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Correlations between Body Composition Components and Bone Mineral Content in Untrained Young Men and Women

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Abstract

PURPOSE: Low bone mineral content has been linked to the development of osteoporosis in humans. Research is divided as to whether fat or lean mass plays a more significant role in the development of bone mass. Physical activity has also been shown to increase bone mineral content in adolescents and combat the loss of bone density with aging. The purpose of this study was to examine correlations that exist between bone mineral content and anthropometric and performance measurements in untrained young men and women. METHODS: 78 untrained males (age 22.3±3.7) and 56 females (age 22.8±3.7) completed a battery of baseline testing measures. Anthropometric measurements such as height, weight, and waist circumference were recorded, as well as a Dual X-Ray Absorptiometry (DXA) scan to determine body composition, bone mineral content and density. In addition, subjects also completed maximal lower body strength and power tests. RESULTS: The primary finding of this investigation was that body mass shows the strongest correlation to bone mineral content in men, women, and the total group (r=0.740, p=0.00; 0.697, p=0.00; and r=0.797, p=0.00, respectively). When examining the components of body mass, lean mass is a stronger predictor of bone mineral content than fat mass in all groups. When examining the men and women grouped together, lean mass showed a correlation of r=0.795 (p=0.00) while fat mass did not correlate to BMC (r=0.263; p=0.00). Though the correlations for lean and fat mass in the separate male and female groups were not strong correlations, lean mass showed a stronger correlation to BMC than fat (r=0.647, p=0.00 and r=0.667, p=0.00 respectively, for lean mass; r=0.553, p=0.00 and r=0.550, p=0.00 respectively, for fat mass). We

found that there were no significant correlations between maximal squat strength and bone mineral content in men or women.

CONCLUSIONS: We found that body mass was the strongest predictor of bone mineral content in untrained men and women. As we expected, lean mass seems to play a greater role in this correlation than fat mass. Contrary to our hypothesis maximal lower body strength did not correlate with bone mineral content in men or women. We believe this to be the result of varied levels of physical activity between subjects, lending that physical activity levels may correlate better with BMC than sheer physical strength.

PRACTICAL APPLICATIONS: Determining the relationships that exist with bone mineral content will allow us to see what might put individuals at risk for developing low BMC and osteoporosis. Despite that we cannot claim that lean mass causes bone mineral content to increase, we can show that there is a relationship between the two that should be explored through future research. In many settings the use of technology utilized in this study, such as the DXA scanner, is not possible. For these instances we used multiple regression analysis to show that using weight, height, and maximal squat strength can account for 77% of the variance in BMC measurements.

Chapter One

Introduction

According to the National Institutes of Health, over 40 million people in the United States either have osteoporosis or are at risk of developing the disease due to having low bone mineral content or density. This bone disease affects men and women of all ages and ethnicities. Older women, especially Caucasian and Asian, are at a higher risk than others but no one is immune to osteoporosis or bone loss. It has been shown through the examination of mono- and dizygotic twins that there is a significant genetic component to the development of bone mineral content and density in humans [1]. Despite the genetic component there are ways to increase, or compromise, the amount of bone mineral content a person can accrue in their lifetime. This includes nutrition, physical activity, and lifestyle choices [2].

Bones have numerous functions including maintaining body shape, protection of internal organs, and allowing for locomotion. Bone fractures can range from minor to devastating and can greatly affect a person's ability to function in daily life. Weak bones are more susceptible to breakage with less traumatic force [3]. Peak bone mass is reached during bone growth in adolescence and young adulthood. This is the most bone mineral a person will ever have because after this time period, bone resorption begins to outweigh bone formation. The hip and vertebrae are the most common sites of bone loss and fragility, thus the most common sites of osteoporotic fracture. Other than increased occurrence of fracture, there is no outward sign to warn an individual that they are losing

bone mass or that their bone quality is poor [2]. If an individual achieves a high peak bone mineral content they may be able to lose bone at a normal rate and still be able to maintain sufficient bone quality and avoid osteoporosis. Thus, it is important to determine what factors will lead to the highest levels of bone mineral content in youth and young adulthood in order to avoid osteoporosis and possibly debilitating fractures in the future.

In order to achieve the high levels of peak bone mass that are necessary to combat osteoporosis, several factors come in to play. Some of these factors are predetermined and cannot be changed by the individual. These include genetic predisposition, gender, race, and age [1, 2]. Men tend to have higher measures of bone mineral content simply due to being taller and heavier than women. Also, women lose more bone with aging than do men because of menopause and the abrupt loss of female sex hormones [2].

Factors affecting bone mineral content that are controllable include, but are not limited to, nutrition, lifestyle choices, physical activity, and body composition. Calcium comprises 95% of the mineral in bone and vitamin D aids in the body's ability to absorb the calcium from the diet [2]. Studies have shown that when a person ingests adequate levels of calcium and vitamin D, additional supplementation does not have an effect on bone mineral content. In contrast, if dietary intake of calcium and vitamin D are insufficient, supplementation increased bone mineral content in children [4]. Physical activity is probably one of the most easily manipulated factors relating to bone mineral content. Being physically active only requires time and dedication by the participant. Through childhood and adolescence the impact forces created from physical activity and

sports participation cause stress and strain on the bone, causing it to remodel and become stronger. Most evidence shows that nonimpact exercise, such as cycling and swimming, do not have a great impact on bone remodeling due to the lack of ground reaction forces. The pull of muscle on bone due to muscle activity alone does not cause enough bone stress to encourage remodeling. Dook et. al. compared women participating in sports with different impact intensities and shows that sports, like basketball, create ground reaction forces 3.9-4.6 times the athlete's body weight. In moderate impact intensity sports, like running, create 2-3 times an athlete's body weight in ground reaction forces [5].

Numerous studies have shown that increased body weight positively impacts bone mineral content [6-11]. This is due to the fact that increased body mass causes increased stress and strain on the bone from daily activities such as walking. When bone mineral content measures are corrected for body weight and are able to be compared across a population with a wide range of body masses, there actually seems to be a negative correlation between obesity and bone mineral content [9]. When the components of body mass are examined independently lean mass seems to have a stronger correlation to levels of bone mineral content than fat mass. The present study examines an untrained population of young men and women to see if correlations indeed exist between the components of body mass and bone mineral content. In addition, we will also look at maximal squat strength to see if correlations exist between lower body strength and bone mineral content.

Statement of the Problem

Therefore, the purpose of this study was to examine correlations that exist between bone mineral content and anthropometric and performance measurements in untrained young men and women.

Chapter Two:

Review of Literature

This review will examine the role body composition and strength play in the development of bone. Evidence in the literature is divided whether fat mass plays a role in increasing or decreasing bone mineral content. There also is controversy regarding whether lean mass or fat mass plays a more active role in predicting bone metabolism. Maximal strength has not been thoroughly examined in regards to bone density but there is a strong correlation between lean mass and strength which allows for some crossover when comparing data. Different types of physical activity and sport participation have been shown to effect bone mass due to the impact forces exerted on the bone and muscular attachments. Also, a majority of the literature focuses on demographic groups not included in this research study. Ample data exists regarding pre- and post-menopausal women, adolescent girls, and racial groups; but little has been presented focusing on healthy young men and women. Though each population is different in their stages of bone development, examining several different populations will allow for a broad overview of the correlations that exist between body composition, strength, power, and bone mineral content.

Bone Mineral Content

Bone mineral content is the amount of bone mineral in the selected area of bone. Bone mineral content is a predictor of osteoporosis and fracture risk, especially in elderly

individuals. Throughout childhood the skeleton increases in size many times. Most people will achieve the maximum amount of bone mass they will ever have between the ages of 18 and 30. This amount of bone is referred to as the peak bone mass [2]. Because the skeleton does not accrue much, if any, more bone mass after the third decade of life, it is extremely important that children and adolescents gain as much bone mineral content as possible. Having a high peak bone mineral content in adolescence may help to offset the effects of bone loss due to aging, and menopause in women [12], [13]. The strength of bones is usually dependent on the amount of bone contained in a cross-section (density), the total cross-sectional area, and the length of the bone itself [14]. Density of the bone is important because more mineral within a given cross-section will make the bone stronger and more resistant to fracture. The greater cross-sectional area of a bone gives it more strength when bending or torque is applied when compared to bones with similar bone mass but smaller cross-sectional area. Finally, long bones are more susceptible to breakage when bending forces are applied than are short bones [14]. This is due to the mechanics of lever systems. For example, imagine taking two sticks of equal circumference but one being twice as long as the other. It would take far less force to break the long stick at the center than it would to break the short stick in the center. The same mechanics apply to bones in the human body.

There are several variables that can have an effect on bone development. In 1987, Pocock et. al. showed that there was a genetic component to bone development [1]. Pocock compared 65 sets of mono- and dizygotic twins in ages ranging from 24-75 years. Figure 2 (below, from [1]) shows a correlation of 0.73 for the monozygotic twins and 0.33 for the dizygotic pairs ($p<0.005$). This study established that there is a significant

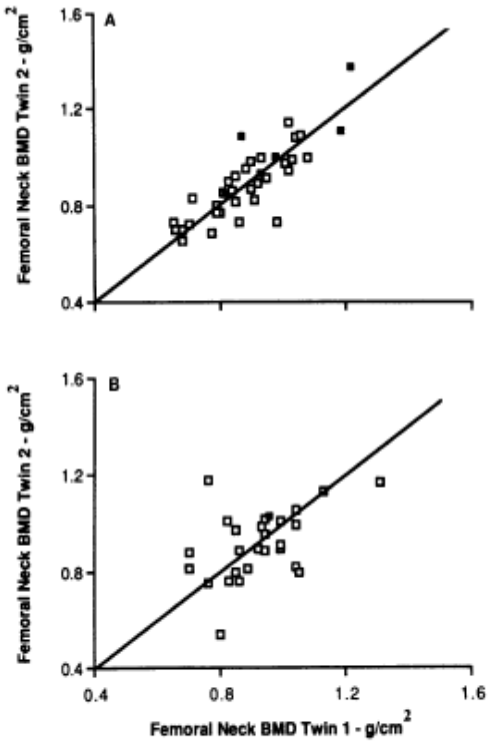

Figure 2. Correlation of femoral neck BMD between (*A*) MZ twins and (*B*) DZ twins showing male (■) and female (□) twin pairs. The line of identity is shown.

genetic component associated with bone mass at the proximal femur and lumbar spine, as well as confirming previous findings of a genetic contribution to bone mass in the upper limb [1]. In addition to genetics, dietary intake plays a role in the development of bone mass, especially in children where bone turnover is at its highest. A meta-analysis by Huncharek et. al. showed that combined data from 21 randomized control trials did not yield a statistically significant increase in total body bone mineral content in dairy calcium supplemented groups versus controls [4]. However, within the data collected in the meta-analysis there were three trials that used subjects with low intakes of dietary calcium. When the subjects with low dietary intakes of calcium were supplemented with dairy calcium there was a statistically significant increase in bone mineral content. This shows that when nutritional minimums of calcium are met, additional supplementation does not affect bone mineral content. In contrast, if the intake of calcium in the diet was less than sufficient, additional supplementation allowed for increased bone growth [4]. Physical activity and gender

both have effects on bone mineral content throughout populations but will be discussed in individual sections of this review.

Gender differences in BMC

There are differences that exist between men and women in terms of bone development. It is generally accepted that on average men are larger in stature than women. Because of this men commonly have higher bone mineral values than do women due to mechanical loading effects. This belief is confirmed by an evaluation of 171 men and 218 women at the age of 50 years old done by Tuck et. al. [15]. The men in the study were both taller and heavier than the women, and had higher values of bone mineral content, bone area, and bone mineral density at all measured sites. However, when the values were corrected for height and weight, the men actually had substantially lower volumetric bone density than did the women [15]. A study conducted on 1120 urban Spanish individuals concluded that women stop accruing bone mass around age 20 whereas men continue to gain bone until about the age of 25 [16]. This study was restricted to an urban Spanish population and thus cannot be generalized to other ethnic populations, but it does give insight to why women are at a higher risk of developing osteoporosis later in life. Due to the fact that men are able to continue adding bone mass for an additional 5 years longer than women, women are not able to reach the same peak bone mass as men and thus begin losing from a lesser value than men.

Focusing specifically on the differences between men and women in bone loss due to aging, women tend to lose more bone as they age than do men. This is commonly

attributed to the onset of menopause and the loss of female sex hormone production. Davis et. al. examined Japanese-American women in Hawaii with ages ranging from 45-81 (mean age=64years), with ninety-two percent of the women being at least 5 years post-menopause. Single-photon absorptiometry was performed on the calcaneus and showed that after the mid-sixties bone loss at the calcaneus remained constant [17]. Though the study does not directly attribute the loss of calcaneal bone mass to the lack of female sex hormones, a study by Dalén et. al. showed that after three years of hormone replacement therapy, women increased their bone mineral content measures by an average of 3% per year when compared to a control group [18]. Osteoporosis in men is a rarely studied phenomenon and thus there is very little literature present regarding bone loss in the male gender. The most common cause of bone loss in elderly men is hypogonadism, augmented by lifestyle choices that also impact the gonads such as smoking and alcohol consumption [19].

BMC in Lean and Obese Individuals

In general, obese individuals tend to have higher crude bone mineral measures due to the fact that the bones of these individuals are constantly loaded with the body weight they are forced to carry. The majority of the literature shows this to be true but also agrees that when the crude numbers are normalized for body mass, obese individuals actually have lower bone mineral values than their lean counterparts. Zhao et. al. presents a compelling study in support of this idea. They found that when bone mineral results are corrected for the mechanical loading effect of total body weight, fat mass has a

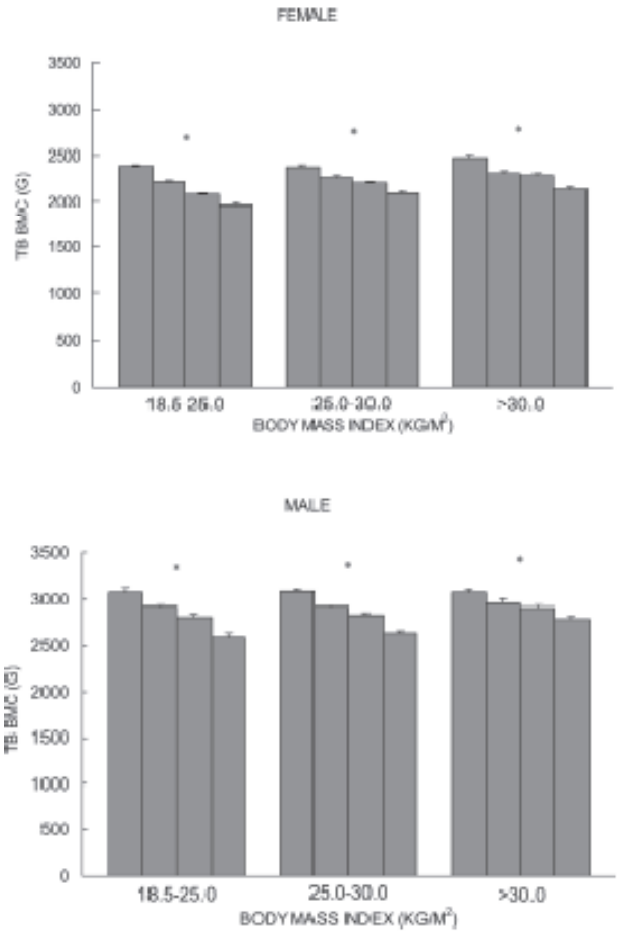

FIG. 1. Least-squares mean (±SE) of the TB BMC stratified by percentage fat mass in normal weight (18.5 < BMI ≤ 25.0 kg/m^2), overweight (25.0 < BMI ≤ 30.0 kg/m^2), and obese (BMI > 30.0 kg/m^2) white men and women. Each bar in each BMI stratum represents quartiles (Q) 1, 2, 3, and 4 (from left to right) of percentage fat mass. A linear mixed model was used with age, weight, exercise, and menopause status as covariates to adjust TB BMC. Familial relationships were treated as random effects in the model. *p < 0.0001.

detrimental effect on bone [9]. Zhao et. al. divided 4489 Caucasian subjects into body mass index (BMI) groups according to the World Health Organization (WHO) cutoff points[20], normal weight (18.5<BMI≤25.0 kg/m^2), overweight (25.0<BMI≤30.0 kg/m^2), and obese (BMI<30.0 kg/m^2). With the groupings, Zhao and colleagues plotted the least-squares means of total body (TB) BMC for both men and women, and within each BMI category broke down the quartiles of percent fat mass (Figure 1, from [9]). The graphs show a significant negative relationship between the percentage of fat mass and bone mineral content within BMI subgroups. Another study conducted by Rocher et. al. showed similar findings in children when a group of 20 obese children were compared to a group of 25 normal weighted controls. In terms of crude bone values, the obese group had BMC and bone area (BA) measures significantly higher than the controls where p<0.05 and <0.01 respectively. When adjusted for total body weight, the data showed the opposite result. Control children had significantly higher BMD, BMC, and BA values where p<0.05,

<0.01, and <0.001, respectively [11]. Finally, El Hage et. al. showed that when crude numbers are corrected for body mass there becomes no difference between the bone mineral values of obese, overweight, and normal weighted adolescent girls [10]. The authors attributed the no difference to the fact that all subjects in the study were controlled for physical activity level (all being sedentary) whereas other papers do not. Activity level has an effect on bone mineral values and thus not controlling for this variable could confound results.

BMC in Physically Active and Sedentary Individuals

Physical activity has also been shown to increase bone mineral content in people of all ages and genders. The American College of Sports Medicine (ACSM) deemed physical activity as an effective way of reducing the risk of osteoporotic fractures [3]. According to the ACSM, "In humans, physical activity appears to play an important role in maximizing bone mass during childhood and the early adult years, maintaining bone mass through the fifth decade, attenuating bone loss with aging, and reducing falls and fractures in the elderly" [3]. The ACSM noted that despite many people's view of exercise focuses heavily on metabolic and cardiovascular training, bone stimulus is caused by the physical deformation of the bone caused by weight bearing activities and to continue adaptations a progressively increasing load must be applied. Dook et. al. examined women that had been consistently participating and competing for over 20 years in high impact, medium impact, or non-impact sports [5]. These women were also compared to sedentary controls. The high impact group consisted of athletes

participating in basketball and netball, sports that involve running and vertical jump-landing sequences. The ground reaction forces estimated for these types of activities was between 3.9-4.6 times body weights. The medium impact group consisted runners and field hockey players. Running has been shown to produce ground reaction forces of about 2-3 times body weights, and since field hockey is a sport that involves running but very little in the way of jump-landing sequences it was also included in the medium impact group. Swimmers made up the final group of active women, those who participated in non-impact activities. Dook et. al. showed that the high impact group had higher whole body and regional leg bone mineral density than both the non-impact and control groups, while the medium impact group showed higher values in whole body bone mineral density and regional leg bone mineral density than the control group [5]. Similarly, Pettersson et. al. compared male sedentary and high level ice hockey players (age ≈ 24years) and found that the highly trained athletes had significantly higher bone mineral density than untrained counterparts [21]. In regards to resistance training and weightlifting, as opposed to general physical activity, Conroy et. al. showed that junior Olympic weightlifters had significantly higher bone mineral density measures at the spine, femoral neck, trochanter, and Ward's triangle than matched controls and adult reference data (untrained men age 20-39) [22]. Significant data shows that individuals involved in physical activity, particularly those creating higher ground reaction forces, have higher bone measures than their sedentary controls.

Different Variables as Predictors of BMC

The literature is diverse when it comes to determining what variables are the strongest predictors of bone mineral values. The results are varied depending on the type of subjects (lean v. obese, active v. sedentary, types of physical activity, male v. female, age groups, etc.). Literature is divided between several outcome variables proposed to be the best predictors of bone mineral values in the chosen subject group.

Body mass: As previously discussed, total body mass is strongly correlated with bone mineral values. However, as Zhao et. al., El Hage et. al., and Rocher et. al. demonstrated, when the mechanical loading effect of total body weight is removed the correlations disappear or even reverse [9], [11], [10].

Lean mass: Madsen et. al. performed a study examining 60 college aged women that were divided into three groups: low weight athletes (LWA), low weight sedentary persons (LWS), and average weight sedentary persons (AWS). The study showed correlations between lean body mass (LBM) and BMD in all groups [23]. Janicka et. al. studied 150 male and 150 female students with ages ranging from 13-21 to determine if fat mass is beneficial in gaining bone mass. They found that regardless of age or gender, there was a positive correlation between the amount of lean body mass measured and the density of bones in the vertebra and lower extremity [24]. In a study of active and sedentary young women it was found that in the sedentary and overweight populations, lean body mass accounted for 52.1% and 61.4% of the variance in leg bone mineral content. However, when comparing sedentary to active populations, the influence of LBM on leg BMC was higher in the sedentary group. Lean body mass accounted for 82.1% of the variance in leg BMC within the sedentary population but only accounted for

50.5% of the variance in the leg BMC values in the active group [25]. This relationship shows, again, that ground reaction and loading forces on bone have a greater impact on bone development than does body composition. Foo et. al. showed that lean body mass is a stronger independent predictor of total body bone mineral content and bone area than fat mass. In their study of 283 girls (average age=15 years) they showed that LBM explained 84% of the variance in total body BMC where fat mass only explained 3-11% of the variance [26]. Foo et. al. also attempted to remove the confounding variable of physical activity by showing that only 27% of participants participated in high impact activities and only 16% were enrolled in organized sports.

Fat mass: In the study by Madsen et. al., when the athlete group was removed from analysis there was a significant correlation between fat mass and total body BMD [23]. This shows that physical activity increases bone density beyond the contribution of body composition alone. In the previously mentioned study by Janicka et. al., when bone mineral values were corrected for lean mass the measures of body fat had either no correlation or a negative correlation with bone mineral values [24]. Lu et. al. chose to search for correlations between percent body fat and percent trunk fat and bone mineral density in a group of 1,147 Chinese, white, and black subjects. They found that even after adjusting values for height, weight, and ethnicity there was still a negative correlation between body fat or trunk fat and BMD [27]. In a mini-review, Reid found opposing results to the previously presented, claiming that fat mass was a more significant determinant of bone density than lean mass. Reid cites stem cell linkage between bone marrow and adipocytes as well as hormonal commonalities in β-cell hormones and insulin resistance [6].

Strength: In the Pettersson et. al. study elite male ice hockey players were compared to sedentary controls. The study showed that in the sedentary group, isokinetic leg strength was a predictor of bone mineral density of the leg and total body [21]. In contrast, muscle strength was not a predictor of BMD at any site for the highly trained group. "Hence it seems that impact forces may be of greater importance in regulating bone mass than muscle strength itself in highly trained athletes" [21]. Conroy et. al. studied junior Olympic weightlifters and showed a strong positive correlation between bone mineral densities of the lumbar spine and proximal femur and total strength [22]. Ribom et. al. studied 64 women and 61 men (age=21 years) to determine if correlations exist between muscle strength and bone mineral density. Handgrip, knee flexion, and knee extension were tested as measures of muscular strength. The study showed that handgrip did not correlate with any BMD values in men nor women. The male participants did not show any correlation between muscular strength tests and BMD, whereas women showed correlations here. In women, leg muscle strength explained about 30% of the variance in total body BMD [28].

Hypotheses

1. Body mass will strongly correlate with bone mineral content for both men and women.

2. Lean mass will be a stronger predictor of bone mineral content than fat mass.

3. Individuals with greater strength will show greater bone mineral content.

Chapter 3

METHODS

Experimental Approach to the Problem

In order to determine if correlations existed between bone mineral content and different anthropometric and strength measures, we examined data collected during the baseline testing protocol of the Resistance Exercise and Protein Supplementation (REPS) study. We used a cross sectional design, utilizing untrained men and women from the general university population. Each subject was given an in depth overview of all the tests and procedures they would be subjected to during participation in the study such as the Dual X-Ray Absorptiometry (DXA) scanner, various surveys, and performance testing. Body composition and bone content were obtained from the DXA scan of each participant. In regards to the performance testing, each subject was provided with a familiarization session during which the tests they would be completing for the study were explained, demonstrated, and practiced. The familiarization sessions were supervised by Certified Strength and Conditioning Specialists (CSCS) who provided feedback and coaching to the subjects to ensure proper technique during the testing protocol. Therefore, this experimental design will examine the correlations that may or may not exist between anthropometric and strength measures and the bone mineral content of participants.

Subjects

This investigation was approved by the University of Connecticut's Institutional Review Board for use of human subjects in research. In an information session, each subject was verbally instructed on the study procedures, as well as the risks, inconveniences, and benefits of participation. At the end of this information session, each subject volunteered their participation via a signed written consent form (See Appendix A). All subjects completed a medical history questionnaire, which was reviewed by our Medical Monitor, and a self-reported physical activity level questionnaire, which was reviewed by study personnel. Each subject, considered healthy, was screened by the Medical monitor as having no medical conditions that would confound any of the study variables. Upon medical clearance and confirmation of the inclusion/exclusion criteria, participants could proceed with the study protocol.

78 untrained males (age 22.3±3.7) and 56 females (age 22.8±3.7) completed a battery of baseline testing measures. Anthropometric measurements such as height, weight, and waist circumference were recorded on the same day as the DXA scan. The DXA scan records information regarding bone mineral content and density of the whole body and regional locations. The DXA scanner also determines body composition in terms of fat mass and fat-free mass (lean mass). In addition, subjects completed performance tests such as maximal lower body strength and power tests.

Procedures

Though subjects enrolled in the Resistance Exercise and Protein Supplementation (REPS) study went through many testing and training procedures, only described are those relevant to the baseline data collection examined in this study.

The following tests were administered during the baseline testing battery. Body composition testing began the morning after an overnight fast using a standardized hydration protocol (urine specific gravity ≤1.020). Subjects were asked to arrive fasted for twelve hours prior to testing and also refrain from strenuous activity for 36 hours prior to testing. Body weight was measured on a calibrated digital scale while the body composition data was collected using a fan-beam Dual X-Ray Absorptiometry (DXA) (Prodigy^{TM} Lunar Corporation, Madison, WI). From the DXA test, data was collected regarding lean and fat mass, as well as bone mineral content and density.

Physical performance tests were performed during a separate day of baseline testing. Prior to performance testing, all subjects were thoroughly familiarized with the performance testing protocol. The participants were guided through a standardized dynamic warm up by individuals working in the Human Performance Laboratory (HPL) qualified as a CSCS. Subsequently the participants were verbally explained, demonstrated, and allowed to practice all of the performance testing exercises. Approximately one week after familiarization, the subjects returned to the HPL for the performance tests. After completing the standardized dynamic warm up participants were given multiple attempts to lift as much weight as possible in the squat and bench press exercises. After the maximal lift tests, the participants also completed a maximal power test by performing sets of non-consecutive loaded squat jumps. The jumps were

completed using 30% of their previously determined squat max. These exercises were performed on a Smith machine (Plyometric Power System, Norsearch, Lismore, Australia) and the kinetics of the lifts were monitored with a computer interface attached to the bar (AccuPower, Advanced Mechanical Technologies Inc., MA).

Statistical Analyses

Pearson's correlation coefficients were calculated to determine the ability of variables to predict bone mineral content within the population. Data met the assumptions for linear statistics. Regression analysis was also performed to determine a possible prediction model for practical settings. Significance was set at $P \leq 0.05$ for this investigation.

Chapter 4

RESULTS

The primary finding of this investigation was that body mass shows the strongest correlation to bone mineral content in men, women, and the total group (r=0.740, p=0.00; 0.697, p=0.00; and r=0.797, p=0.00, respectively). When examining the components of body mass, lean mass is a stronger predictor of bone mineral content than fat mass in all groups. When examining the men and women grouped together, lean mass showed a correlation of r=0.795 (p=0.00) while fat mass did not correlate to BMC (r=0.263; p=0.00). Though the correlations for lean and fat mass in the separate male and female groups were not strong correlations, lean mass showed a stronger correlation to BMC than fat (r=0.647, p=0.00 and r=0.667, p=0.00 respectively, for lean mass; r=0.553, p=0.00 and r=0.550, p=0.00 respectively, for fat mass)

Anthropometrics

Table 4.1: Subject characteristics by group (Men vs. Women)

	Men (n=78)	**Women (n=56)**
Age (years)	22.3±3.7	22.8±3.7
Body mass (kg)	80.5±14.0	65.9±12.0
Height (cm)	177.9±6.6	162.5±6.0
BMI (kg/m^2)	25.3±3.79	24.90±4.34
Fat Body Mass (kg)	18.6±10.4	23.7±9.22
Lean Body Mass (kg)	58.7±6.2	39.4±4.3
Percent Body Fat (%)	21.9±9.10	34.9±7.6
Note: Values are mean±SD		

Correlations

Pearson correlations were calculated and are presented in Table 4.2, below.

			Gender	Height (cm)	Body Mass (kg)	BMI	Lean Mass (kg)	Fat Mass (kg)	%Body Fat	1RM Squat
All subjects, n=134	**BMC**	r	0.626	0.738	0.797	0.472	0.795	0.263	-0.100	0.615
		Sig.	0.000	0.000	0.000	0.000	0.000	0.002	0.249	0.000
		N	134	134	134	134	134	134	134	134
MEN, n=78	**BMC**	r	.	0.499	0.740	0.615	0.647	0.553	0.454	0.273
		Sig.	.	0.000	0.000	0.000	0.000	0.000	0.000	0.015
		N	78	78	78	78	78	78	78	78
WOMEN, n=134	**BMC**	r	.	0.541	0.697	0.507	0.667	0.55	0.433	0.327
		Sig.	.	0.000	0.000	0.000	0.000	0.000	0.001	0.014
		N	56	56	56	56	56	56	56	56

Significance was set at $p \leq 0.05$.

Body Mass

Due to the increase in mechanical loading of the bone caused by increased body mass, we expected a strong correlation between body mass and bone mineral content. Figure 4.1 shows the body mass of all 134 subjects plotted against their measured bone mineral contents. Figures 4.2 and 4.3 show the same plot of bone mineral content against body mass for the male and female groups (n=78, n=56 respectively).

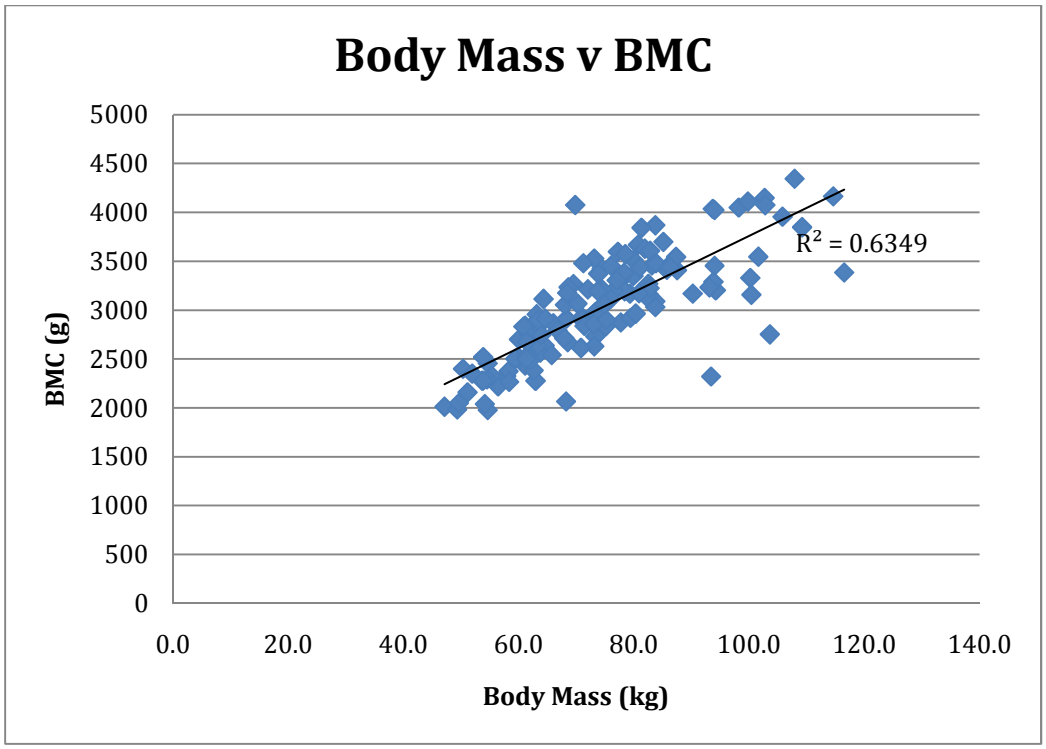

Figure 4.1: Comparison of bone mineral content and body mass in the total group of subjects (n=134) *Significant correlation ($p<0.05$)

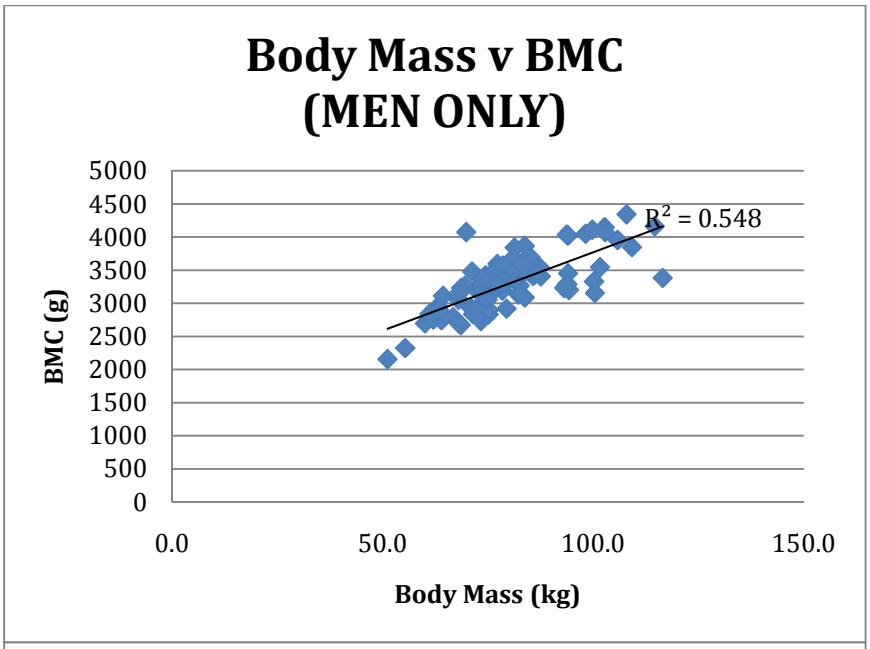

Figure 4.2: Comparison of bone mineral content and body mass in the male participants (n=78) *Significant correlation ($p<0.05$)

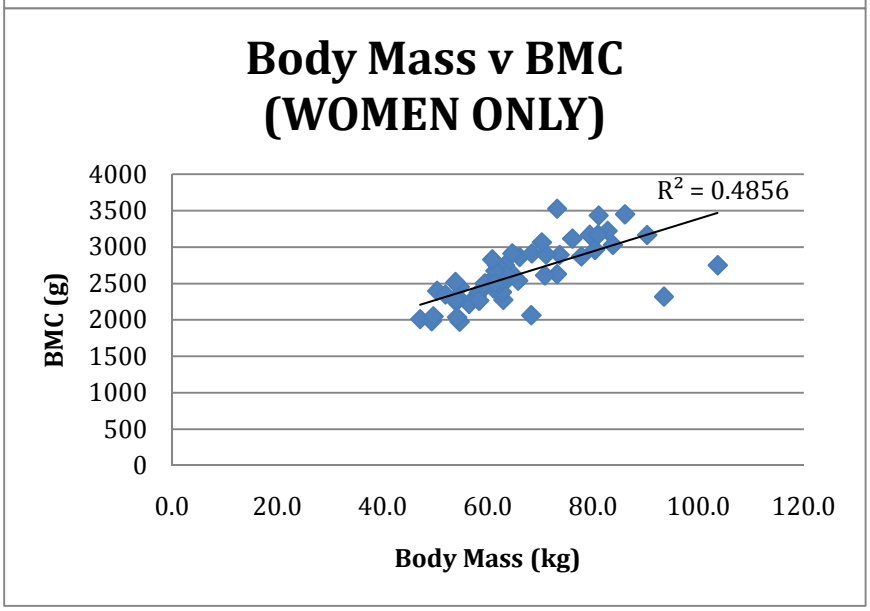

Figure 4.3: Comparison of bone mineral content and body mass in the female participants (n=56) *Significant correlation ($p<0.05$)

Lean Mass v Fat Mass

Figures 4.4-4.7 present plots of lean and fat mass against BMC in all three groups (all, men, and women). The figures allow for the visual comparison of the two variables in terms of their correlation to BMC. The r^2 values shown on the figures show us the amount of variance in BMC that can be explained by the independent variable (lean or fat

mass). Figure 4.4 shows that 63% of the variance in bone mineral content can be explained by lean mass. In comparison, Figure 4.5 shows that only 6% of the variation in BMC measures can be accounted for by fat mass.

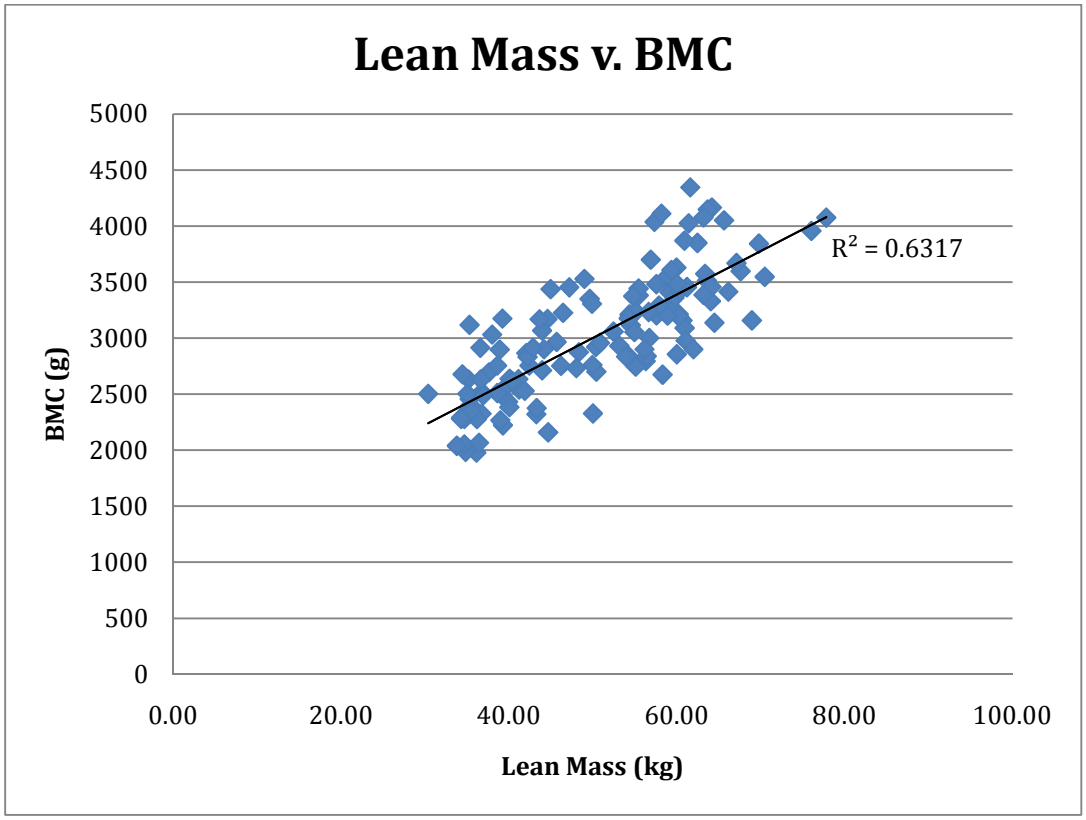

Figure 4.4: Comparison of bone mineral content and lean mass in the total group of participants (n=134) *Significant correlation ($p<0.05$)

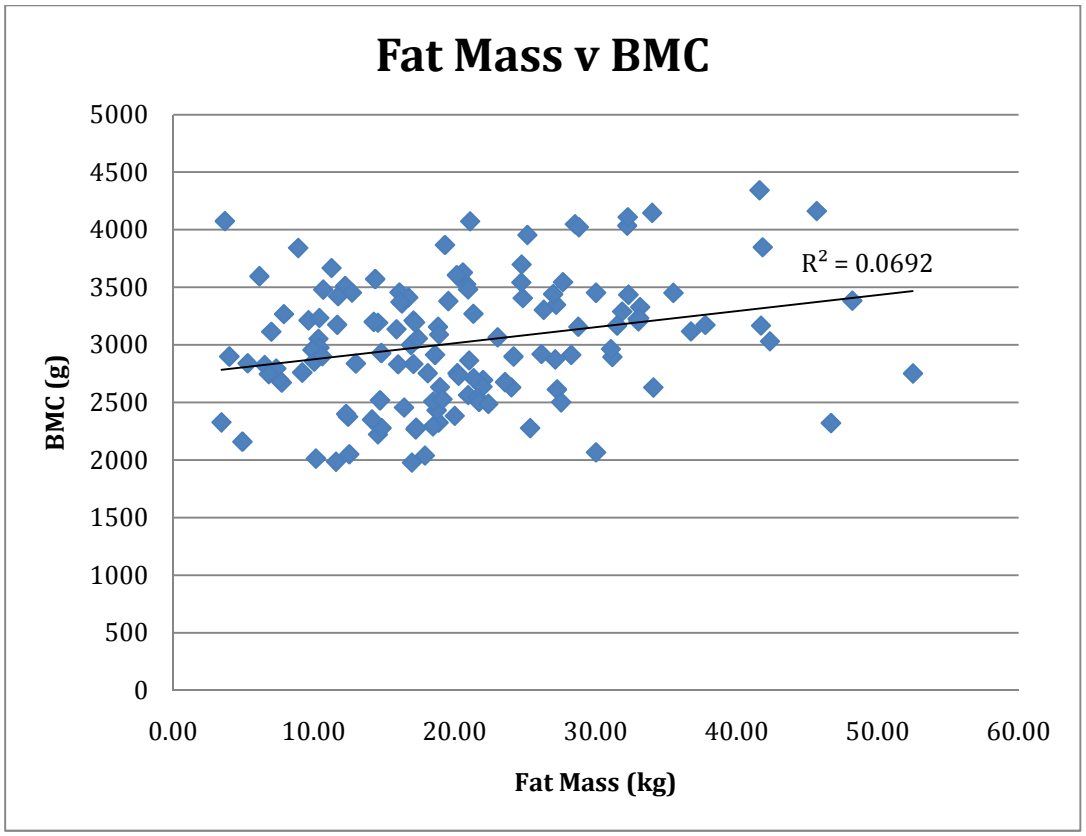

Figure 4.5: Comparison of bone mineral content and fat mass in the total group of participants (n=134) *Significant correlation ($p<0.05$)

Similar correlations exist in the male and female groups with the exception of fat mass showing an increase in correlation to BMC. Figure 4.6 represents data from men only, while Figure 4.7 presents data from only women. In both sets of data lean mass accounts for about 42-44% of the variance in BMC, while fat mass only accounts for about 30% of this variance.

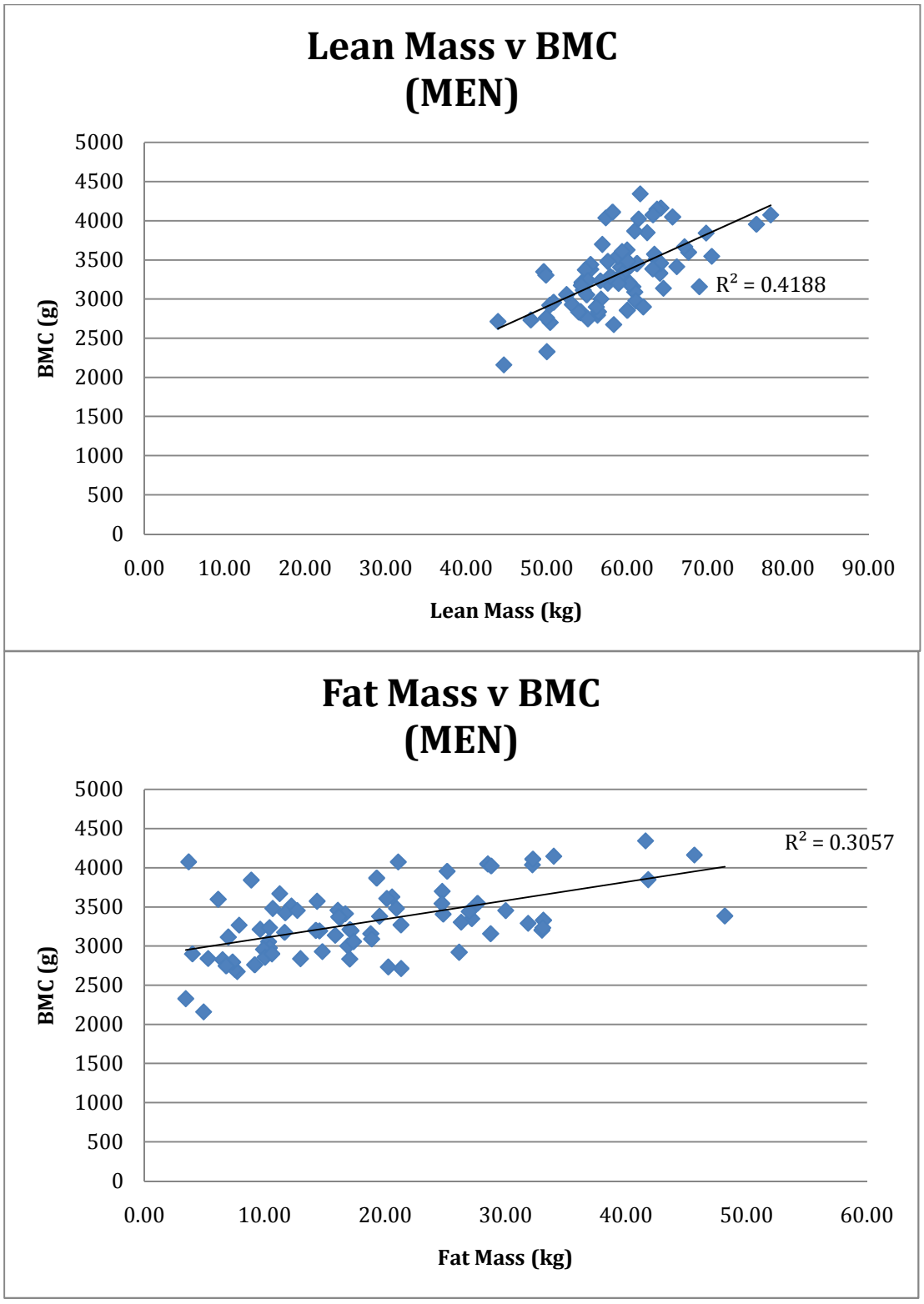

Figure 4.6: Comparison of bone mineral content and lean mass (A) and fat mass (B) in the male participants (n=78) *Significant correlation ($p<0.05$)

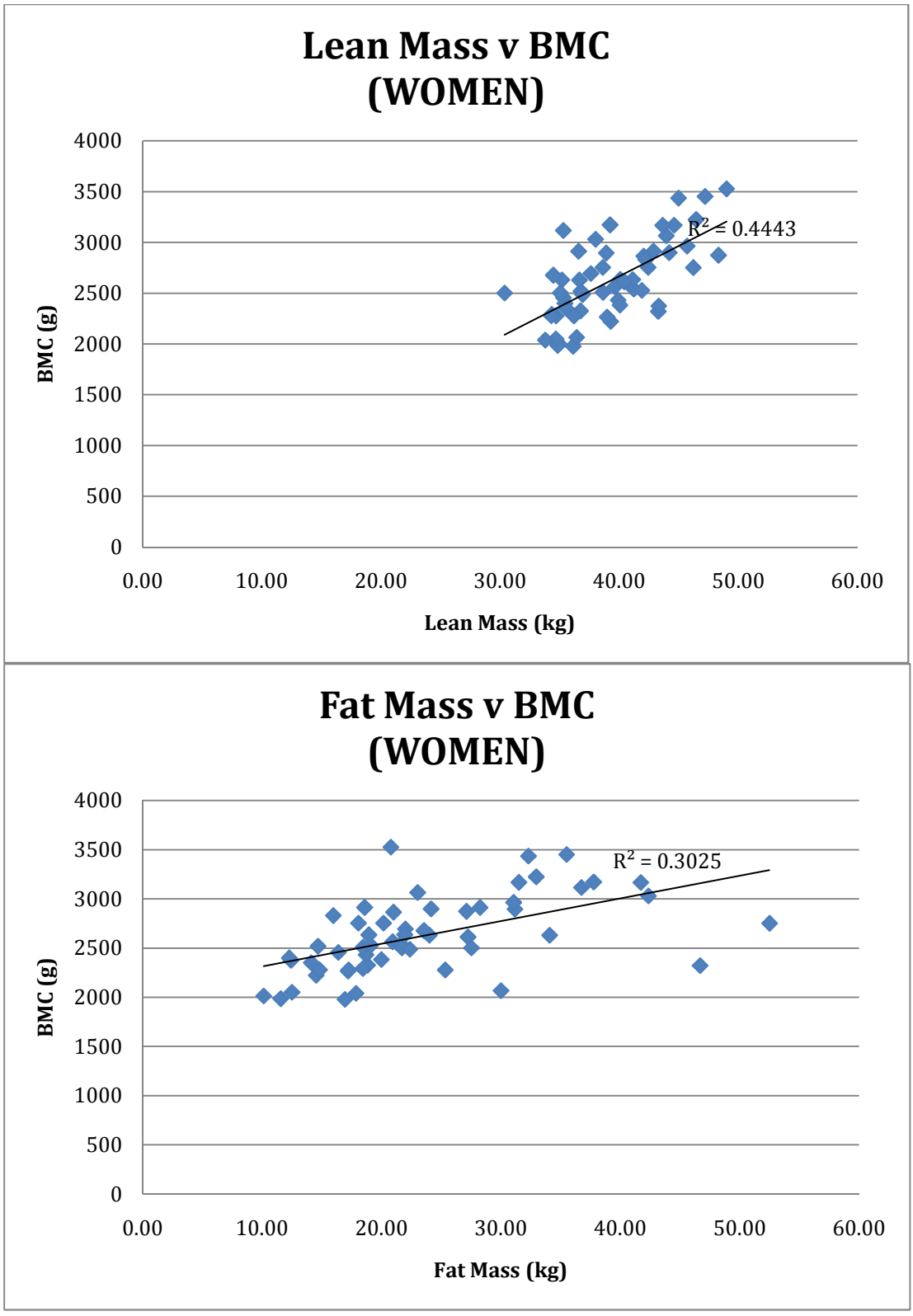

Figure 4.7: Comparison of bone mineral content and lean mass (A) and fat mass (B) in the female participants (n=56) *Significant correlation ($p<0.05$).

Strength

Figure 4.8 displays the strength of the total group against the bone mineral content measurements. The maximal squat strength of the male and female groups alone did not correlate with BMC and thus will not be shown graphically.

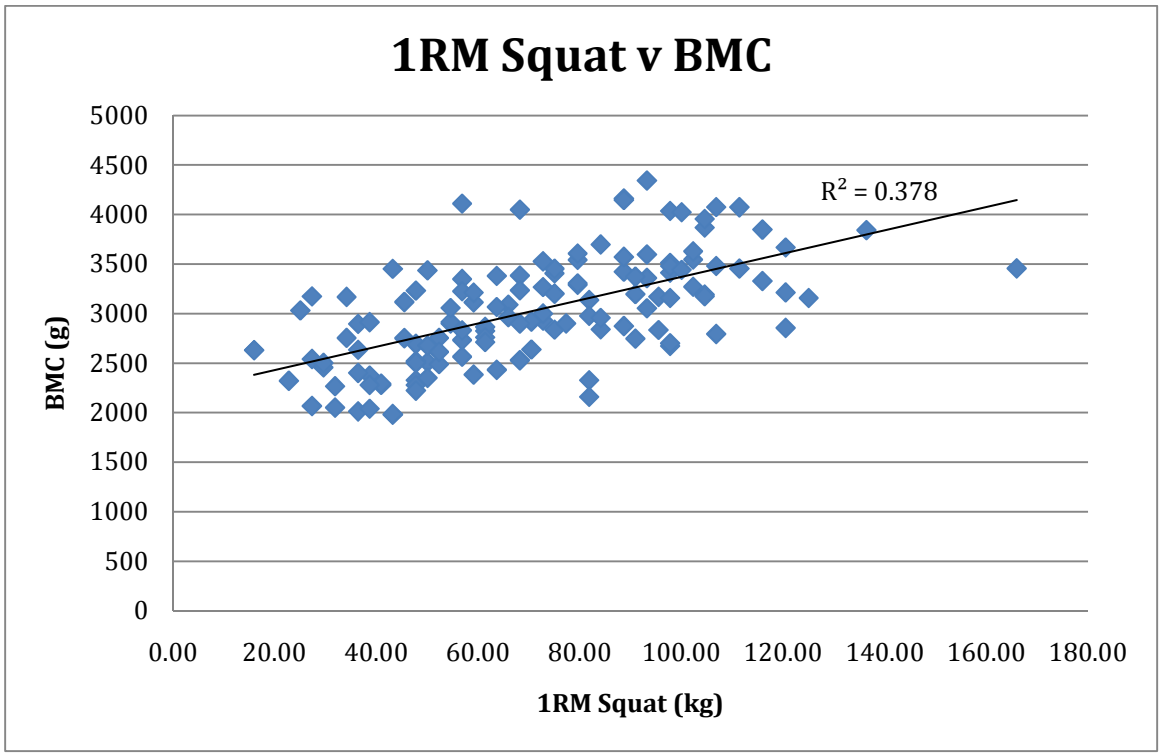

Figure 4.8: Comparison of bone mineral content and maximal squat strength in all participants (n=134) *Significant correlation ($p<0.05$).

Regression Analysis

We preformed a regression analysis on the data and found that body mass was the most significant predictor of BMC in our group of subjects. We also found that when incorporating lean and fat mass in addition to body mass, no increase in the explained variance occurred. We believe that this occurred because body mass already accounts for

the differences in lean and fat mass within a subject. Thinking in terms of a more practical setting where use of a DXA scanner may not be possible, we incorporated variables that could be easily obtained into the regression analysis. This analysis showed that when using body mass, height, and maximum squat strength we could account for 77% of the variance in BMC measurements. Table 4.3 displays the result of the regression analysis. Model one shows using weight as the only predictor, model two uses weight and height, and model three uses weight, height, and max squat to predict BMC.

Model	r	r Square	SE	Significance
1	0.797	0.635	326.693	0.000
2	0.858	0.736	278.854	0.000
3	0.877	0.770	261.421	0.000
a	Predictors: Weight			
b	Predictors: Weight, Height			
c	Predictors: Weight, Height, 1RM SQ			

Table 4.3: Regression analysis

Chapter 5

DISCUSSION

The primary finding of this investigation was that body mass shows the strongest correlation to bone mineral content in men, women, and the total group. When examining the components of body mass, lean mass is a stronger predictor of bone mineral content than fat mass in all groups. When examining the men and women grouped together, lean mass showed a strong correlation to BMC, while fat mass did not correlate. Though the correlations for lean and fat mass in the separate male and female groups were not strong correlations, lean mass showed a stronger correlation to BMC than fat.

The first aim of this study was to determine which component of body mass correlates the most with bone mineral content. Our findings show that between lean and fat mass, lean mass is a better predictor of bone mineral content in untrained young men and women. In the male group lean mass accounted for 42% of the variation in BMC measurements, while fat only accounted for 31% of the variation. Women showed a similar result where lean mass accounted for 44% of the variation in BMC and fat only accounting for 30%. When looking at our total population of both men and women, lean and fat mass actually differentiated themselves even more as predictors of BMC. In the total group, lean mass accounts for 63% of the variation where fat mass accounted for only 7%. All correlations were deemed significant with p-values less than the standard 0.05.

Our data shows that there is a relationship between lean mass and bone mineral content and development of more bone mass in young men and women. Because our study only limited resistance training and did not take into account physical activity we cannot distinguish how the subjects developed their lean mass. We can say that the subjects that had higher lean mass values tended to have higher levels of bone mineral content than those with less lean mass. This statement holds true for both men and women that participated in the study.

The second aim of this study was to determine if there was any relationship between maximal squat strength and bone mineral content. Though our study did not find any correlation between the two in men or women, other studies have proposed that possibly the ground reaction forces incurred through physical activity have more effect on bone mass than pure strength. Pettersson et. al suggested this in their study comparing bone mineral density and muscle strength in young men with different exercise levels. They found that in the high activity group muscle strength was not a predictor of BMD, whereas in the low activity group it was [21]. The subjects that participated in our study were untrained for a minimum of one year in terms of resistance training but were not required to have abstained from physical activity in general. The variations in levels and types of previous physical activity could have confounded the correlation between maximum lower body strength and bone mineral content.

Future research should be conducted in this area to determine the best way to reach a high level of peak bone mineral content and prevent excessive bone degeneration. These studies will be extremely difficult to control because of the many different

variables that effect BMC. Studies would have to choose subjects that all have very similar physical activity backgrounds to normalize the effect that physical activity has on bone remodeling. In a study using adults, the physical activity control would have to date back through childhood, where bone growth is highest and thus physical activity would have more of an impact. Rocher et. al. compared obese and control children and found that after adjusting for body mass, obese children tended to have lower bone mineral content than their control counterparts. However, Rocher noted that the control children reported being significantly more physically active than the obese children [11]. This could confound the results because the increased bone growth in the control children could be attributed to the increased physical activity.

In terms of avoiding the deleterious effects of osteoporosis, lean mass and physical activity are both necessary. The goal of physical activity programs in youth should be to increase lean mass by incorporating weight bearing and resistive exercises. Also, participation in high impact intensity sports has been shown to benefit bone mineral accrual [5]. With aging, physical activity programs should still focus on weight bearing activities in order to protect against bone loss. Resistive exercises should still be incorporated to improve strength and agility to lessen the chance of falls [29].

Practical Applications:

Determining the relationships that exist with bone mineral content will allow us to see what might put individuals at risk for developing low BMC and osteoporosis. Despite that we cannot claim that lean mass causes bone mineral content to increase, we

can show that there is a relationship between the two that should be explored through future research. In many settings the use of technology utilized in this study, such as the DXA scanner, is not possible. For these instances we used multiple regression analysis to show that using weight, height, and maximal squat strength can account for 77% of the variance in BMC measurements.

APPENDIX A

University of Connecticut

Consent Form for Participation in a Research Project

Principal Investigator: Jeff S. Volek and William J. Kraemer

Study Title: The effects of supplementation on responses to resistance exercise

Invitation to Participate

You are invited to participate in this study designed to examine the effects of dietary supplementation with protein versus carbohydrate on responses to resistance training. Resistance training is well known to result in increases in muscle size and strength, but the effects on other health related markers are not as well studied. This project will examine how diet and supplementation with protein and carbohydrate alter responses to 9 months of resistance training in healthy men and women.

Description of Procedures

This research study will take place at the University of Connecticut (UConn) in Storrs and will last approximately 9 months. For this study, you will be required to follow a specific diet and supplementation program and perform resistance training in our facility three times per week for a nine month period. This is specifically what will happen during the research study:

Screening Visit: You will initially be screened, which will include assessment of your medical, nutrition, dietary supplementation, menstrual, and exercise history. We will also determine your height, weight and blood pressure. This visit will take about 30 minutes. We are looking for men and women between 18 and 35 years of age who have not been regularly participating in a high intensity resistance training program. You will be excluded if any of the conditions below are true:

Exclusion Criteria:

1) You have partipated in a resistance training program within the last year.

2) Your body weight is more than 320 pounds.

3) Your blood pressure is more than 150/95.

4) You have diabetes.

5) You regularly use tobacco products.

6) You take cholesterol lowering or blood pressure medications.

7) Your have lost or gained more than 7 pounds in the last 3 months.

8) You are taking anti-inflammatory medication (aspirin, NSAIDs).

9) You consume alcohol more than 3 drinks/day or 18/week.

10) You are pregnant or intend to become pregnant during the 9 mo study period.

11) You have an abnormal menstrual phase.

12) You have an allergy to whey or soy protein.

If you qualify based on the screening visit, we will schedule you for testing. There are a series of tests we will conduct before you start the diet and training portion of the study in order to determine your baseline fitness level. These tests are listed below followed by a brief description of the procedures we will use. We should be able to complete all these tests in three separate visits, but we may need to schedule additional visits depending on your availability.

Testing Measures:

All these tests will be done at baseline and 9 mo of diet and training. In addition, some test will be performed at 3 and 6 months as indicated below. Thus, you will be tested on four separate occasions. We will be asking you to fast for about 12 hours overnight before coming to the laboratory for testing. This means no food or drink that contains calories (including coffee) but you should drink plenty of water. We want you to be well hydrated during all tests. You must also avoid alcohol and strenuous exercise for at least 36 hours prior to coming to the laboratory for testing.

Body weight will be measured on a digital scale.

Body composition (fat, lean, and bone weight) will be determined at four times (baseline, 3, 6, and 9 months) using a machine that will expose you to a small amount of X-ray radiation. You will lie quietly on a table while a scanning arm passes over your body from head to toe. You must remain still for about 5 min during this test. A certified X-ray technician will perform the scan. We will also measure the amount of water in your body by placing two electrodes on your arm and leg while you are comfortably lying down. These tests will take about 1 hour.

Muscle shape will be determined with an ultrasound machine at four times (baseline, 3, 6, and 9 months). We will place a small probe on your upper leg in order to capture various images of the underlying muscle and fat tissues. This test will take about 30 minutes.

Resting Blood pressure will be measured at four times (baseline, 3, 6, and 9 months) by putting a cuff around your arm while you are comfortably seated. Resting blood pressure will take about 15 minutes. We will also attach a monitor that you will wear for an entire day during which time blood pressure and heart rate will be electronically recorded. This will give us an indication of your average blood pressure during the day.

Physical performance will be measured at four times (baseline, 3, 6, and 9 months) by having you lift the most weight in a bench press and squat exercise. Following a standardized warm-up, you will be given multiple attempts to lift as much weight as possible in good form on a specialized machine in our laboratory. Using these same movements, we will assess isometric maximal strength. For this test, you will press up against an immovable bar as hard as possible while we measure your force output. Muscle power will be assessed in the same movements (squat and bench press). We will load the bar with 30% of your previously determined maximum and ask you to perform the movement in an explosive manner to generate as much power as possible. We will also assess your power by having you jump as high as possible off a force platform while you keep your hands on your waist. These tests will take about 1 hour.

Metabolic rate will be determined twice (baseline and 9 months) early in the morning after you have been lying down on a table for 30 minutes. A ventilated canopy will be placed over your head so we can collect your expired breath for about 20 minutes. The expired breath that is collected will be analyzed for oxygen and carbon dioxide content so that we can calculate the amount of energy (kcal) you are burning. During the test you will be required to rest quietly and breath normally but you will not be allowed to fall asleep. We will also ask you to collect your urine in a container for a 24-hour period starting on the morning of the visit for resting metabolic rate testing. This test allows us to determine how many calories you burn during the day while at rest. This test will take about 1 hour.

Blood will be taken from a vein in your arm to assess resting levels of several health related markers (lipids, hormones, etc.). The amount will be equal to about ½ cup. Thus, over the four visits at baseline, 3, 6, and 9 months we will collect 2 cups of blood total. We will be freezing a portion of your blood that may be used at a later point in time to analyze for specific genes affecting your response to the diet and exercise training. We will not share the results of the genetic analysis with you because they have no direct benefit to you. The blood draw will take about 20 min.

An ***Acute Resistance Exercise Test*** will be performed twice (baseline, 3, 6, and 9 months) to assess how your body responds to an exercise bout. For this test, we will put a flexible catheter into a vein in your arm so that we can draw blood before exercise, immediately after exercise, and 15, 30, and 60 min post-exercise. The total amount of blood during this test will be a little more than ½ cup. The exercise bout will consist of a warm up followed by 6 sets of 10 maximal repetitions of squat. This test will only be done at baseline and after 9 months of diet and training and will take 90 minutes. Thus, the total blood from these tests will be one cup. The total amount of blood collected during the whole study including the resting blood will be a little more than 3 cups.

Supplementation and Diet Assignment:

After baseline testing, you will also be randomly (like pulling a number out of a hat) placed into one of 3 groups. You may also request to be in a control group that only performs the testing described above but does not participate in the supplementation and resistance training.

1. Carbohydrate Supplementation + Resistance Training
2. Whey Protein Supplementation + Resistance Training

3. Soy Protein Supplementation + Resistance Training

Depending on your group assignment, you will be provided with a 2-week supply of the supplements and instructed to consume one serving per day with breakfast on non-training days and immediately after exercise on training days. Each serving contains about 190 kcal. Since it is critical you take the supplement every day, we will ask you to record the time you consumed the beverage each day on log sheets.

In addition to being randomized to a supplementation group, we will counsel you to follow a diet that is designed to meet your caloric needs and that contains a specific amount of protein that should remain constant over the 9 months. The diet will follow general diet guidelines (55-60% carbohydrates, 15-20% protein, and 25-30% fat) emphasizing restriction of saturated fat (<7%) and cholesterol (<300 mg/day). Counseling will focus on making healthy carbohydrate choices, encouraging whole-grain products, fruit and vegetable intake, and lean protein sources.
In order to help you with the diet and monitor compliance, we will ask you to complete a 5-day food record every month. You will be given a small scale to weigh food and specific instructions on how to complete the food logs. We will also ask you to attend regular nutrition meetings one time every two weeks. One of the meetings will be a group meeting and the other a one-on-one meeting with one of our study nutritionists. During the meetings, we will provide you with specific diet advice to help you follow the appropriate guidelines and enhance motivation. We will give you educational materials and counseling regarding the diet including specific lists of appropriate foods, recipes, and example meal plans to help you with the diet. To help with motivation and nutrient assessment, we will be providing you with a Personalized Digital Assistant (PDA) with Palm operating system that has nutrient analysis and graphing software. You will be asked to record the food you eat during a 5-day period each month of the study using the PDA. We will provide you with specific training to make sure you feel comfortable with the software and operation of the device.

Resistance Exercise Training:

All groups will perform resistance training. Training will occur three times per week. We will have designated times you can come to our facility in the Human Performance Laboratory. All sessions will be supervised by a certified personal trainer (CSCS). The program will include a variety of exercises to stimulate major muscle groups and provide variation. The entire workout will take approximately 1 hour.

Risks and Inconveniences

Supplementation Protocol. You should not be in this study if you have any major medical problems. If you are unsure, discuss your health history with the Principal Investigator. There are very few potential risks associated with the procedures used in this study. You should inform us if you have an allergy to soy or whey protein in case you are selected to be in one of these supplementation groups.

Blood Draws. Blood draws with a needle may cause discomfort at the puncture site and the development of a slight bruise. You may also experience lightheadedness or fainting during the blood draw. There is a slight risk of infection from these procedures. All possible precautions to avoid infection will be taken including use of sterile disposable needles, drapes and gauze and the practice of aseptic techniques during blood sampling. All blood samples will be obtained by trained people. You should refrain from giving blood during the course of the study.

Body Composition. You will be exposed to a very small amount of radiation by the scanner used to measure your body composition. Exposure to any amount of X-ray radiation, no matter how low, may cause abnormal changes in cells. However, the body continuously repairs these changes and the amount of radiation is very low in this study. The total exposure for a whole body scan is approximately 125 times less than the average radiation from a standard chest x-ray. Thus, the radiation levels are extremely low and the health risk minimal. We don't know what effect the radiation could have on an unborn baby so pregnant women should not be in this study. As a precaution we will ask women to take a urine pregnancy test before the scan. For the muscle shape measures, there are no known harmful effects from the use of ultrasound.

Resistance Training and Testing. Even though the resistance exercise program and testing protocols are designed to be safe, there is the risk that you may become injured. The researchers have an extensive experience in conducting short-term and long-term exercise studies, and they will do everything possible to reduce the chance of injury. Every effort will be made to make the study safe by proper supervision of proper technique during testing and exercise sessions. However, if you experience pain, unexpected discomfort, soreness, headache, loss of concentration, dizziness, vomiting, unusual fatigue or difficulty breathing you should immediately inform one of the supervising members of the research team, who will bring this to the attention of the principal investigators and the medical monitor. The performance of resistance exercise can entail a certain degree of risk from overexertion and/or accident. There are minimal risks for muscle strains or pulls of the exercised muscles. In very rare cases you can experience muscle spasms or tears. Some muscle soreness may be experienced 24 to 48 hours after exercise and this should completely subside with a few days and have no long-lasting effects. The risk of heart attack, although very small, does exist. The chance of any of these events occurring will be minimized by our

screening, selection and monitoring procedures, and by the use of properly conducted research procedures. All the research team members are currently certified in CPR.

Urine Collection: There are no risks associated with the 24 hour urine collection, but this may be inconvenient for you. We will provide you a container that you will be asked to collect all your urine for entire day. You should keep the container refrigerated during the collection period.

Genetic Testing. It is not the purpose of this study to look for or provide you with any medical information or diagnoses relating to your present condition or any other disease or illness. Thus, we will not share the results of the genetic analysis with you. The risks associated with this study are mainly psychological and social. You might worry about having a possible genetic disorder. Although unlikely, there is a possibility that incidental findings might be made such as your risk for a certain disease. Your gene results could be used against you if some of these genes are ultimately shown to predict future disease. This could lead to discrimination, potential loss or difficulty in obtaining employment or insurance. For this reason, your DNA sample will be identified by a code number, and all other identifying information will be removed. The Principal Investigator will keep a code sheet which links the sample code number with your name locked separately and this will be destroyed after two years. This information will not be disclosed to third parties except with your permission.

Benefits

The results of this study will help to determine the role protein supplementation has on responses to weight training and general health, and therefore contribute to a better understanding of dietary recommendations to enhance health. You will be provided with a facility to train under supervised conditions for 9 months during the study. You will also learn your body composition and will most likely improve your fitness and health status.

Economic Considerations

If you complete all training and testing you will receive a stipend of $400 at the end of the study. The stipend will be prorated if you do not complete the study: $50 after

completion of baseline testing, $100 after completion of 3 month testing, and $100 after completion of 6 month testing.

If you are selected for the control group that only performs testing (no training) you will receive $200 for completion of all testing sessions. The stipend will be prorated for those who do not complete the study: $25 after completion of baseline testing, $50 after completion of 3 month testing, and $50 after completion of 6 month testing.

Confidentiality

All the data collected will be kept for a minimum of five years and remain confidential and you will never be identified by name in any reporting of results. Further, the results will not be shared with any person outside the investigation without your consent. The results of this study will be kept in locked cabinets under the supervision of Dr. Volek and Dr. Kraemer. You should also know that the UConn Institutional Review Board (IRB) and the Office of Research Compliance may inspect study records as part of its auditing program, but these reviews will only focus on the researchers and not on your responses or involvement. The IRB is a group of people who review research studies to protect the rights and welfare of research participants.

Confidentiality of your genetic information will be of high priority to protect the DNA samples from falling into unauthorized possession. All blood samples for gene testing will be identified by a code number, and all other identifying information will be removed. The code number will be linked to the physiological data already obtained from you. The genetic information will be kept at a separate facility where the genetic testing will be done. This information will be kept electronically and/or in locked files. The code sheet which links your sample code number with your name will be kept in a locked file and office in a different location at the University of Connecticut. This information will be in hard copy form only and not electronic. The code sheet will be destroyed after two years. Your genetic information will not be disclosed to third parties except with your permission.

In Case of Illness or Injury

In the event you become sick or injured during the course of the research study, immediately notify the principal investigator or a member of the research team. If you require medical care for such sickness or injury, your care will be billed to you

or to your insurance company in the same manner as your other medical needs are addressed.

If, however, you believe that your illness or injury directly resulted from the research procedures of this study, you may be eligible to file a claim with the State of Connecticut Office of Claims Commissioner. For a description of this process, contact the Office of Research Compliance at the University of Connecticut at 860-486-8802.

Voluntary Participation

You do not have to be in this study if you do not want to. If you agree to be in the study, but later change your mind, you may drop out at any time. There are no penalties or consequences of any kind if you decide that you do not want to participate.

Do You Have Any Questions?

Take as long as you like before you make a decision. We will be happy to answer any question you have about this study. If you have further questions about this project or if you have a research-related problem, you may contact the principal investigator, Jeff S. Volek at 860-486-6712. If you have any questions concerning your rights as a research subject, you may contact the University of Connecticut Institutional Review Board (IRB) at 860-486-8802.

Authorization:

I have read this form and decided that _____ will
(name of subject)

participate in the project described above. Its general purposes, the particulars of involvement and possible hazards and inconveniences have been explained to my satisfaction. My signature also indicates that I have received a copy of this consent form.

_____ _____ _____

Participant Signature: Print Name: Date:

Relationship (only if not participant):_____

- I agree that my blood sample may be used for gene testing in this study:
  Initials of participant: _____ YES or _____ NO

- I agree that my blood sample and gene data may be used for unspecified future studies:
  Initials of participant: _____ YES or _____ NO

_____ _____ _____

Signature of Person Print Name: Date:

Obtaining Consent

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