BMD is defined as Z-score between  $-1$  and  $-2$ , and osteoporosis is defined as a BMD Z-score below  $-2$  standard deviations, see Figure 6 (4). We suggest using similar cut-points in males.

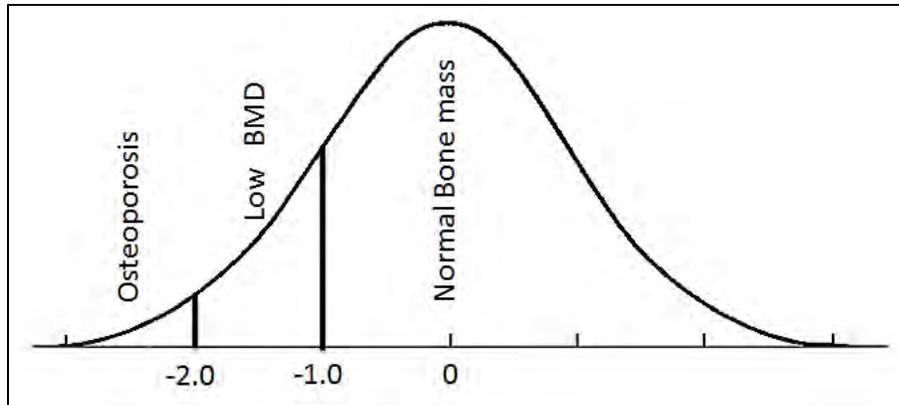


Figure 6: Bone Mineral Density Classification on a standard bell-shaped curve.

### *Energy Availability*

To determine energy availability, a measure of caloric intake, caloric expenditure, and lean body mass will need to be completed. Several methods are available to estimate caloric intake including a written three-day food record. A three-day written food record was previously demonstrated to accurately estimate caloric intake (99). Upon completion of the three-day record, data should be coded and analyzed using food processing software to evaluate the average caloric intake over the days recorded. To help improve accuracy, verbal instruction and written instruction as well as an example of a completed food record should be provided to ensure adequate detail.

In order to quantify energy expenditure from physical activity, an objective measure of physical activity should be used such as an accelerometer. Accelerometers provide data on the velocity of movement completed over a predetermined interval of time (141). Triaxial accelerometers, such as the ActiGraph GT3X+, are used to

conveniently measure acceleration in three planes: horizontal, vertical, and anterior-posterior. Accelerometers have previously been used and validated in human subjects (91, 93). We suggest the objective measure of physical activity should be completed for the same three days as the food record and subjective measure of physical activity.

An estimate for lean body mass is available from the total body scan from the DXA and can be used along with the diet and exercise data to calculate energy availability.

**Energy Availability**= mean dietary energy intake (kcal consumed) - mean exercise energy expenditure (kcal expended during exercise) / fat free mass (FFM), with a unit of kcal/kg FFM.

Previous research on females has demonstrated a significant decrease ( $P < 0.05$ ) in luteinizing hormone pulse frequency when EA falls below 30 kcal/kg FFM ( $P < 0.05$ ) (78). The inhibition of the pulsatile release of LH causes a suppression of ovarian steroidogenesis. Loucks and Thuman further theorize that the brain relies on a constant supply of glucose to maintain homeostasis and if the supply of glucose to the brain is not sufficient, subsequent decreases in the levels of sex hormones will result. In a second study, Ihle and Loucks (79) found significant ( $P < 0.05$ ) decreases in biomarkers related to bone formation (breakdown and remodeling) when EA fell below 30 kcal/kg FFM. As a result, suboptimal EA is defined as  $EA < 30$  kcal/kg FFM. Emerging literature on female athletes suggests that in order to maintain optimal skeletal health EA should be kept at 45 kcal/kg FFM (77, 136). In general, low energy availability is caused by restricting dietary energy intake, increasing exercise energy expenditure, or a combination of both (4)

### *Testosterone Level*

In accordance with the recommendations set forth by the Endocrine Society, a morning measurement of both total and free testosterone should be collected when screening men at risk for androgen deficiency (66). In healthy young men, the lower limit of normal total testosterone is 300 ng/dL and free testosterone is 9 pg/ml (66). An osmolality lab should also be ordered in conjunction with blood hormone tests to ensure values are not falsely elevated due to concentrated (dehydrated) blood.

### *Cardiovascular Health- Lipid Panel*

To assess aim cardiovascular health, fasting (at least 9 hours) serum concentrations of total cholesterol (TC), high-density lipoprotein (HDL) and triglycerides should be completed. Low-density lipoprotein (LDL), cholesterol can be calculated using the Friedewald's equation (142). Friedewald's equation estimates LDL using the equation:

$$\text{LDL} = \text{TC} - \text{HDL} - (\text{triglycerides}/5).$$

This is the standard LDL estimate used in many medical facilities. Results should be compared to recommendations set forth by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) guidelines in order to determine those who fall outside the optimal range (120). Table 7 provides reference values for blood lipids based on the NCEP ATP III guidelines.

<b>Category</b>	<b>Optimal</b>	<b>Low</b>	<b>High</b>
Total Cholesterol	<200 mg/dL		≥200 mg/dL
HDL Cholesterol	≥40 mg/dL	<40 mg/dL	
LDL Cholesterol	<100 mg/dL		≥100 mg/dL
Triglycerides	<150 mg/dL		≥150 mg/dL

Table 6: Blood lipid classification based on the National Cholesterol Education Program Adult Treatment Panel III (120).

### **Clinical Implications**

Health care professionals, including but not limited to physicians, registered dietitians, certified athletic trainers, physical therapists, exercise physiologists and sports psychologists, working with male endurance athletes should be aware of MEAT. Screening for MEAT may be difficult as overt health consequences may not be readily evident. However, health care professionals working with male runners, who consistently train for significant mileage, should consider screening for MEAT. BMD testing can serve as a starting point when screening for MEAT, especially in those with a previous history of stress fractures. Additionally, a registered dietitian could estimate EA and make dietary recommendations specific to training demands. Lastly, a fasting morning blood test could be completed to screen for blood lipids as well as total and free testosterone. Treatment of MEAT will vary depending on which component(s) is/are present. As the number of males participating in endurance sports continues to rise health care professionals need to begin screening for MEAT.

## Chapter 5: Integration

### Summary of Hypotheses

**H1.** Male runners will have low bone mass,  $Z$ -score  $\leq -1$ , in at least one of the four measured sites: total body; lumbar spine; non-dominant hip or non-dominant forearm. Table 7 provides a summary of BMD measurements for the total body, non-dominant femoral neck, lumbar spine, and non-dominant ultra-distal radius. Of the 41 participants, 18 had suboptimal  $Z$ -scores in at least one measured site. Overall, results from the study support hypothesis of inadequate BMD in male endurance runners.

Body Site	Mean Z-score $\pm$ SD	Low BMD $Z \leq -1.0$ to $-2$	Osteoporotic $Z \leq -2.0$	Sum of Suboptimal BMD
Total body	1.22 (1.0)	0/41	0/41	0/41 (0%)
Femoral neck	0.35 (0.96)	5/41	0/41	5/41 (12.2%)
Lumbar spine	-0.22 (1.13)	11/41	2/41	13/41 (31.7%)
Ultra-distal radius	-0.62 (1.18)	13/41	5/41	18/41 (43.9%)

Table 7: Mean BMD  $Z$ -scores and the number of subjects ( $n=41$ ) presenting with low BMD or osteoporosis for each of the four measured sites

**H2.** Male runners will have low energy availability,  $< 30$  kcal/kg FFM.

Of 40 male runners with energy availability data, 19 of the athletes' intakes were lower than 30 kcal / kg FFM, while 30 consumed less than 45 kcal / kg FFM. Overall, results from the study support the hypothesis of low energy availability in male endurance runners.

**H3.** Male runners will have low serum testosterone values, total testosterone  $\leq 300$  mg/dL and/or free testosterone  $\leq 9$  pg/m.

Of the 41 runners, 6 had total testosterone values below the total testosterone threshold ( $\leq 300$  mg/dL ) and 10 had free testosterone below  $\leq 9$  pg/m. Four runners had both low total and free testosterone. Overall, results from the study support the hypothesis of low serum testosterone values in male endurance runners.

**H4.** Male runners will have a suboptimal fasted blood lipid profiles defined as above the optimal range in at least one of the following categories: total cholesterol; low-density lipoprotein; triglycerides; and/or below the optimal range for high-density lipoprotein. Table 8 provides a summary of the number of individuals with optimal and suboptimal blood lipids. Of the 41 participants, 24 had at least one blood lipid value above the optimal range. Overall, results from the study support the hypothesis of suboptimal fasting blood lipid profiles in male endurance runners.

<b>Category</b>	<b>Optimal</b>	<b>Low</b>	<b>High</b>	<b>Frequency of suboptimal blood lipids</b>
Total Cholesterol	36		5	5/41 (12.2%)
HDL Cholesterol	36	5		5/41 (12.2%)
LDL Cholesterol	17		24	24/41 (58.5%)
Triglycerides	40		1	1/41 (2.4%)

Table 8: The number of subjects presenting with optimal and suboptimal blood lipid values

Components of the Male Endurance Athlete Tetrad (MEAT) exist within this cohort of trained male distance runners. Though all four components (decreased bone mineral density (BMD), decreased energy availability (EA), low testosterone, and dyslipidemia) did not often exist concurrently, various combinations of the four components were present with concerning frequency. Suboptimal results from any of the components should not be dismissed, but rather warrant further investigation into the underlying cause. Figures 7 and 8 depict the number of individuals presenting with low BMD (lumbar spine (n= 13) and distal radius respectively (n= 18)), inadequate EA (n= 19), low testosterone level (total (n= 6) and/or free (n= 10)), and/or cardiovascular disease risk (suboptimal blood lipid profile (n=24)).

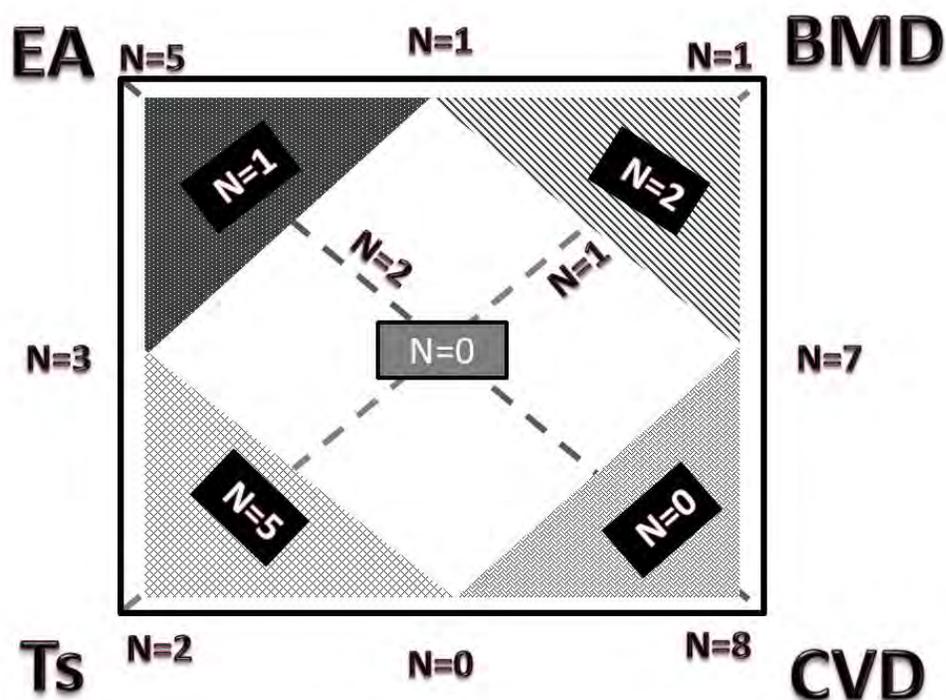


Figure 7: Visual representation of the findings of our study regarding low BMD, inadequate EA, low testosterone level (free and/or total), and suboptimal cardiovascular health with respect to the lumbar spine. The numbers located at the points of the square represent the number of individuals with suboptimal measurements in each singular category. The numbers located along the sides of the square represent the number of individuals with suboptimal measurements in the two categories. The numbers inside the triangles represent the number of individuals with suboptimal measurements in three categories. The number in the center of the square represents the number of individuals with suboptimal measurements in all four categories.

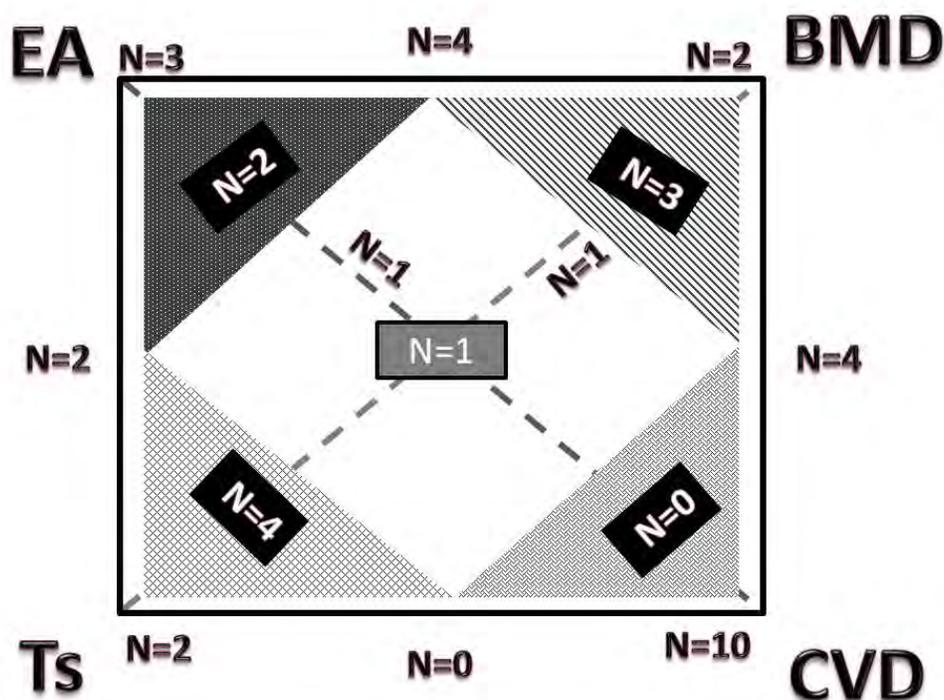


Figure 8: Visual representation of the findings of our study regarding low BMD, inadequate EA, low testosterone level (free and/or total), and suboptimal cardiovascular health with respect to the lumbar spine. The numbers located at the points of the square represent the number of individuals with suboptimal measurements in each singular category. The numbers located along the sides of the square represent the number of individuals with suboptimal measurements in the two categories. The numbers inside the triangles represent the number of individuals with suboptimal measurements in three categories. The number in the center of the square represents the number of individuals with suboptimal measurements in all four categories.

The clear existence of the various components of the triad (tetrad) in various combinations resembles the results of FAT studies. DiPietro and Stachenfeld have questioned the existence of FAT citing that the three conditions do not appear with great

frequency as a common cluster (143). The authors offer the opinion that over-exaggerating the risk of FAT symptoms suggests that the risk of exercise outweighs the benefits (143). Thus perhaps, suggesting that females should not exercise. The commentary drew great attention from the research community with follow up response by Loucks (144). While the triad (or tetrad) may not always occur as a single condition, ignoring the potential long-term deterrents of not eating sufficient calories should not be ignored. Targeted educational materials could be developed by sports dietitians to help address this issue. Rather than suggesting abstaining from exercise, the message could include fueling (eating enough calories) to maintain sufficient calorie balance (or energy availability). Based on our data, the same message would seem to hold true for men as well.

## **Discussion of Hypotheses**

### *Bone Mineral Density*

**H1.** Male runners will have low bone mass,  $Z$ -score  $\leq -1$ , in at least one of the four measured sites: total body; lumbar spine; non-dominant hip or non-dominant forearm.

Overall, results from the study support the finding of inadequate BMD in male endurance runners, especially in the lumbar spine and forearm. Similar to previously reported studies, nearly one-third (31.7%) of all subjects presented with inadequate lumbar spine BMD. However, no significant correlations were found between lumbar spine  $Z$ -score and any variables of interest (height, weight, BMI, body fat, mean energy intake, mean exercise energy expenditure, fat free mass, mean energy availability, serum vitamin D, total testosterone, free testosterone, and cortisol). Ackerman et al recently

found a strong correlation ( $r=0.57$ ,  $p=0.002$ ) between free estradiol and lateral spine BMD (21). Some researchers propose that estrogen may be just as important for bone health in males as in females (107, 119). This study did not measure estrogen levels, so we are unable to draw similar parallels.

Based on our BMD results, total body BMD screening does not appear to be adequate to assess overall bone health. Because our skeleton is comprised of mainly cortical bone (80%) compared to trabecular bone (20%), total body scanning does not capture those with inadequate trabecular BMD due to the low proportion of trabecular bone in the total skeleton. This is important to consider because the three most common sites of osteoporotic fracture in males are composed of trabecular bone (hip, lumbar spine, and forearm). If only total body BMD screening was used as the surrogate measure of bone health, we would not have identified any subject with inadequate BMD due to the total body scan serving mainly as a measure of body composition. Similar results were found by Buell et al in a group of recreational female runners, where only one subject out of 125 presented with inadequate total body BMD(145).

In the present study, after including the non-dominant hip, lumbar spine, and non-dominant forearm BMD measurements, 5 individuals were identified with an inadequate femoral neck, 14 with an inadequate lumbar spine and 18 with an inadequate forearm. These results are surprising given that one would expect approximately 16% of subjects to fall below -1 SD. We had nearly twice as many below -1 SD when measuring the BMD of the lumbar spine (31.7%) and more than two and a half times as many when measuring the BMD of the ultra-distal radius (43.9%). It is unknown if low BMD in young males translates into a higher risk of fracture as it does in older adults.

Though this study is cross sectional, the results may depict a pattern of potential bone redistribution or bone shifting. Although total body BMD appeared adequate, BMD was decreased at the trabecular sites. The BMD measurement at the ultra-distal radius was the lowest, followed by the lumbar spine, and then finally the hip. In our cohort of trained male runners, compared to the total body, bone mass appears to be lower in trabecular sites that receive little to no mechanical stimulation during running (spine and forearm). The concept of bone redistribution or “steal phenomenon” was first described by Smith et al in 1984 while studying the effects of exercise on bone density in women 35-65 years of age (133). The “steal phenomenon” refers to the redistribution of bone from non-loaded sites to loaded sites (134). Smith et al found that the bone density in the forearm of an aerobic exercise group decreased at a greater rate than that of the control group over the first year (133). Other researchers have noted similar redistribution of bone (21, 134, 135). Previous research supports the redistribution of bone to reflect the stress of the particular exercise and Frost’s Theory of form following function. It is not known what lifestyle factors contribute to or help resolve these site-specific issues.

A multivariate stepwise regression model was developed to explain the variance of BMD Z-scores at the ultra-distal radius using SAS. We first examined univariate models with the ultra-distal BMD Z-scores as the outcome and variables of interest (height, weight, BMI, age, age category, running mileage category, body fat, mean energy intake, mean exercise energy expenditure, fat free mass, mean energy availability, total body BMD Z-score, femoral neck BMD Z-score, serum vitamin D, LDL, HDL, triglycerides, total testosterone, free testosterone, cortisol, free T4, thyroid stimulating hormone, ADAM questionnaire, appearance evaluation total score and appearance

orientation questionnaire total score) as predictor variables. Composite variables were also created for cardiovascular risk (included z-score for total cholesterol:HDL ratio), nutrition (sum of z-scores for energy availability, mean calcium intake and serum vitamin D) and hypothalamic pituitary axis (sum of z-scores for free T4, cortisol and total testosterone). Despite using an age-adjusted Z-score, age was the first variable significantly explain the modeling sequence thus it was modeled alongside each variable of interest as a bivariate model to predict the BMD Z-scores in the ultra-distal radius. Age was evaluated as a continuous variable and as a categorical variable where subjects were parsed by decade, and the categorical variable was used in further modeling. After evaluation of all bivariate models to include age category, body fat was selected as the next strongest predictor. Trivariate models were then created using age category, body fat as combined with each of the other variables of interest. Age category, body fat and mean energy availability provided the strongest model. Modeling to identify a fourth variable left the trivariate model as the best model. To test the underlying assumptions of the regression model, we then plotted the model residuals to ensure they were normally distributed. Using the Shapiro-Wilk test statistic, we were able to determine that the residuals were normally distributed ( $p=0.538$ ). Lastly, we tested for multi-collinearity within the selected model to ensure that the variables were not highly correlated with each other by examining the variance inflation factors (VIF). The VIF values were low indicating a lack of collinearity between variables. Age category, body fat and mean energy availability were able to significantly explain 28.2% of the variance in the ultra-distal radius BMD Z-score as demonstrated in Table 7.

<b>R<sup>2</sup>=0.28217 p= 0.0071</b>	<b>Estimate</b>	<b>Standard error</b>	<b>t Value</b>	<b>p</b>
<b>Intercept</b>	0.401	0.890	0.45	0.6547
<b>Age Category</b>	0.773	0.231	3.35	0.0019
<b>Body Fat %</b>	-0.088	0.040	-2.20	0.0341
<b>Mean energy Availability</b>	-0.037	.017	-2.21	0.0333

Table 9: SAS output when modeling age category, body fat and mean energy availability to explain the variance of BMD Z-scores at the ultra-distal radius.

While this may not be an exceptionally great proportion of the variability, epidemiological data suggest that 60-80% of the variance in bone density is related to genetic factors (128). Therefore, in terms of BMD, this model predicts much of the remaining variability not attributed to genetic factors. There is also the potential that not all variables have a linear relationship with BMD and consequently result in a weaker model. Interestingly, two of the three model variables are modifiable (body fat and energy availability) which may be important to future studies aimed at improving BMD.

We expected to see stronger relationships between running mileage, cortisol and thyroid function (free T4 and thyroid stimulating hormone). Running mileage was limited, as only categorical data were available from the prescreening questionnaire. Including a follow up question, where subjects enter a numeric value for their weekly running mileage, could improve the relationship between weekly mileage and BMD, especially at the lumbar spine and radius. Stress hormones have previously been identified as a risk for osteoporosis; however, we did not find any relationship between

cortisol and BMD. Several stress hormones exist, but cortisol was selected due to the relationship with the hypothalamic-pituitary axis. Markers of thyroid function (free T4 and TSH) had no relationship with BMD. All subjects fell within the normal limits for both thyroid hormones and consequently may not be a sensitive marker for male runners.

No relationships were found between androgen deficiency, body image and BMD. Measurements for these were the Androgen Deficiency in the Aging Male (ADAM) and two subscales of the Multidimensional Body Self Relations Questionnaire (MBSRQ). The ADAM questionnaire was originally developed as a screening tool to identify hypogonadism in males 40 years of age and older. The questionnaire is composed of ten of the most common symptoms reported in men with low free testosterone levels and has been reported to have high sensitivity (88%) and acceptable specificity (60%) (146). Results from the ADAM questionnaire were transformed into a dichotomous variable (at risk and no risk). Since there was no strong relationship between measures of testosterone (total and free testosterone) and BMD, it is not surprising that results from the ADAM questionnaire did not stay in the model.

We used 2 subscales of the (MBSRQ) (147) to assess body image, appearance orientation and appearance evaluation. Appearance orientation (AO) is the overall investment in appearance by an individual and determines how important physical appearance is to that subject. A high AO score indicates importance on appearance, presentation and grooming; those with low scores are uninterested in appearance and do not spend much time to "look good". The second subscale we evaluated was appearance evaluation (AE), which serves as an overall measure of how critical one is of his or her appearance characteristics. A high AE score indicates positive and satisfied feelings with

appearance, whereas a low score indicates unhappiness with physical appearance. These subscales have high reliability (Cronbach's alpha = .80-.90) and are widely used to assess the perceptual and attitudinal aspects of body image. They have been utilized in numerous studies of behavioral aspects of body image, including eating, exercise, and substance use (148-151). In general, our subjects had high AO and AE scores and thus a positive body image.

Incorporating peripheral quantitative computed tomography (pQCT) into future studies may help to further explain differences in bony architecture in runners compared to controls. QCT would be able to measure volumetric density ( $\text{mg}/\text{cm}^3$ ) of both cortical and trabecular bone and produce three-dimensional images of bone, determine bone geometry, and subsequently predict bone strength. Some research suggests that bone geometry is the most accurate way to predict bone strength (69-71). Recent literature examining athletes demonstrates a change in shape of the bone where three dimensional volume and density of the bone would improve bone strength (72-74). However, whether similar results would be seen in runners training at high mileage is not clear.

### *Energy Availability*

**H2.** Male runners will have low energy availability, < 30 kcals/kg FMM

No previously published studies have linked low energy availability (EA) to low BMD in males. In female runners, research demonstrated that luteinizing hormone pulse frequency significantly decreases when EA falls below 30 kcals / kg FFM (78). Loucks theorized that the brain relies on a constant supply of glucose in order to maintain homeostasis and if the supply of glucose to the brain is not sufficient, subsequent decreases in the levels of sex hormones will result (78). Also, decreases in biomarkers

related to bone formation have been reported when EA falls below 30 kcals / kg FFM in females (79). It has been suggested that an EA of at least 45 kcals / kgFFM is necessary for optimal bone and hormonal health (78, 79). Our results indicate that on average males consumed 32.1 kcals / kg FFM. Interestingly, nearly half of all subjects 19/40 (47.5%) consumed less than 30 kcals / kg FFM and 75% (30/40) consumed less than 45 kcals/kg FFM. However, no relationships were found between EA and BMD. Decreases in BMD are likely to occur over a long period of time and only collecting three days of food and activity records is likely not an accurate representation of long-term EA. Future studies should aim to determine if a similar threshold for EA and biomarkers of bone formation are present in men.

### *Testosterone*

**H3.** Male runners will have low serum testosterone values, total testosterone  $\leq 300$  mg/dL and/or free testosterone  $\leq 9$  pg/m.

Previous studies have demonstrated a decrease in testosterone in males engaged in prolonged endurance activities (24, 25, 27, 28). We found similar results in our cohort of runners. Nearly one third men, (29%) 10/41 subjects had low levels of either total and/or free testosterone. Hypogonadism has previously been identified as an accepted risk factor for secondary osteoporosis in men (107-110). Prior studies show testosterone deficiency in 50–66% of elderly men with hip fractures (111, 112) and 20% in men with vertebral fractures (109, 110, 113). Hackney and colleagues (106) propose that endurance trained males may have a lower “set-point” for circulating levels of testosterone. Although we did not find a strong correlation between testosterone (total

and/or free) and BMD, researchers are still not clear whether a similar risk for fracture in younger men is present.

#### *Cardiovascular Health*

**H4.** Male runners will have a suboptimal fasted blood lipid profiles defined as above the optimal range in at least one of the following categories: total cholesterol; low-density lipoprotein; triglycerides; and/or below the optimal range for high-density lipoprotein.

We observed suboptimal blood lipid profiles in 24 out of 41 subjects (58.5%). LDL was the most common suboptimal blood lipid, with a mean LDL of 107 mg/dL. An optimal level of LDL was defined as < 100 mg/dL (120). Previous studies on runners have found similar results. Tomaszewski et al presented the lipid profiles of 67 male ultramarathon runners and reported that mean LDL level was 122 mg/dL. In a cohort of 108 male runners (22 elite vs 86 recreational) mean LDL was 123 mg/dL regardless of level of training (124). High LDL cholesterol is a major risk factor coronary heart disease (120). According to the NCEP ATP III, for every 30 mg/dL increase in LDL, above 40 mg/dL, is related to approximately a 30% increase in relative risk for developing coronary heart disease (120).

#### **Impact and Future Directions**

Male runners participating in prolonged endurance training appear to be at similar skeletal risks as female runners. We have shown that components of MEAT exist with a cohort of trained male runners. Components of MEAT appeared as combinations rather than a singular component. For example, in Figure 7, more than half of all subjects

(22/40) presented with 2 or 3 components of the tetrad. Most commonly, subjects presented with either low BMD and cardiovascular risk or low testosterone, low EA, and cardiovascular risk. Taken together, treatment methods should address which component(s) is/are present within each individual.

To our knowledge, our male runner data represent the first attempt to evaluate EA, BMD, testosterone level and cardiovascular health into a single study. Such data will provide the foundation to guide future interventions to assess the impact of long-term endurance training and BMD as well as other components of the tetrad. Further studies are needed to confirm and build on this preliminary work and to identify new potential causal and preventive factors within larger populations.

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## Appendix A: Questionnaires used

### Initial Screening Questionnaire:

We prefer to be in contact with you electronically and ask for you to please enter your email address\_\_\_\_\_.

1. On average, how many *miles per week* do you run?

A. < 40 miles B. 40-50 miles C. 50-60 miles D. >60 miles

2. On average, how many *days per week* do you run?

A. 1 B. 2 C. 3 D. 4 E. 5 F. 6 G. 7

3. How many *months* have you been training at this level?

A. < 12 B. 12-23 C. 24-35 D. 36-47 E.  $\geq$ 48

4. How many *years old* are you?

\_\_\_\_\_ years

5. Have you ever been told you have *low bone mass*?

A. Yes B. No

6. How do you self-identify?

A. Caucasian (non-Hispanic) B. African American (non-Hispanic) C. Hispanic D.

Asian or Pacific Islander

E. Native American or Alaskan Native

7. Are you a current **tobacco** user (any tobacco product: cigarettes, smokeless tobacco, cigars, pipes, etc.)?

A. Yes      B. No

8. Have you ever been told you have **diabetes** (type 1 or type 2)?

A. Yes      B. No

9. Have you ever been told you have **chronic kidney disease** (elevated creatinine or decreased GFR) ?

A. Yes      B. No

10. Have you ever been told you have a **thyroid disorder** (hypothyroidism or hyperthyroidism)?

A. Yes      B. No

11. Are you currently taking any **prescription medications to lower your cholesterol?**

A. Yes      B. No

12. Are you currently taking any **prescription medications known to influence bone mass** such as corticosteroids?

A. Yes      B. No

*Qualifying Answers*

Those with the all following answers were invited to participate in the full study.

1. B or C or D

2. D or E or F or G

3. C or D or E

4. 20-50

5. B

6. A

7. B

8. B

9. B

10. B

11. B

12. B

*Disqualifying Answers*

Those with any of the following answers were excluded from the study.

1. A

2. A or B or C

3. A or B

4. < 19 or >50

5. A

6. B or C or D or E

7. A

8. A

9. A

10. A

11. A

12. A

### Multidimensional Body-Self Relations Questionnaire (MBSRQ)

	<i>Definitely disagree</i>	Mostly disagree	<i>Neither agree nor disagree</i>	Mostly Agree	<i>Definitely agree</i>
My body is sexually appealing.	1	2	3	4	5
I like my looks just the way they are.	1	2	3	4	5
Most people would consider me good looking.	1	2	3	4	5
I like the way I look with my clothes on.	1	2	3	4	5
I like the way my clothes fit.	1	2	3	4	5
I dislike my physique	5	4	3	2	1
I am physically unattractive.	5	4	3	2	1
Before going out in public, I always notice how I look.	1	2	3	4	5
I am careful to buy clothes that will make me look my best.	1	2	3	4	5
I check my appearance in a mirror whenever I can.	1	2	3	4	5
Before going out, I usually spend a lot of time getting ready.	1	2	3	4	5
It is important that I always look good.	1	2	3	4	5
I use very few grooming products.	5	4	3	2	1
I am self-conscious if my grooming isn't right.	1	2	3	4	5
I usually wear whatever is handy without caring how it looks.	5	4	3	2	1
I don't care what other people think about my appearance.	5	4	3	2	1
I take special care with my hair grooming.	1	2	3	4	5
I never think about my appearance.	5	4	3	2	1
I am always trying to improve my physical appearance	1	2	3	4	5

Body image is a critical component of eating disorders or disordered eating. Body image includes one's mental conception of the body, which is divided into three domains; perceptual: attitudinal: and behavioral. Within each of the aforementioned domains are two subscales, orientation and evaluation. Orientation refers to the degree of importance and attention to the domain as well as behaviors related to improvement or maintenance. As opposed to, evaluation which refers to extent of liking, attainment and satisfaction. Behaviors such as calorie restriction or intense physical activity often serve as means to control weight or body shape. Therefore, we will use 2 subscales of the Multidimensional Body Self Relations Questionnaire (MBSRQ) (147) to assess body image. The first subscale we will use is Appearance Orientation. Appearance Orientation is the overall investment in appearance by an individual and indicating how important physical appearance is to that subject. Those with a high score place an importance on appearance, presentation and grooming; however those with low scores are uninterested in appearance and do not spend much time to "look good". The second subscale we will use is Appearance Evaluation. Appearance Evaluation is a global measure of how critical an individual is of his/her appearance characteristics (one's attractiveness and satisfaction with one's looks). Those with a high score on appearance evaluation feel positive and satisfied with their appearance, whereas those with a low score are unhappy with their physical appearance. These subscales have high reliability (Cronbach's alpha = .80-.90) and are widely used to assess the perceptual and attitudinal aspects of body image. They have been utilized in numerous studies of behavioral aspects of body image, such as eating, exercise, and substance use (148-151). The data collected can examine the impact of body image on fueling patterns within the male distance runner population. Questions

1-7 represent the AE domain and questions 8 – 19 represent the AO domain. Note when scoring the MBSRQ questions 6, 7, 13, 15, 16, and 18 are reversed scored.

## The Exercise Addiction Inventory (EAI)

	<i>Strongly disagree</i>		<i>Neither agree nor disagree</i>		<i>Strongly agree</i>
Exercise is the most important thing in my life	1	2	3	4	5
Conflicts have arisen between me and my family and or my partner about the amount of exercise I do	1	2	3	4	5
I use exercise as a way of changing my mood (e.g. to get a buzz, to escape, ect.)	1	2	3	4	5
Over time I have increased the amount of exercise I do in a day	1	2	3	4	5
If I have to miss an exercise session I feel moody and irritable	1	2	3	4	
If I cut down on the amount of exercise I do, and then start again, I always end up exercising as often as I did before	1	2	3	4	5

The Exercise Addiction Inventory (EAI) was used to identify study participants at risk for exercise addiction. Rewarding behaviors, including exercise, when practiced obsessively could trigger severe negative consequences. In the case of exercise, negative consequences may include mood disturbances, hypercortisolemia and/or decreased libido as well as destructive behaviors such as anorexia nervosa (152). The EAI uses six components of behavioral addiction, which are applied to the measurement and identification of exercise addiction. The EAI can distinguish between exercise addiction

and exercise commitment. The inventory also shows excellent concurrent validity compared with the Obligatory Exercise Questionnaire ( $r = .80$ ) and the Exercise Dependence Scale ( $r = -.81$ ), two previously validated questionnaires. Note the scoring on the Exercise Dependence scale is reversed compared to the other two questionnaires. Additionally, content and construct validity have been established for the EAI. When scoring the EAI a sum of scores is used to categorize subjects into one of three categories:  $\geq 24$  points = at-risk of exercise addiction, 13-23 = symptomatic, and  $< 13$  points = asymptomatic.

## **Androgen Deficiency in the Ageing Male (ADAM) Questionnaire**

1. Do you have a decrease in libido (sex drive)?

A. Yes      B. No

2. Do you have a lack of energy?

A. Yes      B. No

3. Do you have a decrease in strength and/or endurance?

A. Yes      B. No

4. Have you lost height?

A. Yes      B. No

5. Have you noticed a decreased “enjoyment of life”?

A. Yes      B. No

6. Are you sad and/or grumpy?

A. Yes      B. No

7. Are your erections less strong?

A. Yes      B. No

8. Have you noted a recent deterioration in your ability to play sports?

A. Yes      B. No

9. Are you falling asleep after dinner?

A. Yes      B. No

10. Has there been a recent deterioration in your work performance

A. Yes      B. No

The Androgen Deficiency in the Aging Male (ADAM) questionnaire was originally developed to be a screening tool to identify hypogonadism in males 40 years of age and older. The questionnaire is composed of ten of the most common symptoms reported in men with low free testosterone levels and has been reported to have high sensitivity (88%) and acceptable specificity (60%) (146). A positive screening is defined as a “yes” to questions number 1 or 7 or any three other questions, see page 86.

## Appendix B: Raw Tetrad Data

Subject_ID	LDL	TC	Total Ts	Free Ts	Total Z-Score	Neck Z-Score	L1-L4 Z-Score	Radius UD Z-Score	Mean_EA
1	141	224	420	9.77	0	0.6	-0.4	-1.9	48.89
2	74	134	217	6.84	-0.1	0.1	-2	-0.4	38.08
3	93	168	832	20.82	-0.2	-0.1	-1.9	-2.2	47.02
4	120	174	293	12.29	2.1	0.6	0.2	-0.3	28.63
5	119	179	662	13.74	1.8	0	-1.1	1.5	17.15
7	84	145	359	11.55	1.8	1	1.9	-1.1	23.47
8	99	180	359	9.54	2	1.2	1.2	0.1	35.25
9	134	192	499	10.42	1.3	-0.3	0.9	-2.3	26.33
10	94	163	346	8.16	0.4	0.3	0	-0.5	23.71
11	86	140	422	11.04	0.3	-1.4	-1.4	-0.2	25.32
13	98	152	623	13.68	0.4	-0.3	-0.3	-1.2	28.53
14	188	303	743	22.87	2.7	0.7	-1.6	0.3	31.40
15	117	188	658	20.81	1.7	1.8	0.4	-1.1	36.46
16	111	190	652	12.86	1.2	1.1	0	0.8	32.11
17	122	184	581	14.53	1.3	-0.9	0.3	-0.9	47.05
18	84	139	348	8.45	2.1	1.6	-0.4	-1.4	13.87
19	118	188	506	12.37	2	0.1	-1.6	0.6	32.62
20	110	174	671	13.12	2.3	1.3	0.3	2.5	43.72
21	142	202	535	13.15	0.8	-0.1	-1.6	-0.3	30.84
22	92	154	208	9.71	3.2	2.5	0.5	2	14.84
23	97	140	552	9.45	2.9	1.8	1.4	0.3	21.18
26	122	188	534	9.94	1.2	1.4	-1.4	-0.5	37.58
27	67	124	343	11.67	0.4	-0.1	-0.6	-0.6	47.01
28	93	179	634	12.7	1	0.6	0.7	-1.8	43.00
29	100	146	591	14.42	2.9	1.2	1	0	
30	165	226	242	6.5	2	0.5	-0.3	0	24.43
31	100	161	608	14.48	0.6	0.6	0.8	-1.7	12.50
32	77	141	292	4.15	0.7	0.2	-1	-1.6	18.18
34	113	200	258	8.25	1.2	0.5	0.1	-0.8	26.56
35	95	189	487	12.31	2.7	0.4	2.9	-1.5	27.34
36	117	176	376	11.13	0.2	-1	-2.2	-2	29.56
37	105	190	671	15.34	1.7	2.3	0.2	-0.8	36.80
38	109	160	522	10.5	-0.4	-1.1	-1.5	-1.9	48.13
39	106	197	546	10.94	-0.6	-1.2	-0.8	-1.8	50.30
40	86	163	457	10.07	0.5	-0.3	0.6	-1.1	29.43
45	110	171	387	9.45	0.3	-1.3	-1.2	-2.1	45.54
46	122	193	453	8.22	1.9	-0.6	0	-0.1	29.16
47	101	190	492	10.37	2.1	0.7	-1.2	1.2	35.43
48	109	187	323	8.09	0.2	-0.2	-0.6	-2	23.92
54	80	158	457	8.59	1.1	0.3	0.5	0.5	30.70
55	95	166	334	8.39	0.5	-0.3	0.1	-1.4	43.30

- 0 components = 2
- 1 component = 9
- 2 components = 19
- 3 components = 10
- 4 components = 1