Short Term Time-Course Skeletal Responses to High Intensity Physical Exercise

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(ABSTRACT)

The purpose of this randomized controlled trial was to investigate temporal skeletal responses to short-term high intensity physical activity. Twenty-eight normal active females [age: 20.7 +/- 2.1 yr (mean +/- SD)] were randomized into exercise (EX, n = 15) or control (CN, n = 13) groups. The exercise group trained 6 days/wk for 6 wk, which consisted of maximal isokinetic knee flexion/extension 3 days/wk, combined with 3 days/wk running. The purpose was to expose the tibiae to a period of abruptly increased loading forces. Tibial bending stiffness (EI_{MRTA}), and serum concentrations of biochemical markers of bone formation [osteocalcin (OC)], and bone resorption [n-telopeptide of type I collagen (NTx)] were measured at baseline, 2 wks, 4 wks, and 6 wks. Isokinetic concentric knee extension/flexion peak torque, as well as total body and site-specific bone mineral density (BMD) were measured at baseline and 6 wk. After training, the exercise group significantly increased ($p < 0.05$) isokinetic concentric peak torque for the dominant (13.6%) and non-dominant (5.7%) quadriceps, as well as dominant (7.7%) and non-dominant (9.5%) hamstrings, compared to the controls. No differences for total body or site-specific BMD were noted. A two-way multivariate repeated measures ANOVA revealed no time•group interactions for composite tibial bending stiffness [(EI_{MRTA}); $p = 0.57$] or the biochemical markers of bone turnover [(OC and NTx); $p = 0.15$] across the four sampling periods. While there were no main effects for group, a trend for time ($p = 0.051$) for composite EI_{MRTA} was observed. The exercise group demonstrated a 20% increase in EI_{MRTA} from baseline (74.8 +/- 22.3 Nm^2) to 6 wk (89.8 +/- 24 Nm^2), compared to controls who demonstrated a 4% increase (Baseline 86.5 +/- 23.8 Nm^2; 6 wk 90 +/- 23.7 Nm^2). Significant group differences ($p = 0.05$) were noted for OC, but not NTx. Differences ($p < 0.05$) for OC were observed at baseline [13.2 +/- 2.4 ng/ml (CN), 15.6 +/- 2.7 ng/ml (EX)], and follow-up ANCOVA revealed no differences for subsequent sampling periods. Main effects for time were found for OC and NTx ($p < 0.001$). Main effects for time in OC were attributable to changes in the exercise group ($p < 0.01$) and NTx ($p < 0.01$), but not the control group.

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Chapter I

INTRODUCTION

Exercise is generally considered to impart positive benefits on the skeleton (6, 16, 27, 104), which include increased bone mineral density (BMD) (10, 98) and bone mineral content (BMC) (10, 54, 56) decreased risk for osteoporotic fractures (88), and increased bone strength (60). The notion that exercise promotes skeletal health finds support in the consequences of immobilization, the increased bone mineral density of athletes, and the results of exercise intervention trials (75). For these reasons, exercise has been prescribed to counteract the detrimental effects of exposure to the microgravity environment on skeletal integrity (31) as well as a modality for slowing bone loss associated with disuse osteoporosis (11, 101) and post-menopausal osteoporosis (15, 91, 116). In addition, exercise is considered essential in developing peak bone mass (PBM) during adolescence (5, 16, 71). There remains little debate that physical activity is important for the development and maintenance of a healthy skeleton throughout the lifespan.

Bone tissue is remodeled subsequent to an activating event caused by systemic hormonal or local load bearing demands (105). Physical exercise has been implicated as a load bearing demand resulting in bone remodeling, however, the mechanisms by which exercise leads to such changes in bone metabolism are not fully understood (117). Possible exercise mediated osteogenic processes include activation of a mechanotransduction mechanism resulting from increased mechanical loading associated

with weight bearing exercise (46) or escalated release and uptake of growth potentiating factors associated with the exercise stimulus (19).

Various modes and intensities of exercise have been shown to alter the normal bone remodeling process, (52, 70, 89), however the exercise mode and dose most likely to enhance skeletal development and maintenance is not known. In addition, it appears that males and females at different life-stages respond to an exercise stimulus differently (6, 17, 99). Thus, there is little doubt that the optimal exercise stimulus will differ for various populations. However, it is clear that mechanical loading is important for positive skeletal adaptations (74).

Bennell et al, (10) suggested that the most effective form of exercise for promoting osteogenesis is dynamic weight-bearing activity such as aerobics, or running. On the other hand, it has been suggested that exercise, which results in high impact forces such as gymnastics, as opposed to running, may impart the greatest (75). Although disagreement exists on the optimal mode of exercise stimulus, it appears that selected modes are dose dependent, and site-specific (74). That is, extended duration and/or very high intensity exercise may be detrimental to skeletal integrity. Supporting this position is (57) who reported that elite runners had significantly less bone mineral content in the lumbar spine than non-running controls. Additionally, high incidences of stress fractures (43, 62, 93), and decreased indices of bone formation (41) have been reported in military recruits soon after initiating a demanding physical activity regimen (50) and during high volume physical training periods. Thereby suggesting that too great of an exercise dose may be maladaptive, and lead to a state of compromised skeletal integrity, especially in the lower extremities. Compromised skeletal integrity could thus result in an increased

vulnerability for tissue failure, especially if exposure to the exercise stimulus is sustained. Yet, it appears that the vulnerable window for skeletal failure is transient (25) and withdrawal or diminution of the potentially injurious exercise dose during this period allows for constructive bone remodeling and subsequent increased bone strength.

Compromised skeletal integrity is also apparent following exposure to the microgravity environment, and appears to be site specific. Following six months of space flight, a marked decrease in tibial trabecular and cortical bone was observed, however no changes in the radius was detected (30). Vico et al, (114) suggests that bone deficits experienced in microgravity appear to be a consequence of the support function of each bone at normal gravity. The bones of the legs, which serve a much greater support function in normal gravity than those of the arms, experience much greater bone loss in the microgravity environment. Space flight results in bone loss, which could be a limiting factor for long duration missions, such as, a Mars expedition or extended occupation of a space station (67). The effects of the microgravity environment could put astronauts at increased risk for fractures when they return to earth (58).

Traditionally researchers have employed dual x-ray absorptiometry (DXA) and/or the biochemical markers of bone turnover to assess bone adaptations to exercise or microgravity mediated short-term site-specific bone strength changes. Although these tools are useful, they provide an incomplete picture of skeletal dynamics. Dual-energy x-ray absorptiometry is useful in quantifying changes in BMD and BMC, although useful in this context, BMD as assessed by DXA is but a surrogate measure for bone strength (71). Bone strength, and subsequent fracture resistance, is dependent on the quantity, as well as the quality and macrogeometry of the bone (39). Dual-energy x-ray absorptiometry only

provides measures of bone quantity, therefore, two important determinants of bone strength, microarchitecture and macrogeometry, remain unclear when DXA is used alone.

The biochemical markers of bone turnover provide useful information related to osteoblastic and osteoclastic cellular activity, and indicate bone formation and resorption dynamics, thereby allowing inference at the tissue level. That is, increased markers of bone formation accompanied by decreased or constant markers of bone resorption would lead to an inference of a net gain in bone tissue. Still, biochemical indicators of increased bone formation do not equate to increased bone strength, nor does increased bone resorption equate to decreased bone strength. Although these markers are useful in understanding osteoblastic and osteoclastic cellular activity, and may contribute to identifying a window of transient vulnerability, they are not accurate measures of bone strength.

Mechanical response tissue analysis (MRTA) is a measure, that was developed by the Stanford University Mechanical Engineering School (107), in collaboration with the National Aeronautic and Space Administration (NASA). It is designed to assess, *in vivo*, the mechanical properties of long bones in humans. These mechanical properties include the cross-sectional bending stiffness (E), cross-sectional moment of inertia (I), and an index of bone "sufficiency" (i.e., a ratio of axial load capability to body weight). The EI_{MRTA} (E x I) yields a measure of long bone structural integrity, which is related to the composition, geometry, and internal architecture of the bone (76, 87, 97, 107). Although limited to measurements of long bones with limited overlying soft tissue, i.e. tibia and ulna; MRTA is valuable in assessing the true strength of these bones.

The inclusion of mechanical response tissue analysis (MRTA) with DXA and the biochemical markers of bone turnover may identify the time-course by which adverse skeletal changes develop prior to the subsequent adaptive response, thereby elucidating the window of transient vulnerability. Additionally, an understanding of the cellular and systemic changes that occur during this window of transient vulnerability will contribute to the development of exercise countermeasures that may maintain or promote skeletal integrity during exposure to adverse stimuli such as, microgravity or postmenopausal and disuse osteoporosis.

Statement of the Problem

The osteogenic effect of weight-bearing exercise is undisputed, however, it appears that there exists a transient vulnerability during the bone remodeling cycle, where bone (specifically of the lower legs) is at a greater risk for fracture. Numerous studies investigating skeletal injury in military recruits have demonstrated the detrimental effects of a high-intensity exercise regimen on skeletal integrity (43, 93), leading to loss of training time, and significant medical costs. In addition, the magnitude and rapidity of bone loss during space flight is alarming, it is recognized that an unloading of the skeleton in zero gravity leads on average to a 1%-2% reduction in bone mineral density at selected skeletal sites per month (58). Wronski et al, (119) reported a 4% decrease in calcaneous bone mineral density (BMD) of Skylab crewmembers after 84 days of orbital flight. Bone loss occurs in weight bearing bones first and later in less weight-bearing bones (115), with the greatest losses occurring in the lumbar vertebrae, pelvis, and legs (51, 92). Coincidentally, the sites of greatest bone loss are weight bearing sites,

suggesting the absence of mechanical loading on specific sites leads to bone degradation, thus, implicating a mechanosensory mechanism in microgravity osteoporosis. Although microgravity bone loss is likely related to the mechanosensory mechanism, alternative possibilities must be explored. However, currently there is no method by which skeletal integrity can be ascertained quickly, and inexpensively.

Assessment of the biochemical markers of bone metabolism can provide information on the rate of cellular bone turnover; however, they do not provide a measure of actual bone strength. Bone mineral density (BMD) measurements have previously been used as a means of estimating bone strength, however, BMD does not provide a complete assessment of fracture risk.

Therefore, the aim of the present study was to quantify the short-term time course skeletal changes that may occur with a high-intensity exercise regimen, and identify a transient vulnerable state, if in fact such a state exists. The variables of interest in the current study include tibial stiffness as assessed with mechanical response tissue analysis (MRTA), bone turnover as assessed with serum biochemical markers of bone formation (osteocalcin) and resorption (n-telopeptide of type I collagen breakdown products), and bone density mineral density as assessed by dual-energy x-ray absorptiometry.

Specific Research Objectives

To measure the effects of high-intensity physical activity on skeletal remodeling responses by measuring:

1. serial changes in blood variables including osteocalcin, and n-telopeptide type I collagen breakdown products;

2. bone mineral density (BMD), and bone mineral content (BMC) for the total body, as well as site-specific BMD and BMC of the total hip, lumbar spine, and forearm;
3. bone stiffness of the tibiae;
4. changes in strength of the muscles in the dominant and nondominant legs that act upon the tibia

Delimitations

The following delimitations were imposed

1. The sample size was limited to 30 college-age female volunteers.
2. Responses to six weeks of isokinetic resistance training, coupled with six weeks of run training was evaluated
3. Subjects completing at least 80% of the exercise sessions were included in the statistical analysis
4. Only females that reported no involvement in a structured exercise program within the past 12-months, reported no fractures in the past 12-months, reported no cardiovascular, pulmonary, or metabolic disease, were included as subjects.

Limitations

1. Subjects were selected in a nonrandom fashion. All subjects were volunteers
2. Results from this study can only be applied to females possessing the physiological characteristics of the subjects evaluated in this study.
3. Results from this study cannot be applied to exercise modalities other than those employed in this study.

Definitions Terms and Symbols

Bone Mineral Content, (BMC): The concentration of bone mineral deposition into the organic collagen matrix, expressed as g.

Bone Mineral Density, (BMD): The concentration of bone mineral deposition into the organic collagen matrix, which is an areal-density expression in g/cm^2

Bone Stiffness, (EI): A structural property of bone, which is the product of Young's Modulus of Elasticity (E), and the cross-sectional moment of inertia, and is expressed in Nm^2. Normal values for EI are dependent on the parameter model chosen for analysis. The nine or twelve parameter model will be used for the analysis in this study. Bending stiffness values using the nine and twelve parameter model will be reported for the first time in this paper.

Isokinetic Resistance Training: Accommodating resistance training, in which maximal force can be exerted, throughout the entire range of motion, with either a concentric or eccentric contraction.

Peak Torque: The greatest amount of force produced, at any angle, throughout the range of motion, using an isokinetic dynamometer. Peak torque is expressed in ft. lbs.

Mechanical Response Tissue Analysis (MRTA): A noninvasive method for determination of the mechanical properties of long bones *in vivo*. The MRTA measurement variable is EI, which is the product of Young's Modulus of elasticity (E), and the cross-sectional moment of inertia (I).

N-telopeptide (NTx): N-telopeptide of collagen cross-links, which are a collagen breakdown product, and are a biochemical marker for bone resorption, and are expressed in nanomoles Bone Collagen Equivalents per liter (nM BCE). The reference range for NTx in healthy adult females is 6.2-19 nM BCE.

Osteocalcin, (OC): Single chain polypeptide biochemical marker for bone formation, which accounts for 25% of non-collagenous protein, and 1-2% of total bone protein. Small quantities of osteocalcin is released into the serum during bone formation Osteocalcin levels are expressed in ug/ml. The normal range for OC in premenopausal females is 3-13 ug/ml.

Chapter II

REVIEW OF LITERATURE

The literature pertinent to this investigation is presented in two major sections. The first section provides general aspects of bone physiology most relevant to this research problem. The second section addresses skeletal adaptations to exercise, with an emphasis on literature that characterizes a period of transient vulnerability in bone following periods of marked increases in weight bearing physical activity. In addition, the second section reviews literature about skeletal adaptations that occur in the microgravity environment, with the premise that MRTA may be used to ascertain bone strength changes and assess true fracture risk on return to normal gravity following extended space travel.

Bone Organization

Bone is a self-renewing tissue, and at the cellular level, is comprised of three primary cell types; osteoclasts, osteoblasts, and osteocytes. In the normal skeleton, these cell types function in concert with one another to balance bone formation and degradation, and maintain skeletal homeostasis throughout the lifespan.

Osteoclasts are multi-nucleated bone-resorbing cells, which are derived from hemopoietic (macrophages, monocytes) stem cells in the marrow. Osteoclastic activity is primarily influenced systemically by parathyroid hormone, 1,25-dihydroxyvitamin D, and calcitonin. Local factors that may play a role in osteoclastic activity are the interleukins, and growth factors. The primary role of osteoclasts is to resorb bone tissue, however these cells are only capable of resorption when in direct contact with bone tissue. In the inactive state, lining cells surround osteoclasts, which prevent direct contact

with the underlying bone matrix. A series of events leading to retraction of the lining cells are essential for the stimulation of osteoclast activity, and subsequent bone resorption.

Osteoblasts are derived from the mesenchymal cell line, and are bone forming cells. Osteoblasts may be classified as either active or inactive, and change shape dependent on activity status. Active osteoblasts are large single-nucleated "plump" cells, which synthesizes the bone matrix, structural proteins, non-collagenous proteins, and regulatory factors. An osteoblast is defined as a cell that produces type I collagen, is responsive to parathyroid hormone, and produces osteocalcin when stimulated by 1,25 hydroxyvitamin D. An inactive osteoblast is a flat quiescent cell, which may be involved in producing enzymes that assist with matrix degradation, and osteoblast/osteoclast coupling.

Osteocytes are the most abundant of the three cell types, and are essentially mature osteoblasts imbedded deep within the bone matrix. Osteocytes are far removed form the bone surface, and are not directly involved in degrading or forming bone tissue. However, osteocytes appear to communicate with adjacent osteocytes and osteoblasts through numerous cell processes that extend through the canaliculi. This intercellular communication between osteocytes and osteoblasts may be important for relaying stress/strain signals throughout bone tissue, which play a role in regulating bone modeling and remodeling.

Mineral Homeostasis

In addition to a support function, the skeleton also serves as a mineral reservoir for calcium, phosphorous, and magnesium ions, and helps to regulate the concentration of

these ions in the extracellular fluid. Undoubtedly, calcium is the most abundant, as well as physiologically vital of these ions. Approximately 50% of the calcium concentration in the extracellular fluid is of the ionized form, which is in equilibrium with the storage form of calcium in bone. During bone degradation and formation, calcium is either being liberated into the extracellular fluid, or stored back into bone.

Normal extracellular calcium concentration is accomplished through gastrointestinal absorption, renal excretion, and calcium exchange with bone. Primary endocrine regulators of the calcium balance are parathyroid hormone (PTH), vitamin D, and calcitonin.

Parathyroid hormone is secreted by the parathyroid gland during periods of hypocalcemia. Parathyroid hormone, serves to increase extracellular concentrations of calcium through a number of processes. Primarily, these processes are renal conservation, vitamin D mediated increased gastrointestinal absorption, and increased bone degradation. The actions of PTH on renal conservation, and bone degradation respond rapidly, whereas, the actions on vitamin D associated intestinal absorption occur much slower. These processes are reversed when normocalcemia is achieved.

Vitamin D plays a powerful role in regulating exrtracellular calcium concentrations. Vitamin D is obtained from dietary sources, as well as from the skin when exposed to ultraviolet light. Increased gastrointestinal absorption is the primary function of vitamin D in regulating extracellular calcium concentration. In addition, vitamin D can also promote differentiation of stem cells into mature osteoclasts, thereby increasing bone resorption and subsequent exrtracellular calcium concentration.

Calcitonin is secreted by the C-cells of the thyroid gland in response to elevated extracellular calcium concentrations. The primary function of calcitonin is to decrease bone demineralization by inhibiting osteoclastic activity. This action decreases mobilization of calcium from bone tissue to the extracellular fluid, and thus lowers extracellular calcium concentration.

The Gonadal Hormones also play a role in regulating mineral homeostasis. Adequate levels of estrogen are essential for optimizing peak bone mass (PBM) in young adult females. In addition, hormone replacement therapy (exogenous estrogen) is widely prescribed as a therapeutic modality to delay or prevent the onset of osteoporosis in post-menopausal women.

Bone Remodeling

Bone remodeling is a surface event that is initiated when active osteoblasts (stimulated by PTH) retract, or inactive osteoblasts secrete proteolytic enzymes, which disrupts the osteoid layer. The role of mechanical loading in initiating this process may be attributed to three potential mechanisms; streaming potentials, fluid shear stress, and mechanical strain (11). Regardless of the initiating mechanism, disruption of the osteoid layer leads to activation of the osteoclasts and subsequent bone degradation as described above. Following bone degradation, osteoblasts, which are closely coupled to the osteoclasts, initiates bone formation on the resorped site. Osteoblasts synthesizes type I collagen fibrils which eventually become the deposition sites of calcium hydroxyapatite, leading to mineralized bone.

The osteoclasts become activated only after enzymes secreted by the inactive osteoblasts disrupt the osteoid layer, or the active osteoblasts retract in response to

parathyroid hormone or 1,25 hydroxyvitamin D. The clear zone and ruffled border of the osteoclasts are responsible for the resorbing activity. Once the osteoid layer is disrupted, the clear zone of the osteoclasts attaches to the underlying bone through an integrin receptor, and seals of an area called the sub-osteoclastic space. Once the sub-osteoclastic space is sealed off, excess hydrogen ions are produced within the osteoclasts cell, and are released across the cell membrane by an ATPase pump. The excess hydrogen ions lead to decreased pH within the sub-osteoclastic space leading to heightened solubility of the bone matrix. Hydrolytic enzymes are then released across the ruffled border and matrix degradation ensues. Bone morphogenic proteins (BMP) are released during matrix degradation, and may modulate cellular events in different areas of the tissue.

Osteoclasts and osteoblasts balance bone loss in a coupled homeostatic process of remodeling that renews approximately 25% of trabecular bone annually (59). A remodeling imbalance may occur with enhanced osteoclast activity and increased collagen breakdown or decreased osteoblast activity and collagen synthesis, resulting in a remodeling imbalance, which leads to bone loss. However, when bone formation exceeds bone resorption, an increase in skeletal tissue occurs, as is the case when bone is exposed to mechanical loading (38, 46, 60, 113).

Stress forces presented to bone during exercise are in the form of compressive, tensile, and torsional forces. In addition, combinations of these forces can be presented to bone. Each of these forces may initiate mechanical skeletal responses, however the most common is compressive. Osteoblasts are only capable of forming new bone at a resorption site; therefore the mechanical signal will initially result in bone resorption followed by the subsequent osteoblastic mediated bone formation. Initial bone strength

and biochemical marker assessments may indicate increased resorption and decreased strength due to the coupling of initial resorption (osteoclasts) followed by formation (osteoblasts).

It has been demonstrated that stress applied to bone resulting in deformation can initiate electrical potentials termed stress generated potentials (SGP). These potentials can be of two types; piezoelectricity and streaming potentials. The piezoelectric potentials are produced as a consequence of strain to the organic materials of the matrix; collagen and proteoglycans. A second form of electrical signals, which may mediate bone turnover and strength, are streaming potentials. These are electric potentials generated by the flow of electrolytes produced by bone material strain. It is currently unclear how these potentials may influence bone remodeling.

The precise mechanism by which mechanical loading leads to bone formation is not known, however, a feasible hypothesis has been offered by Frost (46). Mechanical loading on bone may result in cellular and/or tissue responses, which results in increased bone strength, resistance to fracture, and possible delayed onset of age related bone loss. At the cellular level, mechanical loading above the minimal effective strain (MES) (46) results in a deformation of the osteoblast and osteocyte cell membrane, resulting in a cascade of intra and intercellular events, leading to increased osteoblastic activity, and subsequent bone formation.

Skeletal Dynamics

More than a century ago, Julius Wolff hypothesized that the form and function of bone is determined by the demands placed upon it. In the years since, it has become widely accepted that bone responds favorably to mechanical stimuli. Thus, bone exhibits

functional adaptation, and develops enhanced physical and mechanical properties following periods of increased stress (26), whereas periods of decreased use results in compromised mechanical properties (11). However, the skeleton's adaptive processes resulting from external mechanical stimuli are not clearly understood.

A recent instructional lecture by Turner and Pavalko (113) attempted to clarify the process by which the skeleton responds favorably to mechanical loading. The adaptive process has been termed mechanotransduction, suggesting that mechanical forces are transformed into signals, which result in osteogenesis. However, it should be noted that this process is not independent of nutritional and/or endocrine factors (63, 65). It is postulated that the mechanotransduction mechanism detects physical stressors, and transforms the stressors into signals, which are transmitted locally, as well as systemically throughout the tissue. These signals, which may be electrical, hormonal, or mechanical, play a role in regulating bone formation and resorption. Turner and Pavalko (113) suggest that four mutually exclusive stages comprise the mechanotransduction mechanism. These stages include mechanocoupling, biochemical coupling, signal transmission, and cell response. Each of these stages will be briefly explained in the following paragraphs.

Mechanocoupling refers to the process of transforming an external mechanical load into a signal, which is detected by a sensor cell. The mechanosensory cells most likely responsible for mechanocoupling are the osteocytes (1, 22, 38), which are mechanically deformed to the extent of the bone tissue (113). That is, bone tissue is deformed when mechanical loading forces are sufficient enough. The deformation exhibited by bone while exposed to stresses may mechanically alter the shape or

configuration of the osteocytes within the matrix. This altered spatial configuration may propagate a signal from the osteocytes to the osteoblasts which in-turn initiates the functionally adaptive remodeling process. Osteocytes may also generate mechanocoupling signals due to intercellular fluid flow (32) and electrical changes in the surrounding milieu (100).

Biochemical coupling refers to the transduction of the mechanical signal into a biochemical signal within the osteocyte. Although the mechanism is not fully understood, mechanical strain may open stretch activated ion channels in the cellular membrane (38), and permit an influx of calcium ions, leading to a series of events, which increase gene expression or protein activation. An additional pathway for biochemical coupling may be the mechanical deformation of the cytoskeleton-integrin complex, which is linked directly to the DNA. Mechanical loads may directly deform the cytoskeleton-integrin complex which in-turn directly activates gene expression within the nuclear matrix (1). The end result is heightened osteocyte activity, culminating in increased intercellular communication with the effector cells. Intercellular communication via the canaliculi is most likely the source for relaying the biochemical signals generated by the exercise stimulus from the osteocytes to the osteoblasts and/or osteoclasts effectors (36). The mechanosensory osteocytes and bone lining cells detect mechanical signals, and mediate these signals to the bone surface through nitric oxide (NO) and prostaglandins (38), which in-turn stimulates osteoblastic activity. This functional adaptation of bone may lead to increased BMD, and/or enhanced structural properties.

Bone Mineral Density

Bone mineral density (BMD) is an areal measurement of the mineral content in bone, and is expressed in $g \cdot cm^{-2}$. Bone mineral density is important for bone strength, and resistance to fracture (79), with approximately 70% of bone's strength attributable to its mineral density. Peak bone mineral density (PBMD) is the potential upper limit of BMD, and is regulated by genetic and environmental factors. It is widely acknowledged that the development of osteoporosis later in life is related to peak bone mass. The prepubertal and pubertal years are particularly important for acquiring bone mass (13). Hereditary factors contribute approximately 60-80% of bone mass, with environmental factors such as diet, and physical activity accounting for the remainder. Recently, Fujita et al, (49) investigated various factors that contribute to peak bone density in women. Subjects (N = 157) completed a DXA scan to determine total body, lumbar spine, and femoral neck BMD. Environmental factors contributing to PBM that were investigated included; onset of menarche age, presence of menstrual dysfunction, history of exercise, smoking, and alcohol intake. The single genetic factor investigated, was the vitamin D receptor genotype. Results of the investigation demonstrated that significant differences in BMD were observed between groups dependent on the VDR genotype. Additionally, subjects with no self-reported menstrual cycle abnormalities (1.00 ± 0.09 g/cm^2) exhibited significantly greater ($p = 0.0001$) lumbar BMD, than did subjects with self-reported menstrual abnormalities (0.87 ± 0.05 gm/cm^2). Self-reported exercise history was used to group subjects into one of four exercise categories; 1. non-exercisers (NN) 2. positive exercise history beyond 18 years of age (NS) 3. Positive exercise history between the ages of 12-18 (SN) 4. positive exercise history continuously from age 12 to present (SS). The SS group demonstrated significantly greater lumbar, femoral neck, and

total body BMD than did the NN group. There were no differences in BMD observed between any of the other groups. Smoking and alcohol intake were quantified and correlated with site-specific and total body BMD, of which no significant relationships were observed. Multiple regression analysis indicated that exercise history, menstrual dysfunction, and VDR genotype were all independently related to total body, lumbar spine, and femoral neck BMD. Therefore, it is apparent that genetic and environmental factors influence the development of PBM, with the VDR genotype and menstrual history identified as genetic determinants, and physical activity as an environmental determinant.

A clinical investigation by McKay et al (77) explored lifestyle determinants of bone mineral in Asian (n = 58; 30 boys and 28 girls) and Caucasian (n = 110; 56 boys and 54 girls) boys and girls with a mean age of 8.9 years. The purpose of the study was to identify lifestyle determinants of bone mineral density, and to elucidate ethnic differences in bone mineral acquisition. Subjects completed DXA measurements for the proximal femur, lumbar spine, and total body. In addition, subjects completed dietary intake and physical activity questionnaires with the assistance of a parent. All variables were analyzed for ethnicity and ethnicity by gender. There were no differences in physical stature, or soft tissue mass between the ethnic groups. Results of the questionnaires indicated that the Asian children were significantly less active ($p < 0.001$) and had significantly less dietary calcium intake ($p < 0.001$) than their Caucasian counterparts. The Asian boys and girls consumed 41% and 29% less dietary calcium than the Caucasian boys and girls respectively. As a group, the Asian children were 15% less active than the Caucasian children. However, the differences were much more pronounced for the ethnicity by gender interaction, where 14% of the Asian boys reported

involvement in sports outside of school, and 73% of the Caucasian boys participated in sports outside of school. The BMD differences for the ethnic groups indicated that the Asians had significantly less ($p < 0.05$) BMD and BMC of the femoral neck than the Caucasians. No other differences were observed between the groups. There were no differences observed for any of the BMD measures between the Asian and Caucasian females. The differences observed between the Asian and Caucasian males mirrored the differences observed between the ethnic groups, with the Asian males having significantly less ($p < 0.05$) BMD and BMC of the femoral neck. This difference was 6.5%, which contributed to the differences observed between the ethnic groups. No other differences were observed for the lumbar spine or total body. Therefore, the differences noted between the Asian and Caucasian groups can be attributed solely to the differences between the males only, as there were no differences between the females observed. The only body composition and lifestyle differences noted between the groups and genders were the physical activity scores, and calcium intake which were significantly less in the Asian males ($p < 0.001$), compared to the Caucasian males. Therefore, it can be speculated that the physical activity history and calcium intake history were determinants of the significant differences noted in BMD between the Asian and Caucasian males. Hence, in prepubertal children, environmental determinants may be more influential for acquisition of bone mineral density than genetic determinants.

Bone mineral density is the gold standard for assessing skeletal health. The World Health Organization (WHO) defines osteoporosis in terms of BMD, which in itself speaks for the importance of this skeletal parameter. Presently, dual-energy x-ray absorptiometry derived BMD is the predominant method for determining bone strength,

which is apparent in its widespread application in diagnosing osteoporosis. However, BMD is a static measure of bone status related to the mineral content, which is only a single component of bone strength. Additional factors must be considered when evaluating skeletal health, or skeletal changes due to pharmacological or exercise interventions. One such factor of central importance is bone stiffness, which is a measure of the micro, and macro-architecture of bone, where the micro architecture is related to the thickness and connectivity of the trabeculae, and the macro architecture is related to the structural geometry of the bone. Bones that may have similar BMD, but different structural properties will have different strengths (8). In addition, aging bones that lose mineral density may maintain strength by increasing their section modulus (39) and therefore should be included when assessing experimental interventions.

Bone Structural Properties

Approximately 10%-40% of bone strength variability is related to factors other than BMD (14) and attributable to such factors as micro and macro-architecture of the composite material. A bone's ability to resist a given load is determined by material property and the structural geometry. For long bones (ie. tibia and ulna), the most important geometric properties are the cross sectional area, and the cross sectional moment of inertia (7). Early work by (82) indicated that the cross sectional moment of inertia is the best predictor of stress fractures. While DXA quantifies material density and total content of bone, this information only partially accounts for mechanical attributes; therefore, alternative measurement instruments have been developed to provide information related to the micro, and macro-architecture of bone. Steele (107), at Stanford University developed mechanical response tissue analysis (MRTA) in

collaboration with the National Aeronautic and Space Administration (NASA). Mechanical response tissue analysis measures the impedance of a long bone to low-frequency vibration and assesses structural properties of long bones *in vivo*. These properties include Young's Modulus of Elasticity (E), and the cross-sectional moment of inertia (I). Young's Modulus of Elasticity is a material property that quantifies the stress/strain curve for a given material. A material with a higher Young's Modulus will demonstrate greater resistance to strain for a given stress, and greater resistance to fracture. Young's modulus is quantified by the angle of a stress/strain curve (Figure 1). The cross-sectional moment of inertia (I) is a geometric property that is related to the distribution of material around its central axis. The further away the bone material is distributed around its central axis, the greater is the resistance to bending and subsequent fracture (Figure 2). The EI_{MRTA} (E x I) yields a measure of long bone structural integrity, which is related to the composition, geometry, and internal architecture of the bone (76, 87, 97, 107).

Early work with MRTA investigated the relationship of bone mineral content (BMC) and EI_{MRTA} (76) in pre and post-menopausal healthy women. Forty-eight women distributed into two groups (21-30 years, n = 23; 58-80 years, n = 25), completed single-photon absorptiometry (SPA) measurements to assess BMC and bone width of the ulna, as well as ulnar bone stiffness measurements with MRTA. The SPA and MRTA measurements were completed for the dominant and non-dominant arms. In the dominant ulna of the young women, EI_{MRTA} was significantly correlated with BMC (r = 0.59), and bone width (r = .067). However, when BMC, ulnar width, and body weight were entered into a stepwise multiple regression, bone width was the only independent

predictor of EI_{MRTA} (r^2 = 0.45). In the non-dominant arm, EI_{MRTA} was significantly correlated with BMC (r = 0.52) and bone width (r = 0.78). In addition, stepwise multiple regression revealed that bone width was the only independent predictor of non-dominant ulnar EI_{MRTA}. Measures of the dominant ulna in the older women revealed that EI_{MRTA} was significantly correlated with BMC (r = 0.72), but not with bone width. These correlations also were computed for the non-dominant arm, where EI_{MRTA} was significantly correlated with BMC (r = 0.60), but not bone width. In addition, BMC remained the only independent predictor of EI_{MRTA} in the dominant and non-dominant ulna of the older group. Thus, the results of the study indicate a decrease in bending stiffness with age, along with clear differences in the predictors of bone stiffness between the age groups. Therefore, it is evident that MRTA provides additional information regarding skeletal status, beyond that which is available with measures of bone mineral content, and bone mineral density alone.

A follow-up investigation with MRTA assessed forearm bone mass, and ulnar bending stiffness in healthy men (87). Ninety healthy men (aged 19-89 years) completed SPA measurements to determine BMC and bone width of both ulnae, and radii, as well as MRTA measurements to determine bone stiffness. In addition, grip strength was measured for each arm. For statistical analyses, the results were analyzed as continuous variables and examined for age-related effects; in addition the results were analyzed with the subjects divided into four age subgroups (19-30 years n = 15; 31-40 n = 28; 41-51 n = 21; >60 n = 25). There were no significant changes in BMC for the radius and ulna when the results were analyzed continuously across the age subgroups. Ulnar width increased significantly with age (r = 0.27), however no changes were observed for the radius. The

ratio of BMC with bone width (BMC/BW) decreased significantly with age. When the subjects were divided into age groups, the BMC/BW was significantly lower in the oldest group compared with the three younger groups. However, there were no differences in EI_{MRTA} when analyzed across age, or between groups. Stepwise multiple regression revealed that ulnar width was the best independent predictor of EI_{MRTA} when analyzed across age. Stepwise group analysis indicated that BMC was the only predictor of EI_{MRTA} in the youngest group, with ulnar width the best predictor in the three older groups. The study demonstrated no age-related decline in ulnar EI_{MRTA} for men, as has previously been reported for women (76). The maintenance of ulnar EI_{MRTA} for the men in this study is the result of no decrease in BMC with age, accompanied by an increase in ulnar bone width, thereby indicating the importance of bone geometry. Skeletal integrity of the long bones can be maintained through re-distribution of the bone material leading to increased cross-sectional moment of inertia, of which can be assessed *in vivo* by MRTA.

The first study to investigate the relationships between activity status, muscular strength and the structural properties of the ulna was conducted by Myburgh and colleagues (86). This study investigated the influence of recreational activity and muscle strength on ulnar bending stiffness in men. Subjects (N = 51; aged 28-61 years) completed SPA measurements to determine ulnar BMC and ulnar width, and MRTA measurements to determine EI_{MRTA}. Maximum grip strength was determined with a handheld dynamometer, and isotonic biceps strength was determined with a one-repetition maximum using Universal® resistance training equipment. All measurements were completed on the non-dominant arm. Subjects were then distributed into three

groups based on self-reported participation in recreational activities and physical exercise during the previous year (sedentary, n = 13; moderately active, n = 18; highly active, n = 20). The sedentary (S) subjects did not participate in any regular exercise during the previous year, moderately active subjects (M) participated in 1-4 weekly sessions of exercise, and the highly active subjects (H) participated in > 5 exercise sessions weekly. Statistical analyses indicated that the highly active subjects demonstrated greater BMC ($p < 0.05$) than either the sedentary or the moderately active subjects. Across all groups, grip strength was significantly correlated with BMC ($r = 0.43$) and ulnar width ($r = 0.36$), whereas biceps strength correlated with BMC ($r = 0.41$) only. The highly active group demonstrated significantly greater ulnar EI_{MRTA} than either the moderately active or sedentary group ($p < 0.01$). Across all groups, EI_{MRTA} correlated significantly with BMC ($r = 0.69$), ulnar width ($r = 0.76$), body weight ($r = 0.31$), grip strength ($r = 0.40$), and biceps strength ($r = 0.52$). Stepwise multiple regression revealed that ulnar width, and biceps strength were the only independent predictors of EI_{MRTA}. The results of this study provide evidence for the usefulness of MRTA in detecting skeletal differences that may be related to muscular status, but not activity history.

Mechanical response tissue analysis has also been used to assess the status of the human tibia (4). Healthy males (N = 48) aged 26-51 years of age completed MRTA and bone density measures for each ulna and tibia. The EI_{MRTA} tibial values differed significantly ($p < 0.05$), which were 158.8 $\pm$ 53 (mean $\pm$ SD), and 174 $\pm$ 55, for the right and left tibia respectively. In addition, the EI_{MRTA} ulnar values differed significantly ($p < 0.01$), which were 47.9 $\pm$ 11, and 41.9 $\pm$ 9 Nm^2 for the right and left ulna respectively. Bone mineral density did not differ for either the right and left tibia or ulna. Ulna EI_{MRTA}

comparisons were also made between nine left-handed and nine right-handed subjects who were matched for age, height, weight, ulnar length, and BMI. The right-handed subjects demonstrated significantly ($p < 0.05$) greater EI_{MRTA} values for the right ulna, than the left. This was the first study to report EI_{MRTA} tibial measures in humans, and it should be noted that the authors reported an intratest (multiple measures without re-positioning of the limb) and intertest (measures with re-positioning of the limb) coefficient of variation (CV) of 3.2% and 5-12% respectively.

A study by Stussi and colleagues (108), investigated the effects of 15 weeks of military recruit training on tibial bending stiffness in 559 male Swiss army recruits. An alternative method for determination of bending stiffness; the SWING method was used in this study. Although the SWING instrument differs from MRTA, the concept of a bone's response to a vibratory wave is similar in both instruments. The SWING method employs two accelerometers that are fixed to the facies medialis of the tibia, and an electromechanical hammer that strikes the tibia at mid-diaphysis and introduces a vibratory wave. Dispersion analysis is then used to determine wave dispersion from the hammer strike to the accelerometers, of which bending stiffness is calculated. Reliability of this method was demonstrated by comparing *in vivo* SWING measurements of bending stiffness in 21 human cadaver tibiae with ex-vivo 3-point bending stiffness, which yielded a correlation of $r = 0.96$. In the military recruits, 15 weeks of standard Swiss Army training resulted in a 25% increase in tibial bending stiffness, accompanied by only a 1.8% increase in bone mineral content. The relationship of tibial bending stiffness with BMC at baseline, and post-recruit training yielded non-significant correlations of $r \sim 0.1$, respectively. The results of the study demonstrate that dramatic increases in bending

stiffness can be obtained with only minimal increases in BMC. Therefore micro and/or macro-architectural changes may be the underlying cause for the dramatic increase in bending stiffness over the relatively short intervention period.

Recently, MRTA measurement was refined by Roberts and colleagues (97) with a non-human primate tibia model. Previous investigations with the MRTA had been based on a 7-parameter mathematical model of the skin, soft tissue, musculature, and bone (107). A refined 6-parameter model that consists of bending stiffness, damping, and mass of both the soft tissue and bone, was developed and validated using monkey tibias. Twelve rhesus monkeys underwent MRTA measurement of both tibias *in vivo*. Measurements were analyzed with the six and seven parameter mathematical model. The monkeys were scheduled for necropsy due to illness; following the MRTA measurements the monkeys were euthanized and both tibiae excised. The tibias were then tested to failure in three-point lateral bending stiffness, which was converted to cross sectional bending stiffness. Cross-sectional bending stiffness obtained from the three-point bending stiffness test was then correlated with $EI_{MRTA.}$ The six-parameter model demonstrated a much stronger relationship with three-point bending stiffness (R^2 = 0.947) than the seven-parameter model (R^2 = 0.645). Thus, it is evident that the six-parameter mathematical model possesses improved representation of the behavior of the skin, soft tissue, and musculature. Following this published study, Steele further refined (106) the mathematical modeling to include nine-parameter and twelve-parameter algorithms. Presently there are no published investigations that have used these improved mathematical models.

A recent experimental trial by Adami et al, (2) investigated the effects of six months of strength training on BMD, bone structure, and bone geometry of the ultradistal radius in postmenopausal women. Subjects (N=250) were apparently healthy postmenopausal (>5 years) females, aged 52-72 years old, and were randomized to either 6-months of exercise training (n=125), or control (n=125). Control subjects were encouraged to maintain their normal lifestyle. The exercise program was designed to specifically stress the radius, and included upper body presses, wrist curls, and volleyball, 2 days per week in a group setting. In addition, subjects were encouraged to repeat the exercises for at least 30 minutes daily at home. Bone mineral density was assessed by DXA for the lumbar spine, femoral neck, ward's triangle, trochanter, and ultradistal radius. Bone geometry of the ultradistal radius and proximal radius was assessed with peripheral quantitative computed tomography (pQCT). Geometric variables assessed included cross-sectional area, volumetric bone density, and bone mineral mass. All measurements were completed at baseline, and again following the 6-months of exercise training. No changes in BMD were observed for either group from baseline to 6-months at any of the observed sites. In addition, no geometric or structural changes were noted for the proximal radius for either group. However, cortical bone cross-sectional area significantly increased, which was accompanied by decreased trabecular bone area in the ultra distal radius for the exercise group. In addition, cortical bone mineral content significantly increased, and trabecular bone mineral content significantly decreased in the exercise group as well. No such changes were noted for the control group. These results indicate that BMD did not change as a result of the exercise intervention, however

significant structural and geometric changes occurred. Consequently, the observed changes suggest that bone strength increased due to the increased cortical area, and BMC.

In a prospective study, Beck et al, (7) investigated DXA derived structural geometry of the femur, tibia, and fibula as a means of predicting stress fractures in male U.S. Marine recruits. Subjects (N=625) underwent DXA scans of the femur, tibia, and fibula, as well as a series of anthropometric measures, which included femur length, tibia length, bi-iliac breadth, femur bicondylar breadth, neck girth, waist girth, thigh girth, and calf girth. The cross-sectional area, cross-sectional moments of inertia, and bone width, as well as BMD were derived from the DXA scans for the femur, tibia, and fibula. Recruits were then followed throughout their 12-week initial entry basic combat training for the development of stress fractures. Twenty-three recruits developed stress fractures during the 12-week training period, with the most common fracture site being the tibia. Due to the low number of diagnosed stress fractures, all fractures were pooled for the statistical analysis. Based on the anthropometric measurements, subjects that developed stress fractures weighed less, were shorter, and had a smaller neck, waist, thigh, and calf girth, as well as shorter tibial length. The DXA derived structural properties revealed that recruits who developed stress fractures had significantly smaller cross-sectional moment of inertia, section modulus, bone width, as well as BMD for the femur and tibia as compared to the non-stress fracture recruits. However, when body weight was controlled, there were no differences in BMD between fracture and non-fracture recruits. The only significant differences observed when body weight was controlled are the cross-sectional area, section modulus, and bone width for the tibia. Thereby indicating that

factors other than BMD, which are related to true bone strength may be more important for predicting fracture resistance.

Biochemical Markers

Biochemical markers of remodeling (turnover) provide a minimally invasive measure, which reflects the cellular events in bone, and allow for repeated measures within a single subject. One of the principal advantages of the biomarkers is, they can readily detect acute changes in skeletal metabolism (111). The rate of formation or degradation of the bone matrix can be assessed either by measuring a prominent enzymatic activity of the bone forming or resorbing cells, such as the activity of alkaline and acid phosphatase, or by measuring bone matrix components released into the circulation during formation or resorption (34). A high rate of bone turnover is thought to contribute to the micro-architectural deterioration of bone tissue (84), and is therefore useful in assessing the effects of exercise and physical activity on skeletal adaptations.

Osteocalcin (OC), and bone specific alkaline phosphatase (BAP) are two biochemical markers of bone formation that are currently of central interest. Osteocalcin was used to assess bone formation in this study, and will be discussed in the following paragraphs. Osteocalcin (OC) is also referred to as bone gla-protein, and is the most abundant non-collagenous protein found in bone. Osteocalcin is synthesized by the osteoblasts, and incorporated into the bone matrix during bone formation. However, some osteocalcin is released into the circulation where it can be assayed as an indicator of osteoblastic activity, and is related to the rate of bone turnover. Osteocalcin (OC) is a relatively small (49 amino acid) noncollagenous vitamin K dependent protein, which is synthesized during various stages of osteoblastic proliferation and differentiation. During

bone formation, the majority of osteocalcin is incorporated into the extracellular bone matrix (69), with the balance being released into circulation where it can be assessed with radioimmunoassay, thus providing an indication of bone formation. Osteocalcin has been extensively evaluated to assess exercise mediated (18, 40, 117, 120), and microgravity mediated (24, 30, 68, 85) changes in bone metabolism.

The biochemical markers of bone resorption, which are routinely assessed, include hydroxyproline, pyridinoline (Pyd), deoxypyridinoline (Dpd), and the telopeptides of type I collagen (CTx and NTx), which are commonly referred to as the collagen crosslinks. The N-telopeptide of type I collagen was used in this study to assess bone resorption, and will be discussed in the following paragraphs. The assay used for NTx concentrations in this study was developed by Osteomark® (Seattle, WA), and is the only commercially available assay for serum concentrations of the amino terminal of the type I collagen breakdown products (NTx). During type I collagen degradation, the amino terminal cross-linked telopeptide of type I collagen is released into serum, where it can be measured with enzyme-linked immunoassay (ELISA). A number of pharmacological clinical studies have assessed bone resorption with the NTx Osteomark® serum assay kit, however, no exercise studies have been conducted to date. Serum substances that may be influenced by exercise, have been shown to have no effect on the performance of this assay (Osteomark® NTx Kit Insert, Seattle, WA).

When assessing the biochemical markers, seasonal variations (37), circadian variations (34), and menstrual influences must be considered. Recently, Chiu et al (28) investigated the changes in bone turnover during the menstrual cycle in 20 premenopausal apparently healthy females aged 34 $\pm$ 4.6 years (mean $\pm$ SD). The

investigators assessed serum concentrations of the bone formation markers, BAP and OC, as well as serum and urinary concentration of the bone resorption marker DPYR, thrice weekly during a single menstrual cycle. In addition, serum estradiol (E2), and serum progesterone was assessed to identify characteristic cyclic fluctuations. All blood samples were collected between 7am-10am to avoid circadian fluctuations, on Monday, Wednesday, and Friday throughout a single menstrual cycle. The first blood sample was obtained within 4-days of the onset of menses, and the last sample was obtained at the onset of the next menses. Urine samples collected were the first morning void, and were collected on the same days as the blood samples. Results of the serum and urine assays demonstrated no significant variations between the luteal phase and follicular phase for serum concentrations of BAP and OC, as well as urinary concentrations of Dpyr. However, serum Dypr demonstrated a significantly higher ($p < 0.05$) concentration in the follicular phase (268.04 pM) compared to the luteal phase (259.59 pM). The results of this study suggest that markers of bone formation (BAP and OC) do not significantly vary during the menstrual cycle. However, serum concentrations of Dypr varied significantly between the two major phases of the menstrual cycle by approximately 5.6%. Therefore, when assessing biochemical markers of bone turnover, variability related to the menstrual cycle must be considered for at least one of the markers of bone resorption.

Serum OC undergoes a circadian rhythm within a narrow range, with a difference of 15% between peak levels (4 a.m.), and minimal levels (5 p.m.) (35). Subjects were aparently healthy adult males (n = 6) and females (n = 2) aged 25-35 years. Blood samples were obtained every three hours during a 27-hour period. Osteocalcin levels

were assessed, and compared to the 24-hour mean level. A significant diurnal variation was observed ($p < 0.001$). More recently tent sampling time periods are essential when using OC to estimate bone formation rates.

Skeletal Adaptations to Exercise

Bone adapts to added tensile and compressive loads over time by becoming stronger, apparently due to increased mineral content and density (BMD), modified hydroxyapatite crystal and collagen fiber composition, and reorganization of its micro- and macro-architecture, . When these mechanical loads are diminished over time, as in micro-gravity exposure, these effects, including those for bone BMD (102) would seem to be reversed, leading to a weaker bone In the healthy skeleton, there is a balanced coupling between activities of osteoclasts and osteoblasts resulting in a homeostatic maintenance of bone tissue. However, this coupled balance can be altered by an exercise stimulus, which produces a mechanical strain at the cellular and/or tissue level. Turner (112) discussed three rules for bone adaptation to mechanical stimuli; 1. Bone adaptation is driven by dynamic, rather than static loading. 2. Only a short duration of mechanical loading is necessary to initiate an adaptive response. Extending the loading duration has a diminishing effect on further adaptation. 3. Bone cells accommodate to a customary mechanical loading environment, making them less responsive to routine loading signals. It appears that physical exercise and sports participation would present mechanical stimuli that satisfy the above rules for positive bone adaptation. In fact, it is generally accepted that various forms of physical activity and sport participation are positive adaptive stimuli for bone formation; therefore exercise has been promoted as a means to preserve and promote skeletal health (15, 42).

Published studies related to the effects of exercise on skeletal health are generally cross-sectional studies, or randomized controlled trials (RCT). The cross-sectional investigations provide insight into the differences in skeletal status among various levels of exercisers and non-exercisers, as well as differences in skeletal status among participants in a range of sports endeavors. In addition, within-subject studies provides evidence that participants in sport activities such as tennis, may possess greater BMD of the dominant playing extremity versus the non-dominant extremity activity. The following paragraphs will review a number of cross-sectional investigations that provide evidence for differences in skeletal status that may be due to the inherent loading in a particular sport or activity. In addition, a recent within-subject study will also be reviewed. Although these studies are useful in identifying skeletal differences among participants, and differences among dominant and non-dominant extremities, they do not permit concrete inferences on the circumstances leading to these differences. Randomized controlled trials are essential in understanding the effects of exercise on skeletal status. Consequently, a number of RCT's will be reviewed following the cross-sectional review.

Cross-Sectional Investigations

Regular physical activity, in the long-term, leads to decreased bone resorption, and increased bone formation (117), consequently resulting in net bone gain. A number of cross-sectional studies have revealed differences in skeletal status among participants in various sports. A cross-sectional study by Taaffe *et al*, (110) demonstrated that female gymnasts increased regional and total body BMD more so than runners and controls in an 8-month cohort, and swimmers and controls in a 12-month cohort. The 8-month cohort

revealed that gymnasts increased lumbar BMD by 2.8%, and femoral neck BMD by 1.6%, which was significantly greater than runners who demonstrated a 0.2% decrease in lumbar BMD, and a 1.2% decrease in femoral neck BMD. In addition, the gymnasts in the 12-month cohort displayed a 2.3% increase in lumbar BMD, as well as a 5% increase in femoral neck BMD. In contrast, the swimmers experienced a decrease of .3% and .6% in BMD of the lumbar spine and femoral neck respectively. Gymnastic activity results in ground reaction forces (GRF) that are greater than 10 times the body weight (78), compared to runners who experience considerably less (33), and swimmers who experience no GRF. Thus, the results of this investigation are in accordance with the evidence which supports activities involving high impact forces have a substantially greater osteotropic effect, than lower-impact, or non-impact exercise.

In addition to high loads, Lanyon *et al* (65) suggested that loading should be imposed in unusual patterns, if the osteogenic stimulus is to be optimal. Therefore, exercise that has a rhythmic repeating pattern (i.e. running, rope skipping), may not be as effective in stimulating skeletal responses as an exercise that involves varied loading patterns (i.e., basketball, soccer). Recently Pettersson *et al* (94) investigated the effect of high impact activity on bone mass and size in adolescent females [(mean $\pm$ SD) 17.6 years $\pm$ 0.8], with dissimilar loading patterns. The purpose of the cross-sectional study was to compare the influence of rope-skipping (routine loading) and soccer participation (varied loading) on muscle strength, BMD, BMC, and bone area in late adolescent females. The control cohort (n = 25) participated in vigorous physical activity for approximately one hour per week, whereas the soccer cohort (n = 15) and rope-skipping cohort (n = 10) participated in their activities for five and six hours per week respectively.

Total body and site-specific (lumbar spine, femoral neck, greater trochanter, femur, femur diaphysis, distal femur, proximal tibia, tibia diaphysis, humerus, and radius) bone mineral density was assessed with DXA. Isokinetic strength of the quadriceps and hamstrings was assessed with a Biodex® dynamometer, at an angular velocity of 90°/second. Results of the statistical analyses indicated that the subjects did not differ in age, height, weight, and BMI. However, the control subjects had significantly ($p < 0.05$) more fat mass than the soccer players (21.2 kg vs. 16.9 kg). The DXA measures revealed that the rope-skipping group had significantly greater ($p < 0.05$) BMD for total body, humerus, lumbar spine, greater trochanter, femur diaphysis, tibia diaphysis, and ultra distal radius than the controls. The soccer players demonstrated significantly greater ($p < 0.05$) BMD for the femoral neck, greater trochanter, total femur, femur diaphysis, and tibia diaphysis than the controls. No differences for any of the BMD measures were observed between the rope skippers and soccer players. Sport-dependent skeletal loading differs considerably for the rope skippers compared to the soccer players, yet, no difference in total body or site-specific BMD was observed between these groups. However, the soccer players began participating in their sport approximately 2.5 years prior to the rope-skippers, and had significantly greater lean mass that the rope-skippers. When statistical adjustments were made for these variables, the rope-skippers demonstrated significantly greater total body, lumbar spine, and humerus BMD than the soccer players. In the absence of statistical adjustments, the rope-skippers had significantly greater total body and tibia BMC, than the soccer players. In addition, the rope-skippers had significantly greater bone area (BA) in the lower legs than the soccer players. The researchers speculated that this difference was most likely due to the higher

mechanical forces incurred during rope skipping compared to soccer participation. Thus, the increased bone area in the tibial diaphysis of the rope-skippers is a functional adaptation resulting from the exposure of high mechanical loads (47). Although the rope-skippers participated in a routine loading exercise regimen, compared to the varied loading exercise regimen of the soccer players, the rope-skippers demonstrated a greater functionally adaptive response than that of the soccer players. Therefore, these findings would appear to support Turner's three key rules (112), high loads may be more important than varied loading if bone is to favorably adapt in response to mechanical loading.

Bennell et al, (9) investigated bone mass and bone turnover in power athletes, endurance athletes, and controls in a 12-month longitudinal cohort study. The cohort was comprised of 41 (20 female, 21 male) power athletes, 54 (26 female, 28 male) endurance athletes, and 45 (24 female, 21 male) non-athlete controls, aged 17 to 26 years old. The power athletes and endurance athletes were members of club, state, or national level track and field teams in Australia. The athletes trained at least 3 days per week in their respective sport, whereas the non-athlete controls engaged in physical activity less than 3 hours weekly. Bone mineral density of the upper limb, lumbar spine, femur, lower leg, and foot, as well as total body BMC was assessed at baseline, and again following 12-months of training and competition. Osteocalcin was assessed for bone formation, with pyridinoline and deoxypyridinoline assessed for bone resorption at baseline only. Results of the DXA measurements revealed no significant differences in total body BMC for any of the three female groups at baseline, however the male power athletes (2829g, $p < 0.01$) and endurance athletes (2533 g, $p < 0.05$) were significantly greater in total body BMC

than the controls (2538 g), when statistical adjustments for height and weight were made. In addition, upper limb BMD was significantly greater in the male and female power athletes (.938 g/cm^2, $p < 0.01$ and .814 g/cm^2, $p < 0.05$ respectively) compared to the male and female controls at baseline (.884 g/cm^2 and .766 g/cm^2 respectively). Although upper limb BMD was greater in the male and female power athletes compared to the endurance athletes (.881 g/cm^2 and .775 g/cm^2 respectively), the difference did not reach statistical significance. Lumbar spine BMD of the male and female power athletes (1.244 g/cm^2 and 1.167 g/cm^2 respectively) was significantly greater than that of the male and female endurance athletes (1.095 g/cm^2, $p < 0.01$ and 1.036 g/cm^2, $p < 0.01$ respectively) and controls (1.051 g/cm^2, $p < 0.01$ and 1.020 g/cm^2, $p < 0.01$ respectively) at baseline. Femur BMD was significantly greater in the male power (1.389 g/cm^2, $p < 0.01$) and male endurance (1.333 g/cm^2, $p < 0.01$) athletes compared to controls (1.243 g/cm^2), as well as the female power athletes (1.220 g/cm^2, $p < 0.01$) compared to controls (1.123 g/cm^2). Lower leg (tibia/fibula) BMD was significantly greater in the male and female power athletes (1.282 g/cm^2, $p < 0.01$ and 1.143 g/cm^2, $p < 0.01$ respectively) and the male and female endurance athletes (1.205 g/cm^2, $p < 0.01$ and 1.094 g/cm^2, $p < 0.01$ respectively) compared to the male and female controls (1.109 g/cm^2 and 1.014 g/cm^2 respectively). The only differences noted for any of the biochemical markers of bone turnover were significant greater concentrations of pyridinoline and deoxypyridinoline for the female power athletes [(90.7 nmol:mmol CR (creatinine), $p < 0.05$ and 15.14 nmol:mmol CR, $p < 0.05$ respectively)] compared to the female endurance athletes (71.31 nmol:mmol CR and 11.66 nmol:mmol respectively). Following 12-months of training and competition, all groups for both genders demonstrated a significant increase ($p <$

0.01) in total body BMC, no between group differences were observed. Female controls demonstrated a significantly greater increase (1.9 % , $p < 0.05$) in upper limb BMD than the female power (0.6 %) and endurance (0.5 %) athletes. Lumbar spine BMD increased in all three female groups ($p < 0.01$), however no between group differences were noted. Lumbar spine increased in both the male power (3.0 % , $p < 0.05$) and endurance (0.9 % , $p < 0.05$) athletes, compared to a decline in controls (- 0.04 %). Between-group comparisons indicated significantly greater increase for the male power athletes ($p < 0.01$) compared to the endurance athletes. Femur BMD increases for the female power athletes, endurance athletes, and controls were 2.0% ($p < 0.05$), 1.4% ($p < 0.05$), 1.4% ($p < 0.05$), respectively. No between-group differences were noted. Femur BMD increases for the male power athletes, endurance athletes, and controls were 1.6% ($p < 0.05$), 0.7% ($p < 0.05$), and 1.5% ($p < 0.05$), respectively. No between-group differences were noted. No changes for lower leg BMD was observed for any of the groups. Baseline measurements demonstrated significantly greater BMD at all sites for the male and female power athletes compared to controls, whereas the only differences observed between the male and female endurance athletes and controls was in the legs. In addition, the power athletes had significantly greater lumbar BMD than the endurance athletes. The loading inherent in track and field events for the power and endurance athletes mirrored the differences in BMD at baseline. Power athletes participate in events, which impart mechanical loading at various sites, compared to the endurance athletes where the loading is predominantly on the legs. Skerry *et al* (104) suggests that the primary determinant for effectively increasing bone mass through exercise is high loads, which is in accordance with the results of this study. Muscle forces place greater

load on bones than do gravitational forces associated with normal weight bearing activity (23), therefore exercise involving high muscular forces should promote positive osteogenic responses. Track and field power athletes undoubtedly exert greater muscular forces during training and competition than endurance athletes or controls. Therefore, in addition to greater mechanical loading, increased muscular forces may also be related to the increased BMD observed for this group.

Investigating within-subject differences between the dominant playing arm, and non-dominant arm of athletes, also provides insight into the effects of physical activity on skeletal responses. Unlike cross-sectional studies, where selection bias can confound observed differences, within-subject comparisons that demonstrate significant differences between dominant playing limbs, and non-dominant limbs should reveal the skeletal effects of the loading regimen inherent in that activity. Alfredson et al (3) investigated the regional bone mass of the arm in female volleyball players. The subjects were highly competitive female volleyball players (n = 11) aged 22.0 $\pm$ 2.6 (mean $\pm$ SD), and non-active controls (n = 11) aged 24.6 $\pm$ 3.1 (mean $\pm$ SD). All subjects in both groups were apparently healthy, reported regular menses, and were not taking any medications known to affect bone metabolism. Subjects completed DXA measurements to assess total body (TB), distal radius, and proximal humerus BMD. Results of the DXA measures for between group comparisons, demonstrated no significant differences between the volleyball players and controls for total body BMD, however the volleyball players [9.78 $\pm$ 1.19 (mean $\pm$ SD)] demonstrated significantly greater ($p < 0.01$) BMC (g/cm^2) for the dominant proximal humerus than the non-active controls (8.10 $\pm$ 1.03). No other BMD or BMC differences were observed between the two groups. Within-subject comparisons

revealed significantly greater ($p < 0.01$) BMC of the dominant versus non-dominant proximal and distal humerus in the volleyball players. Bone mineral content of the dominant versus non-dominant arm was 9.6% and 10% greater in the proximal and distal humerus respectively. In addition, the BMD of the distal radius was 5% greater in the dominant versus non-dominant arm in the volleyball players, which corresponded to a significant difference of $p < 0.05$. No within-subject differences for any of the BMD or BMC measures were observed for the non-active controls.

Results of the above cross-sectional and within-subject investigations provide evidence supporting the notion that physical exercise is associated with positive skeletal responses. On the basis of these studies, activities involving high-impact loading may be more beneficial for skeletal adaptation than low-impact or non-impact activities. In addition activities that involve high-muscular forces may be more beneficial in promoting skeletal adaptation than activities requiring lower muscular forces and greater frequency. However, to draw firm conclusions, or identify the optimal exercise stimulus for promoting positive skeletal adaptation, randomized controlled trials are essential.

Randomized Controlled Trials

Randomized controlled trials (RCT)) provide the best evidence for substantiating the treatment effects of exercise on skeletal responses. Although a great number of published RCT's investigating the effects of exercise on skeletal status are available, the results of many of these trials are conflicting. That is, a number of investigations have demonstrated that exercise has a positive impact on skeletal health by increasing, maintaining, or slowing the loss of BMD (53, 90, 109), whereas others have shown no effect (66). Furthermore, a number of studies have demonstrated that exercise promotes

a positive adaptive response in some site-specific regions, but not others (20, 72). These conflicting results can be attributed to a number of factors which differ between studies, including subject age, menstrual status, dietary status, exercise mode, exercise intensity, exercise duration, and intervention period. Given the variability in these factors among the published literature, elucidating the true effects of an exercise intervention on skeletal responses is challenging at best.

Randomized controlled trials can be sub-divided into two broad categories, i.e. those that have investigated: 1 skeletal responses to acute exercise; and 2. skeletal responses to chronic exercise. Within those two categories, effects of various modes, intensities, and duration of exercise have been investigated. Variables of interest for studies investigating skeletal responses to acute exercise usually include the biochemical markers of bone turnover, and/or hormones related to mineral homeostasis. Measures of bone mineral density, bone stiffness, or bone geometric properties are of little or no value when assessing skeletal responses to acute exercise, as the capacity to detect changes in these variables over a short period of time using current technologies is unlikely. Randomized controlled trials, which investigate skeletal responses to chronic or long-term exercise, may include one or more of the above mentioned variables. The following paragraphs will review recent RCT's that have investigated the skeletal responses to acute exercise, followed by a review of skeletal responses to chronic (long-term) exercise.

Acute Skeletal Responses to Exercise

Researchers (17) demonstrated that acute skeletal responses to aerobic exercise, as assessed by the biochemical markers of bone turnover differ between men and women. In this investigation, twenty healthy men (n=10) and women (n=10) participated in a running competition. Fasting blood samples were obtained the day prior to the competition, and again the day of the competition, and two days following the competition. All samples were obtained the same time of day, for each sampling period. Serum concentrations of PICP and ICTP were measured by RIA (Farmos Diagnostika, Oulunsalo, Finland), and serum osteocalcin (OC) was also measured with RIA (CIS bio international, Gif-Sur-Yvette, France). Bone-specific alkaline phosphatase was assessed spectrophotometrically. Data analysis revealed that, in the women, PICP levels decreased from (170 $\pm$ 17 *u*g/l) the day prior to competition on, to 158 $\pm$ 17 ug/l the day following competition, then returned to baseline (167 $\pm$ 19 *u*g/l) two days following the competition. No changes in OC or ICTP were observed for any of the sampling periods in women. Men demonstrated an increased OC concentration at baseline (12.1 $\pm$ 0.9 *u*g/l) compared to women (8.1 $\pm$ 1.0 *u*g/l). In addition, OC levels significantly decreased in men from baseline (12.1 $\pm$0.9 *u*g/l) to 10.3 $\pm$ 1.1 *u*g/l the day following competition. In addition, ICTP increased from 3.67 $\pm$ 0.28 *u*g/l the day following competition, to 3.98 $\pm$ 0.35 *u*g/l. No changes in PICP were observed for the men. There were no changes in BALP for either the men or women at either sampling period. The gender differences in this study indicate that women have lower baseline concentrations of OC than men, which has also been reported elsewhere (44, 99). In addition, this study demonstrated that men and women respond to an identical exercise stimulus differently. Gender

differences in bone biomarker responses to exercise have also been demonstrated by Salveson *et al* (99).

In addition to gender, nutritional status is important in evaluating acute skeletal responses to aerobic exercise. Zanker and Swaine (121) investigated bone turnover marker responses in male distance runners under conditions of energy balance and energy restriction. Eight trained male distance runners [mean 25.1 (SD 5.9)] years, participated in the study. Subjects participated in two trials of three days each separated by 14 days. In one trial, the dietary intake was controlled to ensure 100% of the estimated energy requirement, whereas, in the other trial, dietary intake was restricted to approximately 50% of the estimated energy requirement. On both occasions, the proportion of macro nutrient intake consisted of 25% fat, 15% protein, and 60% carbohydrate. In addition, caffeine and alcohol intake were prohibited during the experimental period. In each of the trials, subjects participated in 60-min of treadmill running, which consisted of four 15-min intervals comprised of four 5-min bouts at 65%, 75%, and 85% of each subject's maximal oxygen uptake (VO_{2max}). Subjects' participated in the exercise regimen on three consecutive days. Blood and urine samples were collected at 0800 hours on the first day prior to exercise, and again at 0800 hours the day following the final treadmill running bout. Blood samples were analyzed for markers of bone formation, which included osteocalcin (OC), and the N-terminal pro-peptide of type I collagen (P1NP). Urine samples were analyzed for bone resorption markers, which included deoxypyridinoline (Dpd), and cross-linked N-telopeptides of type I collagen (NTx). Neither the markers of bone formation or resorption changed significantly as a result of the energy-balanced trial. However, a 15% reduction in the bone formation marker P1NP

was observed as a result of the energy restricted trial. No changes for any of the other biomarkers were observed. The results of this study indicate that the bone formation marker P1CP was influenced by concurrent nutritional status, but not exercise. Therefore, it is apparent that dietary factors must be considered when assessing the acute effects of an exercise bout on the biochemical markers of bone turnover. Previous investigations that have failed to do so the interpretation of biomarker changes may be uncertain in those studies where nutritional status is not reported.

Chronic (long-term) Skeletal Responses to Exercise

Chronic exercise interventions designed to promote skeletal adaptation may include resistance exercise, aerobic exercise, or a combination of both. A recent Medline search limited to the past five years for randomized controlled trials, with the mesh terms "exercise and bone mineral density", and "physical activity and bone mineral density" provided twelve published articles for adult women and men aged 19-44 years. Additional mesh terms such as bone health, and bone geometry, and bone stiffness provided no additional published studies when coupled with physical activity or exercise. However, an identical search for middle-aged, and older women and men produced 34, and 18 published articles, respectively. The majority of the RCT research regarding skeletal responses to exercise is focused on a population that has begun to experience some degree of bone loss. Generalizing the results of those studies to younger apparently healthy adults (19-44 years), where bone loss is not yet apparent, may be problematic. However, the magnitude of literature related to this population cannot be overlooked, and therefore will be included in the following review.

Muscular contraction, rather than body weight accounts for nearly 70% of the bending forces on bone (73). Exercise interventions that maximize muscular contractions, should in-turn maximize positive skeletal responses. In fact, a number of investigations utilizing a resistance exercise intervention have substantiated the positive effects of strength training on skeletal maintenance, and bone formation (48, 80, 88, 98).

Sinaki et al (103) investigated the effects of dose-specified loading and strengthening exercises on spinal and femur BMD of normal active women in a three-year randomized controlled trial. The subjects were 120 apparently healthy premenopausal women aged 30-40 years. Sixty subjects were assigned to an exercise intervention, and the remaining 60 served as non-exercise controls. Prior to initiation of the baseline testing, 24 subjects dropped from the study. All remaining subjects (N = 96; n = 50 exercisers, n = 46 control) completed baseline maximal isometric muscular strength measurements for the back extensors, back flexors, hip extensors, hip flexors, and grip strength of the non-dominant hand. All strength measurements were completed every three months throughout the duration of the study. Bone mineral density of the non-dominant hip, non-dominant mid-radius, and lumbar spine was assessed by DXA. Hip and midradius BMD measurements were repeated again at the completion of the study, whereas spine BMD measurements were repeated at 1 year, and again at the completion of the study. There were no differences in age, height, weight, and physical activity history between the exercisers and controls at baseline. Participation in physical activity was re-assessed every three months throughout the duration of the study. Dietary evaluations were complete at baseline with a 7-day food record, and were repeated every three months throughout the duration of the study. The exercise group participated in

back extension and shoulder girdle weight-lifting exercises thrice weekly for three years. The back extension exercises were performed with a sandbag, which corresponded to 30% of the subjects' baseline body weight, resting on the upper back at the level of the scapulae. The shoulder girdle exercise sessions were isotonic shoulder presses using free weights, which corresponded to 50% of the 10RM (repetition maximum) for the first month, 75% of the 10RM for the second month, and 100% of the 10RM for the third month, after which a strength assessment was completed for a new 10RM. This cycle was repeated throughout the duration of the study. The exercise sessions consisted of three sets of ten repetitions for each of the exercises, and were supervised once weekly by a physical therapist. Of the 96 subjects that began the study, 67 (70%) remained at the end of the three years (n = 32 exercisers, n = 35 control). Exercise adherence for the experimental group, which was calculated as the number of sessions attended compared to possible sessions, at 6 months, 12 months, 18months, 24 months, and 36 months, was 79%, 69%, 63%, 56%, and 48% respectively. In the first 12 months of the study, the control group significantly increased their physical activity ($p < 0.01$) compared to baseline, although the researchers reported giving strict guidelines against doing so. In addition, muscular strength measures indicated that the spine extensors, spine flexors, hip extensors, and hip flexors increased significantly in both groups compared to baseline ($p < 0.01$). The exercise group also demonstrated a significant increase in grip strength during the first twelve months ($p < 0.01$). No between-group differences were reported for the first 12 months of the study, for any of the muscular strength measurements. Muscular strength measurements for the remaining two years of the study revealed a significant decrease in grip strength for the exercisers ($p < 0.01$). No other changes for

any of the remaining strength measures were noted for either group. Results of the BMD measurements revealed no significant changes in lumbar BMD for either the control or exercise group from baseline thru the completion of the study. A slight increase ($p < 0.05$) in the Ward's triangle (absolute values were not provided) from baseline thru the end of the study for the exercise group was the only significant positive change in BMD for either group. A significant decrease ($p < 0.05$) in midradius BMD from baseline thru the end of the study was noted for the exercisers, with no negative changes observed for the control group. Although a number of studies have demonstrated the positive effects of resistance training on skeletal adaptations, the present study failed to do so. Kerr et al (64), demonstrated that resistance training can stimulate site-specific skeletal adaptations in post-menopausal women. The exercise intervention in the present study was designed to stress the muscles of the back, which act directly on the spine, therefore positive spinal BMD responses were expected. The failure to demonstrate positive adaptations in the present study may have been due to the relatively low exercise compliance (< 50% in year 3), the study population, or the selected exercise intervention. The back extension resistance exercises were performed with approximately 30% of the subjects' body weight, which may not have been sufficient enough to produce muscular contractions that were forceful enough to stimulate an osteogenic response. The back extensors and back flexors are required to stabilize the trunk and upper body in most activities of daily living. Therefore, these muscles in normal active apparently healthy women are likely to require a much greater exercise stimulus to promote skeletal adaptation, than what was presented in the current study. In addition, a number of animal and human studies have demonstrated the positive effects of impact loading on skeletal responses. The exercise

intervention in the above study presented no impact loading stimuli, which could be related to the near absence of any positive effects over the three-year period.

A recent study by Bassey et al (6) demonstrated the differences in skeletal responses between premenopausal and postmenopausal women to the same high-impact exercise. Apparently healthy normal active premenopausal women were recruited, and randomized to either exercise (n = 30) or control (n = 25). In addition, normal active postmenopausal women who were receiving hormone replacement therapy (HRT) were randomized to exercise (n = 24) or control (n = 22), and estrogen deplete normal active postmenopausal women were randomized to either exercise (n = 32) or control (n = 45). Postmenopausal subjects were excluded from the study if total body BMD was > 2 SD below, or 1.5 SD above peak young adult values. The exercise intervention group participated in high-impact loading activity, which consisted of six days per week of five bouts of 10 vertical jumps (50 jumps per day) for 6-months in the premenopausal exercise group, and 12-months in the two post-menopausal groups. The investigators cited previous studies in which exercise interventions of 6-months in post menopausal women failed to demonstrate appreciable effects, whereas studies of 12-months demonstrated larger and more consistent effects. The 10-minute exercise sessions were supervised once weekly, and subjects were required to maintain exercise records for the unsupervised days of activity. Investigators reported mean ground reaction forces (GRF) of 301% and 396% of body weight in the pre- and postmenopausal women respectively. Subjects jumped up from the floor, requiring concentric contractions of the leg muscles at take-off, and eccentric contractions upon landing. The investigators reported that the muscles, which act on the hip, were also loaded concentrically and eccentrically,

therefore the hip underwent impact loading from the ground reaction forces, as well as loading from the muscular forces. All subjects were supplemented with calcium throughout the study, with intakes ranging from 1400-1650 mg/day. Subjects completed lumbar spine and hip BMD measurements at baseline, and again following completion of the exercise intervention. Results of the BMD measurements in the premenopausal subjects indicated significant increases ($p < 0.05$) in BMD of the exercise group for all sites, and the control group for the lumbar spine. Between-group comparisons indicated that the exercise group increased significantly ($p < 0.05$) more (2.8%) than the control group at the trochanter. No other significant between group differences were observed for the premenopausal women. In the postmenopausal women, comparisons between the exercise and control group demonstrated no significant changes in BMD at any of the sites for either group. However, the HRT exercise group significantly increased ($p < 0.05$) femoral neck area by 2% compared to the control group. Although the investigators suggested a trend for the HRT group to increase spinal BMD, and the deplete group to lose hip BMD, these observations were not significant. Following completion of the 12-month intervention, 38 post-menopausal subjects continued with the exercise intervention for an additional 6-months. Following 18-months of the jumping exercise, no significant changes in BMD were observed for any site. The exercise intervention in this studied appeared to satisfy the three rules of for bone adaptation to mechanical stimuli discussed by Turner et al (113), however was ineffective in the postmenopausal women; the intervention was dynamic, it involved a relatively short-duration of mechanical loading, and the jumping exercise was novel for the subjects. However, the investigators reported that the exercise sessions were approximately 2-

minutes in duration. Therefore the short-duration mechanical loading rule discussed by Turner et al, may be dependent on some threshold duration, which is greater than 2-minutes.

Heinonen and colleagues (55) investigated the effects of 18-months of high-impact exercise on osteoporotic risk factors in apparently healthy sedentary premenopausal females (N = 98) aged 35-35 years. The subjects were randomized to either exercise (n = 49) or non-exercise control (n = 49). All subjects completed DXA measurements at baseline, 12-months, and 18-months to assess lumbar spine, right femoral neck, trochanter, distal femur, patella, proximal tibia, calcaneus, and dominant distal radius BMD. Subjects also completed muscular strength measurements for the trunk flexors, trunk extensors, dominant elbow flexors, and leg extensors, as well as leg extensor power test. Dietary assessments were completed at baseline, 9-months, and 18-months, using complete 3-day dietary records. The exercise training sessions consisted of 15 min of warm-up, 20 min of high-impact jump training, 15 min of non-impact calisthenics, and 10 min of cool down. The high-impact training alternated bi-weekly between an aerobic jump and an aerobic step program. Ground reaction forces in the two high-impact programs varied between 2-6 times body weight. Every four months throughout the training period, the jumping height was gradually increased, and the number of jumps decreased. During the first two four month periods, the number of jumps was > 200, after which the jumps decreased to 150, 120, and 100 for the following four-month periods. Throughout the study period, the control group was instructed to maintain their normal activity levels. Thirty-nine subjects in the exercise group, and 45 controls completed the study. Compliance for the exercisers for the duration of the study,

was 83%. Results of the DXA measurements indicated no significant differences in BMD for any of the sites measured at baseline between the control and exercise groups. However, following the 18-month exercise intervention, adjusted mean differences for the exercise group compared to the controls were significant at all sites except for the trochanter and distal radius; lumbar spin [0.15 g/cm2, ($p = 0.002$)], femoral neck [0.12 g/cm2, ($p = 0.006$)], trochanter [0.006 g/cm2, (NS)], distal femur [0.17 g/cm2, ($p < 0.001$)], patella [0.007 g/cm2, ($p = 0.036$)], proximal tibia [0.026 g/cm2, ($p < 0.001$)], calcaneus [0.010 g/cm2, ($p < 0.001$)], and distal radius [-0.002 g/cm2, (NS)]. Although absolute percentage changes were not provided, graphical representations indicated approximately 3.5% increase in calcaneus BMD, all other sites increased approximately 1%-2.5%, except for the distal radius, which decreased by approximately 1.5%. Results of the muscular strength tests indicated no significant differences between the two groups for any of the tests. However, the exercise group demonstrated a significant improvement ($p < 0.001$) in lower leg explosive power. The results of this investigation support the findings of cross-sectional studies, where high-impact exercise is related to increased BMD.

Conversely, prolonged aerobic type activity also has been found to decrease bone density (12, 81), and increase bone turnover (57). However, these conclusions have been based predominantly on cross-sectional studies, due to the limited number of RCT's that have investigated the skeletal responses to aerobic type activities. Recently, Humphries et al (insert endnote citation) investigated the effects of exercise intensity on bone density, strength, and calcium turnover in older women. Subjects (N = 65) were apparently healthy women aged 45-65 years who were either taking HRT (n = 23) or not

taking HRT (n = 41) were randomized to either weight training or walking exercise (n = 21 non-HRT weight training, n = 20 non-HRT walking, n = 14 HRT weight training, n = 9 HRT walking). Prior to initiation of the exercise programs, subjects completed isometric strength tests for the knee extensors, a 1RM bench press, 1RM squat, and isokinetic back extension. In addition, subjects completed DXA to assess lumbar spine BMD, blood sampling to measure serum osteocalcin concentrations, and urine sampling to measure deoxypyridinoline concentrations. All measurements were repeated following completion of the 24 wk exercise programs. Subjects participated in either weight training or walking twice weekly, and 100% compliance was required to be included in the fanal analyses. The 24 wk weight-training program was sub-divided into 8-wk blocks of increasing intensity. The training sessions consisted of two sets each of bench press, leg press, squat, lateral pull down, back extension, dead lift, hamstring curl, calf raise, arm curl, triceps extension, and abdominal flexion. The resistance training exercises were performed with either free weights or isotonic resistance machines. The intensity of the training was progressed from 50%-60% of the 1RM for 10-15 repetitions at week one, to 90% of 1RM for 2-4 repetitions in week eight. A qualified trainer supervised all exercise sessions. The aerobic walking exercise served as a control, and consisted of two exercise sessions per week, for 50-minutes per session. Subjects assigned to the walking group were also encouraged to maintain their normal daily activities throughout the 24-wk training period. The investigators reported no attempt to overload the training for the low-intensity walking group. Baseline comparisons revealed no differences between any of the groups for the BMD, or muscular strength measures. Following the 24-wk exercise intervention, there were no significant between group differences for lumbar

spine BMD. However, within group changes indicated that the walking group lumbar spine BMD decreased 1.3% from baseline to post training ($p < 0.05$). A number of significant differences were noted between the weight training and walking group for the majority of the muscular strength measures, however none of the changes were related to BMD or bone turnover. No significant differences for osteocalcin were observed between any of the groups from baseline to post-training. However, the walking group demonstrated a significant ($p < 0.05$) within-group increase (22%) from baseline to post-training. No within or between group differences were noted for deoxypyridinoline for any of the groups. Following completion of the study, the investigators indicated that most likely, a type II error rate contributed to their non-significant findings in BMD changes.

The articles reviewed in this section appear to partially support the three rules for positive skeletal adaptation discussed by Turner et al (112). Cross-sectional studies investigating the skeletal differences between athletes involved in high-impact, or power type activities with those involved in endurance or non-impact activities consistently demonstrated the increased osteogenic effects of the high-impact, high-load activities. This was also supported in the findings of the at least one of the reviewed RCT's (55). Results of animal studies suggest that activities presenting a varied loading pattern have a greater adaptive skeletal response than routine loading, however, current literature has not substantiated this claim in humans. Additionally, Turner discussed that only a relatively short period of mechanical loading is necessary to induce positive changes. However, recent research by Bassey (6) indicated that a novel exercise, which presented a loading period of approximately 2-minutes, failed to show any response in

postmenopausal women. Therefore, it appears that minimum threshold levels exist for the loading period, and possibly the magnitude of impact. Without question, the rules for positive skeletal adaptation as applied to exercise appear to be valid in developing therapeutic programs to optimize, or maintain BMD. However, the most favorable exercise regimen is dependent on factors beyond the rules of mechanical stimulation. Understanding the optimal exercise stimulus, which maximizes the osteogenic response, has important implications, both for the individual and community (10).

Transient Vulnerability

Stress fractures are nontraumatic bone fractures caused by repeated application of loads below the fracture threshold (23). That is, the magnitude of force required in a single application to induce a stress fracture is considerably higher than the forces that will induce stress fracture with repeated exposure are not sufficient to cause tissue failure alone. However, repeated exposure to sub-threshold forces most likely result in fatigue failure, and damage to the bone micro-architecture. Stress fractures are of interest because they can provide insight into how bone strength differs among otherwise healthy individuals (8). The pathogenesis of stress fractures are not clearly understood, however they generally occur following the onset of physical training (83), thereby indicating an inability of the skeleton to adapt rapidly enough to stresses imposed upon it (50). Evidence for a transient vulnerability in bone is supported by literature related to stress fracture incidence in military recruits undergoing high periods of physical activity during initial entry training. It is apparent that stress fracture rates in military personnel are highest at the onset of basic combat training, and lowest near the completion of training.

The onset of stress fractures was tracked in an eight-week basic training program for 109, 296 male and female U.S. Army recruits, over a 4-year period (93). The highest incidence rate of stress fractures for either males or females occurred in week two. A steady decline in stress fracture incidence rate was exhibited by the female recruits throughout the remainder of the training period, whereas the male recruits exhibited a decline in stress fracture incidence rate at week three, followed by an increase at week four, then a subsequent decline for the remainder of the training period. Thereby indicating that the highest incidence of stress fractures occurred in the first few weeks following the onset of high-intensity physical training, thus supporting the premise of transient vulnerability that the bone is unable to adapt rapidly enough to the demands imposed upon it. However, following the second week of training for the females, and the fourth week of training for the males, the decrease in stress fracture incidence suggests that the bone tissue was able to accomplish a positive adaptive response.

Reports of stress fractures in military recruits range from 3-4% in United States Marine Corps recruits (50) to 31% in the Israeli Defense Forces (83). For physically active military personnel, stress fractures of the legs continue to constitute a serious and debilitating problem (45, 95). Stress fractures result in lost training time for the soldier, increased medical care costs, increased training costs, and reduced combat readiness.

It is apparent, that women develop stress fractures at 2-5 times the rate of men. In an 8-week prospective study involving 310 (124 men, and 186 women) U.S. Army basic training recruits, (61) reported a 2.4% and 12.3% incidence of stress fractures in men and women respectively. In addition, women experienced lost training time at the rate of 32 days per 100 person-weeks, compared to men who lost 10 days per 100-person weeks.

Pester et al, (93) reported that female stress fracture incidence rate exceeded the rate of males by nearly 20%. In addition, female recruits developed bilateral stress fractures at twice the rate of male recruits. Hence, it is apparent that regions of the skeleton become compromised when exposed to a maladaptive stimulus, such as a high dose of physical activity concentrated over a relatively short time period, as is the case with military recruits.

Given that the structural geometry of the tibia or ulna are not likely to change over a brief period of high-intensity physical activity, the most probable cause of a transient vulnerability, is compromised bone material property. This may be due to increased bone turnover, which may lead to decreased trabecular thickness and connectivity. In addition, remodeling can occur too rapidly, and not allow adequate repair of microtrauma, resulting in a deterioration of the bone quality. Regardless of the underlying cause, a bone that is in a transiently vulnerable state will have an increase risk for failure.

Skeletal Adaptations to Weightlessness/Microgravity

Compromised skeletal integrity is also apparent in the microgravity environment. Space flight results in bone loss, which could be a limiting factor for long duration missions, such as, a Mars expedition or extended occupation of a space station (67). The effects of the microgravity environment could put astronauts at increased risk for fractures when they return to earth (58) following extended microgravity exposure. Dramatic reductions in total body bone mineral density (BMD) during extended space flight have been reported. However, it appears that rates of bone loss are site specific. That is, bones most stressed by gravity are most affected by weightlessness (11).

Following 6 months of space flight, a marked decrease in tibial trabecular and cortical bone was observed, however no changes in the radius was detected (30). Vico et al (114) suggests that bone deficits experienced in microgravity appear to be a consequence of the support function of each bone at normal gravity. The magnitude and rapidity of bone loss during space flight is alarming, it is recognized that an unloading of the skeleton in zero gravity leads on average to a 1%-2% reduction in bone mineral density at selected skeletal sites per month (58). Wronski & Morey (119) reported a 4% decrease in calcaneous bone mineral density (BMD) of Skylab crewmembers after 84 days of orbital flight. It has been demonstrated that bone loss occurs in weight bearing bones first and later in less weight-bearing bones (115), with the greatest losses occurring in the lumbar vertebrae, pelvis, and legs (51, 92). Coincidentally, the sites of greatest bone loss are weight bearing sites, suggesting the absence of mechanical loading on specific sites leads to bone degradation, thus, implicating a mechanosensory mechanism in microgravity osteoporosis. Thus, it appears that the mechanosensory system that signals bone cells to deposit or resorb tissue in the gravity environment on earth, is a factor in the microgravity environment as well.

The central issue for astronauts is an increased risk for skeletal failure impairing normal daily activities upon return to the full gravitational field on earth. Bone loss does not necessarily result in decreased bone strength. However, presently, researchers are unable to assess true bone strength. Inferences on bone strength must be made based on BMD measurements, although 10-40 percent of the variability in bone strength is unexplained by BMD. Although a bone loses apparent density, it is not necessarily weaker. The redistribution of the bone material in the microgravity environment may

result in no net loss of bone strength. Although the underlying cause of a weakened skeleton most likely differs considerably between astronauts exposed to the microgravity, and athletes or soldiers exposed to a unusually high dose of physical activity, the consequences are the same; an increased risk for skeletal failure.

Summary

Undoubtedly, the structural capacity of the skeletal is influenced by the demands imposed upon it. The role of exercise in promoting skeletal health cannot be disputed, given the magnitude of literature supporting this position. Yet, not all exercise is beneficial for the skeleton. This is apparent in the literature related to stress reaction injuries in athletes and military recruits. In addition, we have shown in our lab, that very high-intensity exercise can suppress osteoblastic activity for up to 24 hours post-exercise (118), indicating a decrease in osteoblastic activity. Therefore it can be speculated that, repeated bouts of high-intensity exercise can lead to compromised skeletal integrity, as has been demonstrated in published literature (17, 57). Moreover, exercise that is progressed too rapidly may be maladaptive as well (9, 21, 23, 29). Nonetheless, manifestations of a harmful exercise stimulus are not apparent until the skeleton fails, as with stress fractures, or until a considerable amount of BMD is lost (~2-4%). Conversely, the beneficial effects of adaptive exercise interventions are not noticeable until considerable BMD gains arise. Presently, DXA is the gold standard for determining the effectiveness of an intervention on the skeletal system. Interventions that increase either total body or site-specific BMD are considered effective, although bone strength may not change (8). For that reason, measures, which assess skeletal factors such as the microarchitecture and macroarchitecture, should be used in conjunction with traditional

procedures in order to obtain an adequate assessment of the skeleton's response to an adaptive or maladaptive stimulus.

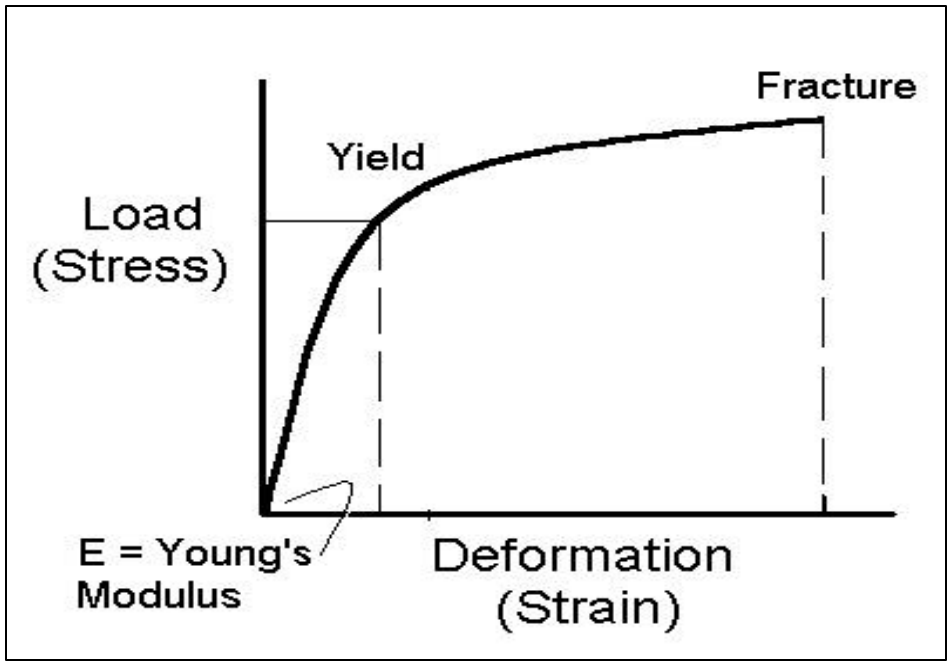

Figure 1. Young's Modulus of Elasticity, which is the value of the angle formed by the stress/strain curve when a material
Deforms under an applied load.

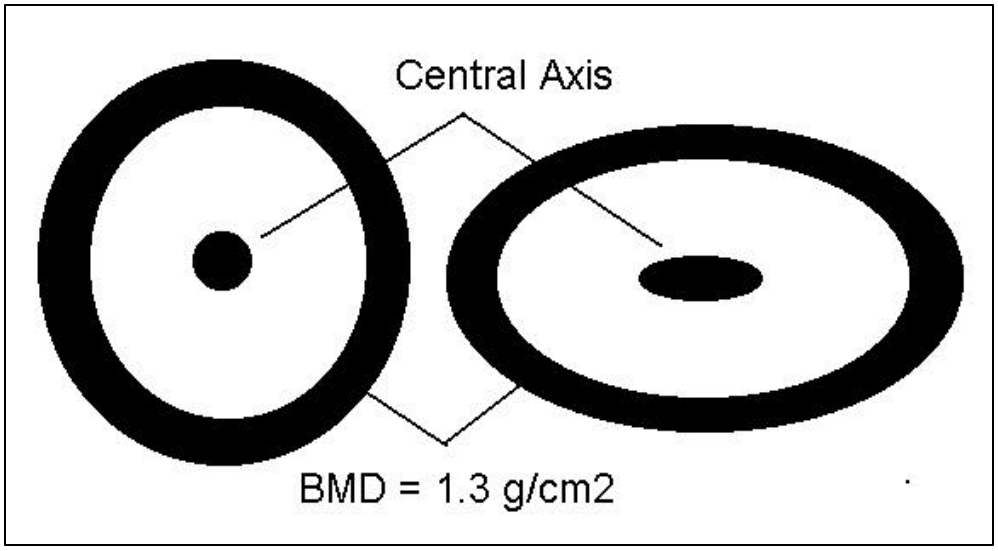

Figure 2 Cross-sectional moment of inertia. A structural property that is dependent on the distribution of a material around its central axis. The further a material is distributed from its central axis, the greater its resistance to bending.

Chapter III

Short-Term Time Course Skeletal Responses to High Intensity Physical Activity

Running Head: Skeletal Responses to Physical Activity

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ABSTRACT

The purpose of this randomized controlled trial was to investigate temporal skeletal responses to short-term high intensity physical activity. Twenty-eight normal active females [age: 20.7 +/- 2.1 yr (mean +/- SD)] were randomized into exercise (EX, n = 15) or control (CN, n = 13) groups. The exercise group trained 6 days/wk for 6 wk, which consisted of maximal isokinetic knee flexion/extension 3 days/wk, combined with 3 days/wk running. The purpose was to expose the tibiae to a period of abruptly increased loading forces. Tibial bending stiffness (EI_{MRTA}), and serum concentrations of biochemical markers of bone formation [osteocalcin (OC)], and bone resorption [n-telopeptide of type I collagen (NTx)] were measured at baseline, 2 wks, 4 wks, and 6 wks. Isokinetic concentric knee extension/flexion peak torque, as well as total body and site-specific bone mineral density (BMD) were measured at baseline and 6 wk. After training, the exercise group significantly increased ($p < 0.05$) isokinetic concentric peak torque for the dominant (13.6%) and non-dominant (5.7%) quadriceps, as well as dominant (7.7%) and non-dominant (9.5%) hamstrings, compared to the controls. No differences for total body or site-specific BMD were noted. A two-way multivariate repeated measures ANOVA revealed no time•group interactions for composite tibial bending stiffness [(EI_{MRTA}); $p = 0.57$] or the biochemical markers of bone turnover [(OC and NTx); $p = 0.15$] across the four sampling periods. While there were no main effects for group, a trend for time ($p = 0.051$) for composite EI_{MRTA} was observed. The exercise group demonstrated a 20% increase in EI_{MRTA} from baseline (74.8 +/- 22.3 Nm^2) to 6 wk (89.8 +/- 24 Nm^2), compared to controls who demonstrated a 4% increase (Baseline 86.5 +/- 23.8 Nm^2; 6 wk 90 +/- 23.7 Nm^2). Significant group differences ($p = 0.05$) were noted for OC, but not NTx. Differences ($p < 0.05$) for OC were observed at baseline [13.2 +/- 2.4 ng/ml (CN), 15.6 +/- 2.7 ng/ml (EX)], and follow-up ANCOVA revealed no differences for subsequent sampling periods. Main effects for time were found for OC and NTx ($p < 0.001$). Main effects for time in OC were attributable to changes in the exercise group ($p < 0.01$) and NTx ($p < 0.01$), but not the control group.

INTRODUCTION

Exercise is generally considered to impart positive benefits on the skeleton (6, 16, 27, 104), including increased bone mineral density (BMD) (10, 98) and bone mineral content (BMC) (10, 54, 56), decreased risk for osteoporotic fractures (88), and increased bone strength (60). In addition, exercise has been prescribed to counteract the detrimental effects in bone that are caused by microgravity (31), as well to counter bone loss associated with disuse osteoporosis (11, 101) and post-menopausal osteoporosis (15, 91, 116). Weight-bearing exercise is considered essential in developing peak bone mass (PBM) during adolescence (5, 16, 71) and there is little doubt that physical activity is important for the development and maintenance of a healthy skeleton throughout the lifespan.

Bone tissue undergoes remodeling in response to stimulation of increased physical activity; with the response likely proportional to incremental load bearing demands in regions specifically affected and associated hormonal responses (105). However, the mechanisms by which exercise leads to such changes in bone metabolism are not fully understood (117). Various modes and intensities of exercise have been shown to alter the normal bone remodeling process (52, 70, 89), however the exercise mode and dose most likely to enhance skeletal development and maintenance is not known. In addition, it appears that males and females at different life-stages respond to an exercise stimulus differently (6, 17, 99).

Bennell et al (10) suggested that the most effective form of exercise for promoting osteogenesis is dynamic weight-bearing activity, such as aerobics or running. On the other hand, it has been suggested that exercise, which results in high impact forces

such as gymnastics, as opposed to running, may impart the greatest (75) osteogenic effect. Although disagreement exists on the optimal stimulus, it appears that there may be specific interactive effects involving mode and intensity that dictate the adaptive response selected modes may be dose dependent. Specifically, sudden exposure to an activity program of unaccustomed repetitive loading of extended duration may be most detrimental to skeletal integrity. A high incidence of stress fractures (43, 62, 93), accompanied by evidence of suppressed bone formation (41) have been reported in military recruits soon after they have initiated demanding physical activity regimens (50). Therefore, a highly demanding exercise dose may be detrimental, and lead to a state of transient susceptibility for exercise induced stress fractures, especially in the lower extremities. Compromised skeletal integrity could thus result in an increased vulnerability for tissue failure, especially if exposure to the exercise stimulus is sustained. Yet, it appears that increased vulnerability for skeletal failure is transient (25) and withdrawal or diminution of the potentially injurious exercise dose during this period allows for constructive bone remodeling and subsequent increased bone strength.

Compromised skeletal integrity also is apparent following exposure to microgravity, and appears to be site specific. Following six months of space flight, a marked decrease in tibial trabecular and cortical bone were observed, however no such changes in the radius were detected (30). Vico et al (114) suggests that bone deficits experienced in microgravity appear to be a consequence of the support function of each bone at normal gravity. Space flight results in bone loss, which could be a limiting factor for long duration missions, such as a Mars expedition or extended occupation of the international space station (ISS) (67). Thus, prolonged exposure to microgravity could

put astronauts at increased risk for stress fractures upon return to earth, particularly in weight bearing bones (58). Various exercise countermeasures have been employed for preventing rapid bone loss in microgravity, as well as restoring bone loss upon return to normal gravity. However, researchers have not been able to adequately assess bone strength changes resulting from exposure to microgravity and application of countermeasures

Traditionally, researchers have employed dual x-ray absorptiometry (DXA) and/or the biochemical markers of bone turnover to describe bone adaptations to various stimuli. Although these tools are useful, they provide an incomplete picture of skeletal responses. Dual-energy x-ray absorptiometry is useful in quantifying changes in BMD and BMC, although useful in this context; BMD is but a surrogate measure for bone strength (71). Bone strength and resistance to stress fracture, is dependent on the quantity, as well as the quality and macrogeometry of the bone (39). Dual-energy x-ray absorptiometry provides information primarily about the quantity and distribution of the mineral content of bone, leaving two very important determinants of its strength, i.e. microarchitecture and geometry unaccounted for.

Concentrations of bone turnover biomarkers, as assayed in the blood, provide useful information concerning osteoblastic and osteoclastic cellular activity and indicate bone formation and resorption dynamics, thereby allowing inference at the tissue level. That is, increased markers of bone formation accompanied by decreased or constant markers of bone resorption infer a net gain in bone tissue. The extent to which changes in blood levels of these markers actually reflect quantitative changes at the cellular source level, i.e. osteoblasts and osteoclasts at bone sites actively undergoing accretion and

resorption remains open to question. Still, biochemical indicators of increased bone formation do not equate to increased bone strength, nor does increased bone resorption equate to decreased bone strength. Although these markers are useful in understanding osteoblastic and osteoclastic cellular activity, and may contribute to identifying a window of transient vulnerability, they are not accurate measures of bone strength.

Mechanical response tissue analysis (MRTA) was developed by Charles Steele at Stanford University (107), in collaboration with the National Aeronautic and Space Administration (NASA). It is designed to assess, *in vivo*, the mechanical properties of long bones in humans. These mechanical properties include the cross-sectional bending stiffness (E), cross-sectional moment of inertia (I), and an index of bone "sufficiency" (i.e., a ratio of axial load capability to body weight). The EI_{MRTA} (E I) yields a measure of long bone structural integrity, which is related to the composition, geometry, and internal architecture of the bone (76, 87, 97, 107). Although this technology limits measurement of EI to long bones with minimal overlying soft tissue, i.e. tibia and ulna, MRTA assess mechanical properties that are directly related to the structural strength of these bones.

The aim of this study was to investigate time course changes in bone vulnerability induced by a 6-week exposure to abruptly increased repetitive mechanical loading with running and resistance exercise. Vulnerability was assessed by examining serial changes in a combination of measures that included tibial bone stiffness, bone turnover, and tibial BMD in a group of untrained healthy young adult women.

Experimental Design and Methods

Recruitment

College women, age 18-26 years, volunteered and were recruited through posters and electronic mail. Volunteers who responded to recruitment solicitation were screened for exclusion criteria. Exclusion criteria included participation in structured resistance training, regular running exercise, and/or participation in a varsity sport within the past 6 month. Additional exclusion criteria were known metabolic disease, bone disease, or bone fractures (including stress fractures) within the previous 12 months. Candidates were also excluded if they had been pregnant, or had irregular menstrual history within the past year, or were using Depo Provera® (Pharmacia & Upjohn, Kalamazoo, MI) or Norplant® (Wyeth-Ayerst, Philadelphia, PA). Following pre-screening, 30 subjects were selected for participation in the study.

The Institutional Review Board (IRB) at Virginia Tech approved this study. Following pre-screening, qualified volunteers completed an informed consent (Appendix B) and medical health history (Appendix C). Subjects then completed baseline testing, and were randomly assigned to exercise training (EX, n = 15) or control (CN, n = 15). The EX group participated in 6 wk of high-intensity isokinetic resistance training, ~ 25 min/d, 3 d/wk, combined with 3d/wk of moderate intensity running, and the control group (CN) maintained normal daily activities throughout the 6 wk study.

Nutritional Evaluation

Subjects completed 3-day dietary logs, which were analyzed with Nutritionist 5 dietary analysis software (version 1.7, San Bruno, CA) to evaluate daily nutritional intake. Subjects who indicated low intake for any of the macronutrients received individual nutritional counseling and were provided with strategies for improving

macronutrient intake. Daily calcium consumption was evaluated and subjects with inadequate calcium intake were supplemented daily with Viactive® calcium supplements (500 mg: Mead Johnson Nutritionals, Evansville, IN) for the duration of the study.

Dependent Measures

Baseline testing consisted of isokinetic strength tests, EI measurement by MRTA, BMD measurements by dual-energy x-ray absorptiometry (DXA), and a blood sample draw from the antecubital vein by a trained phlebotomist. Blood sampling and MRTA tests were repeated at 2-wk intervals throughout the study, whereas the strength test and DXA scans were repeated only at 6 wk.

Bone Mineral Content, and Bone Mineral Density

Standard protocols were used to measure these variables with the Hologic QDR 4500A (Hologic, Inc., Waltham, MA) bone densitometer. Three separate scans were performed for each subject at each evaluation interval of the study. Scans were performed for total body, hip, lumbar spine, and forearm, and. Reference points were established on these site-specific scans at baseline and used again to do these measurements for the scans at 6 wks.

Subjects lied supine on the DXA table for one total body scan to determine BMD and BMC of the total body. Each subject had the non-dominant forearms, lumbar spine, and total hip scanned to determine BMD and BMC of the radius and ulna, femoral neck, trochanter, and Ward's triangle, respectively. Standard total body, forearm, and hip protocols were used during scans. Total body (version 8.25), and forearm (version 8.25) scans were analyzed with Hologic software using the compare function for reference to the baseline scan.

All scans were conducted in the BONE laboratory (Rm 229C Wallace Hall), on the campus of Virginia Polytechnic Institute and State University, by a licensed (State of Virginia) limited radiologic technician, and analyzed by the same technician to eliminate inter-tester variation. Quality control for BMD and BMC was ensured by daily scans of an anthropomorphic phantom lumbar spine prior to any subject testing. The coefficient of variation for phantom spine scanning is 0.39%. Precision for total body BMD measurements is < 1.0% and for femoral neck is < 2.0%. Because soft tissue mass changes occur with resistance training and also impact BMD and BMC, fat-free mass (g), fat mass (g), and percent body fat of the total body as well as regions of interest will be analyzed from total body scans using the total body software (version 825). Precision of percent body fat for the total body with DXA is < 0.80%. Quality control for soft tissue mass was ensured by scans of an external soft tissue bar comprised of aluminum and lucite calibrated against stearic acid and water (Hologic, Bedford, MA).

Measurement of Bone Stiffness of the Tibia

Each subject was tested with mechanical response tissue analysis (MRTA) to determine tibial stiffness (EI) at baseline, 2-wks, 4-wks, and post-training. Measurement procedures were adapted from ulna measurements described by McCabe et al (76).

The major components of the MRTA measurement system are:

1. dual channel dynamic signal analyzer;
2. permanent magnet vibration exciter;
3. impedance head;
4. two charge amplifiers;
5. vibrating shaker/probe.

The vibrating shaker/probe is suspended from a metal support, and is positioned at the midpoint of the tibia (Figure 1). The shaker/probe emits a transcutaneous vibration frequency in the range of 60 to 1600 Hz for very brief periods (<1-2 min). The probe contains the impedance sensor, which detects the vibratory responses of the overlying soft tissue and bone. The force and acceleration responses are relayed to the signal analyzer and microprocessor, which fits the raw data to an EI prediction model.

Upon arrival at the Musculoskeletal Function Laboratory, subject's height and weight were obtained using a standard stadiometer, and balance beam scale. Subjects were then instructed to lay supine for thirty-minutes to allow for fluid imbalances in the lower leg to equalize. During the last five-minutes of this period, the technician palpated the subject's lower leg to isolate the proximal and distal ends of the tibia, before marking points for the medial tibial condyle to the distal medial malleolus; tibial length for each leg then was measured with an anthropometer as the distance between these points (+/- 1 mm). The tibial mid-point was marked on the medial aspect of the anterior tibial crest, to establish the point of MRTA probe placement. The subject was then seated in an adjustable chair with 90°-knee flexion and the posterior upper thigh supported to un-weight the foot and thus allow vibration of the tibia under conditions of minimal axial loading at either the proximal or distal ends of the bone (Figure 2). The MRTA probe was positioned at the mid-point of the tibia, and after 1 minute, the measurement system was activated and five serial measurements were obtained and saved for further analysis. The above procedures were repeated for the opposite tibia.

Determination of EI was a two-step process. The initial step was to complete a multi-model analysis with the 6-, 7-. 9-, and 12-parameter algorithm models for each

measurement. These models provide discrete mathematical modeling of the overlying soft tissue and bone response to the vibratory wave emitted by the shaker. The 7-parameter model consists of the effective masses of soft tissue (m_s) and bone (m_b) in series with linear springs (k_s and k_b) and viscous dash pots (b_s and b_b) and a parallel viscous dash pot (b_p) connected to the skin mass, and was used in early work with MRTA. However, Roberts et al (97)demonstrated improved mathematical modeling of the overlying soft tissue with a 6-parameter model, which consists of the effective mass of the bone (m_b) and springs (k_s and k_b) and viscous dash pots (b_s and b_p) in series. The skin mass (m_s) is derived from the difference in the displacement of the skin, and the displacement of the bone. More recently, Steele (106) developed the 9-parameter and 12-parameter model, to account for displacement at one (9-parameter) or both (12-parameter) ends of the tibia.

All measurements were evaluated with each mathematical model to determine the model that provided the least root mean square (RMS) error for stiffness for each individual measurement. This is an indicator of the actual deviation of predicted modeling to the raw curve. The coefficient of variation (CV) then was computed for all five measurements within a trial, with the 6, 7, 9, and 12-parameter mathematical models. The model that produced the combined least RMS, and CV was selected for the analysis in the second step of the two-step process. In the final step, the individual measurement file that produced the least RMS error within a trial was used to seed for a multi-file batch analysis. The seeded measurement file enabled computational information from the "best" measurement to be carried forward to the four remaining measures. Following the seeded batch analysis, the mean of the three measurement trials that produced the least

CV within the five-measurement trial was selected as the final EI value for the dominant and non-dominant tibia. A composite EI_{MRTA} was then derived from the mean of the dominant and non-dominant EI_{MRTA}.

Response curves were evaluated to determine the quality of each measurement, and were coded for poor, marginal, and high quality. Poor quality measurements were discarded, and treated as missing values. Stiffness (EI_{MRTA}) for the missing values was then predicted by multi-linear regression, with the remaining three sampling period EI_{MRTA} values serving as predictors. This reduced the data set to eleven and twelve subjects for the control and exercise group respectively.

Biomarkers of Bone Turnover

Blood samples were obtained in the early morning, following a period in which subjects had been instructed to abstain from exercise for 24-hour abstention and from food consumption for 10-hour. Samples were collected into 10-ml serum separation tubes and centrifuged at 2500 rpm for 15 minutes. Serum then was transferred into 1.5-ml vials and stored at -80 °C until assayed. Biochemical assays were performed with the technician blinded from knowledge of the experimental condition, which the sample represented. Bone formation activity of osteoblasts was evaluated by total serum osteocalcin using a human-specific radiometric assay (Biomedical Technologies Inc., Stoughton, MA). Bone resorption activity of osteoclasts was evaluated by serum type I N-telopeptide collagen breakdown products using an ELISA assay (Osteomark, Seattle WA). All OC and NTx assays were run in duplicate and repeated if the coefficient of variation (CV) for any sample was < 20%. The intra and interassay CV was 6% and 11.6% for OC and 3.1% and 13.8% for NTx respectively.

Leg Strength

Three days prior to the scheduled strength test, subjects reported to the testing laboratory for a familiarization session with the Biodex® System 2 isokinetic dynamometer (Model 820-200; Biodex Medical, Shirley, NY). Subjects were provided with verbal instruction on the use of the Biodex® system, and performance expectations for the power tests. Subjects then completed three sets of concentric/concentric isokinetic resistance exercise.

Subjects returned to the testing laboratory three days later for the isokinetic muscular power tests of the legs. These tests were performed at baseline, and following the 6-weeks of training. The tests were performed at an angular velocity of 60 degrees/sec for concentric knee extension and flexion. Testing was conducted on each subject's leg in a single testing session. The test order was counterbalanced across subjects, so half of the them were measured first for the non-dominant leg, and the other half with their dominant legs.

. The isokinetic strength test began with 3-min warm up of low intensity stationary leg cycling on a Monark® cycle ergometer followed by 3-5 min of static stretching exercises for the hamstrings and quadriceps muscles. Subjects were positioned on the Biodex® system with the dynamometer axis of rotation aligned with the knee, and 85° hip flexion. All tests began with the knee at approximately 90° flexion, with the initial movement being knee extension to approximately 0°, and the second movement being knee flexion to approximately 90° to complete the first repetition. Subjects completed a warm-up set of six repetitions, followed by a set of six maximal repetitions. The highest values obtained from either of the repetitions for peak torque,

peak torque/body weight ratio, maximal capacity, and total work was recorded. Subjects were verbally encouraged to perform maximally on each repetition for the tests.

High-Intensity Resistance Training

The training period was six wk-, resulting in 18-isokinetic resistance-training sessions. Subjects were required to attend a minimum of 80% of the training sessions to be included in the statistical analyses. The high-intensity resistance training was performed using high-load dynamic seated leg extension and flexion for the dominant and non-dominant legs on the Biodex® System 2 isokinetic dynamometer. At the beginning of each training session, subjects performed 5-8 min of leg warm-up activity on a stationary leg ergometer followed by a prescribed set of static stretching exercises for the leg muscles. The isokinetic resistance training consisted of a warm-up set of six repetitions at approximately 50-75% of maximal effort, followed by five sets of six repetitions of maximal effort for each leg. One minute of rest was provided following each set. The initial training leg was alternated for each session.

Running Program

Subjects participated in the running exercise sessions 3 d/wk on non-consecutive days resulting in 18 total run sessions. Run training and resistance training was not conducted on the same day. The intensity/duration of the running exercise sessions were equivalent to 60-85 percent of the subject's age-predicted maximal heart rate/30 min. Prior to each run session subjects completed a series of static stretches, and were fitted with a commercially available heart rate monitor (Nashbar Model NA-HRM. Canfield, OH). Individual target heart rate ranges were programmed into the monitor, which provided constant feedback throughout the running sessions. Subjects completed 30-min

of self-monitored jogging/running exercise, and then reported to the musculoskeletal lab, where the exercise heart rate and duration data were downloaded and stored from the monitor on a personal computer.

Statistical Procedures

Baseline variables were compared with independent t-tests to reveal any pre-intervention differences between the exercisers and controls. Independent t-tests were also used for pre and post comparisons of the muscular strength, and BMD assessments. A two-way multivariate repeated measures analysis of variance (ANOVA) was used to provide a comprehensive analysis of the comparison group measures, biochemical markers of bone turnover and bone stiffness, taken at all data collection points across time. Time served as the "within subjects" factor, which had four levels (pre-training, 2 wks, 4 wks, and post-training) and the group served as the "between subjects" factor, which had two levels, exercise or control. Primary interest focused on the interaction of group•time effects for bone stiffness, the biochemical markers of bone turnover, and BMD for the two groups. In the repeated measures analysis, main effects were tested for significance using Bonferonni's *post hoc* test procedure. The .05 level of significance was used for all statistical tests. All analyses were completed with the Statistical Package for Social Sciences (version 10.0, SPSS Inc., Chicago, Ill) computer software program.

Results

Following randomization, two subjects assigned to the control group elected to not participate in the study; therefore sample sizes for the control (CN) group (n =13) and exercise (EX) group (n = 15) were unequal. There were no differences between the

groups at baseline for age, height, weight, percent body fat (by DXA), BMI and dietary intake variables (Table1). Dietary records for six exercisers and two controls indicated inadequate calcium intake, and these individuals were provided daily calcium supplements throughout the study. At baseline, OC concentration (ng/ml) was higher ($p < 0.05$) for the exercise group; no other differences were observed between the two groups for any of the other bone related (Table 2), and leg strength variables (Table 3).

There were no significant group•time interactions observed for any sampling period for composite EI_{MRTA} (Table 4). Bending stiffness responses for the control and exercise groups are shown in Figure 3. There were no significant group•time interaction effects for NTx or OC for any sampling period (Table 5). Group differences were observed (Figure 4) at baseline [15.64 ng/ml (EX), 13.28 ng/ml (CN)], however, a follow-up repeated measures ANCOVA revealed no differences for subsequent sampling periods. Osteocalcin and NTx responses are shown in Figure 4 and Figure 5 respectively. There were no group differences ($p < 0.05$) in total body, distal ulna, distal radius, lumbar spine, or hip BMD following the 6 wk training (Figure 6). In addition, there were no significant within group differences ($p < 0.05$) for any of the BMD variables following the 6 wk training.

Strength measurements at 6 wks are shown in Table 6. Significant between group differences ($p < 0.05$) were observed for the isokinetic peak torque measures of the dominant and non-dominant quadriceps and hamstrings following the 6 wk training. The exercise group significantly increased ($p < 0.05$) peak torque values compared to the controls for the dominant quadriceps [135 $\pm$ 7.4 ft·lb (Mean $\pm$ SEM) vs. 104 $\pm$ 5.6 ft.lbs.], non-dominant quadriceps (135.2 $\pm$ 6.3 ft.lbs. vs. 110.8 $\pm$ 4.1 ft.lbs.), dominant hamstrings

(68.5 $\pm$ 3.5 ft.lbs. vs. 56.6 $\pm$ 2.2 ft.lbs) and non-dominant hamstrings (67.6 $\pm$ 3.4 ft.lbs vs. 54.9 $\pm$ 1.9 ft.lbs) (Figure 7). In addition, the exercise group demonstrated significant ($p < 0.05$) within group strength increases for the dominant hamstrings, dominant quadriceps, non-dominant hamstrings, and non-dominant quadriceps. No significant ($p < 0.05$) within group changes for peak torque were observed for the control group.

Discussion

The exercise training in this study was designed to expose the bones of the lower legs to a variety of loading forces. The tibiae were exposed to bending forces through isokinetic resistance training, which resulted in compression and tension along the anterior and posterior surfaces. The tibiae of the exercise group were exposed to these forces 3 days/wk. In addition, the run training exposed the tibiae to axial compressive forces 3 day/wk. The subjects selected for this study indicated that they had not been involved in a structured exercise program within the past year; therefore, they were unaccustomed to such loading forces on the tibiae. The exercise intervention in this study appeared to satisfy the three rules of skeletal adaptation discussed by Turner (112); 1. Bone adaptation is driven by dynamic, rather than static loading. 2. Only a short duration of mechanical loading is necessary to initiate an adaptive response. Extending the loading duration has a diminishing effect on further adaptation. 3. Bone cells accommodate to a customary mechanical loading environment, making them less responsive to routine loading signals.

As expected, there were no changes in BMD between groups or within groups over the 6 wks of training. Significant between group differences were observed for OC at baseline. This difference was most likely due to sampling variation in a small

population, as subjects were from a normally distributed population, and were randomized to experimental groups. Although serum OC concentration is influenced by menstrual status (28), circadian rhythms (34), and seasonal variations (37), there is no basis for these factors to have differentially influenced OC in the two experimental groups. Osteocalcin concentration at baseline was 14% greater in the exercise group compared to control, versus a 15% difference at 6 wks. There were no significant within group differences from baseline to 6 wks, indicating that the magnitude of change for the control (3%) and exercise (2%) groups from baseline to 6 wks was nearly identical. As indicated in figure 4, osteocalcin responses did not differ across the sampling periods for the exercise and control groups. In addition, no significant group•time interaction was observed for NTx, indicating no differences in bone resorption between the groups across the sampling periods.

Although no statistical differences in composite EI_{MRTA} were observed between the groups at any sampling period, Figure 3 suggests mean shifts in EI_{MRTA} for the exercise group, which are not apparent for the controls. The control group served as a measure of inter-day reliability, as no changes in EI_{MRTA} values were expected between each sampling period during the 6 wk study. Previous work in our Laboratory for Health and Exercise Science produced unacceptable tibial EI_{MRTA} reproducibility, with interday variations of 1.8%-18%, using the 7-parameter mathematical model for all measurements. The present study hoped to improve on inter-day reproducibility by careful selection of the mathematical modeling algorithm, and applying the improved models (9 and 12-parameter) when warranted. The mean composite EI_{MRTA} interday variations for the control group ranged from 1%-6% for all sampling periods,

demonstrating a considerable improvement over our previous findings. However, careful selection, and application of the refined models produced inter-day EI_{MRTA} correlations which ranged from r = .03 – r = .59 across all four sampling periods. Only baseline and 4 wk EI_{MRTA} measures were significantly related (p = 0.032). While mean EI_{MRTA} interday variations were quite acceptable (1%-6%), intra-subject variations across the four sampling periods were not.

Exercise has been shown to positively affect bone health by maintaining or increasing BMD, thus sustaining the individual above clinical standards currently regarded as important for medical intervention or assessing risk of fragility fracture, i.e. osteopenia and osteoporosis. However, under certain very suddenly imposed and mechanically demanding protocols of exercise, it is apparent that physical activity may induce stress fractures in specific bones. Military personnel experience the highest incidence of stress fractures within the first two weeks of basic combat training (BCT) (93). This time-course of skeletal injuries suggests that the skeleton is unable to respond rapidly enough to the additional demands imposed upon it by the rigors of BCT, resulting in failure. This loss of skeletal integrity has been termed transient vulnerability, however, presently there is no means to assess this pathological condition. Most likely, the transient vulnerable condition is related to changes in the microarchitecture of the bone, leading to decreased quality and subsequent strength. The traditional measure of bone health, BMD, assesses the quantity of bone material, and provides limited information related to the structural capacity or quality of bone. Mechanical response tissue analysis provides a measure of bone status that is related to the structural capacity of bone, and may be useful in identifying the transient vulnerable state. The present study was

conducted in order to assess skeletal adaptations to short-term high intensity exercise, with MRTA as the primary variable of interest. Bone mineral density measures were included to support our position that bone structural changes may occur without concomitant changes in BMD. The strength changes observed in the exercise group in this study indicated that the quadriceps and hamstrings in the exercise group had undergone considerable adaptations during the exercise program.

The exercise intervention in this study was designed to expose the lower legs of young adult females to a variety of loading stressors, which included compression, bending, and tension. It was hypothesized that the lower legs would exhibit a transient vulnerable state, followed by positive adaptations, and increased bending stiffness. The results of the biochemical markers of bone turnover in this study suggest that a transient vulnerable state may occur in skeletal sites that are exposed to a sudden and sustained increase in loading. However, the skeleton can positively adapt over a relatively short period by increasing structural properties that are related to the macro and microarchitecture of the bone, without concomitant changes in BMD. Previous studies using MRTA have demonstrated potential clinical applications in assessing fracture risk in the osteoporotic population, as well as assessing pharmacological or exercise intervention therapies. This new technology can provide information related to skeletal status beyond what is presently available with DXA alone, however the results of the present study suggest further refinements are warranted.

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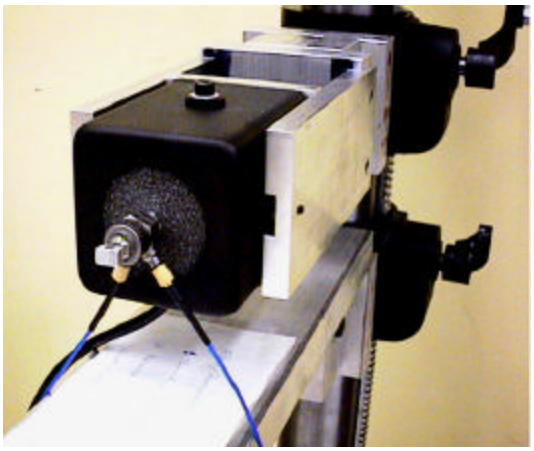

Figure 1. MRTA shaker, sensor, and probe which is positioned at the mid-point of the tibia during measurement.

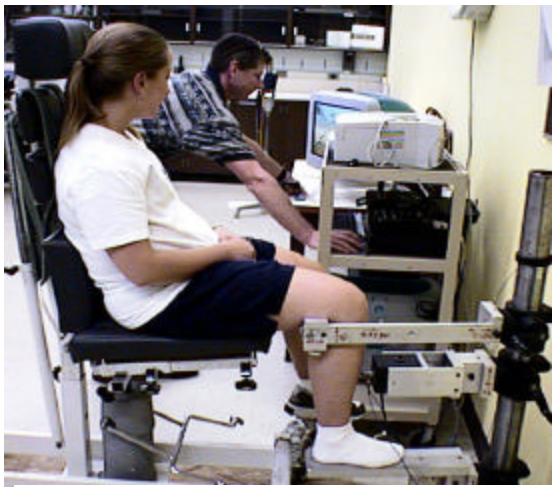

Figure 2. Final positioning on the MRTA for tibial stiffness measurement.

Table 1. Baseline physical characteristics, and dietary intake variables

		N	Mean +/- SD	p value* CN vs. EX
Age (years)	Control	13	20.8 +/- 2.6	NS
	Exercise	15	19.4 +/- 1.2	
Height (cm)	Control	13	165.5 +/- 3.2	NS
	Exercise	15	164.3 +/- 5.8	
Weight (kg)	Control	13	65.8 +/- 8.4	NS
	Exercise	15	66.6 +/- 6.7	
% Body Fat	Control	12	30.7 +/- 5	NS
	Exercise	15	29.7 +/- 4.9	
Body Mass Index	Control	13	24.1 +/- 3.6	NS
	Exercise	15	24.7 +/- 2.3	
Daily Caloric Intake (kcal)	Control	13	1618 +/- 306	NS
	Exercise	15	1737 +/- 469	
Calcium (mg/day)	Control	13	863.5 +/- 395.6	NS
	Exercise	15	999.8 +/- 429	
Vitamin D (ug/day)	Control	13	3.8 +/- 3.0	NS
	Exercise	15	4.7 +/- 3.5	

* Two-sided p value from independent samples t-test comparing control group (CN) with exercise group(EX). NS indicates $p > 0.05$

Table 2. Baseline composite tibial bending stiffness (EI), biochemical markers of bone turnover, and bone mineral density (BMD) variables

		N	Mean +/- SD	p value * CN vs. EX
Baseline Composite EI (Nm2)	Control	13	83.0 +/- 23.8	NS
	Exercise	12	74.8 +/- 22.3	
Osteocalcin (ng/mL-1)	Control	13	**13.2 +/- 2.46	$p < 0.05$
	Exercise	15	15.6 +/- 2.76	
NTx (nM BCE)	Control	13	14.77 +/- 3.22	NS
	Exercise	15	14.55 +/- 3.43	
Total Body BMD (gm/cm2)	Control	13	1.131 +/- .067	NS
	Exercise	15	1.149 +/- .087	
Forearm BMD (gm/cm2)	Control	13	0.551 +/- .034	NS
	Exercise	15	0.568 +/- .036	
Spine BMD (gm/cm2)	Control	13	1.028 +/- .083	NS
	Exercise	15	1.031 +/- .095	
Total Hip BMD (gm/cm2)	Control	13	0.963 +/- .068	NS
	Exercise	15	1.00 +/- .109	

*Two-sided p value from independent samples t-test comparing control group (CN) with exercise group (EX). **Indicates control group is significantly different from exercise group. NS indicates $p > 0.05$

Table 3. Baseline isokinetic strength measurements

		N	Mean +/- SD	* p value CN vs. EX
Dominant Quadriceps (ft. lbs)	Control	13	103 +/- 17	NS
	Exercise	15	115 +/- 19	
Non-dominant Quadriceps (ft. lbs)	Control	13	101 +/- 14	NS
	Exercise	15	110 +/- 17	
Dominant Hamstrings (ft. lbs)	Control	13	52 +/- 13	NS
	Exercise	15	58 +/- 10	
Non-dominant Hamstrings (ft. lbs)	Control	13	50+/-8	NS
	Exercise	15	56 +/- 11	

*Two-sided p value from independent samples t-test comparing control group (CN) with exercise group (EX). NS indicate $p > 0.05$

Table 4. Tibial bending stiffness (EI) for control group and exercise group

			95% Confidence Interval	
	TIME	Mean +/- SD	Lower Bound	Upper Bound
Control	Baseline	86.5 +/- 23.8 Nm2	72.111	100.998
	2 wk	80.9 +/- 19.1 Nm2	67.822	93.941
	4 wk	90.1 +/- 26.5 Nm2	74.611	105.598
	6 wk	90.0 +/- 23.7 Nm2	75.047	105.008
Exercise	Baseline	74.8 +/- 22.3 Nm2	60.954	88.612
	2 wk	69.8 +/- 22.2 Nm2	57.392	82.399
	4 wk	93.7 +/- 22.9 Nm2	78.853	108.522
	6 wk	89.8 +/- 24.0 Nm2	75.487	104.172

No significant between group differences, $p > 0.05$.

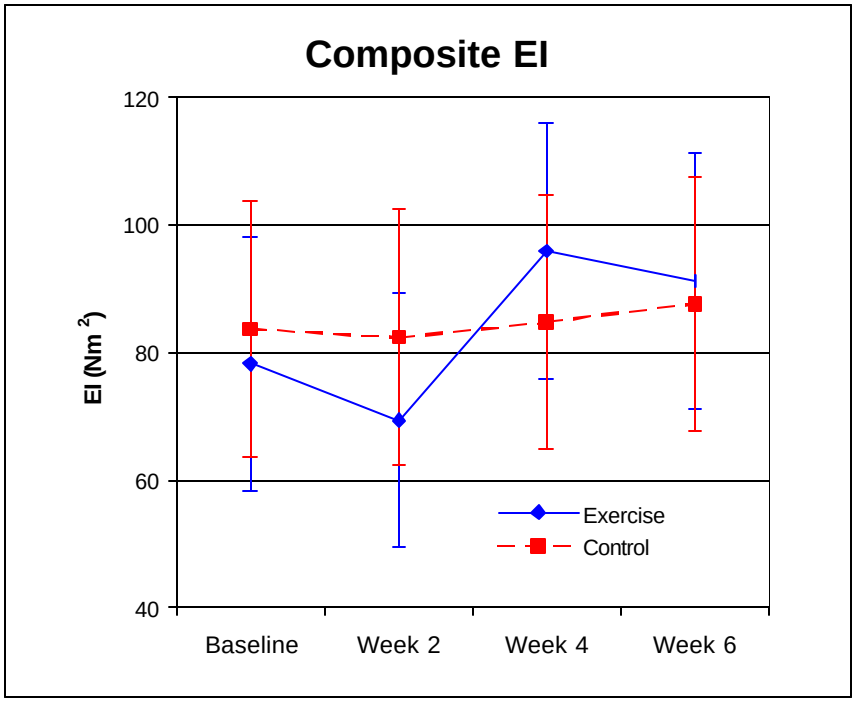

Figure 3 Composite EI values for the control and exercise groups (mean +/- SD). Two-way repeated measures ANOVA revealed no significant (group•time) interaction, or main effects, $p > 0.05$.

Table 5. Osteocalcin and NTx for control and exercise groups.

			Mean +/- SD	p value, CN vs EX	95% Confidence Interval	
					Lower Bound	Upper Bound
OC (ng/ml)	Control	Baseline	13.2 +/- 2.4	**	11.78	14.78
		2 wk	12.3 +/- 2.8	NS	10.85	13.65
		4 wk	12.8 +/- 2.8	NS	11.46	14.18
		6 wk	13.8 +/- 2.7	**	12.18	15.38
	Exercise	Baseline	15.6 +/- 2.8	**	14.25	17.04
		2 wk	13.2 +/- 2.0	NS	11.94	14.54
		4 wk	14.5 +/- 2.0	NS	13.20	15.74
		6 wk	16.0 +/- 2.9	**	14.54	17.51
NTx (nM BCE)	Control	Baseline	14.7 +/- 3.2	NS	12.87	16.67
		2 wk	15.5 +/- 3.7	NS	13.32	17.67
		4 wk	15.9 +/- 4.9	NS	13.80	18.08
		6 wk	16.4 +/- 3.0	NS	14.07	18.78
	Exercise	Baseline	14.5 +/- 3.4	NS	12.78	16.32
		2 wk	16.4 +/- 3.9	NS	14.38	18.43
		4 wk	14.5 +/- 3.0	NS	12.50	16.48
		6 wk	17.4 +/- 4.9	NS	15.25	19.63

**Indicates significant between group differences, $p < 0.05$. NS indicates no between group differences, $p > 0.05$.

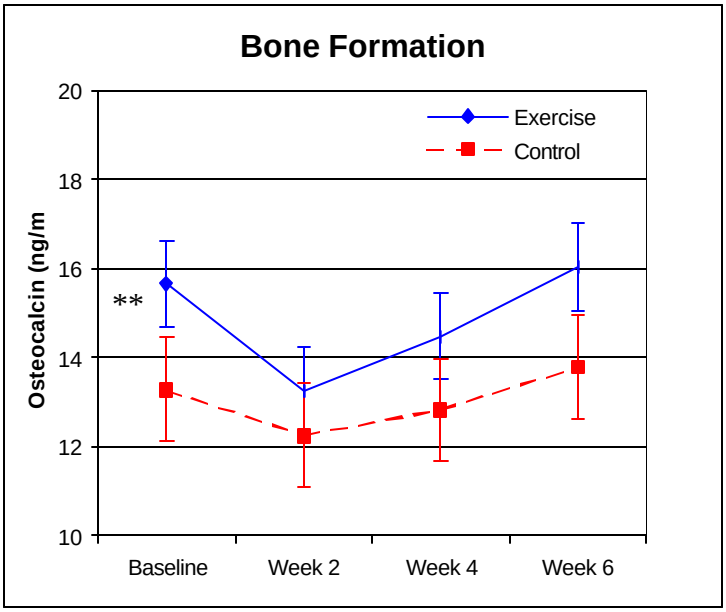

Figure 4. Multivariate repeated measures ANOVA for comparison of bio-marker of bone formation (mean +/- SD) between exercise and control groups. **Indicates significantly different from exercise group ($p < 0.05$). ANCOVA revealed no differences for subsequent sampling periods $p > 0.05$.

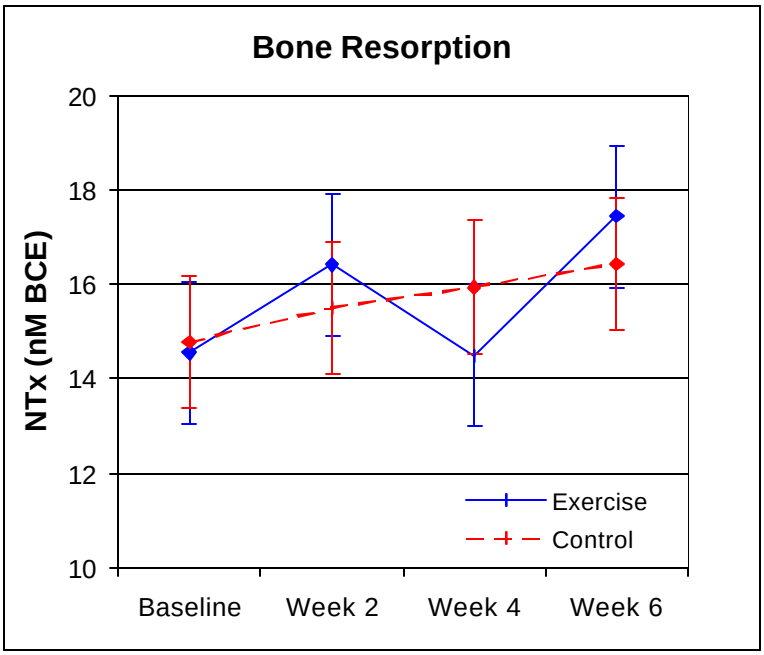

Figure 5. Multivariate repeated measures ANOVA for comparison of bio-marker of bone resorption (mean +/- SD) between exercise and control groups. No significant group•time interaction, or main effects, $p > 0.05$

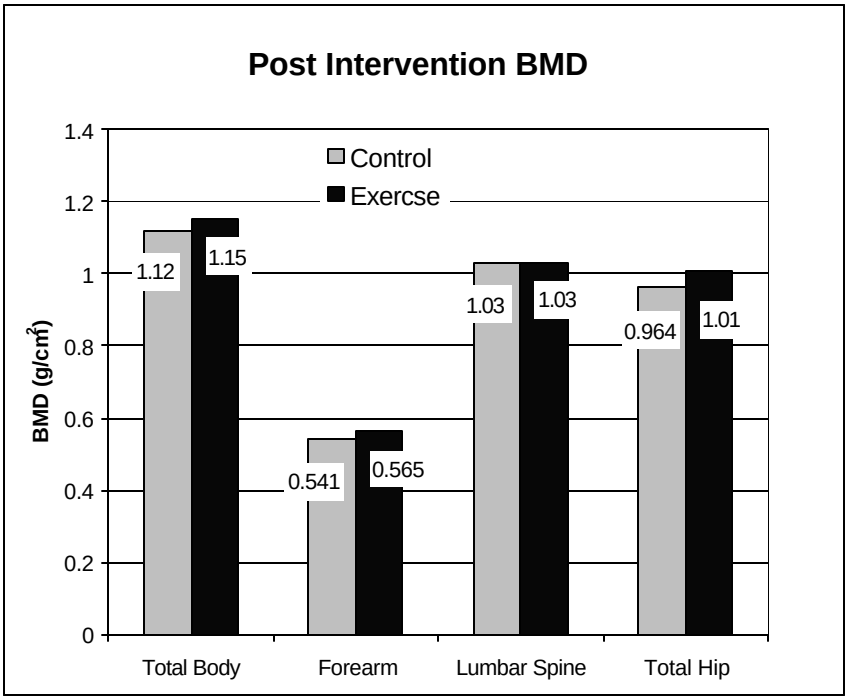

Figure 6. Bone mineral density comparison with control and exercise group following 6 wk training. No group differences were observed.

Table 6. Strength changes following 6 wk training for control and exercise groups

Peak Torque (ft. lbs)		Mean +/- SD	p value, CN vs. EX
Dominant Quadriceps	Control	104.6 +/- 19.4	$p < 0.01$
	Exercise	135.1 +/- 29.0	
Non-dominant Quadriceps	Control	110.8 +/- 14.2	$p < 0.01$
	Exercise	135.2 +/- 23.9	
Dominant Quadriceps	Control	56.7 +/- 7.9	$p < 0.05$
	Exercise	68.6 +/- 12.8	
Non-dominant Quadriceps	Control	54.9 +/- 6.9	$p < 0.01$
	Exercise	67.6 +/- 12.9	

*Two-sided p value from independent samples t-test comparing control group with exercise group.

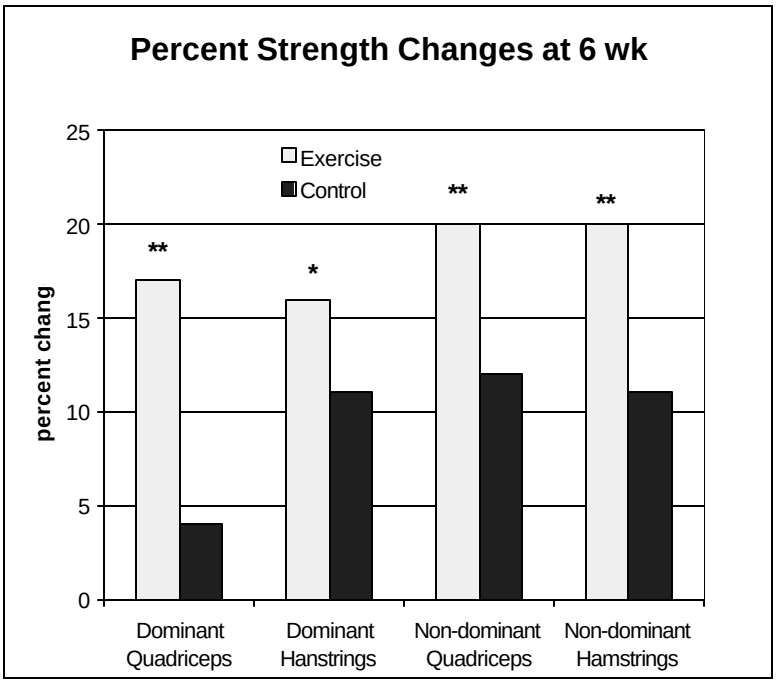

Figure 7. Strength changes in control and exercise group following 6 wk training. **Indicates $p < 0.01$, * indicates $p < 0.05$

Chapter IV

DISCUSSION AND RECOMMENDATIONS FOR FUTURE RESEARCH

Discussion

Skeletal integrity can be compromised because of exposure to stimuli, such as the microgravity environment, decreased bioavailability of gonadal hormones, or an ill-designed exercise program. Compromised skeletal integrity may occur without concurrent changes in BMD (8). Redistribution of bone material, or disruption of the bone microarchitecture may increase susceptibility for failure, however these changes could not be detected by measurements of BMD. Therefore, a measurement of bone structural integrity to complement BMD measures would provide a comprehensive assessment of skeletal status. Mechanical response tissue analysis (MRTA) was developed to assess the structural properties of long bones in vivo (107) and continues to be refined (106). The aim of the present study was to evaluate the effects of a short-term high intensity physical activity program on indicators of skeletal integrity. The high-intensity exercise program was hypothesized to initiate a skeletal response characterized by decreased bending stiffness accompanied by increased bone resorption. This initial response would be followed by a subsequent adaptive response characterized by increased bone stiffness accompanied by increased bone formation. Particular attention was paid to EI_{MRTA} and the biochemical markers of bone turnover. Measures of total body and site-specific BMD were also assessed to support the position that skeletal integrity may be compromised without cocomitant decrements in BMD.

Although there were no between group differences for EI_{MRTA}, a trend ($p = 0.051$) for time efffects was observed. Further analyses revealed significant time effects within

the exercise group ($p < 0.05$), but not the control group (Figure 3). Composite EI_{MRTA} increased from 2 wks (69.8 +/- 22.2 Nm^2) to 4 wks (93.6 +/- 22.9 Nm^2) in the exercise group. No other differences were noted.

There were significant main effects between groups ($p < 0.001$), as well as significant main effects for time for osteocalcin ($p < 0.05$). There were no significant main effects for NTx between the two groups at any of the sampling periods (Figure 5), however significant time effects were observed. Within the exercise group, OC decreased ($p < 0.01$) from baseline (15.64 ng/ml) to 2 wks (13.24 ng/ml), followed by a significant increase ($p = 0.04$) at 4 wks (14.47 ng/ml). This was accompanied by an increase in NTx ($p < 0.05$) from baseline (14.54 nM BCE) to 2 wks (16.40 nM BCE), and an increase ($p < 0.05$) from 4 wks to 6 wks (Figure 1). There were no changes for either OC or NTx at any time-point within the control group (Figure 2).

The OC time-course changes for the exercise group suggest decreased bone formation at 2 wks, followed by increased formation at 4 wks, and a return to baseline at 6 wks. In contrast, NTx increased from baseline to 2 wks in the exercise group. This coupled with the decrease in OC at 2 wks, suggests that osteoblastic activity was suppressed and osteocalstic activity enhanced. This relationship had reversed by 4 wks, with OC and Ntx returning toward baseline in the exercise group.

The pattern of change in the biochemical markers of bone turnover suggests that the skeleton was demonstrating a response by 2 wks into the high-intensity exercise program. This response was no longer apparent at 4 wk. At 6 wks, OC returned to baseline, however NTx was increased 16% above baseline levels, and was significantly higher than 4 wk levels, although not significantly different from controls at 6 wk. This

difference may have been due to seasonal variations (37), as the initial blood draw was obtained in February, and the final blood draw was obtained in May.

Composite tibial EI_{MRTA} did not change in the control group during the 6 wk study. Composite EI_{MRTA} decreased at 2 wk, followed by a subsequent increase at 4 wk, which was consistent with our hypothesis, and followed the pattern of increased NTx accompanied by decreased OC at 2 wk. However, this finding is difficult to interpret, given the variability of EI_{MRTA} measurements in this study.

The main variable of interest for the present study was EI_{MRTA}, as this variable has yet to be assessed in a randomized controlled trial. The performance of MRTA has potential implications for both clinical and athletic applications. The intra-day coefficient of variation (CV) reflects the variability of EI_{MRTA} measures within a single measurement session. The CV of the three trials selected for computing EI_{MRTA} values at each sampling period in the present study ranged from 3.4%-8.4%, which is considerably improved compared to previously reported values of 5%-18% (4). The control group served as a measure of inter-day reliability, as no changes in EI_{MRTA} values were expected between each sampling period during the 6 wk study. Previous work in our Laboratory for Health and Exercise Science produced unacceptable tibial EI_{MRTA} reproducibility, with interday variations of 1.8%-18%. The mean composite EI_{MRTA} interday variations for the control group ranged from 1%-6% for all sampling periods, demonstrating a considerable improvement over our previous findings. Although refinements in the mathematical modeling of the soft tissue and bone have been developed (9-parameter and 12-parameter models), inter-day correlations of EI_{MRTA} values across all four sampling periods ranged from $r = .03 - r = .59$. Only baseline and

4 wk EI_{MRTA} measures were significantly related ($p = 0.032$). While mean EI_{MRTA} interday variations were quite acceptable (1%-6%), intra-subject variations across the four sampling periods was unacceptable. A number of factors may have contributed to the observed intra-subject variation.

The poor reproducibility of our EI_{MRTA} measures may be related to an inability to duplicate subject positioning and achieve similar tension within the muscles that attaches to and surrounds the tibia. The magnitude at which the tibia is free to vibrate is dependent on the constraints of the muscles and tendons that operate on it. The present measurement method provides potential for error in repositioning. With the subject seated in the measurement chair, and the foot of the measurement leg resting on a lower platform, the tester subjectfully unweights the foot by raising a support that is positioned on the posterior thigh. This allows for free vibration of the tibia at both the proximal and distal end. Inter-day variability in the degree of unweighting may occur, which would affect tibial free vibration, and contribute to the poor inter-day reproducibility observed in the present study. The subject is instructed to relax the muscles of the leg, when final measurement positioning is achieved. The ability to relax the measurement leg may vary from day to day, which would affect tibial free vibration, and contribute to poor inter-day reproducibility. In addition, slight variations in probe contact with the tibia may also contribute to measurement variability across days. The present measurement techniques promote similar, but not duplicate inter-day positioning, therefore probe placement is also likely to be similar, but not duplicated. The sensitivity of the MRTA sensor, which is directly attached to the probe, detects the force and acceleration of the tibia's vibratory response to the random vibration emitted by the shaker. The force and acceleration

detected by the sensor will be affected by the probe's angle and pressure of contact with the limb. Presently there are no published RCT with MRTA as a variable of interest. The results of this study suggest that improvements in either the technology or the measurement techniques are warranted before MRTA is useful in a clinical setting.

In addition to EI_{MRTA}, biochemical markers of bone turnover were included to provide an additional dimension of skeletal status. The assays for the biochemical markers of bone turnover performed well in our labs, which is apparent by the low intra, and inter-assay CV. The pattern of response for the biochemical markers of bone turnover was consistent with our hypothesis. Our results demonstrate that within two weeks of inititaing a high intensity exercise regimen, a serum biochemical marker of bone resorption (NTx) was increased, accompanied by a decrease in a biochemical marker of bone formation (OC), indicating acclerated bone loss during this period within the exercise group. This response pattern was not observed in the control group. Pester et al (93) observed that military recruits experience the highest rate of stress fractures during the second week of basic combat training (BCT). Although none of the subjects in the present study experienced stress related injuries, the biochemical markers indicate that the skeleton was undergoing an initial response to the high-intensity exercise stimulus at 2 wk. At 4 wk, NTx returned to baseline, and OC increased toward baseline, indicating that bone resorption had normalized, suggesting that the skeleton was accomodating the additional demands imposed upon it by the high-intensity exercise regimen.

Recommendations for Future Research

Based on the findings of the present study, and relevant literature, the following recommendations appear necessary:

1. A series of tightly controlled studies to determine the source of inter-day variation are warranted. Presently, it is not known if the MRTA instrument or measurement methodology is the source of inter-day variation. Great care is taken to duplicate position from day to day, however subtle differences in limb position and muscular tension may occur. Materials of known stiffness should be obtained, and measured repeatedly with duplicate positioning over a series of days. High inter-day correlations would suggest that the present measurement methodology is the source of inter-day variation. If the materials of known stiffness elicit varying EI_{MRTA} values across different days, this would suggest that the MRTA instrument is the source of variation.
2. A measurement method, which would promote duplicate inter-day subject position, and muscular tension/relaxation is warranted. This may be accomplished by developing a series of restraints, which would allow the subject to fully relax the limb during measurement. Presently, subjects must maintain a degree of muscular tension to prevent the limb from moving once positioned for measurement. Restraints would eliminate the need for subjects to gauge and match the degree of muscular tension on subsequent measurement days.
3. If the source of inter-day measurement variation is identified, and corrected, MRTA has great potential for clinical practice and research. Mechanical response tissue analysis may be used as a screening measure for identifying those at risk for osteoporosis. Normative and diagnostic EI_{MRTA} values can be

established through a series of large clinical trials with healthy and osteoporotic subjects.

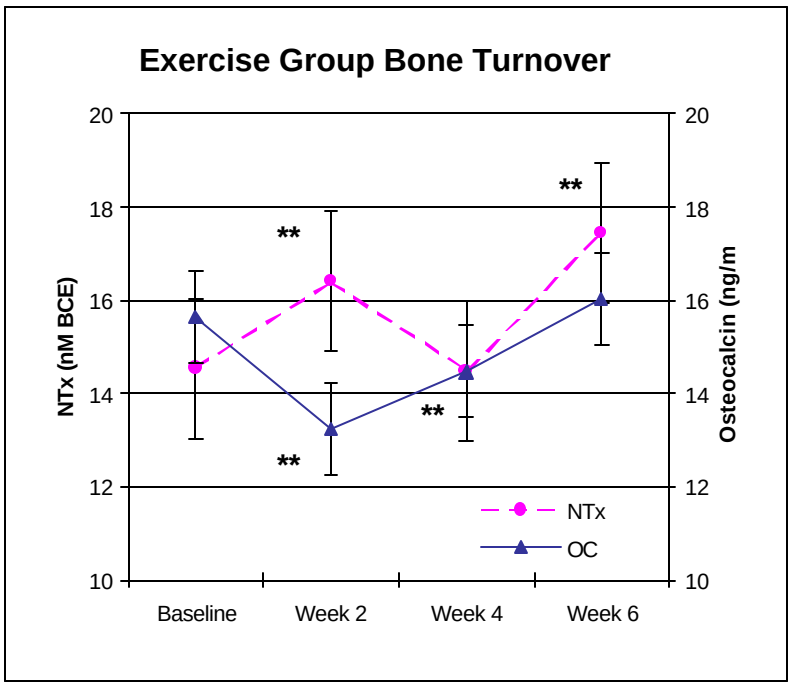

Figure 1. Biomarkerrs in bone turnover for exercise group (mean +/- SD), ** indicates significantly different from previous sampling period, $p < 0.05$.

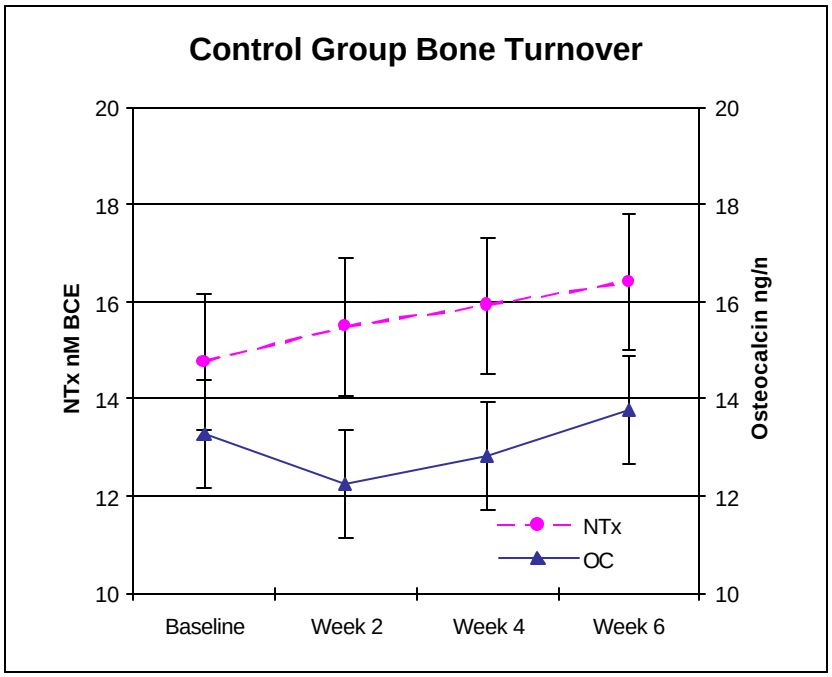

Figure 2. Biomarkers of bone turnover for control group (mean +/- SD). No differences for OC or NTx noted for any sampling period.

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Appendix A

Detailed Methodology

Experimental Design

The present study was approved by the institutional review board (IRB) of Virginia Tech. Female volunteers from the Virginia Tech community were recruited through flyer postings, and e-mail solicitation. Prior to inclusion in the study, subjects completed pre-screening, and an informed consent. Following baseline testing, 15 were randomly assigned to an exercise-training group (EX), and 15 assigned as non-training controls (NTX). The EX group participated in 6 wk of high-intensity isokinetic resistance training, ~ 25 min/d, 3 d/wk, combined with 3d/wk of moderate intensity running. The control group (NTX) maintained normal daily activities throughout the 6 wk study. All subjects completed MRTA testing and blood sampling at baseline, 2 wk, 4 wk, and 6 wk. In addition, subjects completed DXA scans, and isokinetic strength tests at baseline and 6 wks.

Pre-screening

Prior to administration of the informed consent, subjects completed a health-history questionnaire (Appendix B), and underwent pre-screening to determine initial eligibility for inclusion into the study. Pre-screening exclusion criteria were participation in structured resistance training, regular running exercise, and/or participation in a varsity sport within the past 6 months. Subjects who reported participating in recreational activities, i.e. skiing, hiking, canoeing, occasional racquet sports, were not considered disqualifying activities. No potential subjects were excluded based on exercise participation in the previous six months. An additional exclusion criterion was known metabolic or bone disease. One subject indicated a history of skeletal carcinoma, and was eliminated from further pre-screening, no other subjects indicated any history of

metabolic or bone disease. Bone fractures (including stress fractures) within the previous 12 months was also an exclusion criterion, however no subjects were excluded based on this criterion. Candidates were excluded if they had been pregnancy within the previous year, as well as had irregular menstrual history. However, no subject was excluded based on these criteria. Depo Provera® (Pharmacia & Upjohn, Kalamazoo, MI) or Norplant® (Wyeth-Ayerst, Philadelphia, PA) contraception use, was the final exclusion criterion. One subject was excluded based on present Depo Provera® use. Following pre-screening, 30 subjects were selected for participation in the study.

Informed Consent

Following pre-screening, subjects completed an informed consent (Appendix C), with the principal investigator present to answer any questions. After reading through the informed consent, and asking any questions, subjects were instructed to sign and date the informed consent if they agreed to participate in the research study. All 30 subjects agreed to participate in the study, and were provided a copy of the signed informed consent.

Nutritional Evaluation

Subjects completed 3-day dietary logs (Appendix D), which were analyzed with Nutritionist Five (version 1.7, San Bruno, CA) dietary analysis software to evaluate daily nutritional intake. Subjects found to have low intake for any of the macronutrients, received individual nutritional counseling and were provided with strategies for improving macronutrient intake. In addition, daily calcium consumption was evaluated, and subjects found to have inadequate calcium intake were supplemented daily with Viactive® calcium supplements (500 mg: Mead Johnson Nutritionals, Evansville, IN)

throughout the duration of the study. Subjects randomized to the control group were provided with a 6-wk supply of supplements, and were instructed to take the supplements daily throughout the duration of the study. Control subjects were queried periodically throughout the study on their adherence to the calcium supplementation, and all subjects reported taking the supplements as prescribed. Calcium supplements were available in the Musculoskeletal Function Laboratory (War Memorial Hall) for subjects assigned to the exercise intervention. The exercise subjects were instructed to take the calcium supplements daily upon arrival for the exercise sessions.

Testing and Measurement of Dependent Variables

After completion of the informed consent, subjects were scheduled for the baseline testing series, which consisted of isokinetic strength tests, mechanical response tissue analysis (MRTA) test, dual-energy x-ray absorptiometry (DXA) scan, and a blood draw. Blood sampling and MRTA tests were completed at 2-wk intervals throughout the study, whereas the strength test and DXA scans were completed at baseline and post-training.

Bone Mineral Content, and Bone Mineral Density

Standard protocols were used to measure these variables with the Hologic QDR 4500A (Hologic, Inc., Waltham, MA) bone densitometer. Three separate scans were performed for each subject at each evaluation interval of the study. Scans were performed for total body, hip, lumbar spine, and forearm, and. Reference points were established on these site-specific scans at baseline and used again to do these measurements for the scans at 6 wks.

Subjects lied supine on the DXA table for one total body scan to determine BMD and BMC of the total body. Each subject had the non-dominant forearms, lumbar spine, and total hip scanned to determine BMD and BMC of the radius and ulna, femoral neck, trochanter, and Ward's triangle, respectively. Standard total body, forearm, and hip protocols were used during scans. Total body (version 8.25), and forearm (version 8.25) scans were analyzed with Hologic software using the compare function for reference to the baseline scan.

All scans were conducted in the BONE laboratory (Rm 229C Wallace Hall), on the campus of Virginia Polytechnic Institute and State University, by a licensed (State of Virginia) limited radiologic technician, and analyzed by the same technician to eliminate inter-tester variation. Quality control for BMD and BMC was ensured by daily scans of an anthropomorphic phantom lumbar spine prior to any subject testing. The coefficient of variation for phantom spine scanning is 0.39%. Precision for total body BMD measurements is < 1.0% and for femoral neck is < 2.0%. Because soft tissue mass changes occur with resistance training and also impact BMD and BMC, fat-free mass (g), fat mass (g), and percent body fat of the total body as well as regions of interest will be analyzed from total body scans using the total body software (version 8.25). Precision of percent body fat for the total body with DXA is < 0.80%. Quality control for soft tissue mass was ensured by scans of an external soft tissue bar comprised of aluminum and lucite calibrated against stearic acid and water (Hologic, Bedford, MA).

Measurement of Bone Stiffness of the Tibia

Each subject was tested with mechanical response tissue analysis (MRTA) to determine stiffness (EI) of the dominant and non-dominant tibia, at baseline, 2-wks, 4-wks, and post-training. The major components of the MRTA are:

1. dual channel dynamic signal analyzer;
2. permanent magnet vibration exciter;
3. impedance head;
4. two charge amplifiers;
5. vibrating shaker/probe.

The vibrating shaker/probe is suspended from a metal support, and is positioned at the midpoint of either the tibia (Figure 1). The shaker/probe emits a transcutaneous vibration frequency in the range of 60 to 1600 Hz for very brief periods (<1-2 min). The MRTA probe contains the impedance sensor that relays force and acceleration to the signal analyzer. This information is relayed to a microprocessor that fits the raw data to an EI prediction model.

Upon arrival at the Musculoskeletal Function Laboratory, subject's height and weight were obtained using a standard stadiometer, and balance beam scale. Subjects were then instructed to lay supine for thirty-minutes to allow for fluid imbalances in the lower leg to equalize. During the last five-minutes of this period, tibia length was measured from the medial tibial condyle to the distal medial malleolus with an anthropometer to the nearest millimeter for each leg. The mid-point of the tibia was marked with a felt ink marker medial to the anterior tibial crest, to establish the point of MRTA probe placement. The subject was then seated in an adjustable chair with 90°-knee flexion and the posterior upper thigh resting on a support affixed to the MRTA

stand (Figure 2). The support of the posterior upper thigh served to unweight the foot to allow for free vibration of the tibia at the proximal and distal ends. The MRTA probe was then positioned at the mid-point of the tibia, and just medial to the anterior crest.

After the probe was in position for one-minute, the measurement software was activated, and five serial measurements were obtained and saved for further analysis. The above procedures were repeated for the opposite tibia.

Analysis of the measurement response curves for each sampling period was a two-step process. The initial step was to complete a multi-model analysis for individual measurements within the five-measurement trial. Each measurement was analyzed with the six, seven, nine, and twelve-parameter model to determine the model that provided the least root mean square (RMS) error for stiffness for each individual trial. The mean RMS, as well as coefficient of variation (CV) was then computed for all five measurement trials, with the six, seven, nine, and twelve-parameter mathematical models. The model that produced the combined least RMS, and CV was selected for the analysis in the second step of the two-step process. In the final step, the individual measurement file that produced the least RMS error was used to seed for a multi-file batch analysis. The seeded measurement file enables computational information from the "best" measurement to be carried forward to the four remaining measures. Following the seeded batch analysis, the mean of the three measurement trials that produced the least CV was selected as the final EI value.

Lastly, a quality dependent analysis was completed for the measurement response curves. Measurement response curves were visually inspected, and coded for the quality of the measurement response. Measurement response curves were inspected at each

sampling period, and coded as high-quality (Figure 3), marginal quality (Figure 4), or poor quality (Figure 4) measurements.

Blood Draws and Analysis of the Biomarkers of Bone Turnover:

Blood samples were obtained from the antecubital vein after a 24-hour abstention from exercise and 10-hour fast. All blood draws were scheduled between 8am and 10am for each sampling period. A trained laboratory technician collected blood samples into a 10 ml serum separation tube by venipuncture at the antecubital vein and were then centrifuged at 2500 revolution per minute (rpm) for 15 minutes. Serum was then transferred into 1.5 ml storage vials and stored at -80 °C until assayed. Biochemical assays were performed blinded, i.e. without knowledge of the study groups. Bone formation was evaluated by measuring total serum osteocalcin with a human-specific radiometric assay (Biomedical Technologies Inc., Stoughton, MA). The assay was prepared as described in the assay kit insert, and counted in a gamma counter for one minute. Bone resorption was evaluated by measuring serum type I N-telopeptide collagen breakdown products using an ELISA (Osteomark, Seattle WA). The assay was prepared as described in the kit insert. All OC and NTx assays were run in duplicate, and repeated if the coefficient of variation (CV) for any sample was > 20%. The intra and interassay CV was 6% and 11.6% for OC and 3.1% and 13.8% for NTx respectively.

Leg Strength Variables

The isokinetic power testing of the legs was performed at baseline, and following the 6-weeks of training. The tests were performed on the Biodex® System 2 isokinetic dynamometer (Figure 8). The tests were performed at an angular velocity of 60

degrees/sec for concentric knee extension and flexion. Testing was conducted unilaterally on both legs in a single testing session, and were counterbalanced across subjects, so half of the subjects began the testing session with the non-dominant leg, and half the subjects began the testing session with their dominant leg. The variables of interest for the tests were peak torque, peak torque/bodyweight ratio, and total work for the dominant and non-dominant limb.

Three days prior to the scheduled strength test, subjects reported to the Musculoskeletal Function Laboratory within the Laboratory for Health and Exercise Science at Virginia Tech for a familiarization session with the Biodex® isokinetic system. Upon arrival at the testing laboratory, subjects were provided with verbal instruction on the use of the Biodex® system, and performance expectations for the power tests. Subjects then completed three sets of concentric/concentric knee extension and flexion exercise at 60°/sec. Subjects were instructed to complete the first set at approximately 50% of maximal effort, followed by set two at approximately 75%, and the final set at 100%. Three days following the familiarization session, subjects reported back to the Musculoskeletal Function Laboratory for strength testing. The isokinetic strength test began with 3-min warm up of low intensity stationary leg cycling on a Monark® cycle ergometer followed by 3-5 min of static stretching exercises for the hamstrings and quadriceps muscles. The subjects were then positioned on the Biodex® system with the dynamometer axis of rotation aligned with the knee, and 85° hip flexion. The testing began with the knee at approximately 90° flexion, with the initial movement being knee extension to approximately 0°, and the second movement being knee flexion to approximately 90° to complete the first repetition. Subjects completed a warm-up set

of six repetitions, followed by a set of six maximal repetitions. The highest values obtained from either of the repetitions for peak torque, peak torque/body weight ratio, maximal capacity, and total work was recorded. Subjects were verbally encouraged to perform maximally on each repetition for the tests. Upon completion of the isokinetic strength tests, subjects were provided with a visual analog pain scale (VAS) (96) (Appendix E) to be completed for initial post exercise (IPE), and each day thereafter until there was no perceived pain. The VAS is comprised of a 10 cm line, which has "no pain" at 0 cm, and "my pain could not be worse" at 10 cm. Subjects were instructed to draw a slash on the line that corresponded to the level of pain that they perceived immediately following the testing bout, and each day thereafter until they perceived no pain. The scale is scored by measuring the distance from 0 cm to the slash drawn by the subject. Subjects were not permitted to begin the exercise program until they reported no perceived pain. In the present study, subjects reported the most perceived pain on day 3, however, all subjects reported perceived pain < 1 by day 7, for two subjects, who reported no perceived pain by day 8.

Exercise Program

High-Intensity Resistance Training

The training period was 6 wks, resulting in a total of 18-isokinetic resistance training sessions. Subjects were required to attend a minimum of 80% of the training sessions to be included in the statistical analyses. The high-intensity resistance training was performed using high-load dynamic seated leg extension and flexion for the dominant and non-dominant legs on the Biodex® System-2 isokinetic dynamometer (Model 820-200; Biodex Medical, Shirley, NY). The angular speed of the leg exercises

was controlled during training program, so that maximal skeletal strain was promoted. Subjects were encouraged to perform maximally on each repetition for all training sessions. At the beginning of each training session, subjects performed 5-8 min of leg warm-up activity on a stationary leg ergometer followed by a prescribed set of static stretching exercises for the leg muscles. Subjects then performed a warm-up set of six repetitions at approximately 50-75% of maximal effort. Following a 1-min rest period, subjects began the high-intensity resistance training, which consisted of five sets of six repetitions for each leg. Subjects were provided with one minute of rest following each set. The initial training leg was alternated for each session. The resistance training sessions required approximately 30 min. Subjects completed 3 sessions/wk on non-consecutive days. All sessions were supervised to ensure proper form, and to promote compliance.

Running Program

Subjects participated in the running exercise sessions 3 d/wk on non-consecutive days resulting in 18 total run sessions. Run training and resistance training were not conducted on the same day. The intensity/duration of the running exercise sessions were equivalent to 60-85 percent of the subject's age-predicted maximal heart rate/30 min. Prior to each run session subjects reported to the musculoskeletal function laboratory, where they completed a series of static stretches. Subjects were fitted with a commercially available heart rate monitor (Nashbar, Model NA-HRM, Canfield, OH), and individual target heart rate ranges were programmed into the monitor, which provided constant feedback throughout the running sessions. Subjects performed 30-min of self-monitored jogging/running exercise, and then reported back to the

musculoskeletal lab, where exercise heart rates and exercise duration was downloaded from the heart rate monitor and recorded.

Statistical Procedures

Baseline variables were compared with independent t-tests to reveal any pre-intervention differences between the exercisers and controls. Independent t-tests also were also used for pre and post comparisons of the muscular strength and for such comparisons with the BMD results. A two-way repeated measures analysis of variance (ANOVA) was used to provide a comprehensive analysis of the comparison group measures, biochemical markers of bone turnover and bone stiffness, taken at all data collection points across time. Time served as the "within subjects" factor, which had four levels (pre-training, 2 wks, 4 wks, and post-training) and the group served as the "between subjects" factor, which had two levels, exercise or control. Primary interest focused on the main effects for bone stiffness, the biochemical markers of bone turnover, and BMD for the two groups. In the repeated measures analysis, main effects were tested for significance using Bonferonni's *post hoc* test procedure. The .05 level of significance was used for all statistical tests. All analyses were completed with the Statistical Package for Social Sciences (SPSS) 10.0 computer software program (Chicago, Ill).

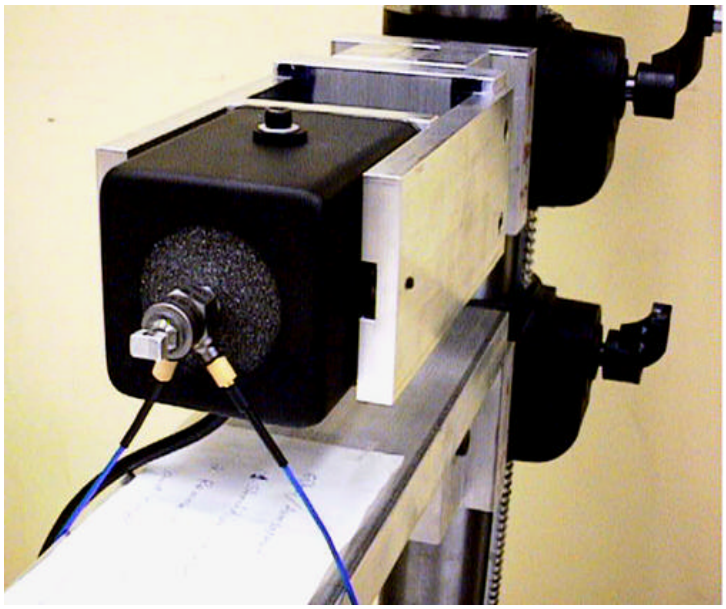

Figure 1. MRTA shaker, sensor, and probe, which is positioned at the mid-point of the tibia during measurement.

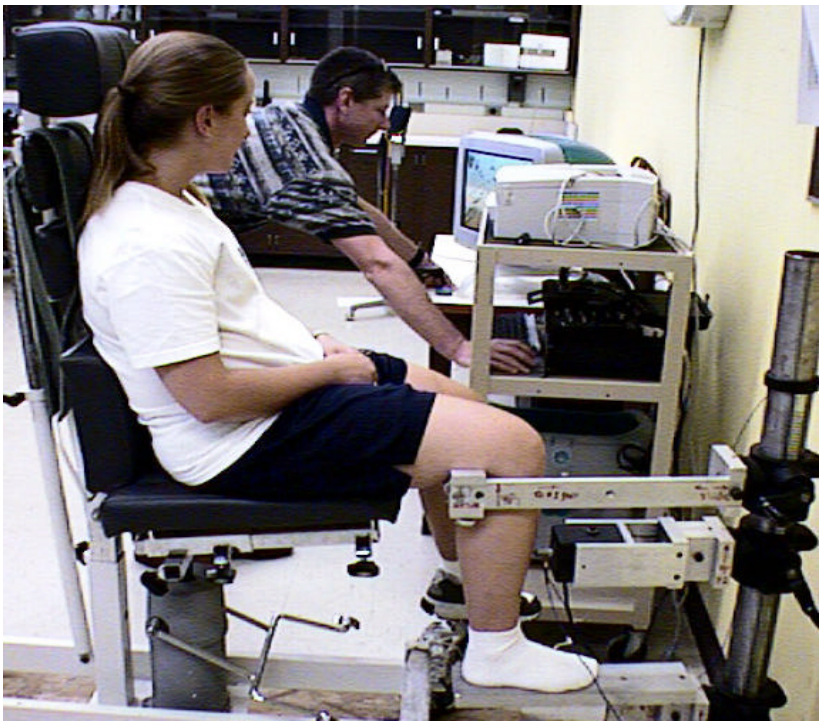

Figure 2. Final positioning on the MRTA for tibial stiffness measurement.

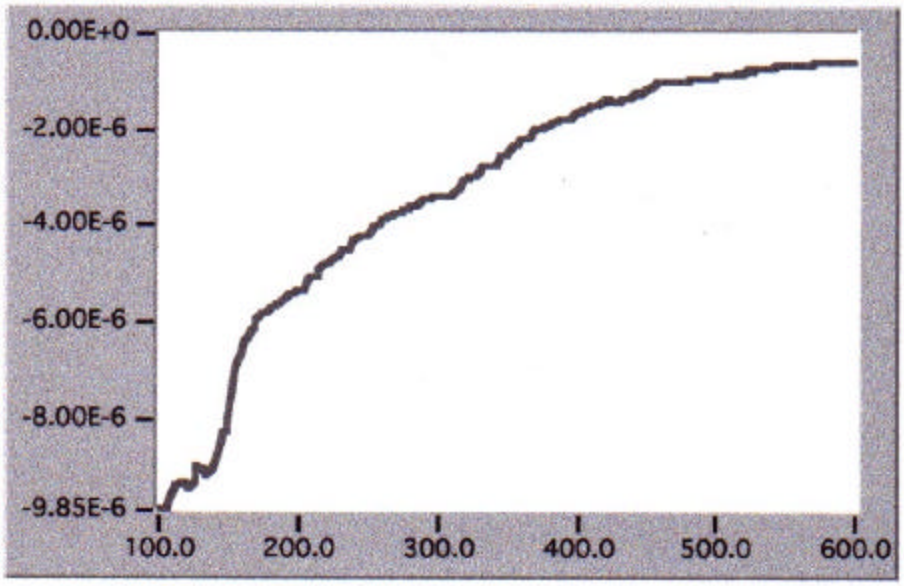

Figure 3. Highest quality MRTA measurement response curve.

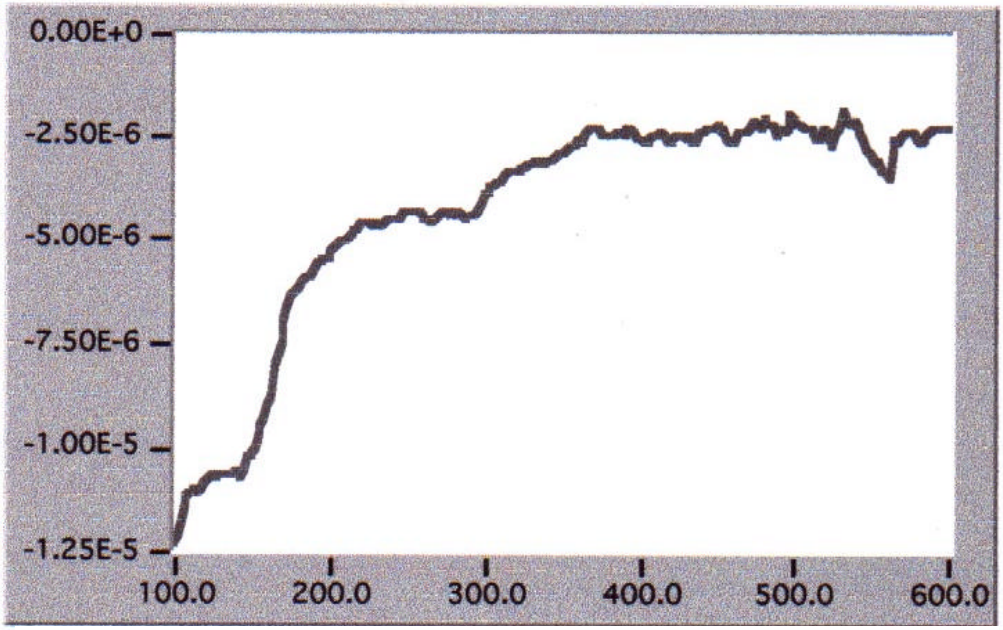

Figure 4. Marginal quality MRTA measurement response curve

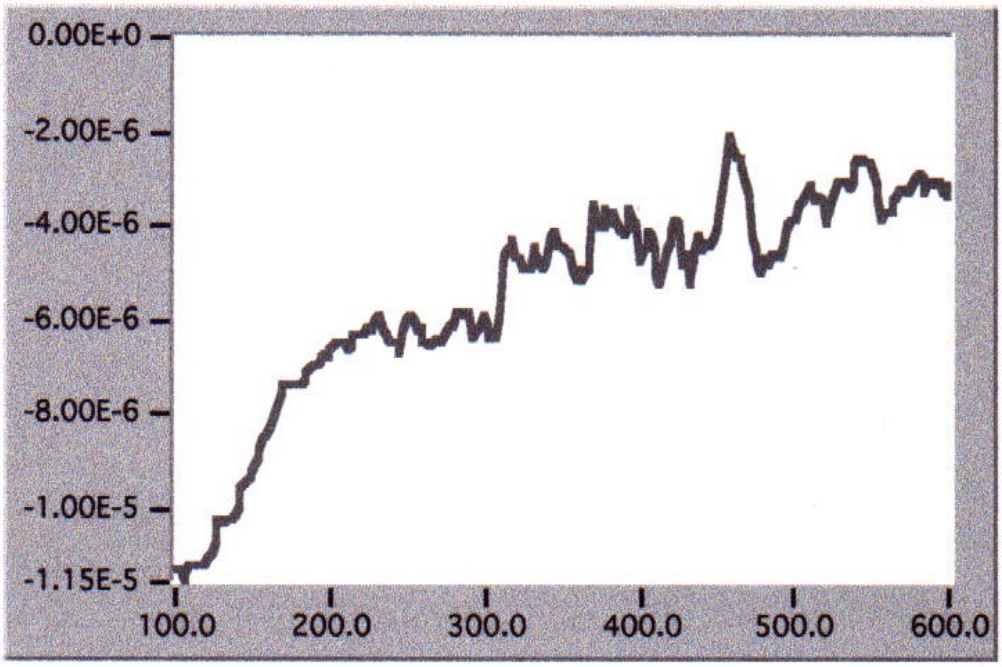

Figure 5. Poor quality MRTA measurement response curve

Figure 6. Isokinetic knee flexion and extension on the Biodex System 2 dynamometer.

Appendix B

Informed Consent

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Informed Consent for Participants of Investigative Project

Title of Study: Short-term Time Course Skeletal Responses to High Intensity Physical Activity

Location of Study: War Memorial Hall (Room 228), Virginia Polytechnic and State University, Blacksburg VA

Principal Investigators: David F. Wootten, MS, William G. Herbert, Ph.D., Warren K. Ramp, Ph.D., Sharon Nickols-Richardson, Ph.D., Ronal Bos, Ph.D., Lawrence Cross, Ph.D.

Purpose of this Research

I am invited to participate in a study that will determine the effects of a high-load weight training program versus an aerobic training program on bone mineral density, bone stiffness, and blood indicators of bone turnover.

Overview

I agree to participate in the study for a period of eight weeks. I understand that I may be assigned to a study group for this period and agree to do only those activities which the researchers assign to me; these might involve either high-load weight training; running; or no organized training at all. If I am assigned to the high-load weight training group, I will be required to participate in a supervised weight training program for approximately 25 minutes per day, 3 days per week for 8 weeks. If I am assigned to the combined running and weight training group, I will be required to participate in a fast walking/running program for approximately 30 minutes per day, 3 days per week for eight weeks, and supervised weight training program for approximately 25 minutes per day, 3 days per week for 8 weeks. I will perform the weight training and running on

alternate days. If I am assigned to a control group, I agree to abstain from participating in any structured vigorous exercise program for eight weeks. By avoiding vigorous activity, we mean avoiding any form of exercise that produces sweating and heavy breathing any more than two times per week, that is sustained for 20 minutes or more per session by running, weight training, cycling, use of gym exercise equipment, etc.

Prior to being included in the study, I will undergo an initial screening to determine my eligibility for participation. If I am included, I will complete a medical/health history, dietary log, and undergo a series of tests: The tests include, a special type (isokinetic) of strength test, a bone density measurement test, a bone strength/stiffness test, and a blood test

Explanation of the Tests

Isokinetic Strength Testing

The strength tests will be done with the legs and are called isokinetic because I will perform a maximal contraction lasting 3-6 seconds against a machine that controls the speed of the movement. The total time for the test will be approximately 10 minutes; the isokinetic strength testing will be conducted with a machine called the Biodex. The testing will begin with a warm up of 3 minutes of low intensity stationary leg cycling. I will then be given practice trials on the Biodex to become familiar with the operation, following which I will perform a maximal knee extension and flexion for each leg. I will then be given a cool down of low intensity cycling on a stationary cycle. I agree to perform an isokinetic strength test at the beginning of the study, mid-way through the study, and again at the completion of the study.

Bone Density Measurements

A bone densitometer will be used to measure the mineral content and density of the bones in my leg. I understand that the bone densitometer is much like an X-ray machine. I understand that the dose of radiation that I will receive with this test is extremely small and no greater than what I receive each day from exposure to my normal environment. The densitometer will scan my entire body very slowly; therefore I will be required to wear a hospital gown, and lie on a table, without moving for approximately 15 minutes, while the densitometer is passed over my entire body. I understand that I will feel no discomfort associated with this test.

Tibial/Ulnar Stiffness

The strength of my arm and leg bones will be measured with mechanical response tissue analysis (MRTA). For these tests, I will have to lie on a padded platform, with my knee flexed at 90-degree angles. A technician will place a device on my lower leg that will produce a mechanical energy wave through my bone. The procedure lasts approximately 15 minutes and produces no unusual sensation or discomfort.

Bone Turnover Markers

I understand that I must have blood samples drawn in order to assess bone turnover markers. I understand that the total amount of the blood that I donate at each of the test intervals will be small, i.e. 20 ml (two standard tubes). A qualified technician will draw the blood samples, and accepted medical procedures will be followed. A laboratory

specialist will examine my blood, look for substances that indicate the rates at which bone mineral is being deposited and removed from my bones. I understand that I will need to abstain from exercise for a period of 24 hours prior to having blood samples drawn. I agree to have blood samples drawn at the beginning of the study, 2 weeks into the study, 4 weeks into the study, 6 weeks into the study, and at the completion of the study.

Risks and Discomforts

I understand that there exists the very remote possibility of adverse changes during the strength tests, strength training sessions, and running sessions. I have been informed that these changes may include abnormal blood pressure, fainting, heart rhythm disorders, stroke, heart attack, and death. The risks of serious problems in maximal exercise that affect my heart, e.g. heart attack, are extremely small (1/50,000 incidents) among young healthy adults who are accustomed to heavy exercise. I have been told that every effort will be made to minimize these occurrences by preliminary examination and by precautions and observations taken during the test. I have also been informed that emergency equipment and personnel are readily available to deal with these unusual situations should they occur. I understand that there is risk of injury, and very small risks for heart attack, stroke, or death as a result of my performance of the test and participation in the training sessions, but knowing those risks, it is my desire to proceed to take the tests, and participate in the training sessions as indicated, herein.

The possible discomforts that I may experience in this study include; pain, bleeding, and local bruising at the site the blood samples. I may also experience muscle soreness

and fatigue resulting from my participation in the strength training and running exercise sessions. I understand that these conditions will most likely diminish after a week or two of regular training. There will be trained personnel present during the strength test, strength training sessions, and the running exercise sessions. These personnel will monitor my condition throughout the testing and exercise training sessions.

Benefits to be Expected

The results of strength tests may or may not benefit me personally. Potential benefits relate mainly to my personal motives for taking the test, i.e., comparing my muscular strength/fitness to that of the general population. Participation in the strength training and running training sessions may or may not benefit my physical fitness or general health. I recognize that involvement in the exercise sessions will allow me to learn proper ways to perform conditioning exercises, and regulate physical effort. These experiences should benefit me by indicating how my physical limitations may affect my ability to perform various physical activities. I further understand that if I closely follow the program instructions, that I may improve my exercise capacity. I understand that these test results will not be made available to me until after I complete my participation; however, should any such test results be obtained that have implications for my health, then I understand that the research staff will so notify me and provide me the option of forwarding such information to the health-care provider of my choice.

I may or may not benefit from the dietary counseling I will receive throughout the study. If I choose, I can make positive changes in my diet as suggested by the study dietitian. I recognize that positive dietary changes may benefit my general health.

The blood tests, bone density tests, and bone stiffness tests may or may not benefit me. Abnormalities in blood parameters or bone density identified during this study will be reported to my physician, the health center, or me. I recognize that reporting of abnormal blood parameter and bone density values to my physician or the health center may lead to medical treatments that may benefit my general health.

Compensation

I understand that I will not be compensated for my participation in this study.

Anonymity and Confidentiality

All information collected during the course of my participation in this study that is personally identifiable with me will be kept strictly confidential. At no time will the investigators release the results of the study to anyone other than individuals working on the research project without my written consent. Representatives from the National Aeronautic and Space Administration may inspect the research records. The information will have my name and identity removed and a subject number will identify me during analyses and any written reports of the research.

Medical Care

I will be screened to ensure appropriate health standards have been met for participation in the program. I have been informed and understand that participation in this study involves potential risk of accidental injury or illness including, but not limited to, tendonitis, sprains, strains, fractures, contusions, abrasions, heart attack, and even the

possibility of death. I have also been informed and understand that there are many other risks of injury and disease which may arise from my participation in this activity and that it is not possible to specifically list each and every individual risk. By signing the consent form notifying me of the injury and disease possibilities, I desire, consent, and voluntarily choose to take part in all such activities. I understand that I may withdraw from the program at any point during the course of the study.

Freedom to Withdraw

My participation in this study is completely voluntary. My refusal to participate in this study will, in no way, affect my standing as a student at Virginia Tech. I also understand that once I agree to participate in the study, I am free to withdraw at anytime without penalty.

Approval of Research

This research protocol has been approved by the Institutional Review Board for projects involving human subjects at Virginia Polytechnic Institute and State University and the Department of Human Nutrition and Foods.

Subject's Responsibilities

I know of no reason I cannot participate in this study. I accept that it is my responsibility to:

1. Accurately report medical history
2. Keep an accurate 4-day dietary log when requested to do so.
3. Refrain from vigorous physical activity for 48 hours prior to having my blood drawn.
4. Arrive to the testing lab, not having eaten or consumed fluids other than water during the 4 hours before the strength test

5. Refrain from consuming alcohol, caffeine, and nicotine products for 12 hours prior to the strength test.
6. Refrain from vigorous activity for 12 hours prior to the strength test.
7. Remain in the testing area for 1/2 hour after the strength test
8. Report to the training areas at arranged training time
9. Report any injuries resulting from participation to the research staff
10. Immediately notify the investigators if during the study I become pregnant or think I might become pregnant

Subject's Permission

I have read and understand the informed consent and conditions of this research study. I agree to undergo all screening procedures described above prior to acceptance into this study.

I understand that it is my right to withdraw from the study at anytime without penalty and that I can be dropped from the study by the investigators without my consent. I also understand the risks of my participation and the nature of any potential benefits.

I have had the opportunity to ask questions. Any questions that I have asked have been answered to my complete satisfaction. I hereby acknowledge the above and give my voluntary consent for participation in this study.

Questions/Responses:_____

_____

_____

_____ _____

Print Name Signature Date

_____ _____

Witness Date

Should I have any questions about this research or its conduct, I will contact:

David Wootten, MS (540) 231-8209
Principal Investigator, Virginia Tech

Sharon Nickols-Richardson, Ph.D (540) 231-5104
Co-Investigator, Virginia Tech

William Herbert, Ph.D. (540) 231-6565
Co-Investigator, Virginia Tech

Warren Ramp, Ph.D. (704) 355-5658
Co-Investigator

Lawrence Cross, Ph.D. (540) 552-6019
Co-Investigator, Virginia Tech

Thomas Hurd, Ph.D. (540) 231-5281
Chair, University IRB, Virginia Tech

Ronal Bos, Ph.D. (540) 231-6565
Co-Investigator, Virginia Tech

Appendix C

Health History Form

VIRGINIA TECH
LABORATORY FOR HEALTH AND EXERCISE SCIENCE
SHORT-TERM TIME COURSE SKELETAL RESPONSES TO HIGH INTENSITY PHYSICAL ACTIVITY

Name: _____ Age:____ Date of Birth: _____

Campus Address: _____

Campus Telephone Number: _____Campus Email Address: _____

Address for Permanent Residence:

_____

Person to contact in case of emergency:

_____

Relationship:_____Daytime Telephone: _____Home Telephone: _____

Primary Care Physician: _____ Telephone: _____

Medical History

Please indicate any current or previous conditions or problems you have experienced or have been told by a physician you have had:

	Yes	**No**
Heart disease or any heart problems:	_____	_____
Rheumatic fever:	_____	_____
Respiratory disease or breathing problems:	_____	_____
Circulation problems:	_____	_____
Kidney disease or problems:	_____	_____
Urinary problems:	_____	_____
Reproductive problems:	_____	_____
Musculoskeletal problems:	_____	_____
Fainting or dizziness, especially with exertion:	_____	_____
Neurological problems/disorders:	_____	_____
High blood pressure:	_____	_____
Low blood pressure:	_____	_____
Low blood cholesterol:	_____	_____
Diabetes:	_____	_____
Thyroid problems:	_____	_____
Eating disorders (bulimia, anorexia):	_____	_____
Allergies:	_____	_____

If "yes" to any of the above please indicate the date, explain, and describe:

_____

_____

_____

Please list any hospitalizations/operations/recent illnesses (Type/Date):_____

_____

_____

_____

Family Health History

Has anyone in your family (blood relatives only) been diagnosed or treated for any of the following?

	Yes	**No**	**Relationship**	**Age**
Heart attack	_____	_____	_____	_____
Heart disease	_____	_____	_____	_____
High blood pressure	_____	_____	_____	_____
Stroke	_____	_____	_____	_____
Kidney disease	_____	_____	_____	_____
Diabetes	_____	_____	_____	_____

Health Habits

Do you add salt to your food? Yes ___ No ___ Are you on any special type of diet?

Yes ___ No ___

If "yes" please describe_____

_____

Do you drink caffeinated beverages? Yes _____ No _____

How many cups per day? _____

Do you drink alcoholic beverages? Yes _____ No _____

How many drinks per week? _____

Do you smoke cigarettes? Yes _____ No _____

Packs per day: _____

Exercise Habits

Do you engage in regular exercise? Yes _____ No _____

If "yes" please list:

Activity	**Frequency (times per week)**	**Duration (minutes)**
_____	_____	_____
_____	_____	_____

Do you ever feel faint, short of breath, or chest discomfort with exertion? Yes: _____
No: _____
If "yes", please explain :_____

_____

Are there any orthopedic limitations you have that may restrict your ability to perform hard running exercise or intense strength-type exercises? (back, hips, knees, ankles)
Yes _____ No _____
If "yes" please explain: _____

_____

Questions Related to Reproductive Function

Do you use birth control? Yes _____ No _____
If "yes" what form of birth control:_____
Date of last menses: _____
Have you had any abnormal menses or absence of menses in the last 12 months? Yes _____ No _____
If “yes”, describe this menstrual problem:

_____

_____

Please list all medications (prescription and over-the-counter) you are currently taking or have taken in the past week: _____

_____

_____

Please sign to indicate the above information is correct:

_____ _____ _____

Print Name Signature Date

Follow Up Review and Interview by:

_____ _____

Signature of Project Staff Member Date

Results of Screening - Routine Findings: Make certain that all questions on this form are properly completed. Query candidate, immediately after they complete this questionnaire, about any items left blank or for which clear answers are not provided. Ask the candidate to complete the authorization form so that their medical record may be secured (usually from Virginia Tech Student Health Center). When the medical record is obtained, examine it and determine if that record can be fully reconciled with the responses to this questionnaire. If no unusual problems are disclosed that may affect the candidate's safety or eligibility for the study, note this finding below and submit file materials to the Research Coordinator.

THIS CANDIDATE QUALIFIES FOR PARTICIPATION IN THE STUDY, SUBJECT TO VERIFICATION BY THE RESEARCH COORDINATOR. Yes: ___ No: ___.

If No, complete next section, below.

Results of Screening - Uncertain Findings: Note (1) the discrepancies between this Health History Form and the medical record or (2) ANY potential health problem listed on the medical record, but not found on this Health History form. Next, contact the candidate for clarification and report outcome to the Research Coordinator. The Research Coordinator will communicate with the investigators and, if needed, the professional designated by the candidate as their health-care provider. **CANDIDATE HAS THE FOLLOWING UNDEFINED/UNCLARIFIED HEALTH PROBLEM(S) THAT WARRANT FURTHER REVIEW AND POSSIBLE EXCLUSION FROM THIS STUDY:**

_____

_____

_____

_____

_____

Appendix D

Dietary Record

Guideline for Food Record

Please write down everything that you eat or drink for two weekdays, and one weekend day. Include beverages (except water), condiments, and snacks. If you record as soon as possible after eating, it is much easier to remember and your food record will be more accurate.

Milk > Specify what kind e.g. non-fat, 1%, 2% etc.

Meat > For chicken, specify the parts, (legs, breast, thighs). Indicate if skin was removed. Specify the cut of beef (sirloin, rib, T-bone). Specify type of fish.

Bread > Specify white, wheat, rye, etc. and number of slices.

Fruit > For canned fruit, indicate if packed in water, its own juices, or syrup. For freah fruit, specify size (small apple, medium banana, etc.)

Cereal > Specify dry or cooked. List brand names.

Vegetable > Specify if canned, frozen, or fresh.

Helpful Hints: Be specific when recording the food you eat. Was it fried, baked, or broiled? Record salt, sugar, mustard, and any other condiments.

- Brand names: write down brand names, or the name of the restaurant chain.
- Portion Sizes: Estimate either volume measures (for example, ½cup, or 2 tablespoons) or weight (for example, 2 ounces or ¼ of an 8 ounce package)
- Ingredients or mixed dishes: estimate the contents

Estimating Amounts:

- A standard scoop of rice, mashed potatoes, or cottage cheese is ½cup
- A one-ounce portion of cheese is a 1-inch cube or the size of a slice of American-type cheese
- A deck of cards is about the size of a 3-ounce portion of meat. One-half chicken breast has about 3-4 ounces of cooked meat.

3-Day Dietary Record

Day of Week Taken: M T W TH F S SU (circle)

What did you eat	Amount	Cooking Method
Breakfast		
Snack		
Lunch		
Snack		
Dinner		
Snack		
Other		

Appendix E

Analog Pain Scale

Pain Analog Scale

Indicate the level of soreness for your legs below

Subject Name_____ Subject #_____

Immediate post exercise Date_____

I do not have any soreness — My soreness could not be worse

Day 1 Date_____

I do not have any soreness — My soreness could not be worse

Day 2 Date_____

I do not have any soreness — My soreness could not be worse

Day 3 Date_____

I do not have any soreness — My soreness could not be worse

Day 4 Date_____

Day 5 Date_____

Day 6 Date_____

Day 7 Date_____

Appendix F

ANOVA Tables

Independent Samples Test for Baseline Strength Measurements

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Concentric Extensi Dominant ft. lbs.	Equal variance assumed	.417	.524	-1.760	26	.090	-11.960	6.796	-25.930	2.010
	Equal variance not assumed			-1.773	25.945	.088	-11.960	6.745	-25.826	1.906
Concentric Extensi Non-dominant ft. lb	Equal variance assumed	.468	.500	-1.484	26	.150	-9.014	6.073	-21.498	3.469
	Equal variance not assumed			-1.506	25.927	.144	-9.014	5.984	-21.316	3.287
Concentric Flexion Dominant ft. lbs.	Equal variance assumed	.944	.340	-1.461	26	.156	-6.322	4.326	-15.215	2.571
	Equal variance not assumed			-1.436	22.757	.165	-6.322	4.402	-15.433	2.789
Concentric Flexion Non-dominant ft. lb	Equal variance assumed	1.066	.311	-1.393	26	.175	-5.253	3.770	-13.003	2.497
	Equal variance not assumed			-1.430	24.856	.165	-5.253	3.672	-12.819	2.313

Independent Samples T-test for baseline physical characteristics and dietary intake

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
ge, years	Equal variances assumed	5.748	.024	1.934	26	.064	1.45	.75	-9.12E-02	2.98
	Equal variances not assumed			1.841	16.217	.084	1.45	.79	-.22	3.11
eight, cm	Equal variances assumed	2.537	.123	.647	26	.523	1.178	1.819	-2.562	4.918
	Equal variances not assumed			.673	22.542	.508	1.178	1.750	-2.447	4.803
eight, kg	Equal variances assumed	.915	.347	-.279	26	.782	-.802	2.872	-6.705	5.102
	Equal variances not assumed			-.275	22.992	.786	-.802	2.919	-6.839	5.236
Body Fat	Equal variances assumed	.274	.606	.488	25	.630	.9417	1.9305	-3.0343	4.9177
	Equal variances not assumed			.487	23.508	.631	.9417	1.9348	-3.0561	4.9394
ody Mass Index	Equal variances assumed	1.611	.216	-.500	26	.621	-.571	1.143	-2.921	1.779
	Equal variances not assumed			-.486	20.393	.632	-.571	1.177	-3.022	1.880
aily Caloric Intake, kcal	Equal variances assumed	1.405	.247	-.782	26	.441	-119.27	152.46	-432.65	194.11
	Equal variances not assumed			-.806	24.293	.428	-119.27	147.97	-424.47	185.93
alcium, mg/day	Equal variances assumed	.631	.434	-.869	26	.393	-136.265	156.866	-458.708	186.178
	Equal variances not assumed			-.874	25.882	.390	-136.265	155.927	-456.848	184.318
tamin D ug/day	Equal variances assumed	.076	.785	-.697	26	.492	-.876	1.257	-3.459	1.707
	Equal variances not assumed			-.705	25.995	.487	-.876	1.242	-3.429	1.677

Independent Samples T-test for baseline EI, biomarkers of bone turnover, and BMD measures

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Baseline Composite EI (nM2)	Equal variances assumed	.195	.663	.891	23	.382	8.251	9.260	-10.904	27.407
	Equal variances not assumed			.894	22.994	.381	8.251	9.234	-10.851	27.354
Osteocalcin ng/mL-1	Equal variances assumed	.208	.652	-2.370	26	.025	-2.3645	.9976	-4.4150	-.3139
	Equal variances not assumed			-2.391	25.973	.024	-2.3645	.9891	-4.3977	-.3313
NTx nM BCE	Equal variances assumed	.019	.891	.176	26	.862	.2227	1.2646	-2.3767	2.8220
	Equal variances not assumed			.177	25.811	.861	.2227	1.2587	-2.3655	2.8108
Total Body BMD gm/cm2	Equal variances assumed	1.013	.324	-.613	25	.546	-1.878E-02	3.0651E-02	-8.2E-02	4.43E-02
	Equal variances not assumed			-.631	24.990	.534	-1.878E-02	2.9788E-02	-8.0E-02	4.26E-02
Forearm BMD gm/cm2	Equal variances assumed	.024	.878	-1.291	25	.208	-1.747E-02	1.3527E-02	-4.5E-02	1.04E-02
	Equal variances not assumed			-1.299	24.188	.206	-1.747E-02	1.3450E-02	-4.5E-02	1.03E-02
Spine BMD gm/cm2	Equal variances assumed	.175	.679	-.098	25	.923	-3.400E-03	3.4870E-02	-7.5E-02	6.84E-02
	Equal variances not assumed			-.099	24.802	.922	-3.400E-03	3.4303E-02	-7.4E-02	6.73E-02
Total Hip BMD gm/cm2	Equal variances assumed	2.301	.142	-1.292	25	.208	-4.673E-02	3.6163E-02	-.12121	2.77E-02
	Equal variances not assumed			-1.360	23.751	.187	-4.673E-02	3.4366E-02	-.11770	2.42E-02

Multivariate Repeated Measures ANOVA for OC and NTx

Effect			Value	F	Hypothesis df	Error df	Sig.
Between Subjects	Intercept	Pillai's Trace	.991	1361.362	2.000	25.000	.000
		Wilks' Lambda	.009	1361.362	2.000	25.000	.000
		Hotelling's Trace	108.909	1361.362	2.000	25.000	.000
		Roy's Largest Root	108.909	1361.362	2.000	25.000	.000
	GROUP	Pillai's Trace	.210	3.319	2.000	25.000	.053
		Wilks' Lambda	.790	3.319	2.000	25.000	.053
		Hotelling's Trace	.265	3.319	2.000	25.000	.053
		Roy's Largest Root	.265	3.319	2.000	25.000	.053
Within Subjects	TIME	Pillai's Trace	.759	11.012	6.000	21.000	.000
		Wilks' Lambda	.241	11.012	6.000	21.000	.000
		Hotelling's Trace	3.146	11.012	6.000	21.000	.000
		Roy's Largest Root	3.146	11.012	6.000	21.000	.000
	TIME * GROUP	Pillai's Trace	.334	1.752	6.000	21.000	.158
		Wilks' Lambda	.666	1.752	6.000	21.000	.158
		Hotelling's Trace	.501	1.752	6.000	21.000	.158
		Roy's Largest Root	.501	1.752	6.000	21.000	.158

Univariate Repeated Measures ANOVA for OC and NTx

Source	Measure		Type III Sum of Squares	df	Mean Square	F	Sig.
TIME	OC	Sphericity Assumed	75.412	3	25.137	6.203	.001
		Greenhouse-Geisser	75.412	2.382	31.662	6.203	.002
		Huynh-Feldt	75.412	2.739	27.533	6.203	.001
		Lower-bound	75.412	1.000	75.412	6.203	.019
	NTX	Sphericity Assumed	80.860	3	26.953	5.823	.001
		Greenhouse-Geisser	80.860	2.337	34.606	5.823	.003
		Huynh-Feldt	80.860	2.680	30.169	5.823	.002
		Lower-bound	80.860	1.000	80.860	5.823	.023
TIME * GROUP	OC	Sphericity Assumed	8.276	3	2.759	.681	.566
		Greenhouse-Geisser	8.276	2.382	3.475	.681	.535
		Huynh-Feldt	8.276	2.739	3.022	.681	.554
		Lower-bound	8.276	1.000	8.276	.681	.417
	NTX	Sphericity Assumed	27.751	3	9.250	1.998	.121
		Greenhouse-Geisser	27.751	2.337	11.877	1.998	.137
		Huynh-Feldt	27.751	2.680	10.354	1.998	.129
		Lower-bound	27.751	1.000	27.751	1.998	.169
Error(TIME)	OC	Sphericity Assumed	316.108	78	4.053		
		Greenhouse-Geisser	316.108	61.926	5.105		
		Huynh-Feldt	316.108	71.213	4.439		
		Lower-bound	316.108	26.000	12.158		
	NTX	Sphericity Assumed	361.053	78	4.629		
		Greenhouse-Geisser	361.053	60.751	5.943		
		Huynh-Feldt	361.053	69.687	5.181		
		Lower-bound	361.053	26.000	13.887		

Repeated Measures ANOVA Tests of Within-Subjects Contrasts for Exercise Group

Source	Measure	TIME	Type III Sum of Squares	df	Mean Square	F	Sig.
TIME	OC	Level 2 vs. Level 1	86.496	1	86.496	19.976	.001
		Level 3 vs. Previous	1.176E-02	1	1.176E-02	.003	.959
		Level 4 vs. Previous	37.005	1	37.005	5.167	.039
	NTX	Level 2 vs. Level 1	51.931	1	51.931	8.528	.011
		Level 3 vs. Previous	14.692	1	14.692	1.373	.261
		Level 4 vs. Previous	78.631	1	78.631	12.882	.003
Error(TIME)	OC	Level 2 vs. Level 1	60.619	14	4.330		
		Level 3 vs. Previous	61.078	14	4.363		
		Level 4 vs. Previous	100.259	14	7.161		
	NTX	Level 2 vs. Level 1	85.249	14	6.089		
		Level 3 vs. Previous	149.827	14	10.702		
		Level 4 vs. Previous	85.457	14	6.104		

Repeated Measures ANOVA for Tests of Within-Subjects Contrasts for Control Group

Source	Measure	TIME	Type III Sum of Squares	df	Mean Square	F	Sig.
TIME	OC	Level 2 vs. Level 1	13.833	1	13.833	1.147	.305
		Level 3 vs. Previous	3.932E-02	1	3.932E-02	.005	.942
		Level 4 vs. Previous	12.834	1	12.834	2.883	.115
	NTX	Level 2 vs. Level 1	6.855	1	6.855	.870	.369
		Level 3 vs. Previous	8.384	1	8.384	1.252	.285
		Level 4 vs. Previous	13.668	1	13.668	2.296	.156
Error(TIME)	OC	Level 2 vs. Level 1	144.765	12	12.064		
		Level 3 vs. Previous	86.166	12	7.181		
		Level 4 vs. Previous	53.412	12	4.451		
	NTX	Level 2 vs. Level 1	94.566	12	7.880		
		Level 3 vs. Previous	80.371	12	6.698		
		Level 4 vs. Previous	71.451	12	5.954		

Repeated Measures for Composite EI

Measure: EI

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
TIME	Sphericity Assumed	4174.730	3	1391.577	2.735	.051
	Greenhouse-Geisser	4174.730	2.813	1484.317	2.735	.055
	Huynh-Feldt	4174.730	3.000	1391.577	2.735	.051
	Lower-bound	4174.730	1.000	4174.730	2.735	.113
TIME * GROUP	Sphericity Assumed	1023.334	3	341.111	.670	.573
	Greenhouse-Geisser	1023.334	2.813	363.844	.670	.564
	Huynh-Feldt	1023.334	3.000	341.111	.670	.573
	Lower-bound	1023.334	1.000	1023.334	.670	.422
Error(TIME)	Sphericity Assumed	32054.065	63	508.795		
	Greenhouse-Geisser	32054.065	59.064	542.703		
	Huynh-Feldt	32054.065	63.000	508.795		
	Lower-bound	32054.065	21.000	1526.384		

Group Contrast for Composite EI

Control or Exercise, 1 = Control 2= Exercise Difference Contrast			Averaged Variable EI
Level 2 vs. Level 1	Contrast Estimate		-4.843
	Hypothesized Value		0
	Difference (Estimate - Hypothesized)		-4.843
	Std. Error		5.194
	Sig.		.362
	95% Confidence Interval for Difference	Lower Bound	-15.645
		Upper Bound	5.959

Composite EI Repeated Measures ANOVA for Control Group

Measure: EI

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
TIME	Sphericity Assumed	620.252	3	206.751	.437	.728
	Greenhouse-Geisser	620.252	2.690	230.599	.437	.708
	Huynh-Feldt	620.252	3.000	206.751	.437	.728
	Lower-bound	620.252	1.000	620.252	.437	.524
	Sphericity Assumed			.	.	.
	Greenhouse-Geisser			.	.	.
	Huynh-Feldt			.	.	.
	Lower-bound			.	.	.
Error(TIME)	Sphericity Assumed	14200.591	30	473.353		
	Greenhouse-Geisser	14200.591	26.897	527.953		
	Huynh-Feldt	14200.591	30.000	473.353		
	Lower-bound	14200.591	10.000	1420.059		

Composite EI Repeated Measures for Exercise Group

Measure: EI

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
TIME	Sphericity Assumed	4757.701	3	1585.900	2.931	.048
	Sphericity Assumed			.	.	.
				.	.	.
				.	.	.
				.	.	.
Error(TIME)	Sphericity Assumed	17853.474	33	541.014		

Composite EI Repeated Measures ANOVA for Tests of Within-Subjects Contrasts for Exercise Group

Measure: EI

Source	TIME	Type III Sum of Squares	df	Mean Square	F	Sig.
TIME	Level 2 vs. Level 1	286.652	1	286.652	.303	.593
	Level 3 vs. Previous	5468.803	1	5468.803	8.166	.016
	Level 4 vs. Previous	1291.342	1	1291.342	1.378	.265
TIME * GROUP	Level 2 vs. Level 1	.000	0	.	.	.
	Level 3 vs. Previous	.000	0	.	.	.
	Level 4 vs. Previous	.000	0	.	.	.
Error(TIME)	Level 2 vs. Level 1	10422.381	11	947.489		
	Level 3 vs. Previous	7366.946	11	669.722		
	Level 4 vs. Previous	10307.982	11	937.089		

Independent Samples T-test for 6 wk BMD Measures

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
otal Body BMD/Pos	Equal variances assumed	1.600	.217	-.735	25	.469	-2.297E-02	3.1261E-02	-8.7E-02	4.14E-0
	Equal variances not assumed			-.754	25.000	.458	-2.297E-02	3.0442E-02	-8.6E-02	3.97E-0
orearm BMD Post	Equal variances assumed	.085	.773	-1.471	25	.154	-2.348E-02	1.5966E-02	-5.6E-02	9.40E-0
	Equal variances not assumed			-1.473	23.821	.154	-2.348E-02	1.5947E-02	-5.6E-02	9.44E-0
otal Hip Post	Equal variances assumed	1.551	.225	-1.386	25	.178	-5.023E-02	3.6255E-02	-.12490	2.44E-0
	Equal variances not assumed			-1.458	23.775	.158	-5.023E-02	3.4461E-02	-.12139	2.09E-0
pine BMD Post	Equal variances assumed	.001	.975	.033	25	.974	1.1333E-03	3.4524E-02	-7.0E-02	7.22E-0
	Equal variances not assumed			.033	23.932	.974	1.1333E-03	3.4438E-02	-7.0E-02	7.22E-0

Independent Samples T-test for comparison of isokinetic strength measurements between exercise and controls at 6 wk

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
oncentric extension p aining dominant leg	Equal variances assumed	1.717	.202	-3.129	25	.004	-30.570	9.770	-50.691	-10.44
	Equal variances not assumed			-3.269	24.368	.003	-30.570	9.351	-49.855	-11.28
oncentric extension p aining non-dominant l	Equal variances assumed	2.162	.154	-3.093	24	.005	-24.381	7.882	-40.648	-8.11
	Equal variances not assumed			-3.213	21.626	.004	-24.381	7.587	-40.132	-8.63
oncentric flexion pos aining dominant leg	Equal variances assumed	3.120	.090	-2.791	24	.010	-11.875	4.255	-20.656	-3.09
	Equal variances not assumed			-2.892	22.044	.008	-11.875	4.106	-20.390	-3.36
oncentric Flexion pos aining non-dominant l	Equal variances assumed	7.854	.010	-3.045	24	.006	-12.702	4.171	-21.311	-4.09
	Equal variances not assumed			-3.184	20.417	.005	-12.702	3.990	-21.014	-4.39

Appendix G

VITAE

CURRICULUM VITA

NAME: **David F. Wootten, Ph.D.**

TITLE: **Research Coordinator**

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EDUCATION:

Institution	Degree	Year Conferred
University of Hawaii at Hilo Psychology	BS	1994
Frostburg State University Human Performance	MS	1997
Virginia Polytechnic Institute Clinical Exercise Physiology	PhD	2001

WORK EXPERIENCE:

2000-Present Research Associate, Effects of Isokinetic Resistance Training and Deconditioning on Bone Stiffness, Bone Mineral Density, and Bone Turnover in Military-Aged Females. Laboratory for Health and Exercise Science; Virginia Polytechnic Institute and State University, Blacksburg VA

1999-2000	Clinical Coordinator, Therapeutic Exercise and Community Health Center, Department of Human Nutrition, Foods, and Exercise; Virginia Polytechnic Institute and State University, Blacksburg, VA
1998-2000	Program Coordinator, Fitness and Wellness Instructional Services, Department of Human Nutrition, Foods, and Exercise; Virginia Polytechnic Institute and State University, Blacksburg, VA
1997-1999	Graduate Teaching Assistant, Department of Human Nutrition, Foods, and Exercise; Virginia Polytechnic Institute and State University, Blacksburg, VA
1996-1997	Laboratory Coordinator, Human Performance Laboratory, Department of Health, Physical Education, Recreation and Dance; Frostburg State University, Frostburg, MD
1995-1996	Graduate Teaching Assistant; Department of Health, Physical Education, Recreation and Dance Frostburg State University, Frostburg, MD

MILITARY SERVICE:

1998-1999	Headquarters 29th Infantry, Virginia National Guard; Roanoke VA
1989-1991	Deutsche Bundesbahn Liason, 27th Transportation Battalion, Bremerhaven, GER
1986-1989	Division Headquarters, G-4; 101st Airborne Division, Ft. Campbell, KY

PROFESSIONAL AND SCIENTIFIC ORGANIZATIONS

American College of Sports Medicine

American Society for Bone and Mineral Research

American College of Sports Medicine, Southeastern Chapter

HONORS AND AWARDS

1999-2000	Raville Outstanding Graduate Student in Human Nutrition, Foods, and Exercise, Virginia Tech
1999-2000	NASA Graduate Student Research Program Fellowship
1996	Distinguished Honor Graduate, Human Performance Program, Frostburg State University
1994	Graduated with highest honors, University of Hawaii at Hilo
1986-1991	Awarded Army Achievement Medal (6 awards) Awarded Army Commendation Medal Awarded Non-Commissioned Officer Leadership Medal Awarded Good Conduct Medal

PUBLICATIONS/ PRESENTATIONS

Developing a Therapeutic Exercise Rehabilitation Program for End Stage Renal Disease Patients. American Nephrology Nurse's Association, Commonwealth Chapter, Annual Conference. Roanoke, VA. Sept. 1999.

Osteocalcin response to lactic acidosis induced by high-intensity cycling exercise. American College of Sports Medicine, Southeastern Chapter, Annual Conference. Charlotte, NC. Jan, 2000.

Mechanical response tissue analysis (MRTA): A pilot study to reliably assess bending stiffness (EI) of the human tibia. American College of Sports Medicine, Southeastern Chapter, Annual Conference. Charlotte, NC. Jan, 2000.

Relationships Between Isokinetic Knee Flexion/Extension Strength, and Tibial Bending Stiffness. American College of Sports Medicine, National Conference. Indianapolis, IN. June, 2000.

The Nature of Mechanical Response Tissue Analysis (MRTA), Body Weight, and Body Mass Index. American Society of Bone and Mineral Research, Annual Conference. Toronto, Canada. Sept. 2000.

Influence of Skinfold Thickness on Non-Invasive Measurement of Tibial Bending Stiffness in Women. International Bone and Hormone Annual Scientific Meeting. North Queensland, New Zealand. Nov. 2000.

Effects of High-Volume Physical Activity on Bone Stiffness, and Bone Turnover in College-Age Females. Bioastronautics Investigators' Workshop. Galveston, TX. Jan. 2001.

Fat-free soft tissue mass predicts tibial bone mineral density in a group of young-adult females. Experimental Biology Annual Meeting. April 2001. Orlando, FL

Bone mineral density (BMD) of the lower legs and tibias in young-adult females with low and high total body BMD. Experimental Biology Annual Meeting. April 2001. Orlando , FL

COMMUNITY AND UNIVERSITY SERVICE

American Red Cross, Montgomery County Chapter. Board of Directors, 1998-1999

American Red Cross, Montgomery County Chapter. Chair of Youth Committee, 1998 1999.

Virginia Tech Adventure Racing Club, President. 1998-1999.

American College of Sports Medicine, Clinical Exercise Physiology Registry, Academic Standards Committee. 2000-present