Mechanical Response Tissue Analysis: Inter- and Intra-Trial Reliability in Assessing Bending Stiffness of the Human Tibia in College Aged Women

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Thanks Mom, Dad and Bessie for your support and encouragement.

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Mechanical Response Tissue Analysis: Measurement Reliability in Assessing Bending Stiffness of the Human Tibia in College Aged Women

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(ABSTRACT)

Mechanical Response Tissue Analysis (MRTA) is an emerging technology for assessing maximal bending stiffness (EI) of human long bones *in vivo*. The MRTA variable, EI, is the product of Young's modulus of elasticity (E) and cross-sectional moment of inertia (I). EI quantifies material and architectural/geometric properties of bone. Published human research using MRTA to measure EI has been limited to the ulna; however, the tibia requires further investigation due to its central involvement in many human activities and exercise-related clinical problems, e.g. stress fracture of the lower leg. To evaluate the inter- and intra-reliability of tibial EI, 22 healthy women (X $\pm$ SD: 20.8 $\pm$ 1.8 yr) were assessed twice daily for three non-consecutive days. Each daily session consisted of five repeated trials. The ulnar EI protocol of McCabe *et al.* [J Bone and Mineral Res. 1991;6(1):53-59] was adapted to assess tibial EI via MRTA. A significant difference was not found in scores for five repeated trials taken consecutively on the same day. Mean scores for EI were higher on day 1 (59.1 $\pm$ 35.5 $N \cdot m^2$, $p < 0.05$), compared to day 2 (46.9 $\pm$ 22.3) and day 3 (49.9 $\pm$ 18.3). Individual trial mean scores for EI on each day (mean of 5 trials) were highly correlated, R^2 = 0.84, 0.62, and 0.79 (set 1 vs. 2, for day 1,2,3, respectively) and the average percent change between sets 1 and 2 on each day was 5.3. The inter-test (between day) reproducibility was found to be low and unacceptable, 11.7, 18.3, and 1.3 %, for day 1 vs. 2, 1 vs. 3, and 2 vs. 3. Poor inter-day reliability may be a result of the inability, at the time of this study, to apply the best computational EI model. It is concluded that tibial bone stiffness measurements with the MRTA are in the range of acceptability for same day inter- and intra-trial reliability when the 7-parameter analytic model of vibratory properties developed by McCabe *et al.* is used.

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Chapter I

INTRODUCTION

Stress injuries of the bone frequently occur in military recruits (Cline, Jansen, & Melby *et al.*, 1998), athletes, recreational athletes, and are associated with repetitive weight-bearing activities (Bennell & Brukner, 1997). It has been proposed that stress fracture development involves both repetitive loading and resulting bone fatigue; however, the direct mechanisms by which exercise leads to changes in bone metabolism are not fully understood. Two fracture theories, the primary microdamage theory, which suggests fractures occur when microdamage production exceeds repair and the primary remodeling hypothesis which proposes irreversible microdamage occurs when the bone is at a susceptible or weakened state during osteoclastic resorption, have been hypothesized (Bennell, Malcolm, Wark & Brukner, 1996). Additionally, individuals may have both intrinsic (bone mineral density, bone geometry, skeletal alignment) and extrinsic (footwear, external loading, surface etc.) risk factors that may predispose or increase their risk for stress injuries (Nattiv & Armsey Jr., 1997; Bennell, Matheson, Meeuswisse & Brukner, 1999).

Several studies have shown that positive bone adaptation occurs with exercise (Fujimura *et al.*, 1997; Heinonen, Oja, Kannus, Sievanen, Mantarri, & Vuori, 1993; Fehling, Alekel, Clasey, Rector & Stillman, 1995); however, too much activity or overuse may lead to structural damage within the bone. Although stress fractures have been detected in all areas of the skeletal structure, fractures occur more commonly in the bones of the lower extremity, specifically the tibia (Bennell *et al.*, 1997). Bennell, Malcolm, Thomas & Wark (1996) investigated female track and field athletes and

found 46% of stress fractures occurred at the tibia. These results are similar to other studies investigating the anatomic distribution of stress fractures in athletes and military recruits (Bennell, Malcolm, Thomas, Wark & Brukner 1996; Courtenay & Bowers, 1990; Beck *et al.*, 1996; Johnson, Weiss & Wheeler, 1994). Running and other repetitive activities lead to a temporary period of bone resorption and inhibition of bone formation (Malm, Ronni-Sivula, Viinikka & Ylikorkala, 1993; Brahm, Strom, Piehl-Aulin, Mallmin, & Ljunghall, 1997; Brahm, Piehl-Aulin & Ljunghall, 1996); a period of transition that may lead to stress fractures with additional activity.

Throughout life bone is continually remodeling by adapting its structure and shape to changes in skeletal loading. Specifically, this is seen with activities involving repetitive movements. This remodeling process occurs by way of specialized cells in bone known as osteoblasts (bone formation) and osteoclasts (degradation). The two mechanical forces that regulate bone remodeling are compressive and tensile strain. Adaptation of bone is a function of the number of cycles, cycle frequency, amount of strain, strain rate, and strain duration per cycle (Frost, 1998). Increased load from activities such as running should increase formation and decrease resorption, while unloading or decreased activity should have the opposite effect. Several studies (Dook, James, Henderson & Price, 1997; Duppe, Gardsell, Johnell, Nillsson & Ringsberg, 1997; Valdimarsson, Kristinsson, Stefansson, Valdimarsson & Sigurdsson, 1999; Madson, Adams & Van Loan, 1998) have shown that physical activity increases BMD and may reduce subsequent fracture risk, however repeated strain (overuse) imposed by chronic weight-bearing activity, such as those found in athletes and military training may cause microscopic fatigue damage or microdamage (MDx)

within the bone. Several studies have demonstrated microdamage following repetitive loading *in vivo* (Frost, 1989; Mori & Burr, 1993; Schaffler, Radin & Burr, 1990).

The strain range in human bones that causes too much MDx to repair called the MDx threshold range is centered around 3000 microstrain (Burr *et al.*, 1997). Strain gauge studies have found strain in the tibia to reach 2000 microstrain, a value well below the fracture threshold. These results suggest that if bone is unable to adapt or compensate to the increased strain (both loading and bending) of specific weight-bearing activities, bone microdamage occurs, and further loading of the bone may result in a stress fracture. Moreover, studies have shown that excessive microstrain decreases the normal stiffness of bone which leads to microcrack accumulation within the bone, impairing the mechanical properties by reducing the bones elastic modulus (Burr *et al.*, 1998).

Additional factors that influence bone to resist fracture includes geometry, material density, quality, and the activity of muscle attachments to bone (Bennell *et al.*, 1999). The geometry of bone is important in determining its overall strength. During weight-bearing physical activity, e.g. running, tension and compression loads are very high. The wider the bone, the greater the surface area to distribute internal forces thus decreasing strain and resisting the probability of stress related injuries.

Investigations using cadaver bone specimens have shown that bones with higher density are stronger (Alho, Torstein & hoiseth, 1988). Carter and Hayes (1977) found the compressive strength of skeletal tissue was approximately proportional to the square of the bone density. This suggests small changes in bone density may lead to large reductions in the overall strength of bone and its ability to withstand excessive

strain. Besides bone density, bone quality plays an integral role in bone strength. The quality of bone refers to the number, spacing, and connectivity of trabeculae within the bone (Bennell *et al.*, 1997). It may be assumed that a more intricate lattice with smaller spacing and dense tissue connections would increase the structural integrity of the bone.

To date researchers have used dual energy X-ray absorptiometry (DXA), an established method of non-invasively estimating bone density and fracture risk. The DXA, elicits a two-dimensional X-ray measuring bone mineral density (BMD), bone mineral content (BMC) and bone width. (Madsen, Jensen & Sorensen, 1998; Sievanen *et al.*, 1996). Although useful, studies have shown that DXA niether precisely determines fracture risk (Gardsell, Johnell & Nilsson, 1989) nor accurately predicts the geometric and mechanical characteristics of bone (Jarvinen, Sievanen, Kannus & Jarvinen, 1998). It has been suggested that low BMD reduces bone strength, thus contributing to the development of stress fractures by increasing the accumulation of microdamage with repetitive loading (Carter, Caler & Spengler, 1981). An association between fragility fractures and low BMD in osteoporotic individuals has been established and bone density has been used to predict the possibility of fracture risk in the clinical setting (Cummings *et al.*, 1993; Melton, Atkinson & O'Fallen, 1993). However, unlike osteoporotic individuals, most active young adults have BMD within normal ranges, if not higher (Bennell, Malcolm & Khan, 1997). Additionally, many studies have reported contradictory results when investigating an association between stress fractures and lower BMD (Lander *et al.*, 2000; Myburgh, Hutchins, Fataar, Hough & Noakes, 1990; Carbon *et al.*, 1990; Bennell *et al.*, 1996; Bennell *et al.*,

1995). From these results, it appears that bone densitometry is not the best screening tool to predict fracture risk in healthy, active individuals.

Bone architecture and bone geometry also affect the mechanical strength of bone (Haapasalo *et al.*, 1996). The amount of load a bone can withstand before breaking is proportional to the cross-sectional area (shape and size factor) of the bone and for bending loads, the cross-sectional moment of inertia and cross-sectional area reflect the strength of the bone (Martin, 1991). Recently, geometric parameters have been shown to provide improved ability to predict fracture risk *in vivo* (Beck *et al.*, 1996). Steele *et al.* (1988) suggests BMC is necessary for proper stiffness, but does not elicit the quality of bone that is indicated by bone stiffness. Studies have shown that a bone's material strength is not highly correlated to mineralization (Currey, 1990). This study implies high or low BMD may not be an accurate indicator of fracture risk and suggests quality of bone to be of greater value when evaluating individual fracture risk. Moreover, there is a greater variation in structural geometry than in BMD properties, including bone mineral density (Martens, Van Audekercke, De Meester & Mulier, 1981). Thus, differences in bone geometry may help answer individual predisposition to stress fractures.

A relatively new technique for bone assessment, the mechanical response tissue analysis (MRTA) is cheaper, requires less space, does not expose individuals to radiation, and is easier to operate than densitometry. Unlike the DXA which measures BMD, MRTA yields the maximal bending stiffness (EI), the load-carrying capacity, and "sufficiency" (S) of bone by measuring the impedance response of a long bone to low-frequency vibration (Roberts *et al.*, 1996; Myburgh, Zhou, Steele, Arnaud &

Marcus, 1992; Steele *et al.*, 1988). The bending stiffness yields a measure of long bone structural integrity, which is related to the composition, geometry, internal architecture, and quality of bone (Roberts *et al.*, 1996; Steele *et al.*, 1988). The bending strength of the tibia or its ability to resist bending moments as measured by area moment of inertia has been found to be a good determinant of stress fracture risk (Milgrom *et al.*, 1989). Furthermore, several studies have shown a high correlation between EI and BMC (Jurist & Foltz, 1977; Orne, Borders & Peterson, 1977). Thus, it appears that EI is an excellent indicator of bone quality and fracture risk.

Currently, studies have focused on techniques that directly measure ulnar bending stiffness with the MRTA (Myburgh *et al.*, 1993; Roberts *et al.*, 1996; Steele *et al.*, 1988; McCabe, Zhou, Steele & Marcus, 1991). A difficulty with the MRTA has been designing a measurement system that produces reproducible results. Steele *et al.* (1988) measured the right and left ulna of 80 subjects and found the BMC and bone stiffness correlated well ($r = 0.81$) and when sufficiency was taken into consideration the association with BMC increased ($r = 0.89$). Furthermore, Steele *et al.* (1988) found inter-test variation to be a 5.3% variation and intra-trial variation (those between same subject and different operator) to be small, 4.3%. This suggests the procedure (involving repositioning of the subject) yields reasonable consistency. Steele *et al.* (1988) also used aluminum test bars of various lengths and diameters to simulate long bones and rubber pads and rubber bands to simulate soft tissue. The results of these tests were reasonable with an error of less than 10 %. The relationship between bone stiffness of the ulna and physical activity has also been investigated. Myburgh *et al.* (1993) examined ulnar EI and BMC in recreational athletes and found highly active individuals ($\geq$ 5 exercise sessions/wk) had

significantly higher EI and BMC values than moderately active and sedentary individuals.

Reliability for ulnar bone stiffness has been demonstrated; however, no studies have looked at tibial bone stiffness and the MRTA. Roberts and Hutchinson (1996) excised non-human primate tibias to directly validate the use of the MRTA to assess long-bone mechanical properties, however intra- and inter-trial reliability for MRTA measurements of the tibia have not been established. Recently, in our human performance laboratory, a pilot study assessing intra-trial reliability (five repeated trials, n=16) yielded high r-values for EI ($\geq$ 0.96). Thus, it appears that the MRTA has the potential to accurately assess the geometric and mechanical properties of human long bone *in vivo* and perhaps provide insight into the role of exercise and the mechanism of stress fractures.

Statement of the Problem

The purpose of this investigation was to determine the intra-trial and inter-trial reliability for MRTA measurements of the tibia *in vivo* in healthy women.

Significance of the Study

For a long time, researchers have been seeking methods to reliably assess bone quality and fracture risk *in vivo*. Currently, the gold standard for non-invasively estimating bone strength is accomplished through the use of absorptiometry; however, studies have shown that DXA does not precisely determine fracture risk (Gardsell, Johnell, & Nilsson, 1989; Steele *et al.*1988; McCabe, Zhou, Steele & Marcus, 1991; Myburgh *et al.*, 1992; Myburgh *et al.*, 1993). Assessing the geometric and mechanical properties of long bone would allow for better clinical evaluation of the potential on

stress fractures associated with bone diseases such as osteoporosis. The MRTA test is appealing clinically because it is cheap, takes up little space, is safe and comfortable for the patient, and takes only seconds to complete.

Stress fractures of the tibia are a major problem among women athletes and military recruits. To note, Pester and Smith (1992) reported a significantly higher stress fracture incidence in women when compared to men in similar basic training. Consequently, the validation of a reliable method of testing the bone strength/fragility of the tibia via the MRTA would be of clinical importance. These findings could have substantial clinical ramifications in military recruits and athletes who have risk factors and/or are prone to stress fractures. Stress fractures in these individuals often result in the loss of training time and an increase in the cost of medical care. The ability to identify individuals who are at high risk could translate into substantial monetary savings and military troop productivity. In addition, identification of individuals who are at high risk for stress injuries should not only help prevent stress fractures, but also decrease the recovery period.

Research Hypotheses

H_o (1): Intra-trial MRTA measurements of the tibia will not differ following five repeated trials without repositioning of the subject.

H_o (2): Inter-trial MRTA measurements of the tibia will not differ following five repeated trials over three non-consecutive days of analysis.

Delimitations

1. Subjects were volunteer women attending Virginia Tech and were between the ages of 18 and 28 yr.
2. Subjects in the sedentary group were not involved in any weight-bearing exercise (defined as < 1 session/wk) for at least 6-mth prior to the study.
3. Subjects in the active group were involved in weight-bearing exercise (defined as $\geq$ 4 sessions/wk lasting at least 30 minutes) for at least 6-mth prior to the study.
4. Subjects must have had no medical problems or taking medications that may have affected bone structure.
5. Subjects were consuming at least 1200 mg of calcium/day.

Assumptions

1. Subjects accurately answered the medical/health history questionnaire and correctly reported their 6-mth physical activity status prior to the study.
2. Subjects complied with all pre-testing instructions, specifically no exercise 12 hr prior to testing.
3. The MRTA was mechanically zeroed before each subject was tested to achieve accurate testing throughout the study period.
4. Subjects were positioned correctly and accurately on seated apparatus when compared to previous trials.
5. Each subject relaxed their limb and did not move after the probe/shaker was positioned on the tibia.

Limitations

1. The design of the study did not allow subjects to be randomly assigned to either exercise or non-exercising groups.
2. Subject group was not representative of the entire Virginia Tech undergraduate student population of women.
3. The study did not separately take into consideration the effect of different types of activity, e.g. running, soccer and/or resistance training, on the stiffness of the tibia.
4. Only measured sedentary and highly active individuals, not moderate or mild activity (2-3 exercise sessions/wk).
5. Subject group was not representative of the male population.

Definitions of Symbols and Terms

1. Accelerometer – measures the response of the bone at the point of excitation.
2. Active subjects – subjects who had involved in an exercise program ($\geq$ 4 day/wk for $\geq$ 30 min/session) for at least 6-mo prior to the study.
3. Anisotropy – Orientation of the bone microstructure with respect to the direction of loading.
4. Area moment of inertia – With respect to bending loads, index that takes into account the cross sectional area and the distribution of bone tissue around a neutral axis (Bennell *et al.*, 1999).
5. Bending stiffness or average cross sectional bending stiffness (EI) – Product of Young's modulus of elasticity and cross-sectional moment of inertia, usually expressed in Newtons per meter squared. Derived from the seven-parameter model

using the relation $K_b = 48EI/L^3$, where K_b is the transverse bending stiffness and L is the length of the ulna or tibia (Roberts *et al.*, 1996).

6. Bone mass – the amount of bone tissue in a bone, usually the volume minus the marrow cavity.
7. Cross sectional area moment of inertia – cross-sectional area and the distribution of the bone tissue around a neutral axis.
8. Dual x-ray absorptiometry (DXA) – a two-dimensional x-ray that measures bone mineral density, bone mineral content, and bone width.
9. Fatigue fracture – any fracture that follows two or more load applications. Usually, these stress fractures follow thousands and thousands of load applications resulting from repetitive-load activities.
10. Impedance (response) Curve – this represents the quantitative fundamental mechanical properties of the bone, i.e. bone stiffness and mass. The ratio of force to displacement, which is obtained from the impedance by multiplying by frequency.
11. Impedance head – measures force and acceleration and relays characteristics to the signal analyzer.
12. Load – any mechanical force on a bone.
13. Microdamage (MDx) – microscopic fatigue damage in bone often caused by repeated strain from repetitive activity. This damage degrades the physical integrity of the tissue's mineralized collagen.
14. Microdamage threshold (MESp) – the strain range that begins to cause too much MDx to repair. Beyond this threshold, MDx begins to accumulate and can cause a fracture; centered around 3000 microstrain.

15. Microstrain – units used to express strain in bone. One-thousand microstrain in compression would shorten a bone by 0.1% of its original length, 10,000 microstrain would shorten it by 1% (Frost *et al.*, 1998)
16. Modal analysis – experimental determination of natural frequencies, mode shape and damping nature.
17. Neutral axis – Axis where stresses and strain on the tibia are measured at zero (Bennell *et al.*, 1999).
18. Osteoblast – a connective tissue cell that forms and builds bone.
19. Osteoclast – a large multinuclear cell of hematopoitic origin that degrades and reabsorbs bone. This is important to the development, growth, maintenance and repair of bone.
20. Quality (of bone) – the number of, spacing and connectivity of trabeculae.
21. Remodeling period – the length of time between resorption of old bone and formation of new lamellar bone. This process usually takes place in 3 months in healthy bone and as long as 1 year in diseased bone.
22. Resorption – the loss of bone by osteoclastic degredation.
23. Sedentary – subjects who had not been involved in structured exercise ($\leq$ 1 day/wk) for at least 6 months prior to the study.
24. Seven parameter model – original model (designed by Steele *et al.*, 1998) used to interpret results using MRTA to determine cross-sectional bending stiffness. The model accounts for the mass, stiffness and damping of soft tissue, skin damping, and the mass, axial stiffness, and damping of bone.

25. Six parameter model – newer model (designed by Roberts *et al.*, 1996) used to interpret results using MRTA to determine cross-sectional bending stiffness. The model consists of the effective bending stiffness, damping, and mass of both the bone and soft tissue.
26. Stiffness – the resistance of bone to strain under a load. Stiff materials strain less than less stiff material under the same load. Dividing the load/stress by the corresponding strain can define stiffness (Young's modulus of elasticity). Stiffness is not the same as strength, i.e. chalk is stiff but weak, while rubber is not as stiff, yet stronger (stiffness is an objects ability to be elastic or flexible).
27. Strain – the deformation or change in shape caused by a load on structural material, e.g. bone. Strain can include stretching, shortening, twisting, and/or bending.
28. Stress – the elastic resistance of the bone (specifically trabeculae) to be stretched by strains. Loads placed on the bone initially cause strain, which in turn causes stress. Types of strain and stress applied to bone include tension and compression.
29. Sufficiency (S) – the load capability divided by body weight, i.e. the number of body weights the bone can support in axial load: $S = P_{cr}/BW$.
30. Vibrating shaker/probe – contains both the vibration source and the impedance head. Emits the frequencies ranging from 60-1600 Hz to the midpoint of the long bone.
31. Viscoelasticity – Characteristic of bone; bone tissue which is loaded more rapidly absorbs more energy than bone which is loaded at a slower rate.
32. Wolff's Law – bone accommodates the loads placed on it by alteration of its mass and distribution of mass. (Marcus, 1999).

33. Young's modulus of elasticity – the stiffness of a material. Found by dividing the load or stress by the corresponding strain; $E = \sigma/\epsilon$, where σ is stress and ϵ is strain.

Summary

Despite the use of absorptiometry to determine fracture risk, additional measurement methods that elicit information regarding the geometry and mechanical properties of bone *in vivo*, may prove to better predict fracture risk. A vibration analysis method, MRTA, is an established method for assessing the mechanical properties of long bone in human ulnas. Currently there is a need to further study the MRTA and its ability to reliably measure the human tibia and provide insight into fracture risk of the lower leg.

Thus, this study sought to determine the intra-trial and inter-trial reliability for MRTA measurement of the tibia *in vivo* in healthy young college-aged women.

Chapter II

Review of Literature

Introduction

This study sought to determine the intra- and inter-trial reliability for MRTA measurement of the tibia, *in vivo*. In addition, the change in bone stiffness among sedentary individuals and recreational athletes was studied. The purpose of this chapter is to provide the reader with: a) a review of the etiology, prevalence, and site-specific occurrence of stress fractures as a result of physical activity; b) a review the mechanisms of bone remodeling and the importance of the geometric properties of bone; c) an examination of current assessment tools for stress fracture risk, and d) an introduction to MRTA, a non-invasive method of determining fracture risk in human long bones.

Stress Fractures and Physical Activity

Stress injuries of the bone frequently occur in military recruits (Cline, Jansen & Melby, 1998), athletes, recreational athletes, and are associated with repetitive weight-bearing activity (Bennell & Brukner, 1997). It is proposed that stress fracture development involves both repetitive loading and resulting bone fatigue; however, the direct mechanisms by which exercise leads to these changes in bone is not fully understood. Currently, two possible scenarios regarding bone fracture are hypothesized. The primary microdamage theory suggests that fractures occur when microdamage production exceeds repair while the primary remodeling hypothesis (see Figure 1; Bennell, Malcolm, Wark & Brukner, 1996) proposes irreversible microdamage occurs when the bone is at a susceptible or weakened state during

osteoclastic resorption (Bennell *et al.*, 1996). Additionally, individuals may have risk factors that increase their risk for stress injuries. These risk factors include menstrual disturbances, inadequate dietary calcium, reduced caloric intake, disordered eating, lower bone density, muscle weakness, female gender, Caucasian, low body weight and intrinsic/extrinsic mechanical factors such as poor skeletal alignment and/or footwear (Nattiv & Armsey Jr, 1997; Bennell, Matheson, Meeuwisse & Brukner, 1999).

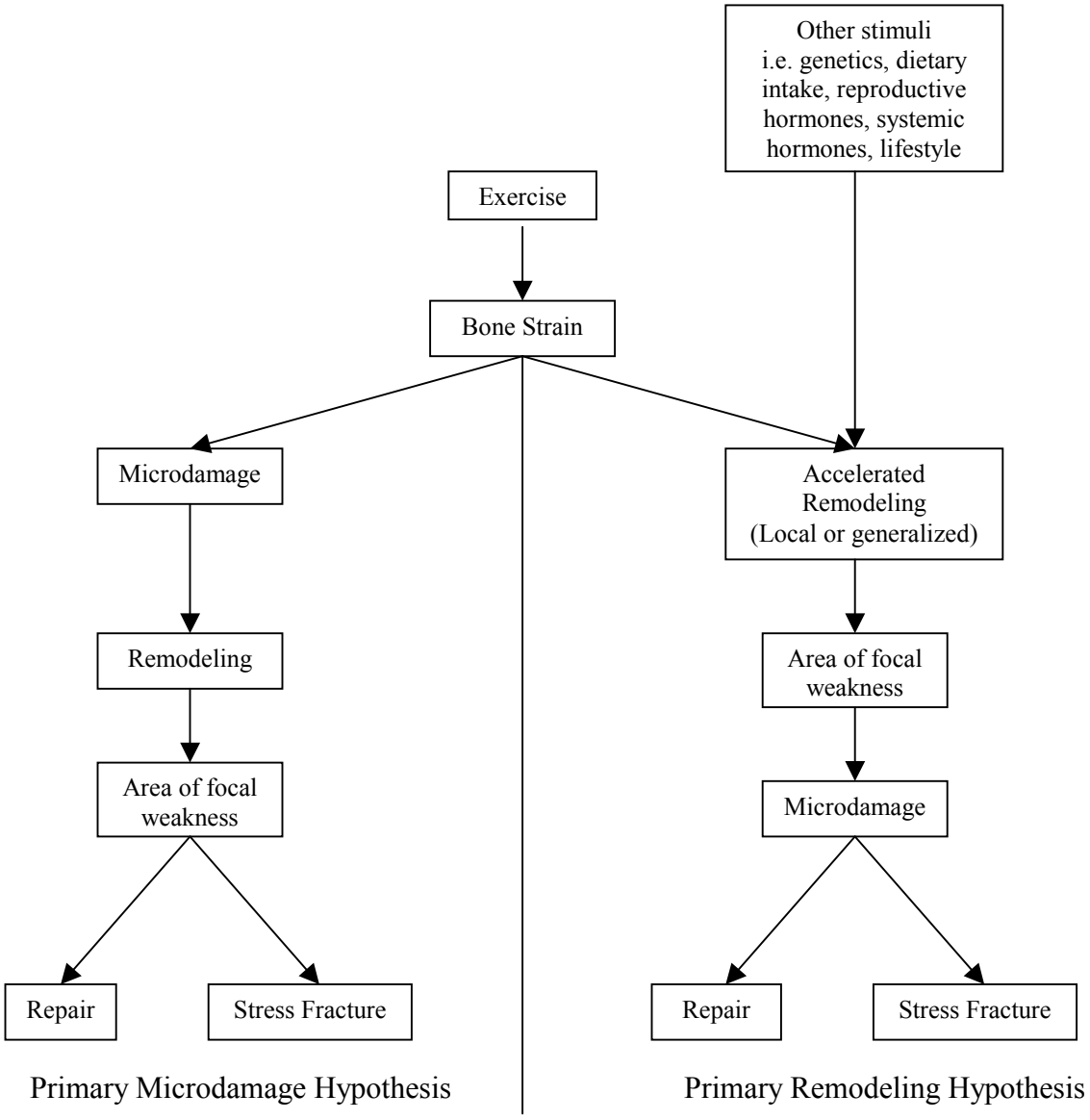

Figure 1. Diagrammatic representation of possible mechanisms for stress fracture development: primary microdamage hypothesis and primary remodeling hypothesis.

A number of studies with varying designs have shown that positive bone adaptation occurs with exercise (Fujimura *et al.*, 1997; Heinonen *et al.*, 1993, Fehling, Alekel, Clasey, Rector & Stillman, 1995). However, it has been suggested that too much activity or overuse may lead to structurally damaged areas within the bone known as stress fractures. Marguilies *et al.* (1986) investigated the effect of intense physical activity on BMC of the tibia in 268 young male and female adults and found high levels of bone loading resulted in a high number of stress fractures. Although stress fractures have been detected in all areas of the skeleton, stress fractures occur more commonly in the bones of the lower extremity, specifically the tibia (Bennell *et al.*, 1997). Matheson *et al.* (1987) analyzed 320 athletes with scan-positive stress fractures and found that 99.4% of all stress injuries occurred in the lower extremities with 49.1% of these injuries appearing at the tibia. These results are similar to other studies investigating anatomic distribution of stress fractures in athletes and military recruits (Bennell, Malcolm, Thomas, Wark & Brukner 1996; Courtenay & Bowers, 1990; Beck *et al.*, 1996; Johnson, Weiss & Wheeler, 1994). On the other hand, Brukner, Bradshaw, Khan, White & Crossley (1996) reviewed 180 stress fractures cases and found metatarsal (23%) fractures a more common site than the tibia (20%); however, the type of sport (running, dancing, rugby, track and field) and population (recreational vs. athletic) in this study varied from that of Matheson (1987). Furthermore, Brukner's group found tibial fractures were more common in athletes participating in track and distance running, events consisting of repetitive loading of bones in the lower extremities. In a study conducted by Bennell, Malcolm, Thomas, Wark, & Brukner (1996) the incidence and distribution of stress fractures in 111 track

and female athletes was evaluated. Twenty-six stress fractures were sustained with the most common site being the tibia (46%). These results are consistent with studies that show runners have a high bone turnover (Hetland, Haarbo & Christianson, 1993) and following long bouts of running, a temporary stimulation of bone resorption and inhibition of bone formation (Malm, Ronni-Sivula, Viinikka & Ylikorkala, 1993; Brahm, Strom, Piehl-Aulin, Mallmin, & Ljunghall, 1997; Brahm, Piehl-Aulin & Ljunghall, 1996). A period of transition that may lead to stress injury with repeated bouts of exercise or loading of the bone.

Bone Remodeling and the Response of Bone to Loading

Throughout life bone is continually remodeling by adapting its bone structure to changes in skeletal loading, specifically to activity involving repetitive movements. This remodeling process occurs by way of specialized cells in bone known as osteoblasts and osteoclasts. Important to the development, growth, maintenance, and repair of bone, osteoclast cells degrade bone tissue by resorption, while osteoblastic cells form the mineralized matrix of bone. This process of replacing old bone with new bone takes 3 months or more, a duration labeled the "remodeling period" (Frost, 1998). In the adult skeleton, osteoclastic resorption and osteoblastic bone formation maintain and balance the amount of bone. Thus, the ability of weight bearing bones to constantly change structure and adapt to mechanical stress suggests mechanical loads from exercise help regulate resorption and formation (Rodan, 1998).

The two mechanical forces that regulate bone remodeling are compressive and tensile strains. Compressive forces are resisted by the mineral component of bone and the tensile forces resisted by the collagen component; an adaptive remodeling process

which is expressed in Wolff's law (Nattiv *et al.*, 1997). Simply, Wolff's law states that bone accommodates to loads by altering its amount and distribution of mass; when loading increases, bone is gained and when loading decreases bone is lost (Marcus, 1999). Adaptation of bone is a function of the number of loading cycles, cycle frequency, amount of strain, strain rate, and strain duration per cycle. Increased load from running, military marching, and other recreational/athletic activities should increase formation and decrease resorption, while unloading or decreased activity should have the opposite effect. However, if excessive strain is applied to bone during physical activity, especially strain involving repetitive motion, a fatigue or stress fracture may occur.

Currently, the etiology of stress injuries is unclear, however, there is building evidence that stress fractures occur when microdamage production (osteoclast activity) exceeds osteoblastic repair (Bennell & Brukner, 1996). Several studies (Dook, James, Henderson & Price, 1997; Duppe, Gardsell, Johnell, Nillsson & Ringsberg, 1997; Valdimarsson, Kristinsson, Stefansson, Valdimarsson & Sigurdsson, 1999; Madson, Adams & Van Loan, 1998) have shown that physical activity increases BMD and may reduce subsequent fracture risk. However, repeated strain (overuse) imposed by chronic weight-bearing activity, such as those found in athletes and military training may cause microscopic fatigue damage or microdamage (MDx) within the bone. Other studies have demonstrated microdamage following repetitive loading *in vivo* (Frost, 1989; Mori & Burr, 1993; Schaffler, Radin & Burr, 1990).

The strain range that causes too much MDx to repair, called the MDx threshold range is approximately 3000 microstrain (Burr *et al.*, 1997). Strain gauge

studies have shown that large compression and tension forces are created in the tibia with walking and running (Lanyon, Hampson, Goodship & Shah, 1975). Burr *et al.* (1996) implanted strain gauges on the tibia in two subjects and found strain in the tibia during running (2000 microstrain) to be well below the fracture threshold. Frost (1989) investigated both clinical and pathological fatigue studies and also suggests this threshold is approximately 2000 microstrain. These results suggest that bone is unable to adapt or compensate to the increased stain of specific weight-bearing activities; bone microdamage occurs, and thus further loading to the bone results in a stress fracture (see Figure 2, McCabe, Zhou, Steele & Marcus, 1991). Moreover, studies have shown that excessive microstrain decreases the stiffness of bone which leads to microcrack accumulation within the bone, impairing the mechanical properties by reducing the bones elastic modulus (Burr *et al.*, 1998).

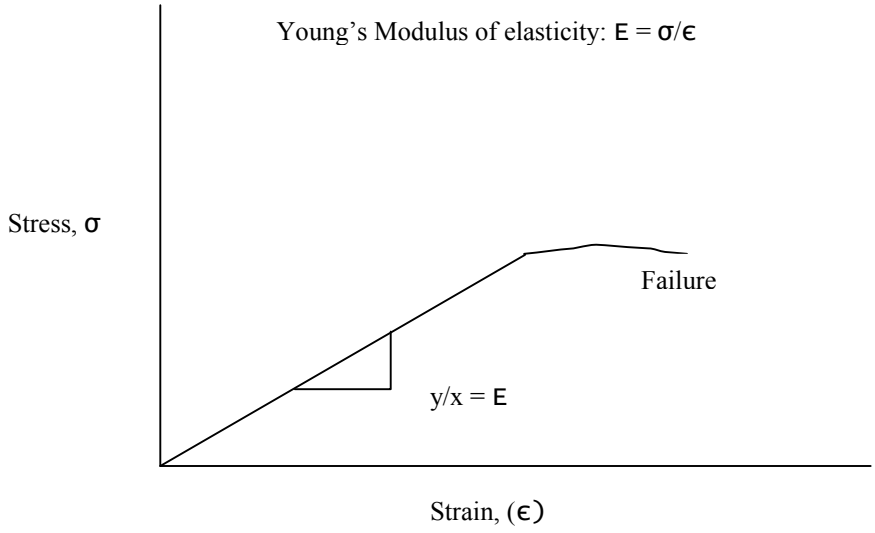

Figure 2. Stress-strain diagram illustrating the deformation (strain) induced by progressive force per unit area (stress). Young's modulus of elasticity defines the slope of this relationship within the region of elastic deformation.

Additional Factors that Influence Bone Loading

Besides the response of bone to loading, a number of additional factors influence the ability of bone to resist fracture. These conditions of bone include geometry, material density, quality, and the activity of muscles attached to bone (Bennell *et al.*, 1999).

The geometry of bone is important in determining its overall strength. During weight-bearing physical activity (e.g. running), tension and compression loads are very high. The compression and bending strength of the bone during weight-bearing activity is proportional to the bone cross-sectional area (Bennell *et al.*, 1999). The larger/wider the bone, the greater the surface area to distribute internal forces, thus, decreasing strain and resisting the probability of stress related injuries. Narrower cross-sectional area should exhibit less resistance. Other important geometric features include the cross-sectional area and the distribution of bone tissue around a neutral axis (axis where stress and strain is the lowest). The area moment of inertia is the index that takes into account the previous factors in respect to bending loads. A larger area moment of inertia is more efficient in resisting bending because bone tissue is distributed farther away from the neutral axis of the bone. Additionally, the length of a bone influences its bending strength. The longer the bone, the greater the magnitude of the bending moment caused by force or the movement when a limb moves through a range of motion (an application of force). Long bones of the lower extremity, especially the tibia are subjected to high bending moments. When a bone is bent, one surface of the bone is subject to compression while the opposite surface to tension. (Bennell *et al.*, 1999).

Investigations using cadaver bone specimens have shown that bones with higher density are stronger (Alho, Torstein & Hoiseth, 1988). Carter and Hayes (1977) found the compressive strength of skeletal tissue was approximately proportional to the square of the bone density. This suggests small decreases or increases in bone density may lead to large changes in the overall strength of bone and its ability to withstand excessive strain. Besides bone density, bone quality plays an integral role in bone strength. The quality of bone refers to the number, spacing, and connectivity of trabeculae within the bone (Bennell *et al.*, 1999).

During the loading of bone *in vivo*, muscles attached to bone contract, which influence the stress magnitude and distribution upon the bone. Nordsletten *et al.* (1994) found intact soft tissue, as well as muscle contractions, substantially increased the structural capacity of the rat tibia. Thus, muscles and other soft tissues may attenuate the strain and force applied to bone. During intense activity when muscles become fatigued, the muscles may alter the distribution of strain within the bone. Yoshikawa and colleagues (1994) found an association between muscle fatigue and increased bone strain. The authors noted that bone strain increased when muscles became fatigued and the strain remained unchanged when the muscle was not fatigued.

Assessment of Risk for Fracture Using Dual Energy X-ray Absorptiometry

To date, researchers have assessed bone adaptation to exercise and fracture risk with dual energy X-ray and single photon absorptiometry (DXA) and quantitative ultrasound measurements (QUS), to name a few. The use of ultrasound has recently been developed to provide information on the architecture and elasticity of bone; however, studies have shown inconsistent results when comparing absorptiometry and

fracture risk (Hans, Schott & Meunier, 1993; Peretz, De Maertelaer, Moris, Wouters & Bergmann, 1999; Prins, Jorgensen, Jorgensen & Hassager, 1998; Jorgensen & Hassager, 1997). The DXA, a more established method of non-invasively estimating bone strength, elicits a two-dimensional X-ray measuring bone mineral density (BMD), bone mineral content (BMC) and bone width. (Madsen, Jensen & Sorensen, 1998; Sievanen *et al.*, 1996).

It has been suggested that low BMD reduces bone strength, thus contributing to the development of stress fracture by increasing the accumulation of microdamage with repetitive loading (Carter, Caler & Spengler, 1981). An association between fragility fractures and low BMD in osteoporotic individuals has been established and bone density has been used to predict the possibility of fracture risk in clinical settings (Cummings *et al.*, 1993; Melton, Atkinson & O'Fallen, 1993). However, unlike osteoporotic and elderly individuals, most active young adults have BMD within normal ranges if not higher (Bennell, Malcolm & Kahn, 1997). Bennell *et al.* (1996) suggests physically active people, compared to less active counterparts whose bones are subjected to lower forces, may require a higher level of BMD to withstand the high magnitude of strain involved with repetitive activity.

Although useful, studies have shown that DXA does not precisely determine fracture risk (Gardsell, Johnell & Nilsson, 1989) nor does it accurately predict the geometric and biomechanical characteristics of bone (Jarvinen, Sievanen, Kannus & Jarvinen, 1998). Giladi, Milgrom & Simkin (1991) compared the tibial BMD of 91 military recruits who developed stress fractures and 198 controls and found no difference between the two. Similar results were found by Cline *et al.* (1998) who

failed to find a difference in BMD between female soldiers with stress fractures and those without. On the other hand, Beck *et al.* (1997) studied 626 military recruits and found that the 23 individuals who developed stress fractures had significantly lower tibial and femoral bone density when compared to the control group. These results may be explained by the significant differences in body weight between the fracture and control groups. It is known that body size and composition are major contributors to BMD (Beck *et al.*, 1997; Cummings *et al.*, 1995; Ensrud *et al.*, 1997). Additional studies have reported contradictory results regarding stress fractures and lower BMD (Lander *et al.*, 2000; Myburgh, Hutchins, Fataar, Hough & Noakes, 1990; Carbon *et al.*, 1990; Bennell *et al.*, 1996; Bennell *et al.*, 1995). From these results, it appears that bone densitometry is not the best screening tool to predict fracture risk in healthy, active individuals. Moreover, DXA measurements are expensive, require extensive operator training and expose the subject to radiation.

Geometric and Material Properties of Bone

Additional characteristics of bone, such as bone architecture and bone geometry also effect the mechanical strength of bone (Haapasalo *et al.*, 1996). In general, the strength/stiffness of a structure for a specific mode and rate of loading is its shape and size and the strength of the material within it. The amount of load a bone can withstand before breaking is proportional to the cross-sectional area (shape and size factor) of the bone. For bending loads, the cross-sectional moment of inertia and cross-sectional area reflect the strength of the bone (Martin, 1991). Recently, geometric parameters have been shown to provide improved ability to predict fracture risk *in vivo* (Beck *et al.*, 1996). Steele *et al.* (1988) suggests BMC is necessary for

proper stiffness but does not elicit the quality of bone that is indicated by bone stiffness. Important measurement parameters of bone quality include the elastic modulus, stiffness, strength, toughness and deformability. Studies have shown that a bone's material strength is not highly correlated to mineralization (Currey, 1990). This study further implies high or low BMD may not be an accurate indicator of fracture risk and suggests quality of bone to be of greater value when evaluating individual fracture risk. Two separate regions of bone may have similar porosity and mineralization, but embody highly variable and different material properties (Martin, 1991).

The geometric properties of long bones vary with age, gender and body size (Miller & Purkey, 1980). Moreover, there is a greater variation in structural geometry than in bone material properties, including BMD (Martens, Van Audekercke, De Meester & Mulier, 1981). Thus, differences in bone geometry may help answer individual predisposition to stress fractures. In a cross-sectional study by Crossely, Bennell, Wrigley & Oakes (1999), no difference was found in tibial BMC and BMD between runners with and without stress fractures. Additionally, those with a stress fracture had a significantly smaller tibial cross-sectional area (adjusting for body mass and height) than those without stress fractures. These finding support the idea that bone geometry plays a significant role in the detection of individuals with stress fractures. In a prospective study of 295 infantry recruits, Giladi and colleagues (1987) measured radiographically the mediolateral width of the tibia at 3 different levels in the bone. The 31% of recruits who developed femoral, tibial, or foot stress fractures had narrower mediolateral tibia widths than those recruits without stress fractures.

The authors suggest the size of the tibia is an indication of the size of the tubular bones. Wider tibial width may be an indicator of a stronger skeletal structure that is biomechanically superior in bending strength and thus may be relatively protected from stress fractures in general.

Mechanical Response Tissue Analysis and Fracture Risk Assessment

A relatively new technique for bone assessment, the mechanical response tissue analysis (MRTA) is cheaper, takes up less space, does not expose individuals to radiation, and is easier to operate than densitometry. Unlike the DXA which measures BMD, MRTA yields geometric properties such as maximal bending stiffness (EI) and mechanical properties such as load-carrying capacity and sufficiency (S) of bone. These attributes of bone are determined by the MRTA via measuring the impedance response of a long bone to low-frequency vibration (Roberts *et al.*, 1996; Myburgh, Zhou, Steele, Arnaud & Marcus, 1992; Steele *et al.*, 1988). Instead of focusing only on resonant frequencies, the MRTA records the complete impedance curve of the bone, which is fitted to a seven-parameter model.

The sufficiency is the number of body weights the bone can support during axial loading (Steele *et al.*, 1988). Sufficiency is independent of size, age, and sex. Physically active individuals have high values, while sedentary individuals have low values. The maximal bending stiffness is the product of Young's modulus of elasticity (E) and the cross sectional moment of inertia (I). The bending stiffness yields a measure of long bone structural integrity, which is related to the composition, geometry, internal architecture, and quality of bone (Roberts *et al.*, 1996; Steele *et al.*, 1988). Milgrom *et al.*, (1989) found the bending strength of the tibia or its ability to

resist bending moments as measured by area moment of inertia is a good determinant of stress fracture risk. Several studies have shown a high correlation between EI and BMC (Jurist & Foltz, 1977). Additionally, Dhoerty, Bovill & Wilson (1974) compared two normal-healthy and one osteoporotic tibia and found the difference between the healthy and osteoporotic bone was 20% in resonant frequency and 80% in EI. The difference is that resonant frequency (ratio of stiffness to mass) decreases in both stiffness and mass in diseased bone. Kiebzak, Box & Box (1999) examined ulnar bending stiffness among normal and osteoporotic women and found the EI of osteoporotic women was 25% lower when compared to non-diseased, normal subjects. Thus, it appears that EI is an excellent indicator of bone quality and fracture risk.

The major components of the MRTA are the dual channel dynamic signal analyzer, the permanent magnetic vibration exciter, impedance head, two charge amplifiers, and vibrating shaker/probe. The flat, curved vibrating shaker/probe with impedance head is slowly applied to the tibia on the skin surface and resonant modes are generated throughout the bone via the dual channel dynamic signal analyzer. The impedance head provides the force and acceleration at the skin surface. The analyzer generates frequencies ranging from 60-1600 Hz on the tibia. Peterson (1977) studied intact non-human primate long bones and concluded a simple model of the intact limb is valid for the frequency range of 40-2000 Hz. The ulna (or any long bone) acts as a beam in bending, the skin as a spring, and the musculature provides heavy dampening. These frequencies (force and acceleration) are detected by the impedance head that relays the information to the signal analyzer and then to a computer where the data are processed. A complete impedance curve of the bone is recorded, on which a seven-

parameter model is fitted. The seven-parameter model accounts for the mass, stiffness, and damping of soft tissue; skin damping; and the mass, axial stiffness, and damping of bone. From the model the average cross-sectional EI of the entire bone is found using the relationship $K_b = 48EI/L^3$, where K_b is the transverse EI at the site of the measurement and L is the length of the long bone measured by the operator. The analyzer yields the impedance curves that are obtained in a few seconds on the Hewlett-Packard 3562A Dynamic Analyzer. Instead of the standard impedance curve, which is the ratio of force to velocity, Steele *et al.* (1988) found it most dependable and easier to work with the stiffness, the ratio of force to displacement, which is obtained from the impedance by multiplying by frequency.

Accurate non-invasive testing of long bone *in vivo* is made difficult because measurement of the bone must take place on the outer surface of the skin. A problem in the interpretation of vibration measurement *in vivo* is the influence of the muscle, skin and joints. Using modal analysis, Van der Perre, Martens & Mulier (1983) identified the different bending modes of the human tibia *in vivo*. When comparing the differences between the natural frequencies *in vivo* with those of the freshly excised tibia, the group found the muscles surrounding the tibia accounted for most of the variation, not the skin and joints. Cornelissen and colleagues (1986) evaluated the influence of soft tissue and joints by modal analysis and similarly found the skin has little effect on the mode of tibial vibration. The author suggests the skin adds little mass and no stiffness to the tibia; however, Roberts *et al.* (1996) finds that the soft tissue does significantly mask the response of the underlying bone. The MRTA adjusts for this by modeling for the behavior of the soft tissue. Originally a seven-

parameter model was utilized (Steele *et al.*, 1988); however, an improved six-parameter model was developed by Roberts and colleagues (1996). The six-parameter model contains a more physiologically realistic representation of the soft tissue when compared to the original seven-parameter model; however, only negligible difference was seen between the two models when compared in humans.

Other difficulties of the skin such as its ability to alter the acceleration signal may influence accurate measurement (Cornelissen *et al.*, 1986; Saha & Lakes, 1977). Applying a spring-loaded pre-load to the accelerometer reduces the effects of the skin. Nokes, Fairclough, Mintowt-Czyz, Mackie & Williams, 1984) first investigated the effect of soft tissue on the signal recorded by a skin-mounted accelerometer and found for a skin thickness of 2 mm the allowable range of pre-load is from 3.8 – 5.2 N with an optimal value at 4.2 N. While valuable this does not take into consideration changes that may occur with a thicker or thinner layer of skin. Steele *et al.* (1988) found that skin mass and the parallel damping element are significant for subjects with a thicker layer of soft tissue. The group designed the weight of the shaker to provide a static pre-load of about 10 N that causes the skin stiffness to be about 2-4 times that of the bone.

Currently, studies have focused on techniques that directly measure ulnar bending stiffness with the MRTA (Myburgh *et al.*, 1993; Roberts *et al.*, 1996; Steele *et al.*, 1988; McCabe, Zhou, Steele & Marcus, 1991). A difficulty with the MRTA has been designing a measurement system that produces reproducible results. Steele *et al.* (1988) measured the right and left ulna of 80 subjects and found that the bone mineral content and bone stiffness correlate well ($r = 0.81$) and when sufficiency is taken into consideration the

association with BMC increases (r = 0.89). Furthermore, Steele *et al.* (1988) found inter-test variation to be $\pm$ 5.3% variation and intra-trial variation (those between same subject and different operator) to be small, $\pm$ 4.3%. This suggests the procedure (involving repositioning of the subject) yields reasonable consistency. Steele *et al.* (1988) also used aluminum test bars of various lengths and diameters to simulate long bones and rubber pads and rubber bands to simulate soft tissue. The results of these tests were reasonable with an error of less than 10 %. The relationship between bone stiffness of the ulna and physical activity has also been investigated. Myburgh *et al.* (1993) examined ulnar EI and BMC in recreational athletes and found highly active individuals ($\geq$ 5 exercise sessions/wk) had significantly higher EI and BMC values than moderately active and sedentary individuals.

Reliability for ulnar bone stiffness has been demonstrated, however no studies have looked at tibial bone stiffness and the MRTA. Roberts and Hutchinson (1996) excised non-human primate tibias to directly validate the use of the MRTA to assess long-bone mechanical properties, however intra- and inter-trial reliability for MRTA measurements of the tibia have not been established. Recently, in our human performance laboratory a pilot study assessing intra-trial reliability (five repeated trials, n=16) yielded high r-values for EI ($\geq$ 0.96). Thus, it appears that the MRTA has the potential to accurately assess the geometric and mechanical properties of human long bone *in vivo* and perhaps provide insight into the role of exercise in the mechanism and etiology of stress fractures.

SUMMARY

In summary, stress fractures frequently occur in athletes and military recruits. These stress injuries are found throughout the skeletal structure; however, they are more commonly detected in the tibia. The direct mechanisms by which exercise leads to changes in bone metabolism are not fully understood, but two theories have been hypothesized; the primary microdamage and primary remodeling theory. Bone adapts to loads imposed on it by altering its mass and distribution of mass; however, excessive stress/strain (even below the human physiological strain range) through repetitive loading can induce bone loss leading to stress injuries. This excessive strain placed upon the bone decreases the strength of the bone, impairing the mechanical properties by reducing the bones elastic modulus. Mechanical properties such as architecture and geometry play an important role in long bones overall strength and have been shown to provide a better understanding about stress fracture risk *in vivo*. The DXA elicits a measurement of BMC and BMC; however, these do not reflect the quality of bone (elastic modulus, stiffness, strength, toughness and deformability) that is necessary to evaluate risk for fracture. Unlike DXA, MRTA yields bending stiffness, a measure of long bone structural integrity that is related to the composition, geometry, internal architecture, and quality of bone. Currently studies have focused on techniques that directly measure ulnar bending stiffness; however, it appears a technique for measuring bending stiffness of the tibia may prove to be beneficial to both athletes and military personnel, as well as to patients with low BMD. The tibia, which is centrally involved in many human activities, may have implications for understanding bone remodeling and exercise-related clinical problems,

e.g. stress fracture of the lower leg. This study investigated test-retest measurement reliability of tibial bending stiffness in humans using MRTA.

Chapter III

Mechanical Response Tissue Analysis: Measurement Reliability for Bending Stiffness of the Human Tibia in College Aged Women

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ABSTRACT

Mechanical Response Tissue Analysis (MRTA) is an emerging technology for assessing maximal bending stiffness (EI) of human long bones *in vivo*. The MRTA variable, EI, is the product of Young's modulus of elasticity (E) and cross-sectional moment of inertia (I). EI quantifies material and architectural/geometric properties of bone. Published human research using MRTA to measure EI has been limited to the ulna; however, the tibia requires further investigation due to its central involvement in many human activities and exercise-related clinical problems, e.g. stress fracture of the lower leg. To evaluate the inter- and intra-reliability of tibial EI, 22 healthy women (X $\pm$ SD: 20.8 $\pm$ 1.8 yr) were assessed twice daily for three non-consecutive days. Each daily session consisted of five repeated trials. The ulnar EI protocol of McCabe *et al.* [J Bone and Mineral Res. 1991;6(1):53-59] was adapted to assess tibial EI via MRTA. A significant difference was not found in scores for five repeated trials taken consecutively on the same day. Mean scores for EI were higher on day 1 (59.1 $\pm$ 35.5 $N \cdot m^2$, $p < 0.05$), compared to day 2 (46.9 $\pm$ 22.3) and day 3 (49.9 $\pm$ 18.3). Individual trial mean scores for EI on each day (mean of 5 trials) were highly correlated, R^2 = 0.84, 0.62, and 0.79 (set 1 vs. 2, for day 1,2,3, respectively) and the average percent change between sets 1 and 2 on each day was 5.3. The inter-test (between day) reproducibility was found to be low and unacceptable, 11.7, 18.3, and 1.3 %, for day 1 vs. 2, 1 vs. 3, and 2 vs. 3. Poor inter-day reliability may be a result of the inability, at the time of this study, to apply the best computational EI model. It is concluded that tibial bone stiffness measurements with the MRTA are in the range of acceptability for same day inter- and intra-trial reliability when the 7-parameter analytic model of vibratory properties developed by McCabe *et al.* is used.

Keywords: mechanical response tissue analysis, bending stiffness, measurement reliability, tibia, mechanical testing

INTRODUCTION

Several studies have shown that bone mineral density (BMD) and bone mass increase as a function of weight bearing exercise (1-3). However, excessive repetitive loading of bone, e.g. running, especially within a short period, may lead to microcracks within the bone and ultimately to stress fractures. Stress injuries frequently occur in military recruits (4-6) and in both recreational and competitive athletes (7). Studies investigating anatomic distribution of stress fractures in both athletes and military personnel reveal fractures commonly occur in the tibia (8-13). To date, researchers have relied heavily on dual energy X-ray absorptiometry (DXA) to assess risk of bone fracture. While useful for assessment of fragility fractures in osteoporotic individuals, planar DXA measurements do not precisely determine fracture risk (14) nor do they accurately predict the geometric and mechanical characteristics of bone (15). Furthermore, studies have shown a bone's material strength is not highly correlated with its mineralization (28), and many studies have reported contradictory results when investigating an association between stress fractures and low BMD (16-20). Bone architecture/geometry (size, shape, and location of tissue within the bone) and bone material properties (bending stiffness and density) have been shown to more accurately predict fracture risk *in vivo* (8). A relatively new, non-invasive technique for long bone evaluation, mechanical response tissue analysis (MRTA), yields the maximal bending stiffness (EI) and the load carrying capacity of bone. The EI is the product of Young's modulus of elasticity and cross-sectional moment of inertia. The EI is a measure of long bone structural integrity, which is related to the composition, geometry, internal architecture, and quality of bone (21,22). The bending strength of the tibia or its ability to withstand bending moments as measured

by area moment of inertia has been found to be a good determinant of stress fracture risk (23). Thus, it appears that EI is an excellent indicator of bone quality and fracture risk.

To date, published human research relating bone stiffness to physical activity has been limited to the ulna. However, the tibia is centrally involved in many human activities, and tibial EI may have implications for understanding bone remodeling and exercise related clinical problems, e.g. stress fracture of the lower leg. Roberts and Hutchinson (21) excised non-human primate tibias to directly validate the use of MRTA to assess long bone mechanical properties. The authors found that *in vivo* scores for EI in the tibia of non-human primates correlated with fracture threshold when placed under stress *in vitro*. To our knowledge, measurement reliability for MRTA values for the human tibia have not been established. Recently in our human performance laboratory, a pilot study with both male and female subjects assessed intra-trial reliability (five repeated trials, n=16) yielded high r-values ($\geq$ 0.96) for EI (31). Thus, it appears that MRTA has the potential to yield consistent values for EI which may provide inferences about geometric and mechanical features of human long bones *in vivo*. Perhaps MRTA will provide a useful tool for the non-invasive evaluation of bone strength in different health and disease states and in response to various experimental treatments designed to enhance bone strength. The ability to use the MRTA as a reliable and sensitive tool for EI assessment would be of clinical significance for both military personnel and athletes. Additional studies investigating the effects of medical treatment of fractures, osteoporosis, and the identification of internal risk factors could be explored.

Thus, the purpose of this investigation was to determine the intra-trial and inter-trial reliability by MRTA measurements of the tibia *in vivo*, in healthy young women.

METHODS

Subjects

Twenty-three healthy female subjects (X $\pm$ SD: 20.8 $\pm$ 1.8 yr) with body mass index (BMI = wt in kg/ht in m^2) of less than 30 volunteered to participate in this study. The Institutional Review Board of Virginia Polytechnic Institute and State University (VPISU) approved the study protocol. All participants were instructed regarding experimental procedures, and written consent was obtained from each subject. Subjects completed a health history, dietary calcium intake, and an activity questionnaire to determine their approximate number of exercise sessions/wk over the previous 6 months. Subjects were screened to exclude individuals with previous injuries (fractures, surgeries, etc.) to the test limb, low dietary calcium intake ($\leq$ 1200 mg/day), and/or the use medications known to alter bone metabolism.

To evaluate the inter- and intra-reliability of tibial EI, subjects reported to the Laboratory for Health and Exercise Science at VPISU once daily for three non-consecutive days. A tibial EI measurement was termed a trial (T) and a group of five trials (T_1, T_2, T_3, T_4, T_5) completed during one measurement session was considered a set (S). Each testing day consisted of the completion of two sets of five serial measurements and was designated a testing session (D). Each set was separated by 3-min of rest in which the test leg did not have contact with the vibrating shaker/probe of the MRTA. During this 3-min period, subjects were repositioned, i.e. the MRTA probe was removed from the leg and then repositioned in contact with the probe after 3-min. The MRTA results are presented as five consecutive readings taken at the same sitting (mean $\pm$ Nm^2). In addition, MRTA results were calculated by discarding the high and low scores from

each set of five measurements and then calculating the mean value of the remaining three scores.

Measurements

Each subject had height, weight, and tibial length measured. Height and weight (to the nearest 0.1 cm and 1.0 kg, respectively) were measured with the subject wearing minimal clothing (short sleeved shirt and shorts) and without shoes or socks. All measurements were taken on the dominant leg and this was determined by asking each subject which leg they used to kick a ball. While subjects sat relaxed with limb resting freely, an anthropometer was used to measure their tibial length from the inferior edge of the medial malleolus to the medial condyle of the tibia. Then, the midpoint of the anterior tibial crest was located, using these distal and proximal points and marked with a pen. The midpoint on the anterior tibial crest was then measured with the anthropometer and marked as the anterior tibial crest site for EI assessment with the MRTA. Subjects then sat with legs resting at body level or assumed a supine position for 30 min to guard against measurement variations due to leg hydrostatic pressure.

Bending Stiffness Testing

Mechanical response tissue analysis [MRTA: Stanford University Engineering Group, Palo Alto, CA (22)] was used to determine tibial EI. The ulnar EI protocol of McCabe *et al.* (24) was adapted to assess tibial EI. Before subject testing, the analyzer was calibrated by zeroing the acceleration signal from the impedance head. Specifically, fitting a flat imaginary (rough) response line over the real (smooth) response line. After calibration of the MRTA, each subject was seated with the knee and hip flexed at 90°, relaxed, slightly restrained by the apparatus with heel/ball of foot slightly in contact with

the resting pad. The vibrating shaker/probe was placed at the midpoint of the tibial crest. The probe was maintained at a pre-load of 7-10g. (Pre-load causes compression of the skin; excessive or inadequate pre-load may cause a poor response curve fit and EI values to fluctuate from actual values). The probe was maintained in place to compress the overlying soft tissue for a period of 1 minute to reduce the elastic effects of the skin on the EI response. At 1-minute the shaker was activated. The placement (angle and location) and pressure of the probe on the tibia was the same for each subject's trials. The probe subjects the tibia to frequencies ranging from 60 to 1600 Hz and an impedance sensor in the shaker relays force and acceleration data to a signal analyzer. This information (force and acceleration) is relayed to a microprocessor that changes the data into a frequency domain which is analyzed by a 7-parameter EI regression prediction model which accounts for mass, stiffness, dampening by soft tissue, skin dampening and mass, axial stiffness, and dampening by bone. From the seven-parameter model, EI of the bone is derived using the following relation: $K_b = 48EI/L^3$, where K_b is the transverse bending stiffness at the measurement site and L is the length of the tibia. EI is expressed in N/m^2. Roberts *et al.*. (21) utilized a 6-parameter model in their study, validating the use of the MRTA on non-human primate tibias. The difference between the two models is the use of frequency weighting (giving different weights different frequency ranges) in the 7-parameter algorithm compared to the 6-parameter mode. The authors indicate that the 6-parameter model elicits a better physiological representation of the soft tissue, however they found insignificant differences between the 6- vs. 7-parameter models, when testing human ulnas *in vivo* (21).

Physical Activity and Calcium Intake Determination

Physical activity status was determined over the previous 6 months using the self-administered Godin Leisure-Time Exercise Questionnaire (25,26). The questionnaire assesses four attributes of activity pattern: weekly frequencies of strenuous, moderate, light activity, and frequency of weekly activity long enough to cause an individual to sweat. The total leisure activity score was calculated as the product of sessions/wk and the metabolic equivalents of the activities, i.e. METs for strenuous, moderate, and light activity were 9, 5, and 3, respectively. To estimate calcium intake, the self-administered Virginia Cooperative Extension Calcium Checklist-Food Guide Pyramid (29) was used. Subjects recorded the number of servings they ate on a typical day in the last week of specific foods found in each of the seven food groups included in the checklist. A calcium value was calculated as the product of number of daily servings using pre-determined calcium values.

Statistical Analysis

Data were analyzed using the Number Cruncher Statistical Systems software package (NCSS-97, Kaysville, UT). A two-way analysis of variance (ANOVA) for repeated measures was used to determine variations in EI values within five-trial sets, testing sessioins, and between days. Statistical significance was accepted if $p < 0.05$. When significant differences were detected by ANOVA ($p < 0.05$), Tukey's HSD test was used to isolate test specific differences. Descriptive statistics were computed and reported for all relevant subject variables at baseline and for the dependent measures. Intra-trial and test-retest reliability coefficients were calculated by trial, set, and day. Dispersion around

the mean values are expressed as ± 95% confidence intervals. Reliability and precision measurements were analyzed through models described by Hopkins (32).

RESULTS

General. The final group included 22 college-aged women. Data for one additional subject was excluded from analysis because the third day of testing was not completed. Physical characteristics of the subjects are presented in Table 1. The daily dietary intake of calcium was 1873 ± 399 mg and the average Godin Leisure Time means were 60.5 ± 31.3. Subjects in this sample typically participated in exercise ≥ 30 min/session. For this group, the mean number of self-reported exercise sessions/wk (± SD) was 6.2 ± 3.4 in moderately vigorous activities that included soccer, running, brisk walking, cycling, aerobics, swimming, and resistance weight training.

Tibial Bending Stiffness. Mean tibial length was 36.4 ± 1.9 cm for these women. The EI values for ranged from 5.1 < EI < 206 $N \cdot m^2$ with a mean EI of 52.0 ± 26.7 $N \cdot m^2$. There were no significant differences in tibial bending stiffness between testing session days (inter-trial) and between the two sets completed on the same testing day. The within trial variation (average of the range found within both sets 1 & 2 , trials $T_1 - T_5$) for all subjects was 9.7 %. Assessment of mean EI yielded high r values for inter-set test-retest reliability (sets of five trials completed on the same testing day), D_1S_1 vs. D_1S_2 (r = 0.92), D_2S_1 vs. D_2S_2 (r = 0.62), D_3S_1 vs. D_3S_2 (r = 0.79), where D is testing session within a day and S is the set (5 trials) within a day. The relationships between EI values for the first, second, and third testing day (Set of five trials completed on each day) are presented in Figures 1, 2, 3, respectively. The percent change for overall mean EI between sets 1 and 2 on each day was 2.9, 4.4, and 8.6, for day 1, 2, and 3, respectively.

When the mean EI for all subjects (testing sessions on each day) was compared between days, a significant difference ($p < 0.05$) was noted between day 1 (59.1 $\pm$ 35.5 $N \cdot m^2$) and both day 2 (46.9 $\pm$ 22.3 $N \cdot m^2$) and day 3 (49.9 $\pm$ 18.3 Nm^2). No differences were found between days 2 and 3. Figure 4, 5, and 6. shows the relationship for inter-trial reliability between days (mean of two sets on each day, 10 trials). The percent change in mean EI (testing sessions/day) was 11.7, 18.3, and 1.3 for day 1 vs. 2, 1 vs. 3, and 2 vs. 3, respectively. Table 2. shows the within-subject variation including the 95% confidence intervals for trials within a day.

When the high and low values of each five-measurement set was excluded and each subject's mean EI value calculated, no differences in scores were found between days 1 through 3 (Table 3.). However, the five-trial 1 and 2 mean EI range (total of 10 trials/day) between all days increased from 9.9 $N \cdot m^2$ to 10.3 $N \cdot m^2$ when using the three median score method from the five-trial mean to represent EI. Table 4. represents the percent variation for trials within a set for each day; both five trial means and three trial means (hi and low excluded) are shown. There was no mean shift in tibial bone stiffness values found between the mean of subjects five serial trials over the three days.

DISCUSSION

The results of this study of college-aged women confirm the intra-trial reliability and precision of MRTA of the tibia *in vivo* in our laboratory utilizing our particular measurement technique. Repositioning of subjects did not reduce the reliability and precision of these measurements; however, we were unable to demonstrate acceptable measurement reproducibility when these same subjects were evaluated on different days under the same conditions. This present study was the first to examine test-retest

measurement reliability of tibial bending stiffness in humans using MRTA. The central purpose of this study was to determine test-retest precision of MRTA within a single day and across non-consecutive days. A secondary purpose was to determine the optimal number of trials for a testing period and to examine how many days of testing are needed for accurate scores.

The mean EI intra-test variation (between sets 1 and 2 on each day) was small, 2.9, 4.4, and 8.6 %. The mean intra-test variation (set 1 vs. 2 for all days) over the three non-consecutive days was agreeable (5.3 %) to ulnar stiffness measurement precision previously demonstrated (22, 24, 27). The within-trial deviation may be a result of slight movement and/or the inability of the subject to relax muscles in the lower limb. The inter-test reliability (Figures 1,2,3) was very similar to results found in ulnar stiffness studies (22). Great care was taken when repositioning the probe on each subject; however, variation as a result of different probe placement (angle and location), pressure of the shaker/probe by the operator, and the subject's inability to relax their leg may have caused a discrepancy in scores. During testing, many subjects complained of discomfort from the probe and, as a result, may have slightly moved during testing to adjust for the uncomfortable position. While discomfort was noted, the mean EI values between set 1 (52.1 $N \cdot m^2$) and set 2 (51.8 $N \cdot m^2$) was not significant. Consequently, it appears that it is necessary to complete only a single set of five trials during a single testing period to obtain accurate EI measurements. However, this recommendation may only be inferred as a result of certain unique aspects of our measurement protocol, i.e. collection of data in two sets/day (subject repositioning) rather than collecting one set of five or more trials. Taking more than five measurements at one time may cause additional discomfort for the

subject at the site of probe positioning on the tibial crest increasing the variation between EI scores.

The mean bending stiffness scores decreased significantly from day 1 to each non-consecutive day, while scores remained similar between days 2 and 3. This inter-test variation is similar to results found in studies evaluating reliability of ulnar stiffness (22). Steele and colleagues (22) found average intra-test variation for inexperienced and experienced operators was not significantly different. In the current study, a single operator was used throughout testing. The variation between intra-test (between days) was very high, sporadic and unacceptable. The standard deviation values for days decreased from 59.1 $\pm$ 35.5 $N \cdot m^2$, 46.9 $\pm$ 22.3, and 49.9 $\pm$ 18.3 for day 1, 2, and 3, respectively; however, the percent change in mean EI (10 trials/day) was 11.2, 18.3, and 1.3 (days 1 vs. 2, 1 vs. 3, and 2 vs. 3). These erratic results are a combination of both operator and subject factors that are different between days. Some subjects may have been able to adjust to the posterior thigh support (finding the most comfortable position) and gained a better understanding/ability to relax the leg muscles while others may not have been able to adapt. While only speculation, the first day of subject measurement should be used as a day to orientate the individual to testing procedures and familiarize them to the MRTA, possibly increasing the precision of MRTA scores between days.

A difference noted from this study of the tibia and previous investigations measuring the ulna is the range of values found for EI. Previous studies of the ulna found values ranging as low as 10 $N \cdot m^2$ and as high as 120 $N \cdot m^2$. The values obtained in this study ranged slightly lower and considerably higher ($5.1 < EI < 206$ $N \cdot m^2$). Stussi *et al.*. (33), a Swiss group using a slightly different vibratory response to obtain tibial EI, found

a range of scores much higher (100 - 200 $N \cdot m^2$) than scores found in this study. The lower scores that were found in this current study are probably not representative of true maximum tibial EI. The low EI values, as well as the low mean EI scores (52.1 and 51.8 $N \cdot m^2$), might be a result of poor measurement technique, i.e. measuring along a short axis of the bone or medial to the anterior tibial crest and/or using a model for EI prediction that does not account properly for added damping effects of the bone created by the support of the upper leg. Higher tibial scores compared to ulnar EI are evident because the tibia is a weight bearing bone that is centrally involved in many daily activities. Repetitive loading is essential for the maintenance of normal bone strength, and with activity, bone strength is increased. Finally, the tibia is a longer bone than the ulna, and the longer the bone, the greater the bending moment caused by the addition of force (30). Hence, the tibia is subjected to higher tensile and compressive forces than the ulna, resulting in higher EI values.

Accurate non-invasive testing of long bone *in vivo* is difficult because measurement of the bone must take place on the outer surface of the skin. Cornelissen and colleagues (29) evaluated the influence of soft tissue and joints by modal analysis and found the skin has negligable effect on the mode of tibial vibration. The authors of this study evaluated the soft tissue of amputated lower limbs, which may not mimic the exact physiological characteristics of soft tissue *in vivo*. If soft tissue does not effect tibial vibration, then the thickness of skin lying over the tibia should not change the MRTA scores between trials and days. From McCabe *et al.* (24) we adapted the protocol for placement of the probe on the long bone (1 min for compression of skin). In this study, skin thickness and the effects of MRTA scores on time placement of the probe on the

tibia were not investigated. Is 1 min long enough to compress overlaying skin for those with thicker subcutaneous layers than subjects' with thin layers? Of future interest would be additional studies investigating skin thickness and whether variation in time placement of the probe is necessary.

The positioning of the limb during testing my have introduced response effects which may require a more elaborate mathematical model to account for factors unrelated to the bending stiffness of the tibia. In this study a posterior thigh support was used to position the tibia and the operator positioned the heel with a minimal amount of weight between the calcaneous and a Styrofoam pad. For some subjects, a vibratory response factor related to the distal end of the tibia not being in a fixed position, might generate additional response factors and require a more advanced analytic model to isolate the EI factor. The current study utilized the 7-parameter model to analyze EI. Roberts *et al.*. (21) developed a 6-parameter model which gives a better physiological representation of the skin; however, the author found negligible differences in EI between the 7- and 6-parameter models when studying the human ulna. Currently, Dr. Steele has developed 9- and 12- parameter models to address response effects and future research may determine whether reliability may be further improved by selecting a specific analytic model which bests fits individual EI response curves for the tibia (personal communication, Charles Steele, Ph.D.)

The results of this study demonstrate that MRTA has excellent potential for *in vivo* assessment of tibial fracture risk in athletes, military recruits, and at risk individuals. Additional studies should be conducted investigating the validity of the MRTA as a tool for testing bone strength/fragility of the tibia. Results from these additional studies could

have significant clinical ramifications in military recruits and athletes who have risk factors and/or are prone to stress fractures. Individuals with stress fractures often result in the loss of training time and an increase in the cost of medical care. The ability to distinguish between individuals who are at high risk could translate into substantial monetary savings and combat readiness of military personnel. The identification of individuals who are at high risk for stress injuries (both internal and external risk factors) may help prevent stress fractures, and if they do occur, decrease time spent in recovery. MRTA might also be used to investigate at-risk patients with low BMD. Changes in EI might be a better indicator of future fracture risk than changes in BMD. An individual might increase their BMD via medication; however, the strength and subsequent fracture risk may not be changed. In addition, studies investigating time placement of the probe and how this might effect MRTA scores is necessary. Studies should also explore the use of different EI parameter models (6, 7, 9, 12) enabling researchers to find the model best suited to the type of positioning used. Intervention studies regarding the role of exercise and the mechanism of stress fractures in the tibia should also be conducted.

In conclusion, it is possible to obtain reliable test re-test tibial measurements when using the MRTA to find tibial EI. To our knowledge this is the first study to examine the measurement reliability of tibial EI scores with MRTA. The range of tibial EI scores was much more expansive than ulnar EI; however, the mean EI of the tibia was found to be similar to previous ulnar studies; however, the range of tibia EI was found to be much lower than other human tibial EI studies. No difference was found between mean EI over days when comparing the three median score method and the average of all test trials. The probe positioning for within-trial scores was found to be highly reliable.

The inter-test (between day) reproducibility was found to be low and unacceptable, a result of operator and subject variables that change between days. The probe positioning used in this study improved technical reproducibility; however, measurements were taken on the short axis (medial tibial crest) of the tibia instead of the anterior crest of the tibia and hence lower EI values. In addition, the unacceptable inter-day reliability by be due to the inability, at the time of this study, to apply the computational model best suited to the type of leg positioning that was used.

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Table 1. Physical characteristics of subjects (means ± SD).

N	Age (yr)	Height (m)	Weight (kg)	BMI (kg/m^2)	Tibial Length cm)
22	20.8 ± 1.8	1.64 ± 0.1	62.5 ± 8.4	23.1 ± 2.3	36.4 ± 1.9

N = number of subjects

Table 2. Within-subject variation between trials on each testing day with confidence intervals.

	Mean value of Subject's Trials			N	Number of Trials	Typical error	Degrees of freedom	95% Confidence Intervals	
Day	Trial 1	Trial 2	Change					lower	upper
1	58.3 $N•m^2$	59.6 $N•m^2$	1.3	22	10	12.5	189	-12.93	15.53
2	46.9	47.7	0.8	22	10	10.9	189	-24.86	26.46
3	51.1	48.7	-2.4	22	10	6.3	189	-14.11	9.31

Typical error = divide the SD of the difference in score (10 trials/day) for each subject by the $root^2$

Degrees of freedom = product of (N-1) and (Number of trials – 1)

Table 3. Subjects mean EI for three non-consecutive days and for sets one and two during each day. Scores are given as five-trial means and mean of three scores after the high and low values have been discarded.

		Count	Ten-Trial mean EI ($N \cdot m^2$)	Ten-Trial mean EI ($N \cdot m^2$) with three median scores[1]
	1	44	59.1*	59.3
Day	2	44	46.9	47.1
	3	44	49.9	49.0
Set	1	66	52.1	52.4
	2	66	51.8	51.2

Count = product of the number of subjects tested and the amount of times tested for each day or set.

*Significantly different from five-trial mean EI of day 1 and 2; $p \leq 0.05$

[1]Three median scores taken from five trials of set 1 and than averaged with five trial median scores from set 2 on the same day

Table 4. Percent variation found within sets 1 & 2 and the overall percent variation for all three days of testing. Percentages are given for within variation for five-trials and within variation after the high and low values have been discarded (three-trials).

		Within variation for five-trials (%)	**Within variation for three-trials (%)**
Day 1	**Set 1**	10.3	5.8
	Set 2	10.4	4.5
Day 2	**Set 1**	8.5	5.0
	Set 2	9.4	5.2
Day 3	**Set 1**	8.2	4.4
	Set 2	11.5	6.4
Mean variation		9.7	5.3

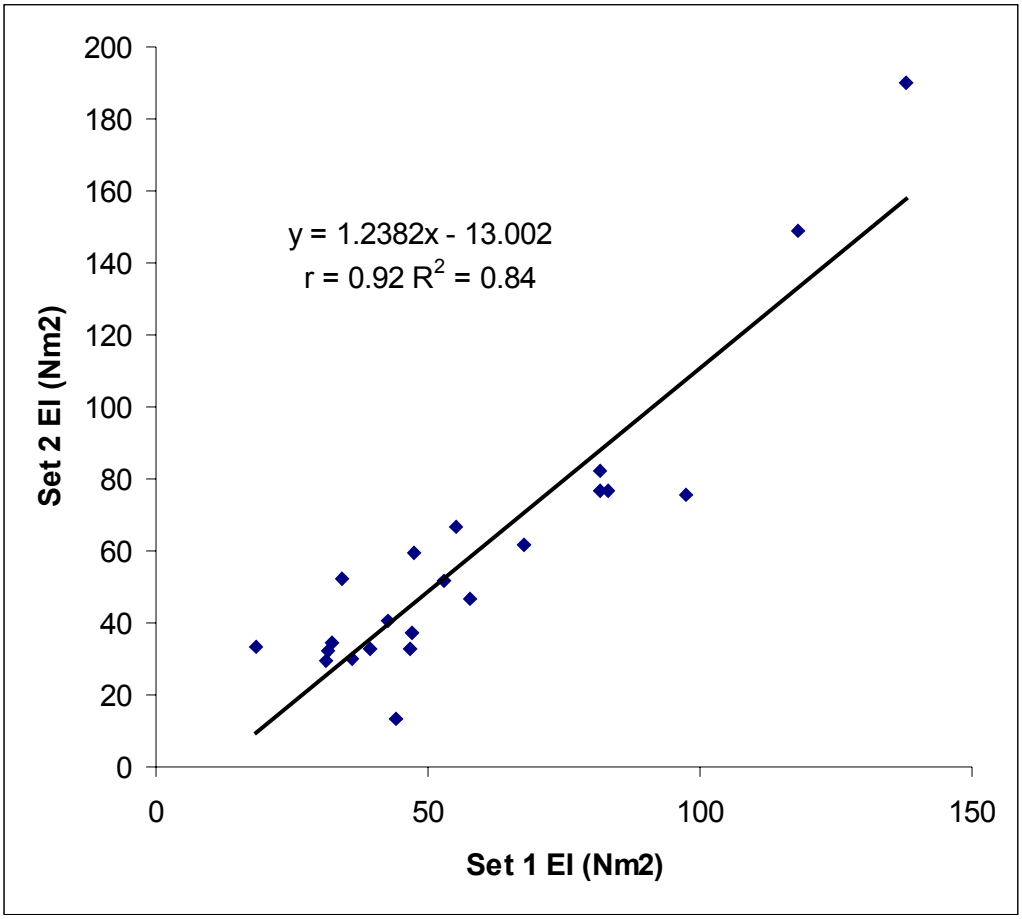

Figure 1. Relationship of subjects mean EI between set 1 and set 2 on the first testing day.

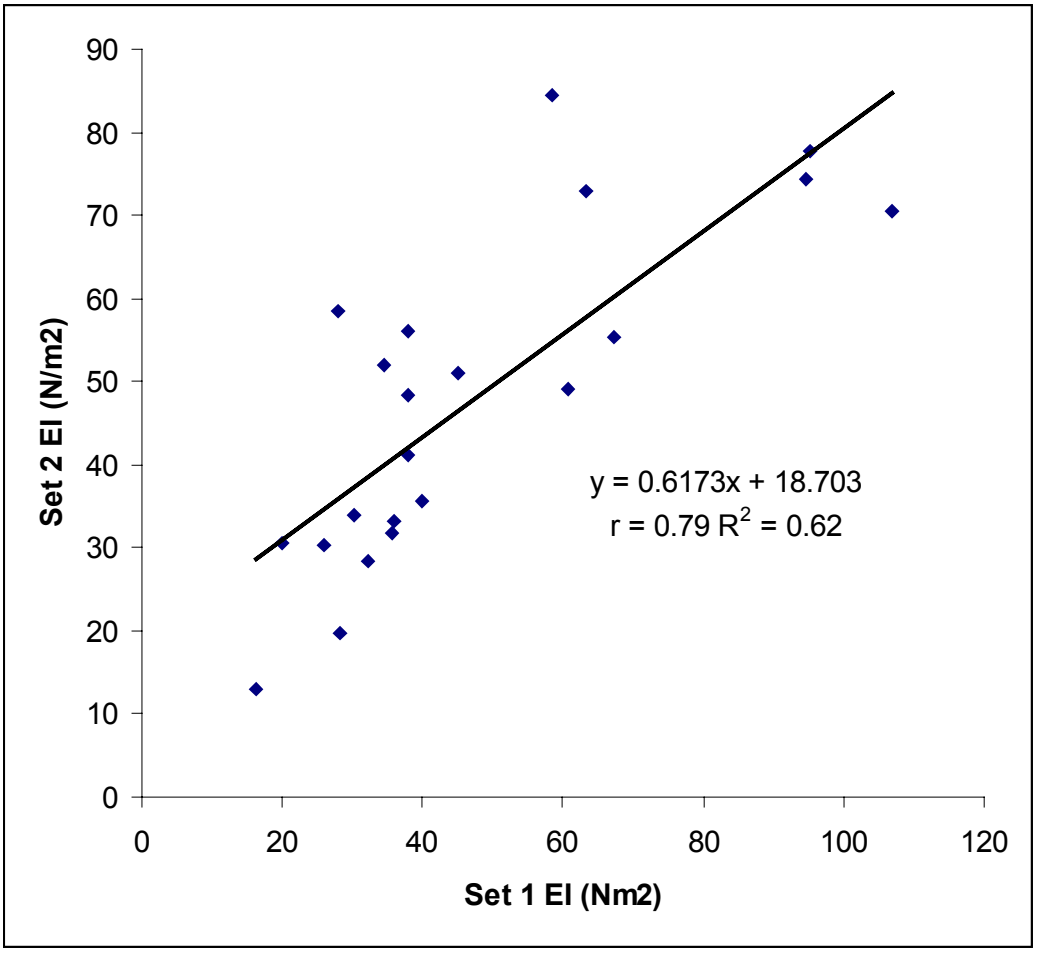

Figure 2. Relationship of subjects mean EI between set 1 and set 2 on the second testing day.

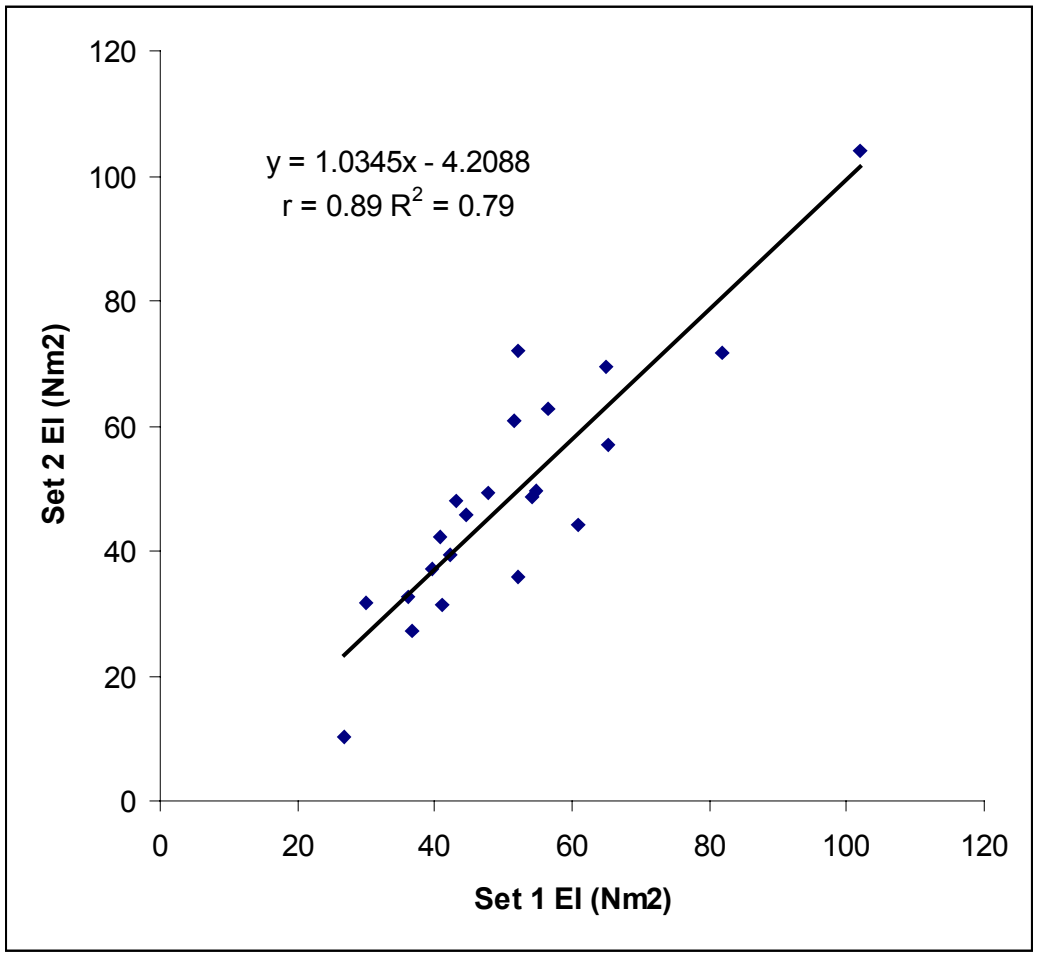

Figure 3. Relationship of subjects mean EI between set 1 and set 2 on the third testing day.

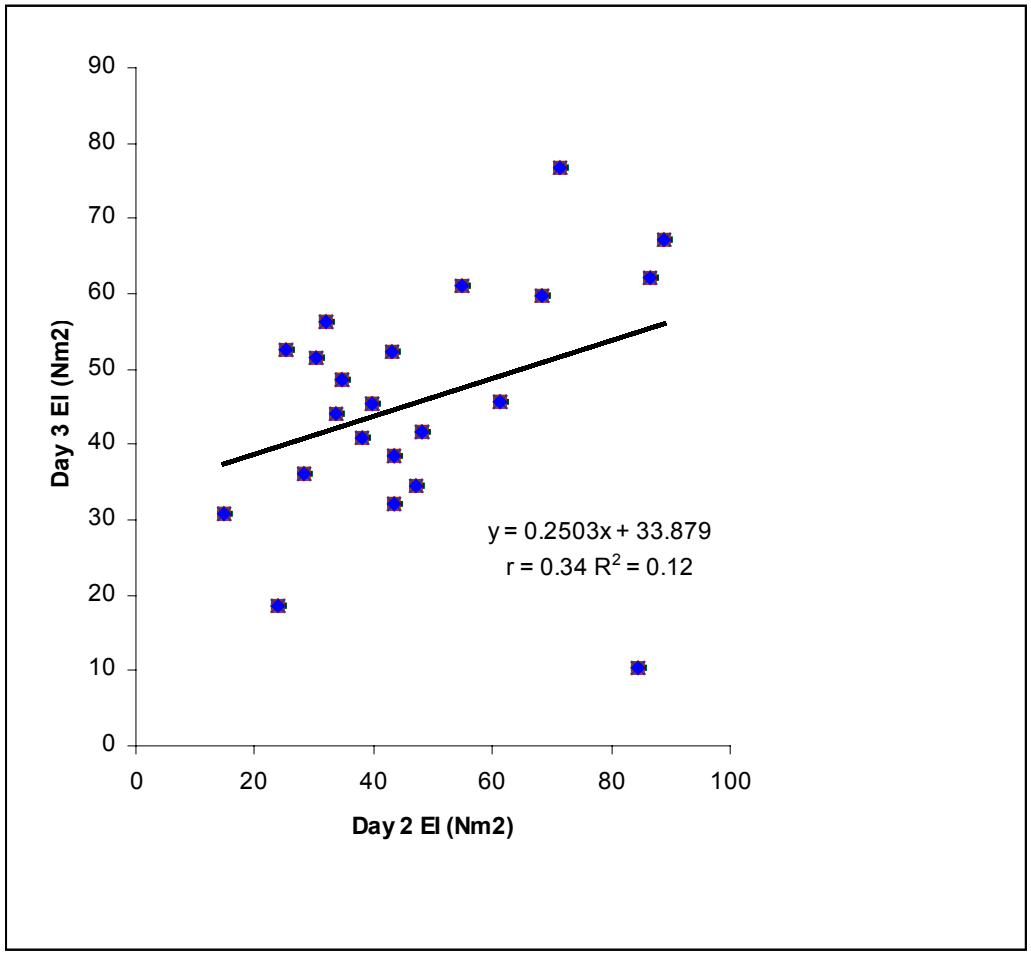

Figure 4. Relationship of subjects mean EI between day 2 (10 scores) and day 3 (10 scores).

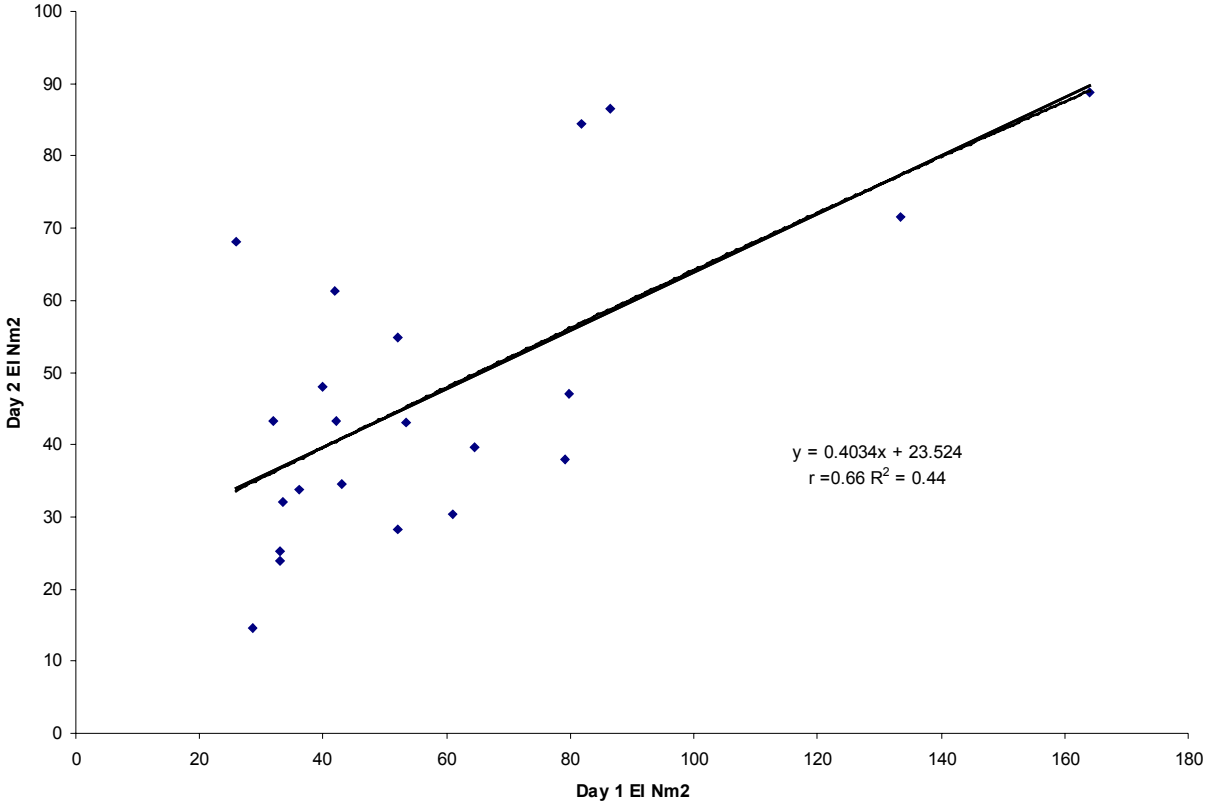

Figure 5. Relationship of subjects mean EI between day 1(10 scores) and day 2 (10 scores).

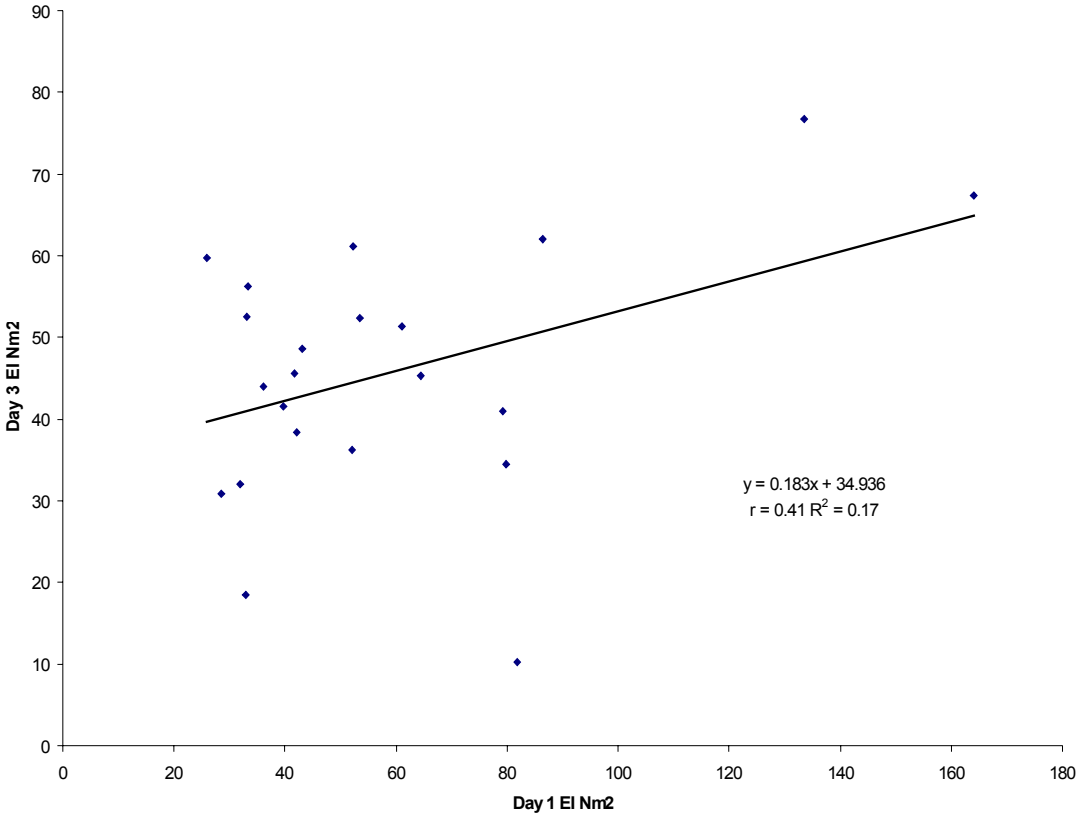

Figure 6. Relationship of subjects mean EI between day 1(10 scores) and day 3 (10 scores).

Chapter IV

SUMMARY, IMPLICATIONS FOR CLINICAL PRACTICE AND RESEARCH

The purpose of this investigation was to determine the intra-trial and inter-trial reliability for MRTA measurements of the tibia *in vivo*, in healthy women. Stress fractures of the tibia are a major problem among women athletes and military recruits. Consequently, the validation of a reliable method of testing the bone strength/fragility of the tibia via MRTA would be of clinical importance.

The range of tibial EI scores in the present study was much larger than for ulnar EI; however, the mean EI for the tibia was found to be similar to previous ulnar studies. In addition, the range of tibial EI was found to be much lower than found in other human tibial EI studies (not using the MRTA). The low scores demonstrated in this study are likely due to poor measurement technique. The lower EI values compared to Roberts *et al.*. (21) suggest the EI measurements were taken across a shorter axis of the tibia instead of the long axis. Furthermore, the correct prediction model for the type of positioning used in this study (upper leg partially supported with minimal weight with the calcaneous and a Styrofoam pad) may not have been used due to new computational models not being available at the time of this study.

The probe positioning for intra-trial scores was found to be highly reliable. These results were very similar to results found in ulnar EI studies (22). The inter-trial (between day) stability was found to be low and unacceptable; a result of operator and subject variables that change between days. Great care was taken to accurately and precisely reposition the probe on each subject; however, variation may have occurred as a result of different probe placement (angle and location), pre-load of the probe applied by

the operator, and subject's inability to relax the leg and minimize movement. There is a need for continued development of the current testing procedure. The unacceptable inter-day reliability may also have resulted because of the inability, at the time of this study, to apply the computational model best suited to the type of leg positioning we used. This study was unable to demonstrate measurement stability over non-consecutive days; however, repositioning of subjects did not reduce the reliability and precision of EI values during trials and sets completed on the same day.

Recommendations for Further Research

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

1. Methodology (probe placement) and easier means of testing subjects needs to be developed. Studies need to focus on the development of additional equipment design and protocols to allow subjects an easier means of relaxing extremities and decreasing the possibility of movements during measurement. Discomfort related to use of the posterior thigh support and the effects of the operator trying to position the heel correctly must be enhanced. A procedure allowing subjects to relax their leg without a thigh support might be developed. This new protocol could be tested against the current method in which the subjects' leg is supported by an uncomfortable thigh support and the where the operator must make sure minimal weight is between the calcaneous and a Styrofoam pad. In addition a study comparing probe placement should be partaken. In the current study, low tibial EI values were obtained as a result of the probe being placed on the short axis of the tibia, medially to the tibial crest. A study should take contrasting probe placement measurements (anterior crest

and medial crest) and compare results. The response curves analyzed to determine which mathematical model fits each subject's results best. The current study utilized the 7-parameter model for tibial EI scores. Currently 6- and 7-parameter models have been used in published studies and Dr. Steele has developed a new 9- and 12-parameter model has been developed (personal communication, Charles Steele, Ph.D.). Using specific models may lead to better interpretation of scores leading to more accurate EI values. Additional studies need to investigate when and how to recognize when a specific model should be used to analyze subject data.

2. Exercise may increase bone strength; however, overuse and repetitive loading may cause injury. Of importance would be intervention studies regarding the role of exercise, the mechanism of stress fractures and how this affects bone stiffness. The study could compare an active group of athletes and sedentary individuals. The active group might consist of both resistance trained athletes and athletes who participate in weight-bearing cardiovascular exercise. In this training study, the damping effect of skin and muscle near the tibia may be investigated. Those athletes with more muscle may affect the ability to place the probe on the long axis of the tibial crest. These studies would be of importance and value for both athletes and military personnel.
3. Accurate non-invasive testing of long bone *in vivo* may also have future implications for clinical use. Currently DXA scans are used to determine the potential for fragility fractures that might occur with osteoporosis; however, MRTA may prove to be a faster, easier, cheaper and a more accurate means of predicting fragility fractures. Of particular interest would be a clinical study investigating MRTA's ability to accurately measure fracture risk. Two groups could be compared. A group of

patients with known fragility fractures and a second group of healthy individuals (no fractures). The groups would be matched for age, gender, BMI and calcium intake. These results may determine that bone strength is a better indicator of fragility fractures than BMD and my offer a better method for predicting them.

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APPENDIX A

Informed Consent

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY
Informed Consent for Participants of Investigative Project

Title of Study: Mechanical Response Tissue Analysis: Measurement Reliability in Assessing Bending Stiffness of the Human Tibia in College Aged Women

Location of Study: War Memorial Hall, Virginia Polytechnic and State University, Blacksburg VA

Principal Investigators: William G. Herbert, Ph.D., Shelly Nickols-Richardson, Ph.D., Warren Ramp, Ph.D.

Purpose of this Research

I am invited to participate in a study that will determine a protocol that elicits reliable data when measuring tibial stiffness via mechanical response tissue analysis (MRTA).

Overview

I agree to participate in the study for a period of 5 days. I understand I will be placed in one of two groups according to my fitness level. Prior to being included in the study, I will undergo an initial screening to determine my eligibility for participation. During the initial screening, I will complete a health history form and a calcium intake questionnaire. If included, I will undergo a bone strength/stiffness test.

Explanation of the Test

I will have my height, weight, body mass index (BMI), body, quadriceps strength and bone stiffness determined. 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 isokinetic strength testing will be conducted with a machine called the Biodex training system. The testing will begin with a warm-up of 3 minutes of low intensity stationary cycling. I will be given practice trials on the Biodex to become familiar with the operation, following which I will perform a maximal knee extension on my dominant leg. I

will 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.

The strength of my leg bones will be measured with mechanical response tissue analysis (MRTA). For this test, I will have to sit in a chair with my hip and knee flexed at 90-degree angles, with the distal tibia and fibula lightly restrained. Tibial length will be measured from the distal medial malleolus to the distal medial condyle with an anthropometer. A technician will place a device on my lower leg that will produce a vibratory sensation through my bone. The entire procedure lasts 10 minutes and produces no unusual sensation or discomfort.

Risks and Benefits to be Expected

The MRTA has been used in many human research studies, and there are no known adverse risks associated with MRTA measurements. However, you may experience slight pressure on the skin above your tibial bone and minor bruising. Benefits associated with the study are related to a single dimension of bone health. I understand the MRTA can provide information on the stiffness, axial strength, and sufficiency of bone, which may be compared to normal values. Upon request, I will be provided with this information which will elicit a greater understanding of skeletal health.

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. 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.

Freedom to Withdraw

My participation in this study is completely voluntary. I understand that once I agree to participate in the study, I am free to withdraw at anytime without penalty.

Approval of Research

The Institutional Review Board for projects involving human subjects at Virginia Polytechnic Institute and State University and the Department of Human Nutrition and Foods have approved this research protocol.

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. Accurately report activity over the past 6 months.
3. Arrive in a timely fashion to scheduled testing sessions.

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:

Robert Thorne (540) 231-4900
Primary Investigator

Dave Wootten (540) 231-4900
Investigator

William Herbert, Ph.D. (540) 231-6565
Human Nutrition, Foods & Exercise

Warren Ramp, Ph.D.

Sharon Nickols-Richardson, Ph.D
Human Nutrition, Foods & Exercise

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

APPENDIX B

Medical/Health History Questionnaire

VIRGINIA TECH
LABORATORY FOR HEALTH AND EXERCISE SCIENCE

Medical and Health History Form

Title of Project: Mechanical Response Tissue Analysis: Measurement Reliability in Assessing Bending Stiffness of the Human Tibia in College Aged Women

Name: _____ Age: _____Date of Birth: _____

Local Address:_____

Telephone Number: _____Email Address: _____

Address for Permanent Residence: _____

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**
Circulation problems:	_____	_____
Kidney disease or problems:	_____	_____
Musculoskeletal problems:	_____	_____
Broken bones (Past 12 months)	_____	_____
High blood pressure:	_____	_____
High blood cholesterol:	_____	_____
Diabetes:	_____	_____
Eating disorders (bulimia, anorexia):	_____	_____

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

_____

_____

_____

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

_____

_____

Health Habits

Are you on any special type of diet? Yes ___ No ___

If "yes" please describe _____

_____

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 (more than one session/week)? Yes _____ No _____

If "yes" please list:

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

Explanation of exercise activities: _____

_____

_____

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

APPENDIX C

Raw Data

Raw Data: Demographics

Group	Age (yrs)	Height (m)	Weight (kg)	BMI	Tibial Length (Cm)	Godin Activity Score	Ca++ (mg)
	21	1.78	66.4	20.96	40.6	85	1900
A							
A	21	1.61	67	25.85	36.3	57	1220
A	20	1.7	61.7	21.35	40	82	1650
A	28	1.66	57.4	20.83	35.8	74	1750
A	20	1.71	66.9	22.88	38.4	56	1720
A	20	1.61	56.6	21.84	36.4	97	2130
A	20	1.56	59.6	24.49	36	60	2160
A	19	1.64	56.5	21.01	36.9	66	2440
A	21	1.74	90	29.73	37.5	52	2430
A	20	1.62	69.7	26.56	36.2	54	1740
A	23	1.6	57.7	22.54	35	101	1890
A	20	1.55	58	24.14	32.3	69	2990
A	20	1.6	55.5	21.68	35.2	138	2020
A	21	1.71	69	23.60	38.4	69	1620
A	22	1.56	48.5	19.93	33.2	62	1930
A	20	1.61	56.5	21.80	35.9	77	1630
A	20	1.64	61	22.68	35.3	47	1910
A	20	1.68	63	22.32	36.6	17	1350
A	21	1.64	56.8	21.12	35.8	9	1290
A	21	1.68	72.9	25.83	37.5	18	1850
A	20	1.63	64.1	24.13	36	27	1600
A	20	1.67	61	21.87	36	15	1810

Raw Data: Godin Leisure Activity Scores.

Group	Strenuous Exercise (Score x 9)	Moderate Exercise (Score x 5)	Mild Exercise (Score x 3)	Total Score (METs)	HR Often (1), Sometimes (2), Never (3)
A	5	5	5	85	1
A	4	3	2	57	1
A	4	5	7	82	1
A	5	4	3	74	1
A	3	4	3	56	1
A	7	5	3	97	1
A	5	3	0	60	1
A	5	0	7	66	1
A	3	2	5	52	1
A	5	0	3	54	1
A	5	7	7	101	1
A	5	3	3	69	1
A	10	6	6	138	1
A	6	3	0	69	1
A	3	4	5	62	1
A	4	4	7	77	1
A	4	1	2	47	1
A	1	1	1	17	2
A	0	0	3	9	3
A	0	3	1	18	2
A	0	3	4	27	2
A	1	0	2	15	2

APPENDIX D

EI Raw Data

Raw Data: Subjects Day, Set and Trial EI Measurements.

Group	Day/Set	Trial 1 ($N•m^2$)	Trial 2 ($N•m^2$)	Trial 3 ($N•m^2$)	Trial 4 ($N•m^2$)	Trial 5 ($N•m^2$)	T1-T5 Mean ($N•m^2$)	Variation T1-T5 (%)	High/Low of T1-T5 Dropped, Mean ($N•m^2$)	High/Low of T1-T5 Dropped, Mean (%)
A	D1/S1	142	134	139	135	140	138	2.5	138	2
	D1/S2	174	186	189	206	193	190	6	190	1.9
	D2/S1	80.5	94.2	98.6	130	130	107	21.1	108	18.3
	D2/S2	74.3	74.2	66.9	70	67.6	70.6	5	70.6	4.8
	D3/S1	60.6	65	65.2	68.8	65.5	65	4.5	65.2	0.4
	D3/S2	69.1	67.4	72.4	69	69.5	69.5	2.6	69.2	0.4
A	D1/S1	34.9	38.9	48.3	64.8	33.2	44	29.6	40.7	16.9
	D1/S2	14.6	12.4	12.1	15	12.1	13.2	11.1	13	10.7
	D2/S1	17.6	17.8	15.6	15.2	14.9	16.2	8.4	16.1	7.9
	D2/S2	13.4	14.4	12.9	12.9	12.2	13.1	6.1	13.1	2.3
	D3/S1	29.3	29.2	29.8	29.8	31.3	29.9	2.7	29.6	1
	D3/S2	32.4	31.2	32.2	31.8	30.4	31.6	2.5	31.7	1.5
A	D1/S1	113	113	121	122	122	118	4.1	119	4.2
	D1/S2	144	149	151	155	146	149	2.9	149	1.5
	D2/S1	57.7	53.3	61.2	58.5	61.2	58.4	5.6	59.1	3.1
	D2/S2	71.9	86	74.1	83.5	107	84.5	16.6	81.2	7.7
	D3/S1	57.6	93.5	84.7	81	92.4	81.8	17.8	86	6.7
	D3/S2	70.2	72.6	69.4	69.1	76.7	71.6	4.4	70.7	2.4
A	D1/S1	46.9	48	48.6	46.3	45.6	47.1	2.7	47.1	1.9
	D1/S2	39.9	38	37.7	35.7	35.3	37.3	5.1	37.1	3.4
	D2/S1	33.6	28.7	27	24.6	26	28	12.5	27.2	5
	D2/S2	60.1	59.9	59.8	57.6	55	58.5	3.7	59.1	2.2
	D3/S1	48.7	41.7	36.3	35.6	35.7	39.6	14.4	37.9	8.7
	D3/S2	38.4	39.3	39.3	34.8	33.7	37.1	7.2	37.5	6.4

Raw Data: Subjects Day, Set and Trial EI Measurements Continued.

Group	Day/Set	Trial 1 ($N•m^2$)	Trial 2 ($N•m^2$)	Trial 3 ($N•m^2$)	Trial 4 ($N•m^2$)	Trial 5 ($N•m^2$)	T1-T5 Mean ($N•m^2$)	Variation T1-T5 (%)	High/Low of T1-T5 Dropped, Mean ($N•m^2$)	High/Low of T1-T5 Dropped, Mean (%)
A	D1/S1	47.8	50.4	46.5	45.2	46.5	47.3	4.2	46.9	1.5
	D1/S2	57	69	63	51.2	56.8	59.4	11.5	58.9	6
	D2/S1	37.5	38.7	37.5	37.7	37.8	37.8	1.3	37.7	0.3
	D2/S2	44.9	45.5	59	43.8	48.7	48.4	12.8	46.4	4.4
	D3/S1	58.7	57.3	51.8	52.6	54.3	54.9	5.4	54.7	4.4
	D3/S2	47.9	51.8	47.8	51.7	49.5	49.6	4.2	49.7	3.9
A	D1/S1	82.9	81.3	80.3	77.7	85.8	81.6	3.7	81.5	1.6
	D1/S2	76.7	73.9	88.8	87.9	83.9	82.2	8.1	82.8	6.9
	D2/S1	102	97.2	80.5	103	89.6	94.6	10.1	96.3	6.5
	D2/S2	74.9	70.6	88.9	71.8	65.6	74.3	11.8	72.4	3.1
	D3/S1	84	107	114	112	94	102	12.5	104	8.8
	D3/S2	97.9	90.8	101	133	97.1	104	16.2	98.6	2
A	D1/S1	63.9	69.4	67.8	65.7	70.7	67.5	4.1	67.6	2.8
	D1/S2	57.2	70.1	61.4	58.3	60.2	61.4	8.3	60	2.6
	D2/S1	40.9	36.5	37.4	37.2	37.7	37.9	4.5	37.4	0.7
	D2/S2	40.7	39.5	40.2	43.7	41.9	41.2	4	40.9	2.1
	D3/S1	46	44.2	44.1	44.1	45.3	44.7	1.9	44.5	1.5
	D3/S2	55.8	52.2	40.2	41.4	39.9	45.9	16.4	44.6	14.9
A	D1/S1	20.2	18.9	19	16.8	16.4	18.2	8.9	18.2	6.9
	D1/S2	36.6	25.9	29.2	37.8	38.3	33.6	16.8	34.5	13.4
	D2/S1	61.4	60.5	61.9	67.7	65.6	63.4	4.9	63	3.7
	D2/S2	66.3	61	80.8	75	81.2	72.9	12.3	74	9.8
	D3/S1	52.1	61	57.4	58	54.8	56.6	6	56.7	3
	D3/S2	59.7	61.9	64.3	66.4	61.2	62.7	4.2	62.5	2.6

Raw Data: Subjects Day, Set and Trial EI Measurements Continued.

Group	Day/Set	Trial 1 ($N•m^2$)	Trial 2 ($N•m^2$)	Trial 3 ($N•m^2$)	Trial 4 ($N•m^2$)	Trial 5 ($N•m^2$)	T1-T5 Mean ($N•m^2$)	Variation T1-T5 (%)	High/Low of T1-T5 Dropped, Mean ($N•m^2$)	High/Low of T1-T5 Dropped, Mean(%)
A	D1/S1	49.5	52.7	53.5	67.9	64.9	57.7	14.2	57	12
	D1/S2	47.7	44.3	46.3	49.2	44.8	46.5	4.4	46.3	3.2
	D2/S1	24.3	20.4	38	23.5	23.2	25.9	26.8	23.7	2.3
	D2/S2	32.7	28.7	30.9	30.3	29.5	30.4	5.1	30.2	2.3
	D3/S1	31.1	40.8	43.5	42.5	47.2	41	14.8	42.3	3.2
	D3/S2	29	29.8	32.6	32.2	33.6	31.4	6.3	31.5	4.8
A	D1/S1	29.2	30.9	29	30.1	28.9	29.6	3	29.4	2.1
	D1/S2	37.9	37.1	34.8	37	36.3	36.6	3.2	36.8	1.2
	D2/S1	20.4	21.9	19.6	21.1	17.2	20	9.1	20.4	3.8
	D2/S2	23.1	34.5	28.8	27.3	39.3	30.6	20.7	30.2	12.6
	D3/S1	53.4	59.7	59.6	65.1	66.4	60.8	8.5	61.4	5.1
	D3/S2	46.4	43.9	43.9	44.7	42.3	44.2	3.4	44.2	0.9
A	D1/S1	55.6	53.1	54.2	55	58.6	55.3	3.7	55	1.3
	D1/S2	61.6	67.6	71.8	66.3	66.3	66.7	5.5	66.7	1.2
	D2/S1	32.5	32.5	31.1	33.1	32.1	32.2	2.3	32.4	0.7
	D2/S2	27.6	29	28.9	28.5	28.7	28.5	2	28.7	0.5
	D3/S1	36.7	58.2	59.4	51.1	65.2	54.1	20.2	56.2	8
	D3/S2	51.1	43.4	58.2	42.8	47.9	48.7	13	47.5	8.2
A	D1/S1	66	85.2	83.2	85.8	87.3	81.5	10.8	84.8	1.6
	D1/S2	77.3	69.1	78.2	77.6	82.4	76.9	6.3	77.7	0.6
	D2/S1	44.6	40.7	40.9	35.7	38.1	40	8.3	39.9	3.8
	D2/S2	32.5	35.1	34.7	33.6	42.4	35.7	10.9	34.5	2.3
	D3/S1	39.9	41.1	44.9	44.1	41.5	42.3	5	42.2	3.8
	D3/S2	38.7	40.4	39.9	39.1	39.3	39.5	1.7	39.4	1

Raw Data: Subjects Day, Set and Trial EI Measurements Continued.

Group	Day/Set	Trial 1 ($N•m^2$)	Trial 2 ($N•m^2$)	Trial 3 ($N•m^2$)	Trial 4 ($N•m^2$)	Trial 5 ($N•m^2$)	T1-T5 Mean ($N•m^2$)	Variation T1-T5 (%)	High/Low of T1-T5 Dropped, Mean ($N•m^2$)	High/Low of T1-T5 Dropped, Mean (%)
A	D1/S1	32.8	33.2	32.8	32.1	31.2	32.4	2.4	32.5	1.2
	D1/S2	34.3	34	34.3	33.7	35.1	34.3	1.5	34.2	0.48
	D2/S1	28.8	29.9	30.1	30.6	31	30.1	2.7	30.2	1.2
	D2/S2	33.3	31.7	33.1	36.6	34.7	33.9	5.5	33.7	2.6
	D3/S1	48.4	54.7	52.5	49.9	51.9	51.5	4.7	51.4	2.7
	D3/S2	66.4	54.1	69.4	61.5	53	60.8	11.9	60.6	10.2
A	D1/S1	38.8	38.8	40.2	38.3	40.7	39.3	2.6	39.2	2.1
	D1/S2	32	33.2	33.2	33.9	32.5	32.9	2.2	32.9	1.3
	D2/S1	34.3	36.8	34.3	36	36.1	35.5	3.1	35.5	2.8
	D2/S2	31	30.8	32.4	32.5	32.1	31.8	2.5	31.8	2.3
	D3/S1	53.7	52.8	52.4	48.9	53	52.2	3.6	52.7	0.6
	D3/S2	33.7	32	35.4	43.4	34.5	35.8	12.4	34.5	2.5
A	D1/S1	38.7	37.3	36.3	36.6	31.8	36.1	7.2	36.7	1.3
	D1/S2	28.3	31.1	31.1	32.4	26.3	29.8	8.3	30.2	5.4
	D2/S1	29.2	28.8	25.7	29.3	28.2	28.2	5.3	28.7	1.8
	D2/S2	19.7	14.8	18.9	22.1	22.9	19.7	16	20.2	8.2
	D3/S1	26.3	27.4	26.8	27.6	25.3	26.7	3.6	26.8	2.2
	D3/S2	15.5	11.2	9.6	10.2	5.1	10.3	36.1	10.3	7.9
A	D1/S1	38.6	43.9	31.1	28	28.9	34.1	20.1	32.9	15.5
	D1/S2	61.1	55.5	33.5	46.9	63.7	52.1	23.5	54.5	13.1
	D2/S1	33.5	37.6	36.9	34.1	36.7	35.8	5.2	35.9	4.4
	D2/S2	28.4	30.1	42.5	32.7	32.6	33.3	16.4	31.8	4.7
	D3/S1	45.4	51.4	47.5	51.5	43.9	47.9	7.2	48.1	6.4

	D3/S2	66.7	63.4	47.4	31.4	37.2	49.2	31.7	49.3	26.8

Raw Data: Subjects Day, Set and Trial EI Measurements Continued.

Group	Day/Set	Trial 1 ($N•m^2$)	Trial 2 ($N•m^2$)	Trial 3 ($N•m^2$)	Trial 4 ($N•m^2$)	Trial 5 ($N•m^2$)	T1-T5 Mean ($N•m^2$)	Variation T1-T5 (%)	High/Low of T1-T5 Dropped, Mean ($N•m^2$)	High/Low of T1-T5 Dropped, Mean (%)
A	D1/S1	50.5	52.5	50.8	51.1	59.4	52.9	7.1	51.4	1.8
	D1/S2	59.5	43.1	45.8	60	49	51.5	15.2	51.4	13.9
	D2/S1	76.3	70	40.4	72.8	44.7	60.8	27.8	62.5	24.8
	D2/S2	44.3	52	51.5	54.3	43	49	10.2	49.3	8.7
	D3/S1	53.9	72.2	74.1	70.7	55.1	65.2	15.1	66	14.4
	D3/S2	47.7	49.2	53.2	67.4	67.2	56.9	17	56.5	16.7
A	D1/S1	59	112	108	106	104	97.6	22.3	106	2
	D1/S2	69.5	72.9	72.8	78.1	83.3	75.3	7.2	74.6	4
	D2/S1	95	97.5	85.2	98.3	99.3	95.1	6.1	97	1.8
	D2/S2	71	68.4	87.8	88.9	72.9	77.8	12.5	77.2	11.9
	D3/S1	52.1	51.9	54.7	51.1	50.8	52.1	3	51.7	1
	D3/S2	65.7	67.4	66.1	69.8	90.8	71.9	14.8	67.7	2.8
A	D1/S1	27.8	29.6	29	35.9	35.8	31.6	12.4	31.5	12
	D1/S2	29.5	29.7	28.2	36	36.8	32.1	12.5	31.8	11.6
	D2/S1	30.7	34.1	37	33.7	37.5	34.6	7.9	34.9	5.1
	D2/S2	49.1	48.6	58.5	53.4	50.6	52	7.8	51	4.2
	D3/S1	37.5	36.8	37.5	33.5	38.2	36.7	5	37.3	1.1
	D3/S2	23.5	24.6	29.6	30	29	27.3	11.1	27.7	9.9
A	D1/S1	47.6	37.6	57.8	40.4	50	46.7	17.2	46	10.9
	D1/S2	39.6	41.6	0	41	41.8	32.8	56	40.7	2.5
	D2/S1	49.8	44.5	43.5	47.3	40.1	45	8.3	45.1	4.4
	D2/S2	51.6	53.4	48	48.3	53	50.9	5.1	51	4.7
	D3/S1	34.5	48	38.9	42.3	40.5	40.8	12.1	40.5	4.2

	D3/S2	40.6	39.1	45	47.4	39.3	42.3	8.9	41.6	7.2

Raw Data: Subjects Day, Set and Trial EI Measurements Continued.

Group	Day/Set	Trial 1 ($N•m^2$)	Trial 2 ($N•m^2$)	Trial 3 ($N•m^2$)	Trial 4 ($N•m^2$)	Trial 5 ($N•m^2$)	T1-T5 Mean ($N•m^2$)	Variation T1-T5 (%)	High/Low of T1-T5 Dropped, Mean ($N•m^2$)	High/Low of T1-T5 Dropped, Mean (%)
A	D1/S1	58.1	62	94.3	106	95.5	83.1	25.9	83.9	22.7
	D1/S2	88.5	73.6	76.6	73.3	70.8	76.6	9.1	74.5	2.5
	D2/S1	39.4	39.1	38.1	36.7	36.7	38	3.3	38	3.1
	D2/S2	50.5	53.1	55.9	59.2	61.4	56	7.9	56.1	5.5
	D3/S1	35.1	34.8	36.7	36.5	37.2	36.1	2.9	36.1	2.4
	D3/S2	41.3	33	32.9	27.7	28.8	32.8	16.3	31.6	7.6
A	D1/S1	30	43.6	45.9	47.9	46.1	42.7	16.9	45.2	3
	D1/S2	38.6	38.9	41.2	41.9	43.2	40.8	4.8	40.7	3.9
	D2/S1	65.3	68.5	67.1	69.6	66	67.3	2.6	67.2	1.9
	D2/S2	58.3	47.9	52.4	52.4	65.3	55.3	12.2	54.4	6.3
	D3/S1	43.1	44.8	48.4	39.7	39.3	43.1	8.7	42.5	6
	D3/S2	46.6	46.7	57	43.5	46.9	48.1	10.7	46.8	0.3

APPENDIX E

Summary ANOVA Tables

Analysis of Variance Report
Days, Sets, and Trials

Analysis of Variance Table

Source Term	DF (Alpha=0.05)	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power
A (Trial)	4	799.551	199.8878	0.27	0.897744	0.107248
B (Set)	1	12.77133	12.77133	0.02	0.895679	0.051884
AB	4	291.4485	72.86214	0.10	0.983032	0.069333
C (Day)	2	17920.6	8960.298	12.07	0.000007*	0.993761
AC	8	272.872	34.109	0.05	0.999958	0.061907
BC	2	463.1396	231.5698	0.31	0.732099	0.097592
ABC	8	722.3851	90.29814	0.12	0.998389	0.083814
S	630	467602.4	742.226			
Total (Adjusted)	659	488085.2				
Total	660					

* Term significant at alpha = 0.05

Means and Standard Error Section

Term	Count	Mean	Standard Error
All	660	51.97759	
A: Trial			
1	132	50.06136	2.371271
2	132	51.55204	2.371271
3	132	52.40333	2.371271
4	132	53.24166	2.371271
5	132	52.62955	2.371271
B: Set			
1	330	52.1167	1.499723
2	330	51.83849	1.499723
C: Day			
1	220	59.13227	1.836778
2	220	46.87168	1.836778
3	220	49.92882	1.836778
AB: Trial,Set			
1,1	66	49.04848	3.353483
1,2	66	51.07424	3.353483
2,1	66	52.55712	3.353483
2,2	66	50.54697	3.353483
3,1	66	52.48545	3.353483
3,2	66	52.32121	3.353483
4,1	66	53.73788	3.353483
4,2	66	52.74545	3.353483
5,1	66	52.75455	3.353483
5,2	66	52.50455	3.353483
AC: Trial,Day			
1,1	44	56.34546	4.107161
1,2	44	45.82954	4.107161
1,3	44	48.00909	4.107161
2,1	44	58.23409	4.107161
2,2	44	45.9925	4.107161
2,3	44	50.42955	4.107161
3,1	44	59.49091	4.107161
3,2	44	46.65909	4.107161

3,3	44	51.06	4.107161
4,1	44	61.10909	4.107161
4,2	44	48.05455	4.107161
4,3	44	50.56136	4.107161
5,1	44	60.48182	4.107161
5,2	44	47.82273	4.107161
5,3	44	49.58409	4.107161
BC: Set,Day			
1,1	110	58.31454	2.597597
1,2	110	46.88427	2.597597
1,3	110	51.15127	2.597597
2,1	110	59.95	2.597597
2,2	110	46.85909	2.597597
2,3	110	48.70636	2.597597

Means and Standard Error Section

Term	Count	Mean	Standard Error
ABC: Trial,Set,Day			
1,1,1	22	53.35455	5.808403
1,1,2	22	47.05909	5.808403
1,1,3	22	46.73182	5.808403
1,2,1	22	59.33636	5.808403
1,2,2	22	44.6	5.808403
1,2,3	22	49.28637	5.808403
2,1,1	22	57.55909	5.808403
2,1,2	22	46.77136	5.808403
2,1,3	22	53.34091	5.808403
2,2,1	22	58.90909	5.808403
2,2,2	22	45.21363	5.808403
2,2,3	22	47.51818	5.808403
3,1,1	22	59.84546	5.808403
3,1,2	22	44.89091	5.808403
3,1,3	22	52.72	5.808403
3,2,1	22	59.13636	5.808403
3,2,2	22	48.42727	5.808403
3,2,3	22	49.4	5.808403
4,1,1	22	60.66364	5.808403
4,1,2	22	48.89545	5.808403
4,1,3	22	51.65454	5.808403
4,2,1	22	61.55455	5.808403
4,2,2	22	47.21363	5.808403
4,2,3	22	49.46818	5.808403
5,1,1	22	60.15	5.808403
5,1,2	22	46.80455	5.808403
5,1,3	22	51.30909	5.808403
5,2,1	22	60.81364	5.808403
5,2,2	22	48.84091	5.808403
5,2,3	22	47.85909	5.808403

Analysis of Variance Report
Set and Day with High and Low Discarded

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A (SET)	1	48.97091	48.97091	0.07	0.795343	0.057424
B (Day)	2	3758.057	1879.028	2.59	0.078828	0.499237
AB	2	244.5332	122.2666	0.17	0.844965	0.075029
S	126	91327.45	724.821			
Total (Adjusted)	131	95379.02				
Total	132					

* Term significant at alpha = 0.05

Means and Standard Error Section

Term	Count	Mean	Standard Error
All	132	51.81818	
A: SET			
1	66	52.42727	3.313931
2	66	51.20909	3.313931
B: Day			
1	44	59.28409	4.05872
2	44	47.13636	4.05872
3	44	49.03409	4.05872
AB: SET,Day			
1,1	22	58.64545	5.739897
1,2	22	47.1	5.739897
1,3	22	51.53637	5.739897
2,1	22	59.92273	5.739897
2,2	22	47.17273	5.739897
2,3	22	46.53182	5.739897

APPENDIX F

Summary Regression Tables

Correlation Report

Comparison of Trials Completed on Days and Sets

Pearson Correlations Section (Pair-Wise Deletion)

	D1S1T1	D1S1T2	D1S1T3	D1S1T4	D1S1T5	D1S2T1
D1S1T1	1.000000	0.910856	0.906957	0.861213	0.913673	0.942644
D1S1T2	0.910856	1.000000	0.958752	0.926065	0.957680	0.878286
D1S1T3	0.906957	0.958752	1.000000	0.976523	0.985236	0.878986
D1S1T4	0.861213	0.926065	0.976523	1.000000	0.967902	0.838519
D1S1T5	0.913673	0.957680	0.985236	0.967902	1.000000	0.895300
D1S2T1	0.942644	0.878286	0.878986	0.838519	0.895300	1.000000
D1S2T2	0.954159	0.886387	0.872271	0.820881	0.881564	0.981951
D1S2T3	0.937023	0.891167	0.862560	0.843910	0.887443	0.962296
D1S2T4	0.960068	0.891541	0.879251	0.833515	0.894172	0.983668
D1S2T5	0.944023	0.899464	0.873159	0.820138	0.885561	0.985206
D2S1T1	0.504512	0.610733	0.562562	0.472736	0.575212	0.502035
D2S1T2	0.528257	0.633646	0.578129	0.490813	0.584694	0.544098
D2S1T3	0.627701	0.707757	0.662484	0.586290	0.669507	0.657999
D2S1T4	0.625020	0.669101	0.621000	0.533988	0.626975	0.633642

D2S1T5	0.661447	0.724771	0.672631	0.590643	0.667492	0.681161
D2S2T1	0.530749	0.561153	0.571290	0.486475	0.580440	0.572242
D2S2T2	0.606537	0.612978	0.631655	0.545919	0.641220	0.665465
D2S2T3	0.392073	0.474546	0.451074	0.374088	0.465722	0.470612

Pearson Correlations Section (Pair-Wise Deletion)

	D1S2T2	D1S2T3	D1S2T4	D1S2T5	D2S1T1	D2S1T2
D1S1T1	0.954159	0.937023	0.960068	0.944023	0.504512	0.528257
D1S1T2	0.886387	0.891167	0.891541	0.899464	0.610733	0.633646
D1S1T3	0.872271	0.862560	0.879251	0.873159	0.562562	0.578129
D1S1T4	0.820881	0.843910	0.833515	0.820138	0.472736	0.490813
D1S1T5	0.881564	0.887443	0.894172	0.885561	0.575212	0.584694
D1S2T1	0.981951	0.962296	0.983668	0.985206	0.502035	0.544098
D1S2T2	1.000000	0.966461	0.980795	0.985874	0.465103	0.517290
D1S2T3	0.966461	1.000000	0.970102	0.962646	0.492679	0.541628
D1S2T4	0.980795 0	0.970102	1.000000	0.990755	0.547285	0.596270
D1S2T5	0.985874	0.962646	0.990755	1.000000	0.543426	0.597772
D2S1T1	0.465103	0.492679	0.547285	0.543426	1.000000	0.982821

D2S1T2	0.517290	0.541628	0.596270	0.597772	0.982821	1.000000
D2S1T3	0.651946	0.659942	0.714001	0.724369	0.891031	0.931986
D2S1T4	0.617577	0.631746	0.698937	0.689652	0.936240	0.974721
D2S1T5	0.683165	0.691705	0.745442	0.751442	0.875622	0.936158
D2S2T1	0.552813	0.551526	0.596842	0.596740	0.803174	0.794429
D2S2T2	0.635406	0.619281	0.672213	0.668375	0.749471	0.731779
D2S2T3	0.438512	0.448964	0.475472	0.495074	0.801582	0.786872

Pearson Correlations Section (Pair-Wise Deletion)

	D2S1T3	D2S1T4	D2S1T5	D2S2T1	D2S2T2	D2S2T3
D1S1T1	0.627701	0.625020	0.661447	0.530749	0.606537	0.392073
D1S1T2	0.707757	0.669101	0.724771	0.561153	0.612978	0.474546
D1S1T3	0.662484	0.621000	0.672631	0.571290	0.631655	0.451074
D1S1T4	0.586290	0.533988	0.590643	0.486475	0.545919	0.374088
D1S1T5	0.669507	0.626975	0.667492	0.580440	0.641220	0.465722
D1S2T1	0.657999	0.633642	0.681161	0.572242	0.665465	0.470612
D1S2T2	0.651946	0.617577	0.683165	0.552813	0.635406	0.438512
D1S2T3	0.659942	0.631746	0.691705	0.551526	0.619281	0.448964
D1S2T4	0.714001	0.698937	0.745442	0.596842	0.672213	0.475472
D1S2T5	0.724369	0.689652	0.751442	0.596740	0.668375	0.495074
D2S1T1	0.891031	0.936240	0.875622	0.803174	0.749471	0.801582
D2S1T2	0.931986	0.974721	0.936158	0.794429	0.731779	0.786872
D2S1T3	1.000000	0.951509	0.978050	0.845513	0.766222	0.789240
D2S1T4	0.951509	1.000000	0.973292	0.787813	0.730675	0.740047
D2S1T5	0.978050	0.973292	1.000000	0.803537	0.734757	0.747678

D2S2T1	0.845513	0.787813	0.803537	1.000000	0.959390	0.945550
D2S2T2	0.766222	0.730675	0.734757	0.959390	1.000000	0.914832
D2S2T3	0.789240	0.740047	0.747678	0.945550	0.914832	1.000000

Pearson Correlations Section (Pair-Wise Deletion)

	D2S2T4	D2S2T5	D3S1T1	D3S1T2	D3S1T3	D3S1T4
D1S1T1	0.477004	0.478750	0.431405	0.509140	0.499885	0.522051
D1S1T2	0.566176	0.513877	0.404215	0.415796	0.439842	0.439479
D1S1T3	0.570757	0.542390	0.298343	0.345869	0.361398	0.362721
D1S1T4	0.496915	0.472986	0.212548	0.266273	0.303594	0.295262
D1S1T5	0.578197	0.543933	0.330491	0.389509	0.418437	0.410489
D1S2T1	0.559173	0.595514	0.425662	0.508170	0.489586	0.513795
D1S2T2	0.523109	0.564339	0.424046	0.486232	0.458431	0.481524
D1S2T3	0.528430	0.557850	0.473393	0.516213	0.524208	0.525253
D1S2T4	0.568003	0.589373	0.445199	0.525248	0.515066	0.532761
D1S2T5	0.571608	0.594818	0.452740	0.513951	0.500055	0.521197
D2S1T1	0.795429	0.645750	0.660492	0.633109	0.681302	0.654697
D2S1T2	0.780126	0.628797	0.655442	0.591994	0.643449	0.621808

D2S1T3	0.800405	0.714811	0.575880	0.530877	0.564594	0.549678
D2S1T4	0.750274	0.614294	0.636087	0.583526	0.624896	0.618917
D2S1T5	0.763783	0.650802	0.591751	0.509667	0.542736	0.538402
D2S2T1	0.959605	0.901676	0.610784	0.558220	0.533873	0.514742
D2S2T2	0.953462	0.932358	0.639945	0.643205	0.595132	0.591023
D2S2T3	0.957365	0.865486	0.674296	0.595660	0.576937	0.566247

Pearson Correlations Section (Pair-Wise Deletion)

	D3S1T5	D3S2T1	D3S2T2	D3S2T3	D3S2T4	D3S2T5
D1S1T1	0.563752	0.492936	0.513099	0.464199	0.437821	0.505333
D1S1T2	0.471613	0.500900	0.545793	0.463687	0.427741	0.599271
D1S1T3	0.409375	0.421277	0.447444	0.397447	0.367877	0.518506
D1S1T4	0.356803	0.336903	0.360148	0.316113	0.291549	0.448297
D1S1T5	0.457155	0.423680	0.450758	0.411004	0.391284	0.534320
D1S2T1	0.565669	0.525119	0.553136	0.479731	0.388057	0.517608
D1S2T2	0.564596	0.514236	0.542578	0.467116	0.367595	0.498184
D1S2T3	0.606331	0.516365	0.540445	0.499915	0.420909	0.536481
D1S2T4	0.587014	0.519144	0.551268	0.512019	0.434662	0.552690

D1S2T5	0.576386	0.557933	0.591904	0.527080	0.425277	0.559809
D2S1T1	0.497957	0.669968	0.723951	0.739011	0.791218	0.844203
D2S1T2	0.476924	0.667910	0.717710	0.734862	0.754256	0.824940
D2S1T3	0.484602	0.644108	0.702825	0.710268	0.668838	0.764250
D2S1T4	0.496126	0.650402	0.695603	0.723726	0.725276	0.788619
D2S1T5	0.464158	0.639807	0.686333	0.693797	0.653117	0.745636
D2S2T1	0.475720	0.586722	0.661765	0.664376	0.651015	0.717891
D2S2T2	0.581575	0.600420	0.682660	0.656584	0.648267	0.740970
D2S2T3	0.499348	0.634108	0.716640	0.673248	0.691104	0.762642

Pearson Correlations Section (Pair-Wise Deletion)

	D1S1T1	D1S1T2	D1S1T3	D1S1T4	D1S1T5	D1S2T1
D2S2T4	0.477004	0.566176	0.570757	0.496915	0.578197	0.559173
D2S2T5	0.478750	0.513877	0.542390	0.472986	0.543933	0.595514
D3S1T1	0.431405	0.404215	0.298343	0.212548	0.330491	0.425662
D3S1T2	0.509140	0.415796	0.345869	0.266273	0.389509	0.508170
D3S1T3	0.499885	0.439842	0.361398	0.303594	0.418437	0.489586
D3S1T4	0.522051	0.439479	0.362721	0.295262	0.410489	0.513795

D3S1T5	0.563752	0.471613	0.409375	0.356803	0.457155	0.565669
D3S2T1	0.492936	0.500900	0.421277	0.336903	0.423680	0.525119
D3S2T2	0.513099	0.545793	0.447444	0.360148	0.450758	0.553136
D3S2T3	0.464199	0.463687	0.397447	0.316113	0.411004	0.479731
D3S2T4	0.437821	0.427741	0.367877	0.291549	0.391284	0.388057
D3S2T5	0.505333	0.599271	0.518506	0.448297	0.534320	0.517608

Pearson Correlations Section (Pair-Wise Deletion)

	D1S2T2	D1S2T3	D1S2T4	D1S2T5	D2S1T1	D2S1T2
D2S2T4	0.523109	0.528430	0.568003	0.571608	0.795429	0.780126
D2S2T5	0.564339	0.557850	0.589373	0.594818	0.645750	0.628797
D3S1T1	0.424046	0.473393	0.445199	0.452740	0.660492	0.655442
D3S1T2	0.486232	0.516213	0.525248	0.513951	0.633109	0.591994
D3S1T3	0.458431	0.524208	0.515066	0.500055	0.681302	0.643449
D3S1T4	0.481524	0.525253	0.532761	0.521197	0.654697	0.621808
D3S1T5	0.564596	0.606331	0.587014	0.576386	0.497957	0.476924
D3S2T1	0.514236	0.516365	0.519144	0.557933	0.669968	0.667910
D3S2T2	0.542578	0.540445	0.551268	0.591904	0.723951	0.717710
D3S2T3	0.467116	0.499915	0.512019	0.527080	0.739011	0.734862
D3S2T4	0.367595	0.420909	0.434662	0.425277	0.791218	0.754256
D3S2T5	0.498184	0.536481	0.552690	0.559809	0.844203	0.824940

Pearson Correlations Section (Pair-Wise Deletion)

	D2S1T3	D2S1T4	D2S1T5	D2S2T1	D2S2T2	D2S2T3
D2S2T4	0.800405	0.750274	0.763783	0.959605	0.953462	0.957365
D2S2T5	0.714811	0.614294	0.650802	0.901676	0.932358	0.865486
D3S1T1	0.575880	0.636087	0.591751	0.610784	0.639945	0.674296
D3S1T2	0.530877	0.583526	0.509667	0.558220	0.643205	0.595660
D3S1T3	0.564594	0.624896	0.542736	0.533873	0.595132	0.576937
D3S1T4	0.549678	0.618917	0.538402	0.514742	0.591023	0.566247
D3S1T5	0.484602	0.496126	0.464158	0.475720	0.581575	0.499348
D3S2T1	0.644108	0.650402	0.639807	0.586722	0.600420	0.634108
D3S2T2	0.702825	0.695603	0.686333	0.661765	0.682660	0.716640
D3S2T3	0.710268	0.723726	0.693797	0.664376	0.656584	0.673248
D3S2T4	0.668838	0.725276	0.653117	0.651015	0.648267	0.691104
D3S2T5	0.764250	0.788619	0.745636	0.717891	0.740970	0.762642

Pearson Correlations Section (Pair-Wise Deletion)

	D2S2T4	D2S2T5	D3S1T1	D3S1T2	D3S1T3	D3S1T4
D2S2T4	1.000000	0.924424	0.574336	0.548075	0.521317	0.499765
D2S2T5	0.924424	1.000000	0.516037	0.560432	0.501018	0.484454
D3S1T1	0.574336	0.516037	1.000000	0.862227	0.863254	0.880300
D3S1T2	0.548075	0.560432	0.862227	1.000000	0.977909	0.973468
D3S1T3	0.521317	0.501018	0.863254	0.977909	1.000000	0.982774
D3S1T4	0.499765	0.484454	0.880300	0.973468	0.982774	1.000000
D3S1T5	0.473255	0.526588	0.789772	0.949691	0.934636	0.928703
D3S2T1	0.580439	0.526928	0.799600	0.810277	0.806494	0.818992
D3S2T2	0.658153	0.615235	0.831678	0.829463	0.814885	0.828934
D3S2T3	0.625403	0.573429	0.803057	0.846171	0.854239	0.831986
D3S2T4	0.617246	0.521911	0.875612	0.886945	0.912412	0.904310
D3S2T5	0.744773	0.642017	0.798404	0.830770	0.846658	0.822143

Pearson Correlations Section (Pair-Wise Deletion)

	D3S1T5	D3S2T1	D3S2T2	D3S2T3	D3S2T4	D3S2T5
D2S2T4	0.473255	0.580439	0.658153	0.625403	0.617246	0.744773

D2S2T5	0.526588	0.526928	0.615235	0.573429	0.521911	0.642017
D3S1T1	0.789772	0.799600	0.831678	0.803057	0.875612	0.798404
D3S1T2	0.949691	0.810277	0.829463	0.846171	0.886945	0.830770
D3S1T3	0.934636	0.806494	0.814885	0.854239	0.912412	0.846658
D3S1T4	0.928703	0.818992	0.828934	0.831986	0.904310	0.822143
D3S1T5	1.000000	0.759804	0.768045	0.794107	0.802933	0.777491
D3S2T1	0.759804	1.000000	0.975510	0.932923	0.848900	0.854184
D3S2T2	0.768045	0.975510	1.000000	0.925798	0.857936	0.900274
D3S2T3	0.794107	0.932923	0.925798	1.000000	0.922290	0.919903
D3S2T4	0.802933	0.848900	0.857936	0.922290	1.000000	0.915073
D3S2T5	0.777491	0.854184	0.900274	0.919903	0.915073	1.000000

Correlation Report
Mean values for Days and Sets

Pearson Correlations Section (Pair-Wise Deletion)

	D1S1	D1S2	D2S1	D2S2	D3S1	D3S2
D1S1	1.000000	0.915663	0.638960	0.552934	0.420033	0.469687
D1S2	0.915663	1.000000	0.648679	0.583790	0.534064	0.524893
D2S1	0.638960	0.648679	1.000000	0.785232	0.607244	0.766606
D2S2	0.552934	0.583790	0.785232	1.000000	0.584744	0.693793
D3S1	0.420033	0.534064	0.607244	0.584744	1.000000	0.891394
D3S2	0.469687	0.524893	0.766606	0.693793	0.891394	1.000000

Cronbachs Alpha = 0.891157 Standardized Cronbachs Alpha = 0.914687

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Physical activity and bending stiffness in human long bones.
Fracture risk assessment via mechanical testing.

PUBLICATIONS

Graduate, R.T., W.G. Advisor. 2000. Test-retest reliability of tibial bending stiffness in humans using mechanical response tissue analysis. *Med & Sci Sports Exerc*. 32(5):s146.

Colleague, D.W., R.T. Graduate, W.K. Colleague, W.G. Advisor. 2000. Relationship between isokinetic knee flexion/extension strength and tibial bending stiffness. *Med & Sci Sports Exerc*. 32(5):s1056.

ABSTRACTS

Graduate, R.T., D.W. Colleague, W.K. Colleague, W.G. Advisor. 2000. Mechanical response tissue analysis (mrta): a pilot study to reliably assess bending stiffness (ei) of the human tibia. American College of Sports Medicine (ACSM) Southeastern Conference, Charlotte, NC.