Reliability of Tibial Measurement with Mechanical Response Tissue Analysis

by

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ABSTRACT

Mechanical response tissue analysis (MRTA) provides a noninvasive means of estimating the cross-sectional bending stiffness (EI) of long bones, and thus can serve as a predictor of bone strength. Estimates of bone bending stiffness are derived from the point impedance response of a long bone to low frequency (70-500Hz) stimulation according to beam vibration theory. MRTA has demonstrated the ability to reliably estimate human ulnar bending stiffness with between-test coefficients of variation of 5%, and *in vivo* measurements of monkey tibiae have been validated with *ex vivo* 3-point mechanical bending tests. Human tibial MRTA measurement has achieved between-trial coefficients of variation of only 12%, so a new physical MRTA configuration and improved computer algorithms have been developed in an attempt to improve upon this level of reliability. The new configuration removes the rigid proximal and distal tibial restraints and models the tissue behavior with a 12-parameter algorithm that accounts for free vibration at the ankle and knee joints. Initial testing with only the hardware changes and application of the 7-parameter model of tissue behavior used in earlier systems yielded unacceptable variation. Subsequent reliability testing with application of 6-, 9-, and 12-parameter models demonstrated modest improvements, prompting the development of the more robust 12-parameter model used in the present study. Evaluation of 110 college-age females (age 20.2±1.8 yr, height 163.3±5.9 cm, weight 60.7±9.3 kg, BMI 22.8±3.1 $kg \cdot m^{-2}$) with the current MRTA system has demonstrated an improvement in within-trial reliability for unsupported tibial EI measurement with a coefficient of variation of 11.2%. These results demonstrate the ability of the system to measure tibial response characteristics when both proximal and distal ends are free of rigid support. Long-term measurement reliability is still problematic with a coefficient of variation of 36.5% for a set of 4 measurements spanning 21 months.

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CHAPTER 1
Introduction

The primary objective in bone strength research is fracture prediction and the tools presently available are not performing adequately; therefore, research and development into new tools and techniques is warranted. Fractures generally fall into four categories: osteoporotic compression fractures, osteoporotic traumatic fractures, stress fractures, and general traumatic fractures. The two categories of osteoporotic fractures involve bones that have low bone mineral density (BMD) and are structurally weak. Bones that are structurally weak will fracture at relatively low levels of stress, such as the compressive stress on the spine experienced while bending over or the trauma experienced during a fall from standing. Stress fractures generally occur in apparently healthy people when they are subjected to unaccustomed levels of repetitive physical activity that exceeds the loading capacity of their bones. General traumatic fractures occur with acute loading beyond the stress levels that a normal healthy bone can tolerate, such as during a collision or a fall from a height. Accurate and reliable measurement of bone strength is important for the identification of individuals that require intervention for either the prevention of osteoporotic fractures or stress fractures, while general traumatic fractures, on the whole, are less related to structural weakness and will not be prevented by screening.

The societal cost of osteoporotic fractures cannot be overlooked with estimates on the order of $17 billion in yearly health care expenses in the United States as of 2001 for an estimated 1.5 million fractures (NOF 2003). These figures are up from 1995 estimates of 1.3 million osteoporotic fractures and yearly costs of $13 billion (Ray *et al.* 1997) and, with the projected aging of the population, the number of yearly fractures and associated health care expenses will continue to grow accordingly. Females over the age of 50 have a 40% chance of sustaining a symptomatic fracture in their lifetime (Melton *et al.* 1992). Hip fractures increase mortality by 10 to 20% depending upon age and sex of the victim (Marcus *et al.* 2001), and five years from the diagnosis, mortality rates associated with vertebral fractures are similar to mortality rates associated with hip fracture (Cooper *et al.*

1993). Clearly, prediction of fracture risk would aid in treatment and prevention, with the potential to avert billions of dollars of health care expenditures, saving suffering and money for both the individuals affected and the society as a whole.

Two primary populations affected by stress fractures are new military recruits and competitive athletes, as both of these groups are under pressure to increase their performance levels. To attain high levels of performance, these groups exercise intensely with motions that place the bones under repeated stress loading cycles. Over time, repeated loading leads to the accumulation of microcracks and for some individuals this accumulation exceeds their tolerance limit and results in a stress fracture, effectively removing them from participation. The lost participation time is costly for athletes, new military recruits, and their respective organizations. It is estimated that the cost of stress fractures to the Department of Defense is in excess of $10 million per year in medical expenses and lost duty time (USAMRMC 1999). Early identification of recruits at risk for stress fractures would allow for placement into alternative training programs designed to strengthen bones without the development of fractures. A method of stress fracture risk assessment may also be of interest to athletic teams who are in the business of pushing players for peak performance, but run the risk of sidelining valuable athletes if they are pushed too far (Bennell *et al.* 1996).

Presently, dual-energy x-ray absorptiometry (DXA) is used to measure BMD for the diagnosis of osteoporosis. BMD is one component of bone strength, but unfortunately, it has a low predictive ability relative to fracture risk (Burr and Martin 1983; Marshall *et al.* 1996). Bone strength is also dependent upon architectural properties of the bone, such as cortical thickness (Augat *et al.* 1996), periosteal diameter (Bouxsein *et al.* 1994; Grutter *et al.* 2000), trabecular connectivity (Vajjhala *et al.* 2000; Keaveny *et al.* 2001), mineral crystal alignment (Currey 1969), and collagen fiber alignment (Martin 1991). Measurement tools that can quantify these architectural parameters hold promise for enhancing the ability to predict fractures. Due to the poor predictive capabilities of current technology, many people are unnecessarily treated with antiresorptive medications and subjected to the potential dangers associated with these

medications. It is suggested that a pharmacologically reduced rate of bone turnover will lead to microcrack accumulation and hypermineralization associated with increased brittleness and fragility of the bone (Currey 1990; Turner 2002). Additionally, with the growing burden of prescription medication costs for an aging population, measures should be pursued to limit the prescription of unnecessary medications. The costs of unnecessary medication reach beyond the affected patient; they contribute to growing insurance costs and burden a medical care system that is in distress.

An additional contribution to be derived from the measurement of bone strength is the step that it can make toward the possibility of long range space travel. In low or zero gravity environments, BMD of the normal weight bearing bones declines rapidly even in the presence of exercise countermeasures (McCarthy *et al.* 2000). Identification of appropriate countermeasures requires improved tools capable of detecting the small changes in bone strength that occur during short durations in low gravity environments and potentially detecting other parameters of bone health. The large size of DXA systems makes them impractical for use in space, so a small device, such as a Mechanical Response Tissue Analysis (MRTA) system brings the potential of following bone adaptations in a microgravity environment.

As progress is made in new areas of research, the potential for offshoots in novel directions grows. MRTA is an example of the novel application of a mechanical engineering technique to bone strength measurement in humans and animals. MRTA represents a technology that has the potential to improve upon the current predictive ability of bone strength measurement tools. If actual bone strength measurement and the prediction of fracture risk can be improved upon, and improvements in diagnosis and the application of pharmacological interventions can be realized, research studies will benefit as they are able to detect more meaningful changes, and cost effective screening will open the door to early detection and treatment for a larger portion of the population.

To understand the importance of MRTA it is helpful to review some basics of bone biology as related to bone adaptation and strength. Wolff's Law, as developed in

1892, states that as the function of a bone changes, the internal architecture and external confirmation will adapt to reflect the new function (Wolff 1986). These functional adaptations are carried out by the remodeling process of bone which involves the continual resorption and formation of bone tissue, allowing for additional deposition of bone in response to increased regional loading, as well as the loss of bone in response to decreased regional loading. The process of remodeling in response to loading follows three rules outlined by Turner (1998). The first rule of bone adaptation is that bone responds to dynamic loading and not to static loading. The second rule is that only a short duration of mechanical loading is necessary for a response, and the third rule is that bones accommodate to regular strains; thus, they require abnormal strains to initiate further adaptation. Turner's rules were established through experimental animal model research (Lanyon *et al.* 1982; Lanyon and Rubin 1984; Rubin and Lanyon 1984; Turner *et al.* 1994), but exercise research involving human subjects has demonstrated mixed results with respect to Turner's rules. Some exercise studies support the bone remodeling rules, while others fail to find significant results with designs that should elicit bone strengthening (Adami *et al.* 1999; Jarvinen *et al.* 1999). The discrepancies between predicted changes in bone strength and measured changes potentially stem from the measurement tools and techniques employed (Jarvinen *et al.* 1999). Exercise studies have often relied upon BMD as a surrogate measure of strength, but as noted by Wolff, architectural changes accompany new loading environments. Exercise interventions that affect a significant strength change through modification of architecture, but do not demonstrate significant BMD changes, will require a measure that is sensitive to the structural strength improvements.

Problem Statement

It is essential to understand the reliability of MRTA with respect to both testing at a single point in time and serial testing over a time span comparable to that covered in typical exercise training studies. Without establishing the reliability of the present MRTA system, including the latest algorithms, the validity of measures will be questionable and any conclusions drawn from the data will be open to criticism. Additionally, a full understanding of the system reliability will allow for appropriate power analysis and experimental design for future research, as well as the comparison of results with other testing modalities.

The most recent version of the MRTA hardware, as found in the Human Performance Laboratory at Virginia Tech, has removed the limb fixation structures seen in previous versions. Limb positioning with fixation of proximal and distal ends of the bone involved substantial technician training and experience, so by eliminating the requirement for limb fixation the amount of technician training and experience can be reduced. The theory is that with the additional computational features provided by the 12-parameter model, fixation of the bone ends is no longer required, hopefully reducing the error introduced by the technician's level of experience. Additionally, the reduction in technician training and experience is necessary for a practical transition to future clinical application.

Specific Aims

1. To determine the short-term (measures taken within one session) reliability of tibial measurement with the current MRTA system and the 12-parameter model.
2. To determine the long-term (serial measurements taken over ~21 months) reliability of tibial measurement with the current MRTA system and the 12-parameter model.

3. To determine the short-term (day-to-day) reliability of tibial MRTA measurement with the current MRTA system and the 12-parameter model.

Methods

Bone bending stiffness data, as measured with MRTA, were collected on 110 college-age females as part of a study on bone adaptation to concentric and eccentric isokinetic training. MRTA measurements were collected at baseline, at 4.5 months of training, at the conclusion of 9 months of training, and at approximately 12 months post training. Subjects were randomized into either concentric or eccentric training groups and then attended three isokinetic training sessions per week for nine months, during which they trained only their non-dominant arms and legs. At each measurement phase, nine successive MRTA measures were taken at the mid-point of the ulna and at the mid-point of the leading edge of the tibia.

The subject was positioned to bring the MRTA probe into transcutaneous mechanical contact with the bone to be measured. Good contact with the bone was established by palpating the proximal and distal end of the bone to check for the transmission of vibration by the probe. After each measurement, raw data were displayed on the computer screen, at which point the technician had the option of accepting or rejecting data based upon visual inspection. Measures that clearly demonstrated significant levels of noise (data do not represent a smooth curve) were rejected and subject contact with the probe was reestablished. Figures 1.1 and 1.2 illustrate data sets that were accepted by the technician (dark lines represent raw data, light lines represent the curve established by the 12-parameter model).

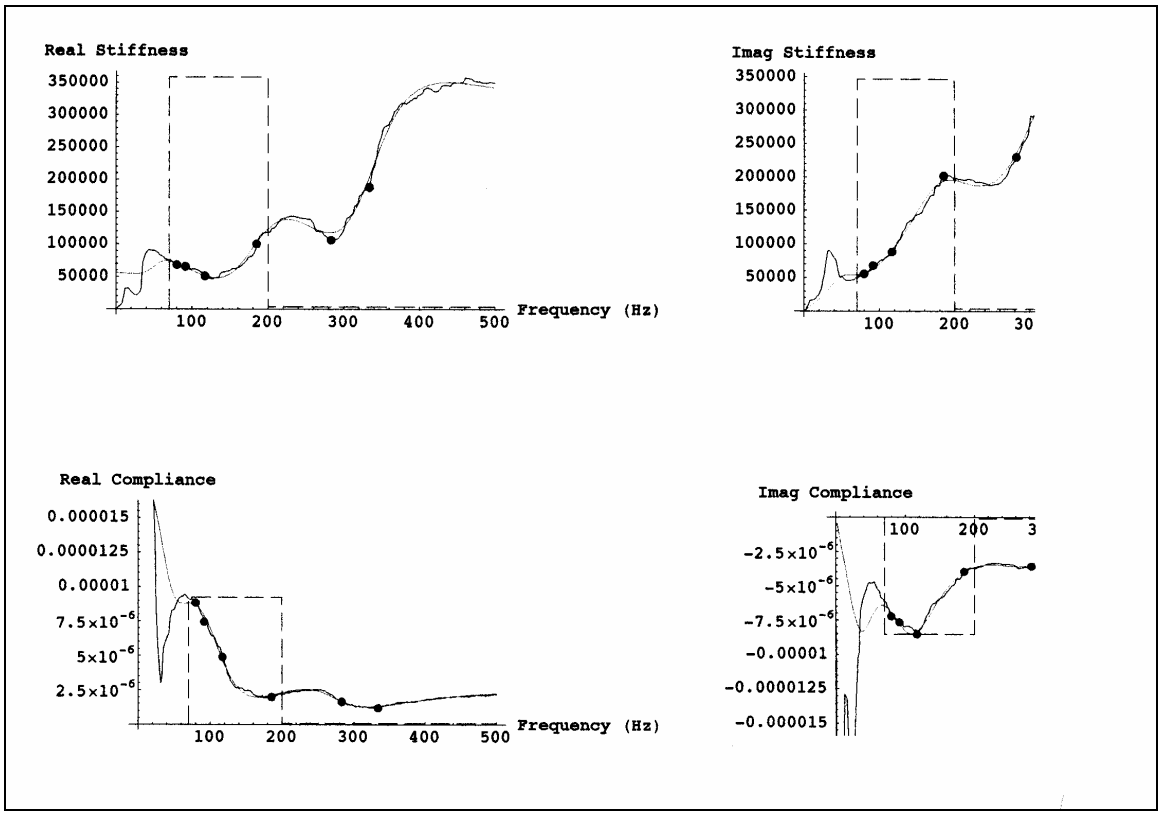

Figure 1.1. Example of 12-parameter model output with good fit to raw data. Measurement meets RMS criteria. The dashed lines show the weighting amplitude with the bias for a good fit in the low frequency range 70-200 Hz. The measurement is not accurate for low frequency 0-70 Hz, and the noise is usually excessive for high frequency 500-1600 Hz.

rmsStiff = 0.028
rmsComp = 0.039
rmsPoleZero = 0.062
rmsEff = 0.032 → Accepted

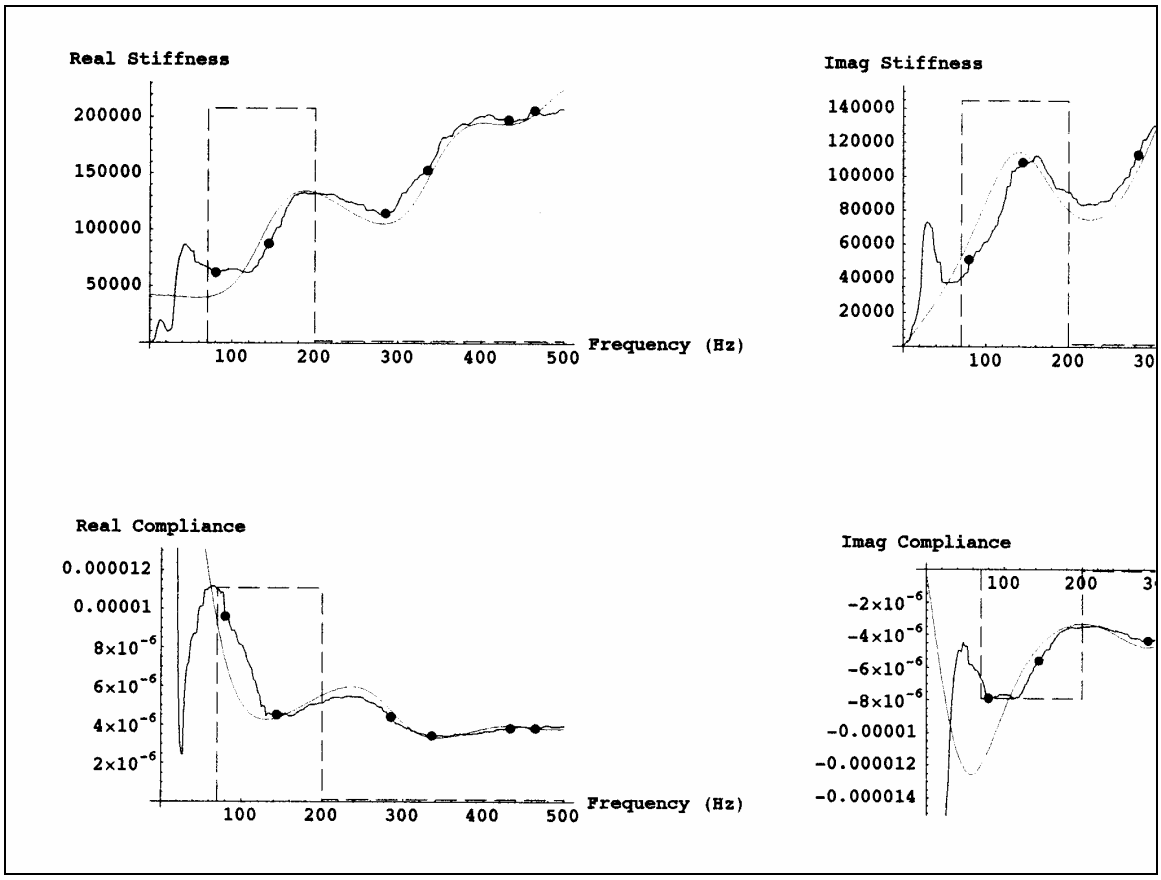

Figure 1.2. Example of 12-parameter model output with poor fit to raw data. Measurement does not meet RMS criteria.

rmsStiff = 0.080

rmsComp = 0.198

rmsPoleZero = 0.506

rmsEff = 0.133 → Rejected

Raw data were analyzed with a 12-parameter model of tissue behavior to predict bending stiffness (EI). This analysis process attempts to match a prediction curve to raw data. The success of the prediction curve in modeling raw data is estimated by the root mean square errors (RMS) between the prediction and raw data. Based upon the RMS values, measurements are accepted if they achieve RMS < 0.10, and the "Best EI" is selected based upon the measure with the lowest effective RMS score.

Short-term (within-trial) reliability involved the within-subject analysis of accepted measures (RMS < 0.10) recorded during one session for each anatomical site. Each site was measured nine times without repositioning, so short-term reliability in this case potentially yielded information about the variability attributable to subject characteristics. The individual subject's CV for each measurement session was calculated as the standard deviation of measurements that achieve effective RMS values lower than 0.1 divided by the mean of the measurements. To control for unequal numbers of measurements per subject a one-way analysis of variance (ANOVA) was applied to compute the mean square error term (MS_E), with subject+bone (each measured tibia) as the factor. The standard error of measurement (SEM) is the square root of MS_E and the CV is computed as the SEM divided by the mean of all measurements: CV% = (SEM/mean)·100. For consistency this method was used to calculate CV for both within-trial and between-trial comparisons, where within-trial CV evaluates nine measurements taken in a row on each tibia and the between-trial CV evaluates the mean measurement value for each tibia from each testing session.

Short-term (between-trial) reliability involved analysis of measurements taken on each subject over the course of three days in a period of less than one week. Long-term (between-trial) reliability involved the within-subject comparison of the average EI obtained on untrained (control) limbs for each measurement site across a series of 4 measurement sessions spread over the course of ~21 months (baseline, 4.5 months, 9 months, 21 months). This analysis established the variation that can be expected over the course of a typical exercise intervention, for similar subject populations.

Hypotheses

1. Within-trial CV of tibial EI and sufficiency (S) measurements was less than 5 percent.
2. Between-trial (long-term: 21 mo) CV of tibial EI and S measurements are less than 12 percent.

3. Between-trial (short-term: 1 wk) CV of tibial EI and S measurements are less than 12 percent.
4. Within-subject variation in EI or S is not a function of EI, body weight, or height.

Delimitations

The following delimitations are inherent to the design of this investigation:

1. The study of short-term (within-trial) reliability and long-term (21 months) reliability is confined to healthy females who are not taking medications that may alter bone metabolism, with the exception of oral contraceptives, 18 to 22 years of age, and recruited from the Virginia Tech student body.
2. The study of short-term (day-to-day) reliability is confined to healthy adults recruited in Blacksburg, VA.
3. Anatomic measurement sites are limited to the mid-tibia on right and left legs.
4. The independent variables are confined to nine repeated measures at each anatomic measurement site for short-term (within-trial) reliability, three measurements for short-term (day-to-day) reliability, and four measurements across 21 months for long-term reliability.
5. The dependent variables for short-term reliability are confined to "EI" and "S" of the tibia, and for long-term reliability are confined to "Average EI" and "S" of the tibia.

Limitations

The limitations of the study are:

1. For the short-term (within-trial) and long-term evaluations, subjects are recruited from a homogeneous subset of the population and, therefore, do not represent the larger population.
2. Habitual activity levels were not measured, so variation due to habitual activity is unaccounted for.

Basic Assumptions

1. The MRTA system was properly calibrated before each measurement session.
2. MRTA technicians followed the same standard procedures for each measurement session.
3. Subjects maintained a steady relaxed posture during each measurement session.
4. Bone stiffness “EI” is a stable short-term measure.

GLOSSARY

3-point mechanical bending tests – test of beam bending stiffness and ultimate load; beam is supported at each end and a force is applied at a central point to deform and potentially break the beam (see figure 2.3); the force applied to the beam (stress) and the displacement of the beam (strain) are plotted to form the stress-strain diagram (see figure 2.2).

12-parameter model – computational model applied to analysis of raw data collected with the MRTA system, with the goal of fitting a mathematical model to the raw data to solve for the bending stiffness of the bone; this model incorporates parameters of mass, stiffness, and damping for the bone, the skin, the proximal, and the distal joints (see figure 2.10).

cross-sectional bending stiffness (EI) – a extrinsic measure of the resistance of a beam to lateral deformation; product of Young's modulus (E) [N/m^2] and the cross-sectional moment of inertia (I) [m^4]; units of Nm^2.

Best EI – the EI value selected from a series of repeated measures during a session based upon the lowest RMS score for the series.

collagen fibers – structural proteins contributing to the toughness of bone

hydroxyapatite – crystal of calcium phosphate; the primary form of mineral in bone; individual crystals are hexagonal and measure ~50 x 50 x 400 angstroms (1μm = 10,000 Å)

reliability – the reproducibility of a value when it is measured two or more times

sufficiency (S) – an estimate of the number of body weights that a bone can support in axial loading

trabeculae – bone in the form of plates or struts with a thickness of ~200 μm; axial length on the order of 1 mm and axial diameter on the order of 0.1 mm

CHAPTER 2

Introduction

This review of the literature will serve as a background supporting the need for further development of the Mechanical Response Tissue Analysis system (MRTA). MRTA serves as a non-invasive tool for evaluating the stiffness of long bones. In humans both the tibia and ulna are accessible for *in vivo* measurement with MRTA. The stiffness of a long bone is predictive ($r^2 > 0.9$) of the maximum strength of the bone (Roberts *et al.* 1996), and therefore actually measures the quality of the bone, which is based upon more than just the size and density of the bone. Microarchitectural parameters, such as the alignment and distribution of collagen fibers and hydroxyapatite crystals, and the connectivity of trabeculae contribute to the strength of the bone, but are not measured or easily quantified by other analysis systems.

A primary objective of MRTA development is to improve its utility as a research tool for the study of how exercise relates to bone health. To understand how MRTA can be applied to exercise physiology research it is important to develop a broad understanding of bone research in relation to various modes of exercise, so an extensive review of past exercise related bone research is included to provide this background. A brief discussion of basic bone physiology, including function, structure, and adaptation, will provide background necessary for a review of exercise interventions, structural mechanics, and measurement tools involved in bone research.

The literature pertaining to the effect of exercise on bone strength will explore findings of cross sectional analysis of various athletic populations, as well as longitudinal studies of athletic populations and randomized control studies of exercise interventions. These human exercise interventions will be followed by examples of animal exercise models and animal models involving various mechanical bone loading or unloading situations. The sum of these bone studies demonstrates that exercise can influence bone adaptation to yield greater bone density and strength, but that there are potential

deficiencies in the measurement techniques that result in non-significant findings in terms of bone density. Animal models that predict bone strength increases under various conditions but are not found under human exercise conditions introduce the possibility that bone density measures are not accurately predicting bone strength increases. If this is the case then a different measure of bone strength, such as bending stiffness, may reveal benefit where bone density is not significantly changed.

A brief review of the structural mechanics of bone will set the stage for a discussion of the various measurement systems used to evaluate bone adaptations. The strength of long bones is dependent upon a variety of factors, from the macroarchitectural components of length, cross sectional diameter and cortical wall thickness to the microarchitectural components of fiber and crystal orientation. The same principles that apply to the mechanics of inert composite beams apply to live bones, and an understanding of these principles is important for the evaluation of measurement systems.

The measurement systems incorporated in bone strength evaluation range from large facility-based systems, such as DXA and quantitative computed tomography (QCT), to smaller more portable systems, such as quantitative ultrasound and MRTA. A review of the strengths and weaknesses of various systems demonstrates where the MRTA system could fill a gap in the spectrum of measurement tools. With the variety of measurement systems utilized in bone strength research, there are a variety of measures attempting to quantify or predict bone strength. For clarity and consistency, these measures will be referred to as measures of bone strength as a group, but will be referred to by their individual measures when they stand alone.

Basic Bone Structural Physiology

Functions

Basic functions of the skeletal system are to provide mechanical support and protection, to act as a sink for plasma calcium homeostasis, and to support hematopoiesis.

The mechanical support function of the skeletal system is regulated to exceed the needs of the general stress and strains placed upon the system. In general, the breaking strength of a bone is ten times greater than the voluntary strain that an individual can exert (Burr *et al.* 1996). The protection function of bone is most clearly demonstrated in the skull, which maintains its integrity in the absence of a load bearing role. For this reason the BMD of the skull is sometimes used as a comparative measure between groups to see if higher BMD in weight bearing bones is related to an overall level of high BMD throughout the skeleton, or if it active subjects have higher BMD at load bearing sites and equivalent BMD of the skull when compared to non-active subjects.

Composition

The osseous components of long bones generally fall into two categories, cortical (or compact) bone and trabecular (or cancellous) bone. Cortical bone is the dense tissue forming the outer shell of bone and ranges in porosity from 5-10% (Martin *et al.* 1998). The skeleton is approximately 75-80% cortical bone by mass with the remaining 20-25% being trabecular bone (Bailey *et al.* 1996). Trabecular bone forms a matrix of thin plates, on the order of 0.1mm thick and 1mm long (Gunaratne *et al.* 2002), which are distributed throughout a space encompassed by cortical bone. Trabecular bone is found in the vertebral bodies, the ends of long bones and in some flat bones and has a porosity of 75 to 95% (Martin *et al.* 1998), giving it a sponge-like appearance. The greater porosity of trabecular bone gives it greater surface area to volume ratio compared to cortical bone; thus, trabecular bone is thought to experience higher metabolic activity and turnover (Bailey *et al.* 1996; Mosley 2000). Additionally, the small diameters of individual trabeculae make them susceptible to complete erosion in the presence of resorptive trends in bone remodeling, and once they are dissociated, it is thought that they cannot be reformed (Parfitt *et al.* 1983).

Both cortical and trabecular bone are composed of mineral, protein, fat, and water, with the distribution of these elements based upon conditions that vary throughout the skeleton. By mass, mineral accounts for approximately 70% of bone tissue and this is

primarily in the form of hydroxyapatite (Marcus *et al.* 2001). Hydroxyapatite is a crystal of calcium phosphate and lends compressive strength to bone. The primary minerals found in bone are calcium, phosphorus, sodium, potassium, zinc, and magnesium (Bailey *et al.* 1996). Collagen fibers, the main form of bone matrix protein, contribute to the toughness and tensile strength of bone.

The primary microstructure of bone is the lamella, or layers, in which collagen fibers are oriented in the same direction. The alternating orientation of adjacent lamella contribute to the strength of the bone by alternately accepting either tensile stresses or compressive stresses depending upon their orientation (Ascenzi 1988). Through the remodeling process, lamellae are formed concentrically around a blood vessel to form an osteon, known as a Haversian system (Martin *et al.* 1998).

Adaptation

The three processes of bone adaptation are growth, modeling, and remodeling. Growth is the genetically determined process of skeletal development until adult size is reached. Modeling is the process in which site-specific adaptation of bone occurs in response to loading conditions. Modeling involves bone formation, the addition of bone mass, without bone resorption, for the development of strength in response to localized strains placed on the skeleton. The process of modeling is primarily associated with growing bone. Remodeling is primarily associated with mature bone and involves a site specific process of bone resorption, closely followed by bone formation.

Bone is controlled by various mechanisms and is continuously undergoing a process of remodeling. The purpose of remodeling is to maintain the function of the bone as it ages (Parfitt 2002). Bone is continuously being placed under strain, which results in fatigue microdamage (Burr 2002). As a structure is repeatedly placed under strain, the elements of the structure will weaken, this is referred to as mechanical fatigue, and as the elements weaken they will develop microcracks. This microscopic damage will accumulate over time and result in a weakness in the overall structure. This is where the

basic multicellular unit (BMU) and the process of remodeling come into play. As fatigued bone is resorbed and then reformed, the microcracks will be repaired, thus offsetting the accumulation of damage.

In the 1960's, Harold Frost proposed the concept of basic multicellular units (BMUs), for maintenance of mechanical integrity and described the skeleton as primarily a mechanical organ. The BMU is comprised of a leading edge of osteoclasts, for bone mineral resorption and a trailing group of osteoblasts that form new bone. From the beginning of resorption through the end of formation the BMU is active for approximately 200 days (Parfitt 2002). On histological evaluation of bone turnover there is a coefficient of variation of greater than 50% and greater than 30% when measured biochemically, indicating that the average rate of turnover is greater than necessary for microdamage repair (Parfitt 2002). Some of this remodeling is targeted to replace microdamaged bone, but there is also a significant amount of remodeling that is not needed for this process (Parfitt 2002).

Influences on the Remodeling Process

As with other physiological systems, genetics plays a strong role in bone status, but at least half of the variability can be attributed to other factors (Krall and Dawson-Hughes 1993). Some of the primary influences are nutrition, hormones, metabolism, age, and mechanical loading. It is important to understand the relative influence of age on the bone formation and remodeling processes, to recognize of how treatments will interact with the natural process. The current literature reveals that a substantial proportion of an individual's total bone mass is accumulated between puberty and the late teenage years. Then there is additional accumulation until peak mass is attained at approximately 30-35 years of age and this bone mass remains constant until about 50 years of age at which point a gradual decline of 0.5-1.5% per year is experienced (Snow-Harter and Marcus 1991). These figures are broad generalizations to establish a basic outline from which to work, realizing that many variables will modify the time frame for bone accumulation and loss.

Of the primary modifiable influences on bone status, only mechanical loading provides a stimulus for bone formation. At present, the other influences can only be maintained in balance to prevent accelerated bone loss. The high impact mechanical loading experienced by gymnasts can even overcome detrimental effects of imbalances in hormonal or nutritional status (Taaffe *et al.* 1997), though this is the exception.

As previously mentioned, the process of remodeling prevents the accumulation of microdamage to bone, but it also allows for the formation of additional bone in areas of high strain. This serves the purpose of gradually changing the shape of the bone to adapt to new mechanical loading patterns. Wolff's Law states that bone will respond to increased loads by favoring the formation of bone over resorption in regions of increased strain (Wolff 1986). Bone responds to strains according to three rules outlined by Turner (1998). The first rule of bone adaptation is that bone responds to dynamic loading and not to static loading. The second rule is that only a short duration of mechanical loading is necessary for a response, and the third rule is that bones accommodate to regular strains; thus, they require abnormal strains to initiate further adaptation.

Demonstrating the first rule of bone adaptation, avian ulna under both non-loaded and statically loaded conditions, lost bone on the endosteal surface and increased intra-cortical porosity, resulting in decreased cross-sectional area (Lanyon and Rubin 1984). In contrast, intermittently loaded bone increased cross-sectional area by 24% (Lanyon and Rubin 1984). The second rule of bone adaptation is demonstrated by the fact that the intermittent load was similar to the static load and was only applied for a short period on a daily schedule. The third rule of bone formation is demonstrated with physiological loads applied in an abnormal strain pattern, resulting in both periosteal and endosteal bone formation on the order of 30 to 40% above the original bone mineral content (Rubin and Lanyon 1984).

These basic studies of bone physiology and the response to various loading environments provide a framework for which human exercise studies can be evaluated.

Bone strength increases, as measured by a variety of techniques, in response to loading environments that follow the three basic rules of bone adaptation. The next section will address the translation of basic mechanical loading to its application in exercise studies.

Bone Response to Loading

The goal of bone strength research is to establish effective interventions for the maintenance or optimization of bone strength in the face of the adverse conditions of aging, intense exercise, and weightlessness. With this goal in mind, the translation of basic laboratory models to realistic human interventions requires an understanding of how mechanical forces generated in the laboratory (e.g., loading forces applied through pins in avian bones) compare to forces generated in practical settings. Additionally, invasive measurements (e.g., *ex vivo* 3-point bending tests) must be approximated with non-invasive tools. To identify effective and practical exercise interventions, it is useful to look to existing modes of exercise for potential intervention models. In researching the bone response to various forms of exercise, an understanding is developed of how the components of exercise fit into the general framework of bone adaptations.

A review of cross-sectional and longitudinal research on exercise and bone strength establishes that exercise is an effective intervention for the purpose of developing increased bone strength. Cross-sectional studies lead this review to establish a general link between various modes of exercise and bone strength. Supporting the cross-sectional findings are numerous longitudinal studies which demonstrate the causal link between exercise and the development of increased bone strength. Further evidence is supplied by literature on animal exercise involving tightly controlled trials.

Numerous cross-sectional studies have demonstrated the correlation between exercise and various measures of bone strength (Slemenda and Johnston 1993; Heinonen *et al.* 1995; Matsumoto *et al.* 1997; Andreoli *et al.* 2001; Heinonen *et al.* 2001; Qin *et al.* 2002). Early work made the connection between intensity of the loading experienced in

an activity and the bone density of athletes. Athletes participating in sports with more intense loading of the legs demonstrated greater femoral bone density, with weight lifters at the top end of the spectrum, and swimmers approximating the bone density of control subjects (Nilsson and Westlin 1971). This early work is supported by subsequent studies using a variety of measurement tools from DXA (Fogelman and Blake 2000) and CT (Haapasalo *et al.* 2000) to peripheral quantitative ultrasound (Lehtonen-Veromaa *et al.* 2000) and MRTA (Myburgh *et al.* 1993). Evidence from the variety of cross-sectional studies brings forth questions of the mechanisms involved in development of bone strength in human exercise. The three rules of bone adaptation articulated by Turner (1998) provide a framework with which exercise can be evaluated.

It appears that several factors are influential, including the volume of strain placed upon the bones (Karlsson *et al.* 2001), the magnitude of strain (Rubin and Lanyon 1985), the loading rate (O'Connor *et al.* 1982), and the loading angles (Rubin and Lanyon 1984). Long distance runners place a large volumes of strain on their legs over time, but this strain follows regular patterns and is of relatively low intensity. Power lifters generate tremendous strains over a moderate loading rate (compared to the impact loading associated with running or jumping) in a consistent pattern. Gymnasts follow a wider variety of loading patterns across their skeletal system with variable intensities of impact and loading rate. Swimmers accumulate high volumes of muscular work, but in patterns that impose low or no impact forces on the skeletal system, with regular loading rates and consistent loading angles.

Additional studies have looked for the connection between bone strength and muscular strength (Snow-Harter *et al.* 1990; Madsen *et al.* 1993; Adami *et al.* 1999; Rittweger *et al.* 2000) or aerobic fitness (Bevier *et al.* 1989; Huuskonen *et al.* 2000). A variety of muscular strength measures have been compared in the search for a simple measure to predict bone strength (Eickhoff *et al.* 1993). Additionally, studies of muscular strength have attempted to link the effect of local muscular forces on producing local bone strengthening responses (Colletti *et al.* 1989). To a certain degree both muscular strength and aerobic fitness measure general activity levels which will influence

bone strength, but strain on the human skeletal system is a multifaceted phenomenon that cannot be quantified with simple parameters. The complex interactions of impact forces, loading rates and loading angles, as well as the variability in training programs, individual exercise history, and hormonal and nutritional status, challenge researchers to design effective interventions. In spite of this variability, several studies (Nichols *et al.* 1994; Stussi *et al.* 1994; Kerr *et al.* 2001) have been successful in demonstrating clear patterns in bone strength development.

Endurance activities, such as long distance running, cross-country skiing and swimming, generally involve a high volume of motions that follow a regular loading pattern. According the rules of bone adaptation, these activities will be limited in the ability to develop bone strength because the majority of time spent in these activities will be beyond the number of loading cycles that yield benefit. Long distance running involves thousands of impact loading cycles per session, so there is no question that there is mechanical stimulus on the bones. If the development of bone strength is dependent upon sufficient mechanical stimulus then long distance running should qualify but the issue of what type of stimulus is required for bone strengthening comes into question. Running satisfies the requirement for impact activity, but it generally follows a uniform loading pattern. Hetland *et al.* (1993) have actually demonstrated a reduced bone mass in male long distance runners compared to controls, and found a significant negative correlation between distance run on a weekly basis and lumbar bone mineral content. Elite runners had 19% lower bone mineral content of the lumbar spine than non-running controls (Hetland *et al.* 1993). Significant correlations remained with adjustments for body mass index and height, but the results lost significance ($p=0.08$) when they were adjusted for body weight, so interpretation becomes problematic.

Pettersson *et al.* (2000) demonstrated significantly higher BMD for 16 year-old female cross-country skiers when compared to non-active controls on the order of 6.9% to 9.3% in the arms and legs. These athletes had been training for an average of 6 years and were presently training for approximately 6 hours per week. Dramatic differences were found in bone mineral apparent density (BMAD) of the femoral neck with cross-

country skiers achieving values 19.4% higher than controls. Of interest are the side to side BMD and BMAD differences found in control subjects, while the cross-country skiing group did not demonstrate right to left BMD or BMAD differences, suggesting that the side-to-side differences found in control subjects are the result of asymmetrical loading during normal activity, and the lack of these differences in athletes is a result of uniform bilateral loading that overrides the asymmetry of normal activity. The BMD of the head was used to evaluate selection bias, and no difference was found between active and control subjects (Pettersson *et al.* 2000).

Children that participate in competitive weight bearing sports (running, gymnastics, tumbling, dance) that generate impact forces of at least three times body weight, demonstrate higher BMD at the femoral neck than children participating in competitive swimming (Grimston *et al.* 1993). Female adult subjects also demonstrate activity specific BMC differences with resistance athletes (body builders) having significantly greater BMC than both endurance athletes (runners, swimmers) and non-active controls, with no significant difference found between the endurance athletes and controls (Heinrich *et al.* 1990). The lack of differences between endurance athletes and controls and the difference in findings between child runners and swimmers and adult runners and swimmers leads to the conclusion that bone mineral measures are not fully quantifying actual bone strength.

Resistance training at competitive levels follows the principle of high strain placed upon the skeleton in short dynamic intervals without excessive daily repetition. Males ranging in age from 14 to 20 years typically have bone mineral densities seen in immature bone (Virvidakis *et al.* 1990; Slemenda *et al.* 1991), but a population of elite junior Olympic weightlifters with a mean age of 17 years was measured to have BMD values of the lumbar spine and femoral neck above fully developed males according to reference data (Conroy *et al.* 1993). In this study, sport specific strength accounted for 30-56% of the variability in BMD. This finding supports previous work demonstrating a high correlation between BMC and strength (Virvidakis *et al.* 1990).

Kirchner *et al.* (1996) found that BMD in the lumbar spine, femoral neck, Ward's triangle, and whole body of former gymnasts was significantly greater than found in controls. Additionally, the differences between college-aged gymnasts and age, height and weight matched controls were even greater (Nichols *et al.* 1994), suggesting that BMD differences peak during active training, but diminish when activity returns to normal levels. Nevertheless, BMD differences of 9-22% resulting from former physical activity lend a tremendous advantage to these individuals as the natural process of bone resorption proceeds. As noted earlier, the benefit of achieving a high peak bone mass as a young adult is that even if activity levels drop to match average population levels, the length of time for bone mass to drop to osteoporotic levels will be greater. Activity levels of former gymnasts were higher than activity levels of controls, but even with these differences controlled for statistically, the BMD differences between groups was still significant (Kirchner *et al.* 1996). Additional evidence to the importance of physical activity is provided by the demonstration that high BMDs were achieved by gymnasts even though calcium intake was below the RDA (Nichols *et al.* 1994; Kirchner *et al.* 1996).

Total body bone mineral density (TBMD) as measured by DXA was used to compare collegiate athletes competing at the national level in the sports of judo, long-distance running, and swimming (Matsumoto *et al.* 1997). Male and female judo athletes were found to have significantly higher TBMD when compared to long-distance runners and swimmers of the same sex, while no significant differences were found between the runners and swimmers. There were no significant weight differences between judo athletes and swimmers, so these bone mineral comparisons reflect true differences, but the runners were significantly lighter than the two other groups, so interpretation of TBMD differences with runners may not reflect true bone strength differences relative to body weight.

Compelling evidence for the effect of activity on bone density is found in studies of unilateral activities. When the bones of one limb are systematically loaded to a greater degree than the bones of the opposite limb, the subject serves as their own control. These

studies have the advantage over traditional cross sectional comparisons, because the experimental limb can be compared to the control limb without the complication of subjects self-selecting for participation in an activity or the potential selection bias due to continued participation relying on sufficient genetically predetermined bone density. A subject selecting to participate in a unilateral activity serves as their own control with respect to self selection or selection bias. The drawback to a subject serving as their own control is that the dominant limb will potentially have greater bone strength for reasons other than the unilateral activity in question. To account for these differences in dominant/non-dominant bone strength, the differences in bone strength should be compared to differences found in control subjects that are not participating in systematic unilateral loading.

Arm dominance was shown to play a significant role in forearm development of men in the general population. Radial width and BMC of the dominant arm were significantly greater ($p=0.0001$) than the non-dominant arm, and dominant ulnar BMC was also significantly larger but to a lesser degree ($p=0.01$) (Myburgh *et al.* 1992). These findings of larger bones in the dominant arm are supported by research on tennis players and controls. Tennis serves as a good example of systematic unilateral loading of the dominant arm. Volumetric bone density at cortical and trabecular sites did not differ between the dominant and non-dominant arms of tennis players (or controls); the asymmetric differences in bone found between the arms was due to an enlargement of the bone (Haapasalo *et al.* 2000). Earlier research on young tennis players also demonstrated side-to-side differences. In a comparison of female tennis players from 7 to 17 years old, BMD differences between dominant and non-dominant arms ranged from 1.6 to 15.7%, while controls displayed differences of -0.2 to 4.6% (Haapasalo *et al.* 1998). The differences between players and controls were not significant until the adolescent growth spurt, at which point total training hours and the number of current training sessions per week became significant in the correlation to side-to-side BMD differences (Haapasalo *et al.* 1998). In addition to greater side-to-side differences, the tennis players also demonstrated greater BMD in the lumber spine than controls beginning later in adolescence, but no difference in BMD of the non-dominant radius, indicating that BMD

increases are site specific for loaded bone (Haapasalo *et al.* 1998). It is important to understand the distinction between areal and volumetric BMD because training responses in growing bone that produce larger bones are fundamentally different than training responses that preserve volumetric bone mineral density. Both changes in bone size and changes in volumetric bone density are important factors in assessing bone strength, but they represent different physical parameters which may respond to different interventions; thus, the two should not be confused when interpreting experimental or descriptive studies.

In comparing power and endurance athletes with controls, the greatest BMD differences between athletes and controls were found at sites loaded by exercise (Bennell *et al.* 1997). Power athletes differed from controls at the lumbar spine as well as the upper and lower limbs, while endurance athletes only differed from controls at the lower limb sites (Bennell *et al.* 1997). An additional finding of interest was that lower limb BMD differences between athletes and controls were greatest at the foot and progressively smaller at more proximal sites, with the tibia/fibula differences smaller than the foot and the differences at the femur smaller than the tibia/fibula differences (Bennell *et al.* 1997). These findings support the notion that ground reaction forces attenuate as they transmit up from the foot, with the result that mechanical strains are lower at proximal sites (Bennell *et al.* 1997). Another possible explanation is that because proximal bones are larger they experience less deformation, or on a percentage basis they do not have to increase BMD as much as a smaller bone to achieve the same gain in mechanical strength. When total body bone mineral content (BMC) was compared, similar levels were found for both female athlete and control groups even though there were site-specific BMD differences. Bennell (1997) suggests that exercise may redistribute bone mineral in response to exercise patterns without increasing the total amount. The lack of difference in total body BMC may just be related to the fact that regional bone mineral increases are relatively small in comparison to the total mineral content of the skeleton and are, therefore, not large enough to result in significant differences.

Further evidence of site-specific bone adaptation to activity is found in a study of adolescent females competing in either rope-skipping or soccer, compared with sedentary controls. Both athletic groups demonstrated greater lower body BMD than controls (Pettersson *et al.* 2000), and rope-skippers demonstrated higher humeral BMD than both soccer players and controls. Competitive rope-skipping involves regular upper body exercise, while soccer generally does not, so the humeral differences in these groups follows the theory of site specific bone adaptation to exercise. Soccer players demonstrated greater BMD of the lower extremities when compared to sedentary controls, but did not display differences in arm BMD (Pettersson *et al.* 2000). As the arms are primarily unloaded in soccer, this comparison of arm BMD values indicates that BMD differences found between groups is not the result of self selection, if one accepts that the likelihood of preferential development of lower body BMD is low. The intuitive conclusion to be drawn from lower body BMD differences in the absence of upper body differences is that the bones have adapted to the site specific loading of the lower extremities in soccer, where ground reaction forces are on the order of 3-6 times body weight (Pettersson *et al.* 2000).

Tai Chi Chuan is a gentle form of exercise that involves no impact, so it provides an interesting situation for comparing bone strength differences between practitioners and non-exercising controls. In a comparison of postmenopausal Tai Chi Chuan practitioners with age matched non-exercising controls, BMD differences of 10.1 to 14.8% in the lumbar spine, proximal femur, and ultradistal tibia were found (Qin *et al.* 2002). All Tai Chi Chuan subjects had been practicing for more than 3.5 hours per week for at least 4 years and had not participated in any regular vigorous physical activity earlier in life. These results lead to the question of whether simple weight-bearing is a sufficient stimulus for improved bone strength maintenance or if there are other mechanisms at work. Several potential sources of the BMD difference found in the Tai Chi Chuan practitioners include self selection, increased muscular strength from standing in a bent knee posture, improved hormonal balance, and the possibility of increased vigor during activities of daily living. This situation self selection for any exercise program over remaining sedentary is a strong confounding influence to a study of postmenopausal

women, but the implications for finding differences in bone strength that are based upon a non-impact form of exercise are profound.

As with any cross-sectional analysis, selection bias is an important consideration. Are participants with a tendency toward lower bone strength eliminated from continued participation in activities that are associated with higher bone strength? Or do participants with higher bone strength tend to seek out activities that are associated with higher bone strength? To address these questions, longitudinal research is necessary to distinguish between selection bias and actual response to training.

Evidence supporting the role of exercise in bone strength development from cross sectional research is supported by numerous longitudinal studies demonstrating the training response of bone. Longitudinal studies of resistance training (Menkes *et al.* 1993; Ryan *et al.* 1994; Sinaki *et al.* 1996; Kerr *et al.* 2001), calisthenics (Ayalon *et al.* 1987; Adami *et al.* 1999), gymnastics (Taaffe *et al.* 1997; Nickols-Richardson *et al.* 1999), and jumping (Heinonen *et al.* 1996; Heinonen *et al.* 2000) support the premise that bone strength is related to exercise, and they also demonstrate the detraining and loss of bone strength principle (Dalsky *et al.* 1988). Additionally, training studies show that the specificity of exercise principle applies to bone strength, with bones that are actively loaded demonstrating gains while non-active bones remain unchanged (Ryan *et al.* 1994).

Evidence that weight-bearing aerobic exercise produces increases in lumbar bone mineral content is found in a longitudinal study of postmenopausal women from 55 to 70 years old (Dalsky *et al.* 1988). After 9 months of exercise consisting of walking and or jogging combined with stair-climbing and additional nonweight-bearing exercise three days per week, study subjects demonstrated a 5.2% increase in BMC, as compared to a loss of 1.4% in control subjects (Dalsky *et al.* 1988). An additional 13 months of training resulted in additional gains in BMC up to the total of 6.2% above baseline, but the BMC gains primarily represent a temporary change, as 13 months of detraining saw a drop in BMC back to a level of only 1.1% above baseline values (Dalsky *et al.* 1988). In contrast to Dalsky's results, a one year program of brisk walking was not able to prevent bone

loss in postmenopausal women (Cavanaugh and Cann 1988). This program involved progressive exercise beginning with 15 minute walking sessions and advancing to 40 minute sessions 3 days per week. Exercise intensity was based upon 60 to 85% of maximal age adjusted heart rate. The differences in results between the programs of weight-bearing exercise for postmenopausal women indicate that walking is not a sufficient stimulus for bone loss prevention, but potentially stair-climbing or jogging are responsible for the gains observed in BMC without the need for the walking component.

Gymnasts have been shown to display higher levels of BMD than sedentary controls and other athletic populations, and this cross-sectional evidence is supported by longitudinal data. College age gymnasts, runners, and non-athletic controls were followed for eight months and the gymnasts experienced significantly greater increases in lumbar spine and femoral neck BMD than the other two groups (Taaffe *et al.* 1997). A second cohort of gymnasts was also studied in comparison to swimmers and non-athletic controls over one year, with a similar result of significantly greater increases in lumbar and femoral neck BMD for the gymnasts when compared to swimmers and controls (Taaffe *et al.* 1997).

Resistance training serves as an ideal model for an exercise intervention, because it is readily controlled, and it meets the bone adaptation requirement for producing dynamic strains over a relatively short period and can introduce a variety of strain patterns with the proper instruction. A sixteen week strength training program resulted in a 2.8±0.6% increase in BMD of the femoral neck, while other traditional BMD sites were not significantly altered (Ryan *et al.* 1994). A six month strength training program targeting the forearm (with bending forces – partially supinated 500 g weight lifting – 10 minutes twice per week + at home, push-ups, volleyball) in postmenopausal women, aged 52-72 years, resulted in site specific changes in bone structure and geometry without a corresponding increase in mass of the segment studied (Adami *et al.* 1999). The cortical area of the ultradistal radius increased primarily at the endosteal surface, with a corresponding decrease of the trabecular area (Adami *et al.* 1999). These results are

consistent with the responses of bone found in animal studies (Lanyon *et al.* 1982; Lanyon and Rubin 1984).

Power athletes (sprinters, jumpers, hurdlers, and multievent athletes) were followed in a 12 month study comparing bone mass and bone turnover to endurance athletes and controls ranging in age from 17 to 26 years old. The results of this study demonstrate that while both athletes and controls experienced increases in total body BMC and femur BMD, power athletes gained significantly more density at the lumbar spine than either the endurance athletes or the control subjects (Bennell *et al.* 1997).

The significantly higher BMD values, mentioned earlier, found in Tai Chi Chuan practitioners when compared with non-exercising controls, are bolstered by longitudinal data comparing rates of bone loss between these two groups. A 12 month study following Tai Chi Chuan practitioners and controls demonstrated that while both groups continued to lose bone, the Tai Chi Chuan exercise group displayed a slower rate of bone loss at both trabecular and cortical sites in the tibia (Qin *et al.* 2002).

In support of the cross sectional and human longitudinal research on bone strength, longitudinal animal studies provide additional evidence for the response of bone to increased loading. Studies of standard exercise training demonstrate the standard patterns of bone strengthening results shown by human studies. The evidence base is expanded through the use of animal models, in which well controlled experimental designs can be employed and unique situations of loading are studied.

Adult beagles were fitted with jackets that allowed them to carry various weights during treadmill exercise. Over time the jackets were increased to 130% of body weight and then the dogs exercised with this weight 75 $min \cdot day^{-1}$ for 5 $days \cdot week^{-1}$ over 48 weeks. When compared to a group of control beagles, both groups experienced increases in tibial mineral content, but the loaded exercise group experienced a significantly greater increase (Martin *et al.* 1981). Another animal model involved immature swine that exercised on a treadmill for 12 months at a pace resulting in heart rates of 65 to 80

percent of maximum heart rate. At the conclusion of the 12 month program, femoral cross-sectional properties increased significantly in the exercising swine as compared to a group of non-exercising swine (Woo *et al.* 1981). Femoral cortical thickness increased with the resulting increases in cortical cross-sectional area and the maximum and minimum moments of inertia. Bone density and biochemical contents of the exercise and control group bones were similar, so the primary measured effect of exercise was limited to the macroarchitectural changes.

Exercise forces are often simulated through mechanical loading of animal bones allowing for tight regulation of strain rates, angles, and magnitudes. O'Connor and Lanyon (1982) measured strain patterns in the radius and ulna of sheep as they walked and ran on a treadmill, then based upon these patterns the bones were mechanically loaded to various levels for 6 weeks. Bones that were loaded to levels greater than the peak strains experienced during treadmill exercise achieved the largest gains in BMC, while bones loaded to low strain levels lost BMC. Turner and Robling (2003) mechanically loaded the right ulna of rats for 16 weeks with resulting increases in areal BMD and BMC of 5.4% and 6.9%, respectively. These modest bone mineral gains actually resulted in large increases in mechanical test properties with a 64% increase in the maximum stress the bone can withstand (the ultimate force) and a 94% increase in energy to failure (the amount of energy absorbed by the bone before failure). This second example of bone loading demonstrates the fact that bone mineral changes may be small and difficult to detect in human exercise studies, where there is greater variability between subjects and the loading that they experience, but that actual mechanical improvements may be large.

Cross-sectional data makes the case for a positive bone strength response to exercise with the greatest bone strengths seen in populations that undergo the largest forces and strain rates. Longitudinal data supports the findings of cross-sectional studies by demonstrating the link between exercise and bone strength gains over time. The longitudinal data from human studies is further bolstered by animal research that clearly shows the relation between loading and bone changes. It is the tightly controlled

environment of mechanical loading of animal bones that brings home the point that minor changes in bone mineral have dramatic effects on bone strength. The fact that some human exercise studies fail to show significance in bone mineral measures when they are expected may be a reflection that bone mineral changes are too small to detect even though significant improvements in bone strength have occurred.

Structural Mechanics

Bone strength depends upon both macro- and microarchitectural characteristics, so a brief review of these characteristics will serve as a background for understanding bone strength measurement. The primary macroarchitectural components are bone length, diameter, and cortical thickness. Additionally, the cross-sectional shape and distribution of bone mass within this cross-section are important (Martin 1991). At the microarchitectural level, fiber and crystal alignment along with trabecular spacing, connectivity, and alignment are the important components that determine bone strength (Martin and Ishida 1989; Turner 1992).

Long bones generally can be approximated as beams, with their length being substantially greater than their width. When viewed in cross-section, along the majority of their shaft, long bones have a thick shell of cortical bone with an essentially hollow core, meaning that tissue in the core does not provide mechanical support.

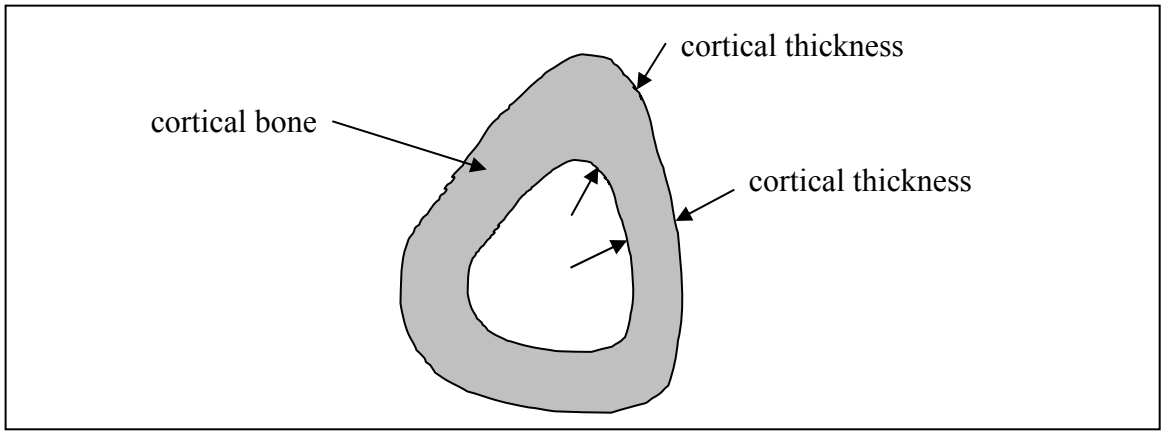

Figure 2.1. Tibial shaft cross-section. Note that cortical thickness can vary from point to point in response to increased stimulus experienced in regions of maximal stress.

To understand how the macroarchitectural structure of a long bone influences strength, it will be helpful to become familiar with the stress-strain diagram (Figure 2.2).

Stress (σ) – force per unit area (load/cross sectional area) [N/m^2]
Strain (ε) – deformation divided by total length (δ/L); a dimensionless measure
(new length – original length)/(original length)

Young's Modulus (or the elastic modulus) (E) – stress/strain [N/m^2]; slope of the stress-strain relationship in the elastic response range

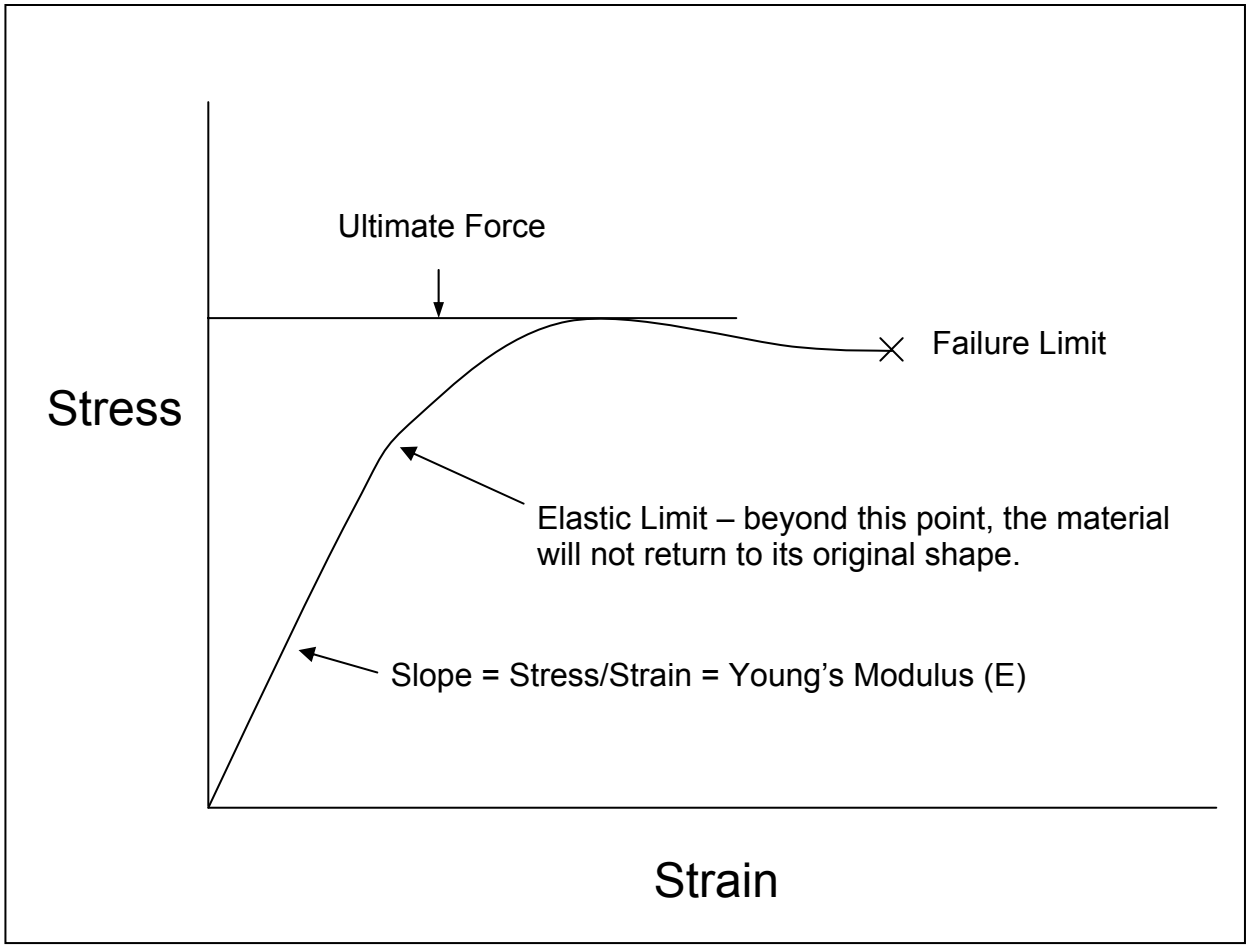

Figure 2.2. Stress-strain diagram.

The stress-strain diagram depicts the deformation behavior of a material as it is placed in a loading situation. Initially, materials behave elastically and follow a linear relationship between stress and strain according to Hooke's Law ($\sigma = E\varepsilon$) (Beer and Johnston 1981). Young's modulus (E), or the modulus of elasticity, is an intrinsic material property defined by the linear stress-strain response and serves as a measure of stiffness. Beyond the elastic limit, as defined by the transition from linear to nonlinear behavior, the material undergoes permanent, or plastic, deformation and eventually reaches a failure point. Bone typically fails when the ultimate force, or maximum stress, is reached (Turner and Burr 1993), but some materials follow varied deformation patterns and actually fail at stress levels below the ultimate force, as shown in Figure 2.2. Bones can be loaded in 3-point bending machines (Figure 2.3) to generate stress-strain graphs and measures of bending stiffness and ultimate force.

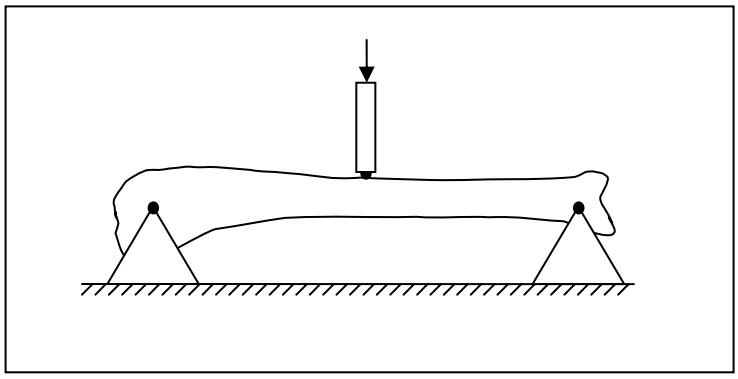

Figure 2.3. Tibia in 3-point bending configuration.

To evaluate the functional stiffness at the level of the bone, and not just the material level, Young's modulus is coupled with the cross-sectional moment of inertia (I) to yield the extrinsic stiffness, or the cross-sectional bending stiffness, EI (Turner and Burr 1993). The cross-sectional moment of inertia for a homogeneous material clearly depends upon the simple geometry of the structure, but heterogeneous materials such as bone are also influenced by the quantity, distribution, and orientation of bone mineral and collagen fibers (Martin and Ishida 1989) around the bending axis (Figure 2.4). The effective stiffness of bone is also influenced by trabecular connectivity, alignment and

number, as well as the alignment of bone minerals and collagen fibers. These complex factors are difficult, if even possible, to quantify with non-invasive measures, so the ability to directly measure extrinsic stiffness (EI) avoids this problem.

Area Moment of Inertia (I) – distribution of material around a central axis [m^4]

$$I_x = \int_A y^2 \, dA \qquad I_y = \int_A x^2 \, dA$$

I_x = moment of inertia with respect to the x-axis

I_y = moment of inertia with respect to the y-axis

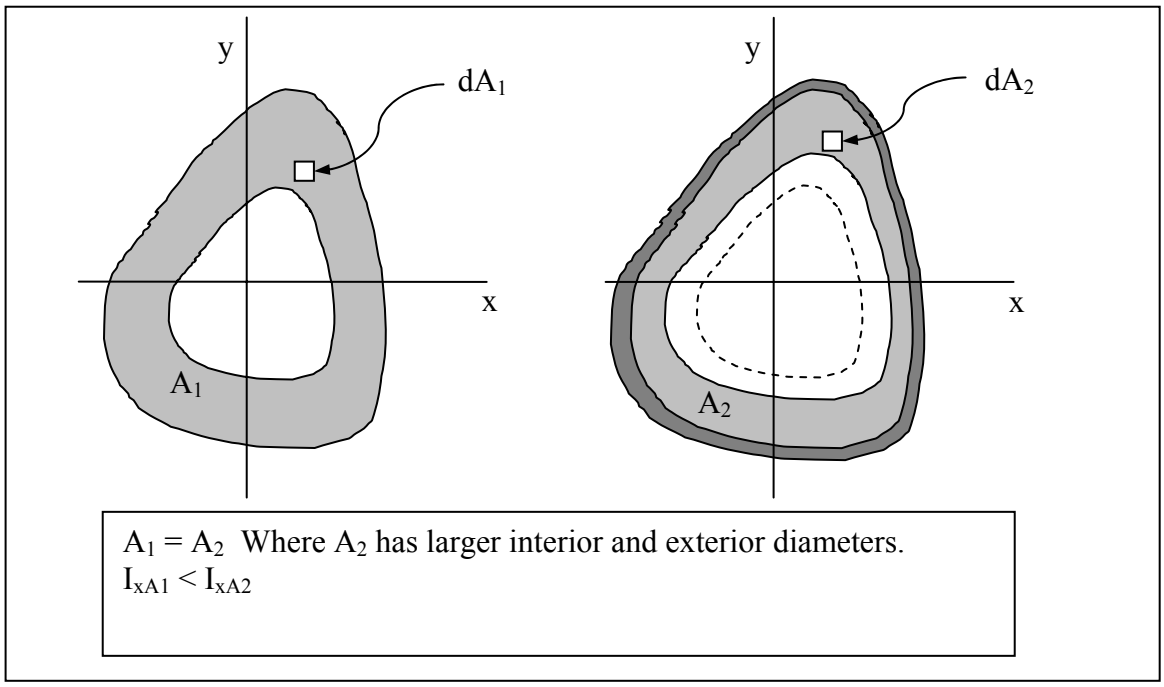

Figure 2.4. Area moment of inertia comparison for two hypothetical objects with the same total area, yet different distribution patterns. I_{xA2} is greater that I_{xA1} because the sum of distances to the elements (dA_2) from the x-axis is greater than the sum of distances to the elements (dA_1).

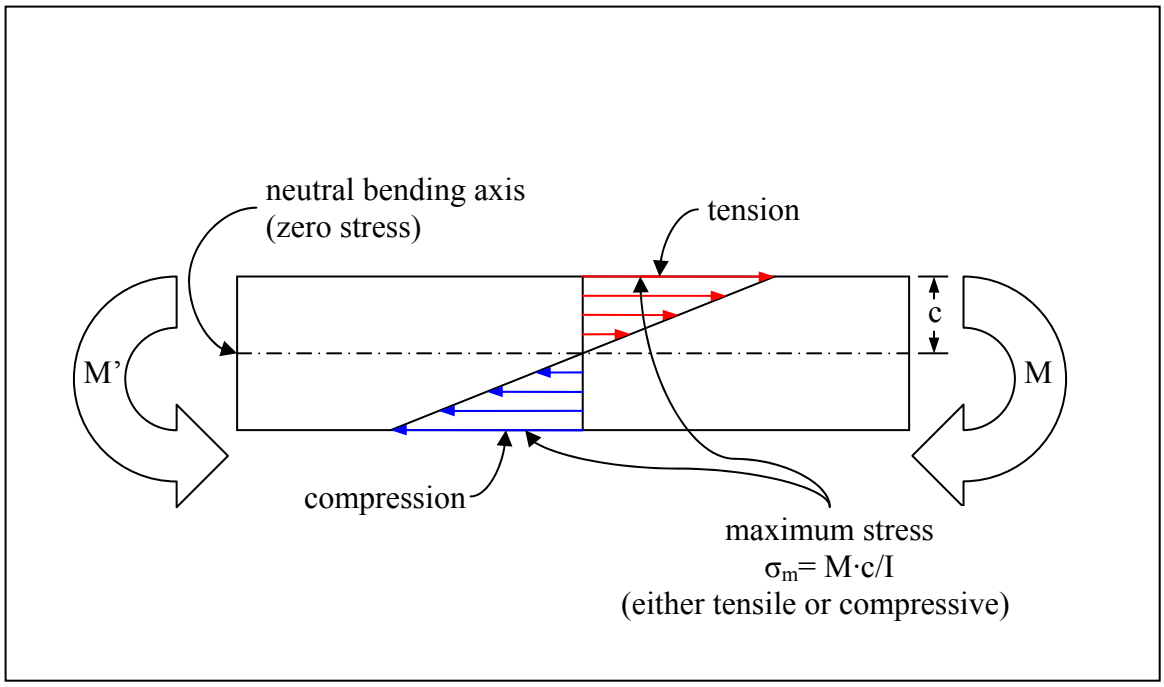

Figure 2.5. Side view of uniform beam demonstrating the compressive and tensile forces associated with a bending moment, M [Nm]. Tensile and compressive stresses, σ_m [N/m^2], reach maximum values at the furthest distance from the neutral axis (for linear elastic materials), and these forces are dependent upon the cross-sectional moment of inertia, I [m^4].

Bone Strength Measurement Tools

To understand fully the importance of the mechanical response tissue analysis (MRTA) system requires background information on the other measurement tools used in bone strength research. This section contains a brief review of both the radiation based systems of dual x-ray absorptiometry (DXA) and quantitative computed tomography (QCT), as well as the vibratory methodologies of quantitative ultrasound (QUS) and flexural wave velocity measurement. The strengths and weaknesses of the various tools will be discussed along with how they complement each other.

The primary objective of bone measurement is to identify individuals at high risk for fracture. To address the issue of fracture risk prediction, several techniques and

measurement tools have been utilized with varying levels of success. One category of analysis is to look at the amount of bone mineral present in the region, as this serves as a good predictor of bone strength. A second category of analysis measures the spatial elements of the structure, either cortical wall thickness or trabecular spacing, from which a strength analysis can be performed. The third method of analysis is to measure the vibrational properties of the bone and to predict the modulus of elasticity and strength of the bone.

The strong correlation between bone mineral density (BMD) and bone strength makes the measurement of BMD an obvious starting point in the search for a predictor of fracture. The systems used for quantifying bone mineral in a region are the DXA scan for measuring BMD in units of g/cm^2, and QCT for measuring BMD in units of g/cm^3. The most commonly used tool in bone strength research is DXA. The World Health Organization's (1994) definition of osteoporosis is based upon DXA measurements of BMD and DXA measurements can explain 50-60% of site specific bone failure loads (Eckstein *et al.* 2002). DXA provides reliable measures of BMD with a fairly low radiation exposure. The radiation dose involved in a full body scan is less than the background radiation exposure received during the course of a full day (Bonnick and Lewis 2002). DXA produces a two-dimensional measure of BMD in units of g/cm^2 which is often referred to as areal BMD. DXA measures bone mineral content (BMC) and the two-dimensional area as defined by the silhouette of the bone, the combination of these parameters then yields the areal BMD. The problem with areal BMD is that when three-dimensional changes in bone size are measured in two-dimensions, interpretation of the results can be problematic. For instance, in growing bone serial measures will detect proportionally greater changes in BMC than bone area, so areal BMD will increase while the actual volumetric density (g/cm^3) may actually remain constant (Bailey *et al.* 1996). Additionally, minor structural changes in cortical wall thickness that are not detected with DXA measurement may have truly significant effects on bone strength, while areal BMD changes result in statistically nonsignificant findings (Adami *et al.* 1999; Jarvinen *et al.* 1999; Khan 2001). In addition to the two-dimensional measurement problem, other drawbacks to DXA are the high cost of the device and, therefore, the cost of

measurements and the large size of the system. DXAs strengths are that it is widely used and there are established population data sets for comparison.

QCT improves on the measurement capability of DXA by being able to measure in three-dimensions and thus assess volumetric bone density (Cavanaugh and Cann 1988) and accurately evaluate bone growth. QCT is able to discriminate between cortical and trabecular bone yielding a greater ability to differentiate between normals and vertebral fracture patients than DXA. The ability to measure additional architechtural parameters allows for more sensitive measurement of bone changes (Adami *et al.* 1999; Jarvinen *et al.* 1999). The precision of QCT is on the order of 2 to 4% with accuracy errors of 5 to 15% for trabecular measurements of the spine (Genant *et al.* 1996). To achieve the extra dimension of measurement capability QCT has to employ approximately 30 times as much radiation as experienced during DXA scanning. As with DXA, prohibitive cost and large size are drawbacks. Peripheral QCT (pQCT) measurement is an alternative that allows for a reduced radiation dose by limiting measurement sites to the limbs, but forfeits the ability to measure at the hip and spine (Genant *et al.* 1996).

Quantitative ultrasound (QUS) measures speed of sound through bone and broadband ultrasound attenuation which appear to independently measure characteristics of bone strength (Genant *et al.* 1996). Currently, QUS is most reliable for calcaneal measurement, which has poor predictive ability for the prevalent sites of osteoporotic fractures. QUS is an attractive alternative to DXA and QCT because it involves no radiation exposure and it is portable and inexpensive. At present, QUS is being used as a community screening tool and as an accessory research tool.

Low frequency vibrational analysis techniques have been under development for more than 25 years (Young *et al.* 1976), and are benefiting from continual advances in computing power. Flexural wave velocity measurement (FWVM) and mechanical response tissue analysis (MRTA) use low frequency stimulation of long bones to estimate mechanical properties. These techniques share the characteristics of being non-invasive, non-radiological, and employ relatively inexpensive and compact equipment. The

strength of a bone is based upon both the quantity of its material composition, the distribution pattern of this material, and the microarchitectural alignment of material. Bones are of a heterogeneous composition and vary in this composition throughout a single skeleton, as well as throughout regions in a single bone (Keaveny and Hayes 1993; Adami *et al.* 1999; Hengsberger *et al.* 2002). Bone mineral and its distribution are of major importance to the strength of bone, and while some systems are able quantify bone mineral and its distribution (Genant *et al.* 1996), they fall short of being able to establish the quality of bone based upon its microarchitecture. The FWVM and MRTA systems bypass the quantification of mineral distribution and attempt to measure the stiffness of the material, which is a good indicator of fracture strength (Steele *et al.* 1988; Stussi *et al.* 1994).

FWVM is based upon the principle that the speed and attenuation of waves in a beam is related to the mechanical properties of the beam (Fah and Stussi 1988). By applying a mechanical impulse to the tibia and measuring the response of the bone with two or more accelerometers, the mechanical characteristics of the bone can be estimated (Fah and Stussi 1988; Stussi and Fah 1988). Validation of one version of FWVM, referred to as SWING, involved analysis of 21 human cadaver tibia followed by pQCT assessment and 3-point mechanical bending tests to failure. The results of this validation are quite impressive with bending stiffness measured by SWING correlating to bending stiffness measured by 3-point bending at a level of 0.96 (Stussi *et al.* 1994). As for the comparison with mechanical fracture limits the correlation was r=0.86, though the researchers mention that with these results the standard error could be high. Beyond the mechanical validation of the system, a study of 20-year-old tank recruits demonstrated increases in bending stiffness of 25% over the 15 week training period, while bone mineral content only increased by 1.8%. Following the training period, bending stiffness declined by 6% over the course of 24 months, while BMC continued to increase at approximately the same rate as seen during the training period. This interesting finding may suggest that bending stiffness reflects rapid changes in bone strength that are not matched by BMC changes.

Mechanical Response Tissue Analysis

Mechanical response tissue analysis (MRTA) is a technology that predicts the bending stiffness (EI) of long bones on the basis of their response to low frequency vibration. Bone bending stiffness is a surrogate measure of strength with correlations of 0.9 and higher (Roberts *et al.* 1996), so the ability to predict bending stiffness with a simple non-invasive technique provides a significant contribution to bone health research. This section will describe MRTA and it development, review the primary research that has been conducted, and summarize the various systems and respective reliability levels that have been reported.

Early work in the field of long bone vibration testing attempted to predict bone strength on the basis of the resonant frequency detected when the bone was subjected to vibration (Jurist 1970a; Jurist 1970b). This prediction was based upon the fundamental equation for axial vibration of a bar ($F_0L = KC$), where F_0 = resonant frequency, L = length of bar, C = velocity of elastic wave propagation, and K = a proportionality constant, equal to ½ for free ends. $C = \sqrt{(E/\rho)}$ where E is the average value of Young's modulus and ρ is the average density. Assuming the bone shape and boundary conditions (elastic properties of the joint capsule, surrounding muscle tissue, soft tissue between the bone and the measurement device) are constant, F_0L is proportional to $\sqrt{(E/\rho)}$ and F_0L was compared across subjects. Jurist (1970b) found significant differences between osteoporotic women residing in a nursing home and normal age matched women from the community during initial testing, but was subsequently unable to reproduce this result in an outpatient population (Jurist 1973). The conclusion was that geometric or other systematic differences resulted in a resonant frequency bias that is not truly predictive of osteoporosis. This finding led to the pursuit of alternative vibration analysis methods (Campbell and Jurist 1971; Young *et al.* 1976).

MRTA differs from the resonant frequency approach by incorporating information collected across a range of frequencies and does not depend solely on one

resonant frequency. Resonant frequency is the ratio of stiffness to mass which is problematic for the detection of osteoporosis because both stiffness and mass decrease with osteoporosis (Steele *et al.* 1988). Work by Doherty *et al.* (Rennie and Johnson 1974) found differences of 80 percent in bending stiffness (EI) between one osteoporotic and two normal tibiae, while only a 20 percent difference was detected in resonant frequency. Subsequent studies have demonstrated the ability of MRTA to detect EI changes during trials of experimental disuse (Young *et al.* 1983; Hutchinson *et al.* 2001) and measure EI differences between various populations including exercise groups (Myburgh *et al.* 1993; Hutchinson *et al.* 1994), disease states (Smith *et al.* 1994; Kiebzak *et al.* 1999), and age groups (McCabe *et al.* 1991).

The basic components of the MRTA system are the mechanical shaker, the impedance head, the contact probe, the system control and measurement software, and the analysis software. The mechanical shaker, impedance head, and contact probe are connected in series (Figure 2.6) with the shaker physically driving the impedance head-probe with a randomly generated frequency from 0 to 1600Hz, as specified by the controlling software. The impedance head transmits both force and acceleration data to the measurement software, and the probe (<1 cm^2) provides transcutaneous contact with the bone at the mid-point of either the ulna or tibia. When contact with the bone has been established measurements can be taken; each measurement involves approximately 5-10 seconds of data collection. Real-time data is converted with Fourier transforms to the frequency domain for analysis. The analysis software decomposes the response data into real and imaginary components of stiffness and compliance, from which further computation attempts to match a mathematical model to the raw physical data (see Figure 2.7).

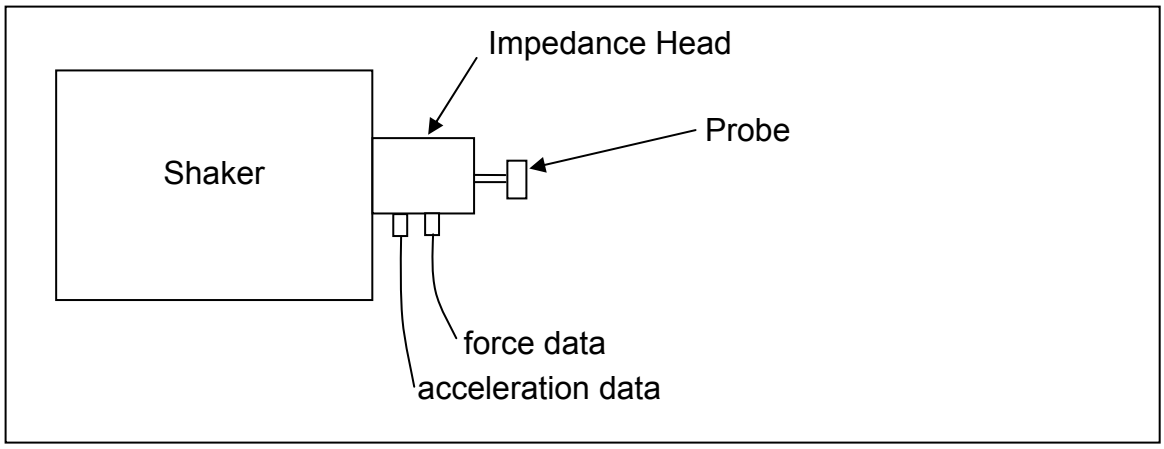

Figure 2.6. Shaker-impedance head-probe hardware.

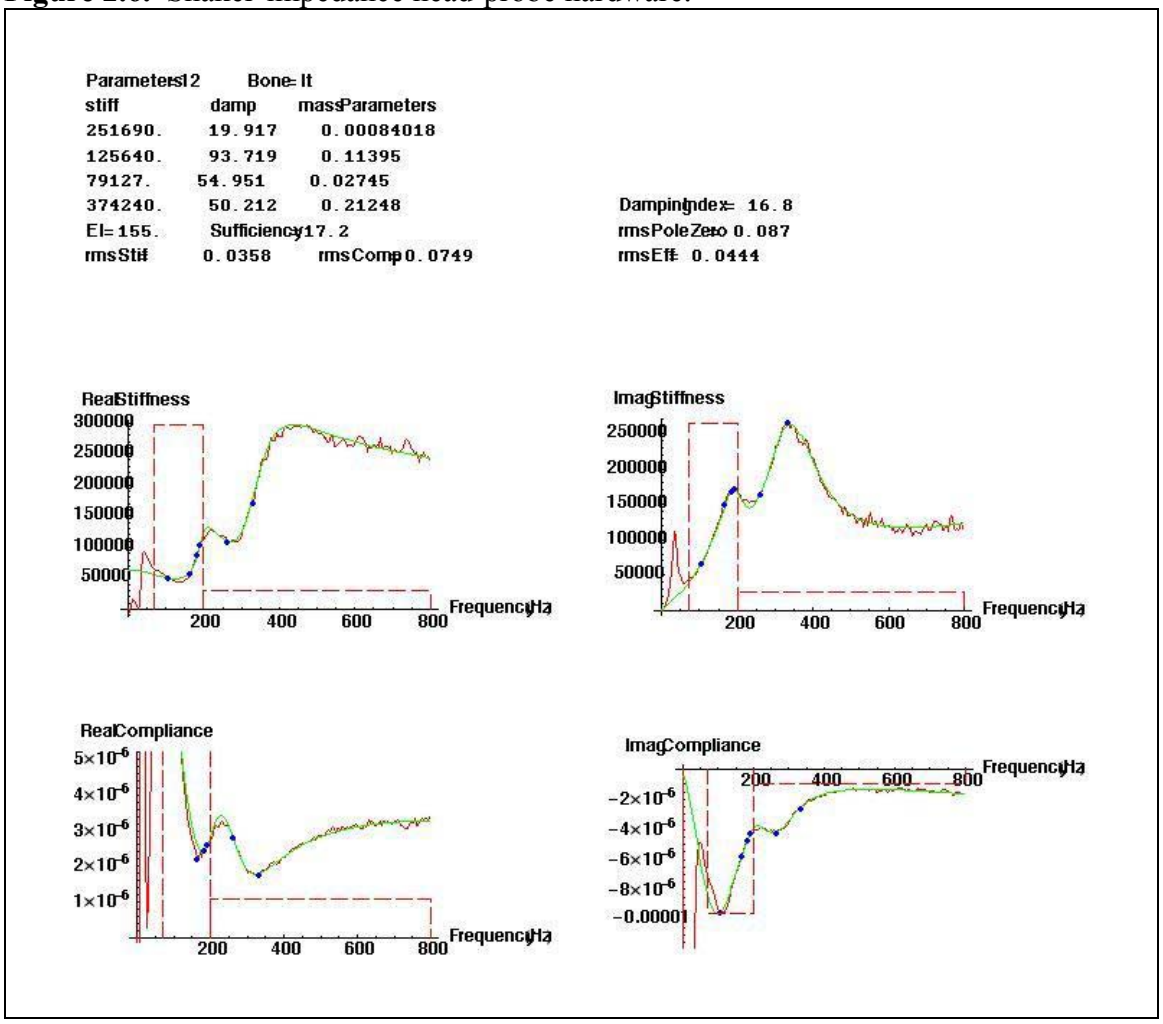

Figure 2.7. MRTA output graphs**:** The primary information on bone bending stiffness is contained between 70 and 500 Hz. The smooth lines represent the mathematical model and the rough lines are the raw data points.

Various mathematical models have been developed to account for the masking effects of overlying tissue and the restraint conditions at the ends of the bone. Human ulnar measurements have been successfully analyzed with a 7-parameter model (see Figure 2.8) of tissue behavior (Steele *et al.* 1988). The 7-parameter model has a closed form algebraic solution allowing for almost instantaneous data analysis, allowing the MRTA operator to evaluate the quality of each measurement as it is taken. Human ulnar measurements are reliably analyzed with the 7-parameter model with coefficients of variation on the order of 3-5% (Steele *et al.* 1988).

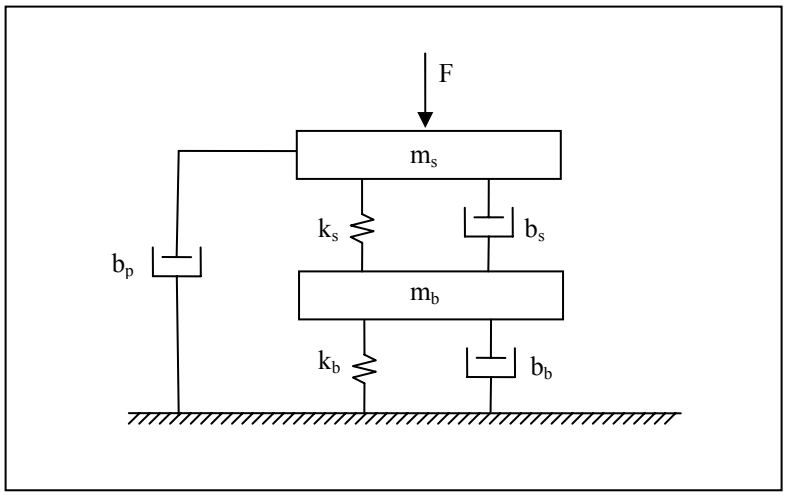

Figure 2.8. 7-parameter model of tissue behavior (reproduced with permission from the authors: Steele *et al.* 1988). Elastic behavior of the skin and bone are denoted k_s and k_b respectively, the damping behavior of the skin and bone are denoted b_s and b_b respectively, and a 7^{th} parameter b_p represents the damping behavior of the skin in relation to the position of the bone.

When the more flexible monkey tibiae were measured, it was found that new model of tissue behavior was necessary for reliable analysis. Monkey tibiae were clamped at both ends, so a 6-parameter model (see Figure 2.9) that accounts for fixation of both proximal and distal ends of the bone was developed (Roberts *et al.* 1996). This 6-parameter model has demonstrated a strong relationship (r^2=0.95) between *in vivo*

assessment of EI by MRTA and *ex vivo* assessment of EI by 3-point mechanical bending of monkey tibiae (Roberts *et al.* 1996). The 6-parameter model does not have a closed form algebraic solution as in the 7-parameter case, so an iterative process was developed to make an initial approximation of the raw data and then step through refinements that attempt to model accurately the measured response. The iterative process is considerably slower than the 7-parameter solution, so during the measurement process it has not been practical for the technician to evaluate the quality of the measurement or the ability of the 6-parameter model to analyze the raw data.

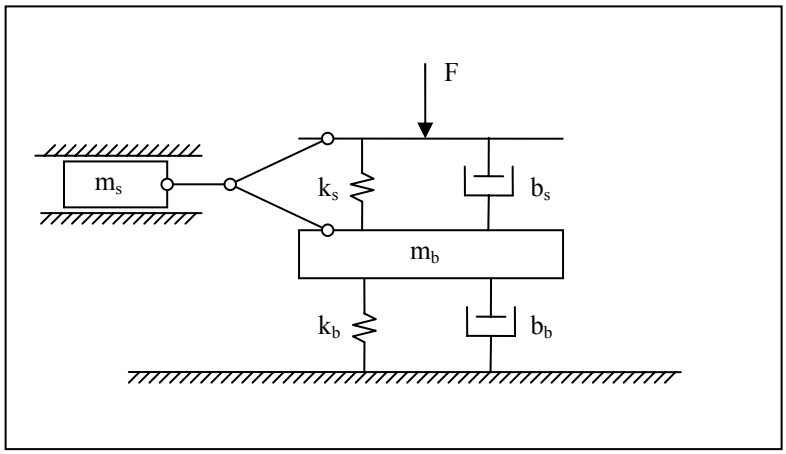

Figure 2.9. 6-parameter model of tissue behavior (mass, stiffness, and damping for both the bone and the skin) (reproduced with permission from the authors: Roberts *et al.* 1996). In this case the bones are firmly clamped on both ends.

The ulna has a fairly uniform structure, which approximates a long cylindrical tube and has been successfully analyzed with the 6 and 7 parameter models. The human tibia is not radially uniform in cross section (Figure 2.4), the orientation of the cross section varies longitudinally, and the bone is slightly curved.

As MRTA progressed to evaluation of human tibiae, a 9-parameter model was developed to account for conditions in which one end (the ankle) is free to vibrate, requiring the addition of 3 parameters (the mass, stiffness, and damping at the ankle joint) to the 6-parameter model. Further development has since led to a 12-parameter model (Figure 2.10), taking into account the condition of both ends (the ankle and knee) being

free to vibrate, thus 3 additional parameters (the mass, stiffness, and damping at the knee joint) have been added. The development of the 12-parameter computational model allows for both ends of the bone to be free, eliminating the need for supporting hardware. In theory the elimination of supporting hardware will simplify measurement and reduce error introduced by the level of technician training and experience. Refinements to the 12-parameter model over the last year have improved it to the point of being the model of choice, though there is evidence that occasionally the 6 or 9 parameter models will outperform the 12 parameter model (Steele 2001). The performance of the models may depend upon anatomical variations in the joints of subjects.

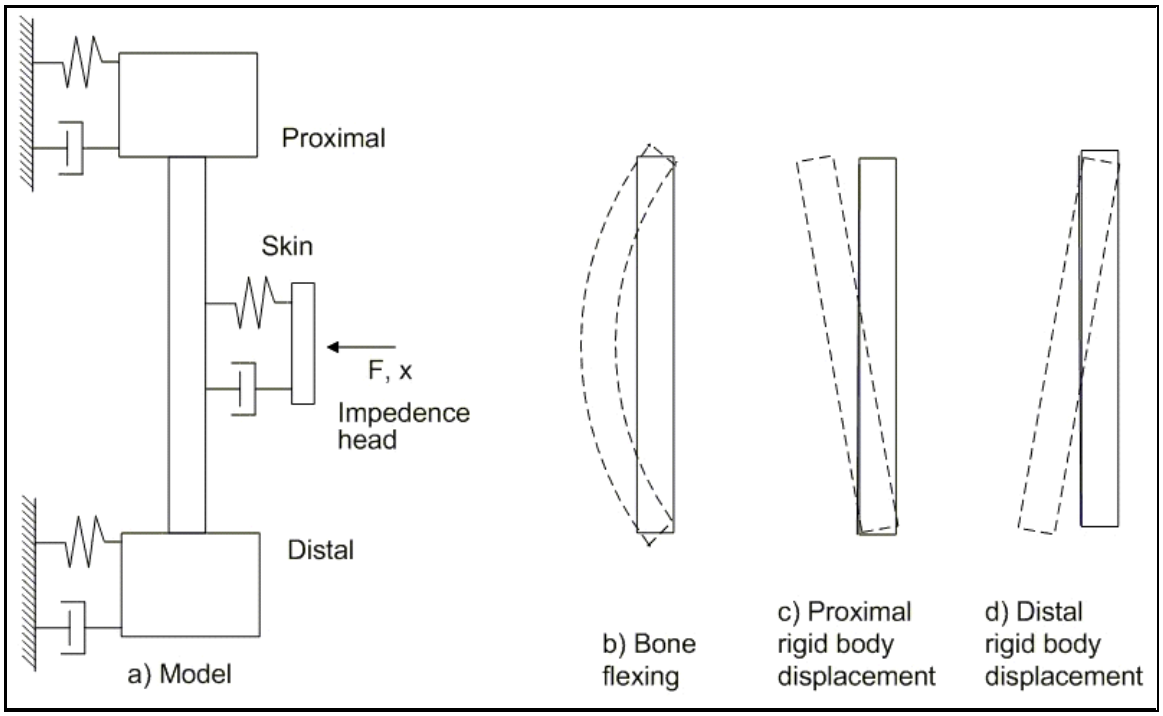

Figure 2.10. Representation of the 12-parameter model of tissue behavior, which accounts for vibration within both the proximal and distal joints. The 9-parameter model involves pinning either (c) the distal or (d) the proximal end (reproduced with permission: Steele 2001).

The data obtained with MRTA is used to calculate cross-sectional bending stiffness (EI) of the bone in units of Nm^2, the mass of the bone, the axial load capacity (Euler buckling load, P_{cr}), and the bone sufficiency (ratio of P_{cr} to body weight). These

variables have been applied in several research studies and demonstrated the ability to discriminate between various populations in cross-sectional human studies (McCabe *et al.* 1991; Myburgh *et al.* 1993; Kiebzak *et al.* 1999) and detect changes in a longitudinal study of disuse and recovery in monkeys (Young *et al.* 1983). These findings demonstrate the past success of MRTA, yet they leave open the question of how well does MTRA detect longitudinal changes in human exercise interventions.

Disuse Osteoporosis

In a comparison of MRTA and BMC analysis during a trial of experimental disuse osteoporosis in the monkey, MTRA demonstrated a greater sensitivity to changes in bone quality than found with BMC. During six months of semireclined restraint, BMC losses of 23-31% were measured in the proximal tibiae, while there was a decrease in bone stiffness of 36-40% (Young *et al.* 1983). Following the restraint, bone bending properties returned to normal after 8.5 months of recovery, but even after 15 months of recovery, bone mineral content did not return to baseline levels (Young *et al.* 1983). These results indicate that MRTA is detecting adaptations in the bone that are not quantified by BMC changes. The BMC losses that were not recovered by 15 months are possibly being compensated for through mechanisms such as improved hydroxyapatite crystal and collagen fiber alignment that lend stiffness to the bone without the need for additional mineral.

Osteoporosis

MRTA has been used to compare bending stiffness (EI) values of normal, osteopenic, and osteoporotic women, as classified by World Health Organization criteria. The findings of this study reveal that the osteoporotic women displayed EI values 25% lower than the mean value for normal women ($p=0.0001$) and 17% lower than osteopenic women ($p<0.05$) (Kiebzak *et al.* 1999). No difference in EI was found between the osteopenic and normal women, which could imply that the BMD changes precede actual stiffness changes, though this is contrary to the experimental findings by Young *et al.*

(1983) in the study of monkeys and disuse osteoporosis, where bending stiffness changes were dramatic. The conflict here might arise from the difference in environment, the BMD changes displayed by osteopenic women may be the result of age related BMD losses in the face of normal continuous daily stress versus the BMD losses experienced by monkeys in a disuse situation. Continuous daily stress may result in either a preferential retention of the bone mineral that offers the greatest contribution to bending stiffness or a compensatory adaptation of other components of bending stiffness, such as collagen fiber alignment or trabecular connectivity. It is anticipated that the slow loss of BMD with age allows sufficient time for the preferential retention of bone stiffness elements. In the case of disuse osteoporosis, there are no stresses to stimulate preferential retention of bone stiffness elements, and bone loss proceeds in a more uniform fashion taking both elements of high and low importance. This also follows the results of the return to use situation in which monkeys recovered bending stiffness more rapidly than BMD. In theory, the bone mass that provides the greatest contribution to bending stiffness will return first, followed by bone mineral that plays a smaller role in stiffness.

Age

MRTA detects an age-related decrease in the bending stiffness (EI: 27.7± 1.3 vs. 21.3±1.1 Nm^2 ($p<0.005$):young 25±0.6yr vs. older 64±1yr) of female human ulnae (McCabe *et al.* 1991), but a study of ulnar bending stiffness and its relationship to age in men, no correlation was found with age (Myburgh *et al.* 1992). These contrasting findings actually reflect the nature of long bone response to aging in men and women. Ulnar width increased with age and ulnar BMC did not decrease with age in men (Myburgh *et al.* 1992), indicating that the maintenance of bending stiffness may be related to an increase in bone width to compensate for losses in other factors contributing to bending stiffness, such as microarchitectural changes in collagen fiber alignment or trabecular connectivity. Men tend to retain the integrity of their long bones with age and this study demonstrates that MRTA is capable of measuring this non-invasively.

Activity

In a study of bending stiffness and activity, MRTA was able to demonstrate that a group of male athletes displayed significantly higher ulnar bending stiffness than age matched subjects of lower habitual exercise levels (Myburgh *et al.* 1993). The measures of bending stiffness correlated muscular strength, ulnar width and bone mineral content. Specifically grip strength and bicep strength were predictive of ulnar bending strength, which lends evidence to site specific adaptations to bone strength. The correlation between ulnar width and bending stiffness is not a surprising finding, because for the case of a circular cross section, increased width increases the area moment if inertia (I) as a function of the change in width to the fourth power. The actual effect on I, *in vivo,* may be slightly less because the cross section will not be uniformly circular. The ulnar width to bending stiffness relationship found in this study provides evidence that MTRA is measuring bending stiffness. Again, the correlation between BMC and bending stiffness is not surprising because as BMC increases, either density increases, which will result in an increase in E, or the cross-sectional area increases which will result in an increase in I, and either of these will result in an increase in bending stiffness, which is more evidence that MRTA measurements are valid. Two additional pieces of information from this study provide interesting material for future research. Bending stiffness differences were not detected between sedentary individuals and subjects exercising at moderate levels, which may indicate that these moderate exercisers were not reaching a threshold level necessary to realize bending stiffness improvement. Adding to this information is the knowledge that the subjects in the high level exercise group, which demonstrated significantly higher ulnar bending stiffness, were also participating in exercises that more heavily loaded the arms.

Validation

Validation trials of MRTA demonstrate a predictive relationship ($r^2 = 0.95$) between cross-sectional bending stiffness (EI) as measured *in vivo* by MRTA and as measured *ex vivo* by 3-point mechanical loading (Roberts *et al.* 1996). Initial validation was determined by testing the MRTA system with cylindrical aluminum bars of varying cross-sections. A rubber pad was placed between the MRTA probe and the bar to

simulate soft tissue. The value of Young's modulus (E) was known for the bars so theoretical values of EI were calculated based upon the radius of each bar. $EI_{(MRTA)}$ was strongly associated (r^2=0.99) with $EI_{(theoretical)}$ for the aluminum test bars and $EI_{(MRTA)}$ values were within 5% of the calculated values. Subsequently, on a population of 12 monkeys, scheduled for necropsy due to illness, *in vivo* tibial MRTA measurements were conducted for comparison with *ex vivo* 3-point mechanical loading tests. Beyond the strong association between $EI_{(MRTA)}$ and $EI_{(3\text{-point bending})}$, there is also a strong association (r^2 = 0.92) with the maximum load and $EI_{(MRTA)}$.

Reliability

Human ulnar measurements were analyzed with a 7-parameter model for curve fitting and produce good reliability demonstrating average variations of 5.3% and 7.8% with repositioning between trials (Steele *et al.* 1988). Ulnar measurements on a version of the MRTA system that used an additional pad for limb restraint yielded precision errors of 2.9% (McCabe *et al.* 1991). Additional studies with limb repositioning resulted in variability of less than 5% for ulnar measurements, but tibial measures ranged in variability from 5% to 12% (Arnaud *et al.* 1991).

Preliminary studies of the tibia with the 6th generation MRTA hardware (no proximal or distal restraints) have shown poor between-trial reliability with high coefficients of variation (CV), on the order of 30% (Thorne 2000; Wootten 2001; Miller 2003). The high CVs were associated with earlier versions of the MRTA software employing the 7-parameter model, as well as a combination of 6-, 7-, and 9-parameter models. Subsequent enhancements to the analysis software has brought between-trial CV to the range of 15% with the most recent version of the 12-parameter model (Miller 2003).

Comparisons of CVs between studies have to be made cautiously because there is insufficient information on the methodology applied in each research project. Subject repositioning between measurement series may involve pulling the subject away from the equipment or it may involve having the subject leave the laboratory and return on another

day. After measurements have been collected, it is possible to subjectively drop apparent outliers or allow the computer to objectively select acceptable measurements based upon predetermined criteria. A third source of error between studies can arise if differing methods of calculating CV are used. The group CV can be calculated as the average of individual CVs or it can be calculated in a more conservative manner with the mean square error term from an analysis of variance (Gluer *et al*. 1995). Any of these differences in methodology may result in artificial differences in CV between studies.

Table 2.1. Reliability of various MRTA systems.

Year	Investigators	Equipment/ Analysis Model	Bone/Test Specimen	Reliability
1977	Petersen	1st generation	Human ulna (male) Monkey ulna Monkey tibia	10-15% variation <5% variation 5-7% variation
1988	Steele *et al*	4th generation	Human ulna (male)	5.3% variation 7.8% variation
1988	Ernst *et al*	4th generation	Human ulna (female)	6.5% variation
1991	McCabe *et al*	5th generation	Human ulna (female)	2.9% variation
1991	Arnaud *et al*	5th generation	Human ulna Human tibia	<5% variation 5-12% variation
1996	Arnaud *et al*	5th generation	Human tibia (male) Monkey tibia	5.8% variation 4.8% variation
1999	Thorne	6th Generation 7-parameter	Human tibia (female)	30% variation
2001	Wootten	6th Generation 6, 7, and 9 – parameter	Human tibia (female)	30% variation
2002	Djokoto	5th generation	PVC tube Human Ulna	7% variation
2003	Miller	6th Generation 12-parameter	Human tibia (female)	15% within-trial 20% between-day

Conclusion

MRTA has demonstrated its ability to reliably measure ulnar bending stiffness and has demonstrated that these measures discriminate between various groups (physical activity in men (Myburgh *et al.* 1993), young vs. old women (McCabe *et al.* 1991), normal vs. osteoporotic women (Kiebzak *et al.* 1999)) and detect changes in stiffness in response to both activity and inactivity (Young *et al.* 1983). The ulna approximates a fairly uniform cylinder, which lends itself to repeatable measurement and stable results from the bending stiffness calculations. The next challenge is to bring tibial assessment with MRTA to a level of acceptable reliability. As mentioned throughout this review, the skeleton is a heterogeneous structure that adapts to the regular strains that it experiences. Therefore, while it is not possible to measure all of the regions that are of particular interest with MRTA, we would like to apply MRTA as a possible tool to yield the best prediction of various fracture risks. Clearly, forces experienced by the femur are more likely to be predicted by the forces experienced by the tibia than forces at the ulna, as demonstrated by comparison of BMD of soccer players who approximate normals in upper extremity BMD but exceed normals at the tibia and femur. Reliable measurement of tibial bending stiffness will potentially allow for more accurate prediction of hip fracture.

CHAPTER 3

JOURNAL MANUSCRIPT I

Tibial stiffness measurement reliability with mechanical response tissue analysis

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Abstract

Mechanical response tissue analysis (MRTA) provides a noninvasive means of estimating the cross-sectional bending stiffness (EI) of human long bones and, thus, can serve as a predictor of bone strength. The purpose of this study is to evaluate the within-trial and between-trial reliability of tibial measurement with MRTA. Estimates of EI are derived according to beam vibration theory from the impedance response of a long bone to low frequency (0-1600Hz) stimulation. MRTA has demonstrated the ability to reliably estimate human ulnar EI with between-test coefficients of variation of 5%, and *in vivo* measurements of monkey tibiae have been validated with *ex vivo* 3-point mechanical bending tests. Human tibial MRTA measurement has only achieved between-test coefficients of variation of 12%, so a new physical MRTA configuration and improved computer algorithms have been developed in an attempt to enhance this level of reliability. The new configuration removes the rigid proximal and distal tibial restraints and models the tissue behavior with a 12-parameter algorithm that accounts for free vibration at the ankle and knee joints. Initial testing with only the hardware changes and application of the 7-parameter model of tissue behavior used in earlier systems yielded unacceptable variation. Subsequent reliability testing with application of 6-, 9-, and 12-parameter models demonstrated modest improvements, prompting the development of the more robust 12-parameter model that was used in the present study. Evaluation of 110 college-age females (age 20.2±1.8 yr, height 163.3±5.9 cm, weight 60.7±9.3 kg, BMI 22.8±3.1 $kg \cdot m^{-2}$) with the current MRTA system has demonstrated an improvement in within-trial reliability for unsupported tibial EI measurement with a coefficient of variation of 11.2%. These results demonstrate the ability of the system to measure tibial response characteristics when both proximal and distal ends are free of rigid support. Long-term measurement reliability is still problematic with a coefficient of variation of 36.5% for a set of 4 measurements spanning 21 months.

Introduction

Mechanical response tissue analysis (MRTA) is a non-invasive tool for evaluating the cross-sectional bending stiffness (EI) of long bones, and in humans both the tibia and ulna are accessible for *in vivo* measurement. The measurement of EI is important for bone research because it addresses aspects of bone quality that are directly related to bone strength. The primary objective of bone strength research is fracture prediction but the tools presently available are not performing adequately. Therefore, research and development into new tools and techniques is warranted. Presently, dual-energy x-ray absorptiometry (DXA) is used to measure bone mineral density (BMD) for the diagnosis of osteoporosis. BMD is one component of bone strength, but unfortunately, it has a low predictive ability relative to fracture risk (Burr and Martin 1983). When osteoporotics and normals, as defined by BMD, are compared against actual fracture incidence there is considerable overlap between the groups, indicating that DXA alone is an inadequate predictor of fracture risk (Marshall *et al.* 1996). Measurement of BMD by DXA is widely available and there is a large body of research demonstrating the correlation of BMD with exercise but minor increases in BMD which are statistically insignificant can actually result in significant bone strength improvements (Jarvinen *et al.* 1999). Bone strength is also dependent upon architectural properties of the bone such as cortical thickness (Augat *et al.* 1996), periosteal diameter (Bouxsein *et al.* 1994; Grutter *et al.* 2000), trabecular connectivity (Vajjhala *et al.* 2000; Keaveny *et al.* 2001), mineral crystal alignment (Currey 1969), and collagen fiber alignment (Martin 1991). Measurement tools that quantify these architectural variables hold promise for the improvement of fracture prediction and the accurate targeting of interventions. Treatment with antiresorptive medication is an intervention that has demonstrated the ability to reduce fracture risk, but due to the poor predictive capabilities of current technology many people are unnecessarily treated and are exposed to potential side-effects. It is suggested that a pharmacologically reduced rate of bone turnover will lead to microcrack accumulation and hypermineralization associated with increased brittleness and fragility of the bone (Currey 1990; Turner 2002). Additionally, with the growing burden of

prescription medication costs for an aging population, measures should be pursued to limit the prescription of unnecessary medications.

Mechanical response tissue analysis (MRTA) is an evolving technology that provides an alternative approach to bone strength measurement by estimating bone bending stiffness and material damping. Bone bending stiffness and material damping are correlated to *in vitro* fracture strength and these measures demonstrate changes seen with interventions on a much larger scale than seen with BMD measurement (Dimarogonas *et al.* 1993; Roberts *et al.* 1996). Measurement of bending stiffness with MRTA has been reliable for the human ulna with coefficients of variation (CVs) on the order of 3-5% (Steele *et al*. 1988; Arnaud *et al*. 1991; McCabe *et al*. 1991). During measurement of the human tibia, CVs have ranged from 5-12% in the best case to 20-30% with the present hardware configuration (Arnaud *et al*. 1991; Thorne 2000; Wootten 2001; Miller 2003). The most recent step in MRTA development involves improved computer algorithms that address the analysis of the human tibia under conditions in which neither end of the bone is rigidly supported. The aim of the present study was to evaluate the short and long-term measurement reliability of the present MRTA hardware and analysis algorithms.

Methods

Subjects

In order to assess the within-trial and between-trial reliability of MRTA when applied to human tibiae that are not rigidly supported, data from 110 female subjects (age 20.2±1.8 yr, height 163.3±5.9 cm, weight 60.7±9.3 kg, BMI 22.8±3.1 $kg \cdot m^{-2}$) participating in a 21 month isokinetic exercise training-detraining study were evaluated. The data set includes nine MRTA measurements of the tibia at each measurement session. Measurement sessions were conducted at baseline, 4.5 months, 9 months, and 21 months. Within-trial reliability was computed from the nine measurements taken at baseline for all subjects on both legs. The training protocol involved three isokinetic exercise sessions per week of only the non-dominant leg for 9 mo, followed by a 12 mo period without structured training. Between-trial reliability was assessed in this study only using results for the control (dominant) leg of subjects that completed the study and

had measurements taken at all four time periods. From the initial 110 subjects, only 33 were present during all 4 measurement sessions and 6 of those subjects were dropped from the between-trial analysis when measurements were rejected.

Equipment

The basic components of the MRTA system are the mechanical shaker, the impedance head, the contact probe, the system control and measurement software, and the analysis software. The mechanical shaker, impedance head, and contact probe are connected in series (Figure 3a.1) with the shaker physically driving the impedance head-probe with a randomly generated frequency from 0 to 1600Hz, as specified by the controlling software. The impedance head transmits both force and acceleration data to the measurement software, and the probe (<1 cm^2) provides transcutaneous contact with the bone at the mid-point of the tibia. This equipment is based upon the vertical test stand system with a single axis self-aligning probe as described by Steele (1988). MRTA has evolved from early versions of equipment that involved various means of fixation of the long bones and a manual frequency sweep to generate an impedance curve (Young *et al.* 1976; Peterson 1977; Young *et al.* 1979) to more recent versions that incorporate computer controlled frequency generation and additional long bone support structures (Steele *et al.* 1988; Roberts *et al.* 1996; Hutchinson *et al.* 2001). The current version of the MRTA hardware supports the foot but does not provide rigid support of the tibia at either the proximal or distal end.

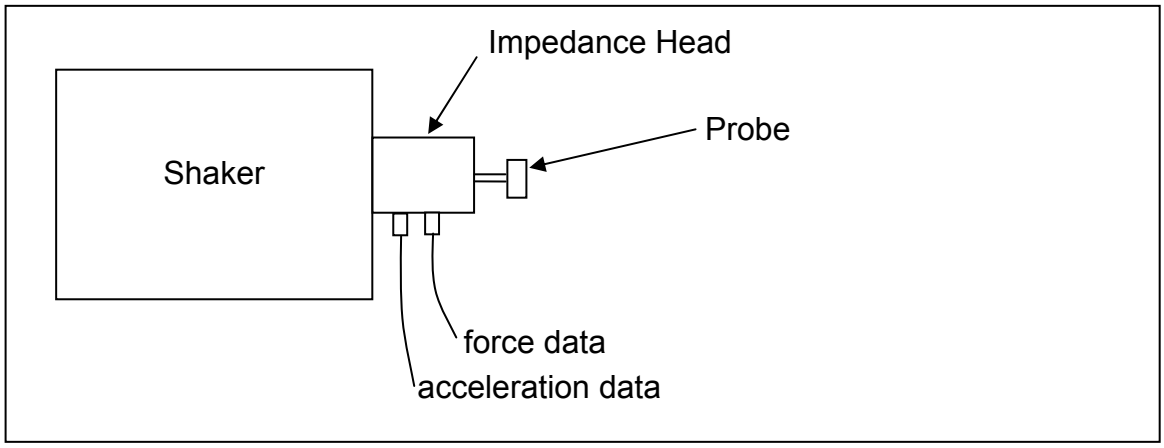

Figure 3a.1. MRTA measurement hardware.

Measurement Procedure

Subjects were seated with one foot lightly supported on a platform, the tibia vertically positioned, and the knee bent to 90 degrees. The MRTA probe is positioned on the anterior crest of the tibia at a point halfway between the proximal medial condyle and the distal edge of the medial malleolus. The probe is driven by an electromechanical shaker in a random pattern through a low frequency range (0-1600Hz), and transcutaneous contact with the tibia is evaluated by palpating the proximal and distal tibia for the presence of this vibration. Force and acceleration data are measured by an impedance head positioned between the probe and the shaker and collected by a MacIntosh G3 desktop computer. After positioning, nine measurements are taken with each measurement requiring 5-10 seconds of data collection. Presently, the data collection and data analysis functions are separated, requiring the technician to rely upon visual inspection of the raw data to make a decision on accepting or rejecting a measurement before taking the next measurement. Measurements that display considerable levels of noise (Figure 3a.2) are rejected and minor repositioning of the subject is required to establish sufficient contact between the probe and the bone. The visual inspection process leads to the acceptance of some raw data that does not meet the requirements of the analysis software, therefore, not all subjects have nine measurements per session.

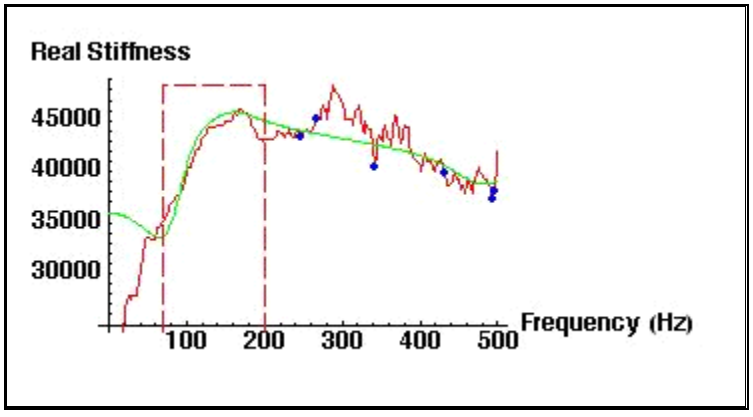

Figure 3a.2. Example of raw data with excessive noise indicating that the technician should reject this measurement.

Measures

MRTA provides three measures of interest for the estimation of bone strength and the evaluation of changes occurring with exercise training. Cross-sectional bone bending stiffness (EI) is the product of Young's modulus of elasticity (E) and the cross-sectional moment of inertia (I) and is measured in units of Nm^2. Sufficiency (S), a unitless quantity, provides a relative measure of bone strength to body weight and is calculated as the maximum axial load, $P_{cr} = EI\ (\pi/L)^2$, divided by body weight. The damping index (DI) is measured in units of $kg{\cdot}m{\cdot}\ s^{-1}$, and represents the maximum lateral force that the bone can withstand.

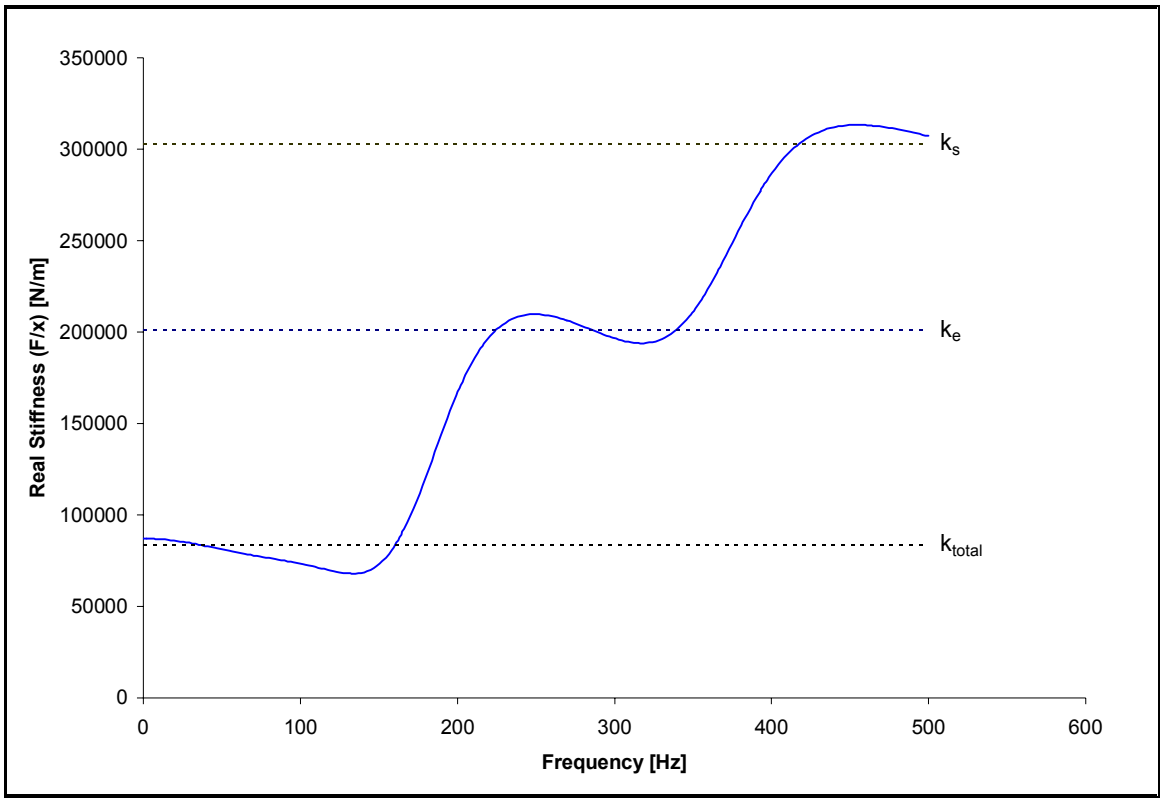

Figure 3a.3. Impedance curve with simplified approximations. The plateaus give roughly the skin stiffness [k_s] for high frequency, the skin plus bone [$k_e = (k_s^{-1} + k_b^{-1})^{-1}$] for the intermediate frequency, and the total [$k_{total} = (k_s^{-1} + k_b^{-1} + k_{end}^{-1})^{-1}$] for the low frequency, where k_{end} is the effective stiffness of the end support. Generally, however, there is strong coupling, so this approximation is not used in the curve fit algorithm.

MRTA generates a curve fit (Figure 3a.3) to the raw impedance data and approximates a value for bone stiffness (k_b) from this curve fit. From bone stiffness and the tibial length (L) the value of cross-sectional bone bending stiffness (EI), the axial load capacity (Euler buckling load, P_{cr}), and the bone sufficiency (ratio of P_{cr} to body weight) are calculated.

$$EI = k_b \cdot L^3/48 \qquad \text{(Eqn 3.1)}$$

$$P_{cr} = EI \cdot (\pi/L)^2 \qquad \text{(Eqn 3.2)}$$

$$S = P_{cr} / (\text{body weight}) \qquad \text{(Eqn 3.3)}$$

Data analysis

Real-time force and acceleration data is converted with Fourier transforms to the frequency domain for analysis across the range of 70 to 500Hz. The analysis software decomposes the response data into real and imaginary components of stiffness and compliance from which further computation attempts to match a mathematical model to the raw physical data by identifying maximum and minimum values and then following an iterative least root mean square error method of approximating the raw data. Proper identification of the true maximum and minimum values can fail due to noise in the raw data which can lead the iterative curve fitting process astray. To improve on the identification of maximum and minimum values, the curve fitting procedure performs an initial analysis of all the measurements taken on a bone within one trial and selects the measurement with the best curve fit on the basis of the lowest root mean square (RMS) error between the raw data and the curve fit. The maximum and minimum values from this measurement are then used as the starting point, or reseeding value, for the iterative curve fitting of all other measurements on the bone for this trial. Goodness of fit with the raw data is specified by the RMS error, and the requirement for an acceptable fit is an RMS error of less than 10 percent. The reseeding procedure has improved the automated curve fitting process from a prior yield of 70% acceptable curve fits to 89% in this study. This level of measurement acceptance results in 95% of subjects having acceptable data that meets the 10% RMS error level (see Figure 3a.4).

A 12-parameter model of tissue behavior that takes into account the mass, stiffness, and damping for the bone, skin, and proximal and distal joints has been most successful in analysis of the tibia when both the proximal and distal ends are not rigidly supported (Steele 2001). Evaluation of a previous 12-parameter model, as used by Wootten (2001), has led to enhancements resulting in the current 12-parameter model. The data from this study was analyzed with this most recent version of the 12-parameter model of tissue behavior, which is computationally intensive, so data analysis was completed on a MacIntosh G4.

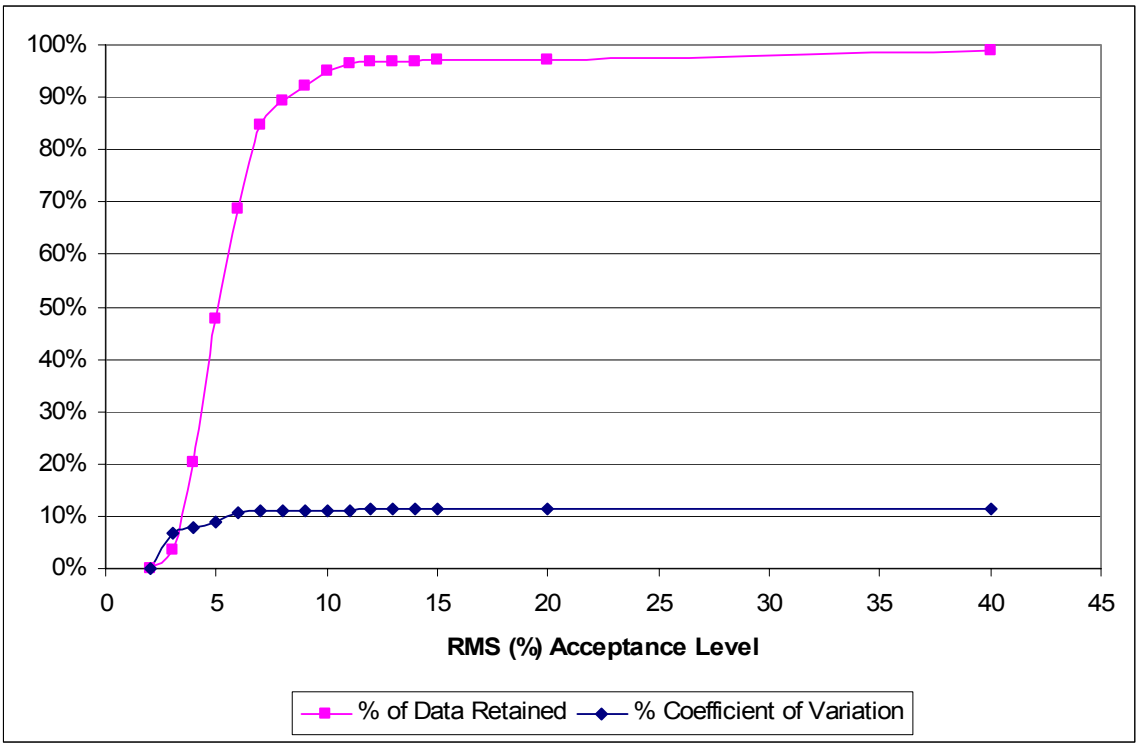

Figure 3a.4. RMS acceptance level versus the percent of subject data retained and the within-trial percent coefficient of variation for baseline data of all subjects.

Statistics

Initial analysis of the data involved calculation of the coefficient of variation (CV) for within-trial and between-trial measurements. The data collection and data analysis procedures generally result in the rejection of some measurements, therefore, the number of measurements per session is unequal across subjects. CV is typically calculated as the standard deviation divided by the mean, and for a group of subjects the average of the

individual CVs is an estimate of the group CV. However, taking the average of individual CVs may underestimate the actual CV by up to 25% (Gluer *et al.* 1995). To control for a non-normal distribution of individual CVs and unequal numbers of measurements per subject a one-way ANOVA is applied to compute the mean square error term (MS_E), with subject+bone (each measured tibia) as the factor. The standard error of measurement (SEM) is the square root of MS_E and the coefficient of variation is computed as the SEM divided by the mean of all measurements: CV% = (SEM/mean)·100. For consistency this method is used to calculate CV for both within-trial and between-trial comparisons, where within-trial CV evaluates nine measurements taken in a row on each tibia and the between-trial CV evaluates the mean measurement value for each tibia from each of the four measurement sessions.

Results

Within-trial reliability was assessed for the nine measurements taken on the control legs for each session. Table 3a.1 presents information on the breakdown of subject attrition and data loss for each measurement session as well as the combination of only the subjects measured across all four sessions. Failed measurement for a subject at any of the four sessions resulted in exclusion of that subject from analysis. Table 3a.2 presents the mean±SD for the tibiae included in the calculation of CV for each session. Note that baseline data is presented for all of the tibiae measured, as well as the subgroup of tibiae that served as controls during the study. Data for the three remaining sessions is only presented for tibiae of the control legs in subjects that were available for testing and had data that met the acceptance criteria (RMS <0.1). Table 3a.3 presents the mean±SD at each session for only those control tibiae that had acceptable data at all four time periods. Table 3a.4 presents the within-trial CVs at each of the four time periods as well as the between-trial CVs for subjects with complete data sets.

Table 3a.1. Data loss due to subject attrition and failed measurements.

	Number of Subjects		
	Measured	Failed	Accepted
Baseline	81	6	75
4.5 months	63	2	61
9 months	60	3	57
21 months	43	3	40
All 4 sessions	33	6	27

Table 3a.2. MRTA output descriptive statistics (Mean±SD) for control legs with acceptable measurements at each measurement session.

	N	Bending Stiffness [$N \cdot m^2$]	Sufficiency	Damping Index [$kg \cdot m \cdot s^{-1}$]
Baseline				
(all legs)	208	114.6 ± 55.5	14.2 ± 7.0	14.4 ± 6.1
(control legs)	75	122.1 ± 42.0	16.0 ± 5.7	15.5 ± 5.7
4.5 months	61	131.1 ± 54.0	17.1 ± 7.0	15.1 ± 4.6
9 months	57	135.8 ± 56.8	17.3 ± 6.2	15.6 ± 4.7
21 months	40	123.0 ± 43.1	16.9 ± 6.2	15.2 ± 6.5

Table 3a.3. MRTA output descriptive statistics (Mean±SD) for control legs with acceptable measurements at all four measurement sessions. These means correspond to the data used in calculation of the between-trial CV.

	N	Bending Stiffness [$N \cdot m^2$]	Sufficiency	Damping Index [$kg \cdot m \cdot s^{-1}$]
Baseline	27	118.0 ± 43.6	16.1 ± 6.5	14.0 ± 4.7
4.5 months	27	145.1 ± 55.6	19.8 ± 7.7	15.5 ± 4.6
9 months	27	141.0 ± 68.9	18.3 ± 7.2	15.9 ± 4.1
21 months	27	127.7 ± 45.3	17.5 ± 6.8	15.0 ± 7.3
All Sessions	108	133.0 ± 54.6	17.9 ± 7.1	15.1 ± 5.3

Table 3a.4. CV percentage for within-trial and between-trial measurements.

	N	Bending Stiffness	Sufficiency	Damping Index
Within-trial				
Baseline				
(all legs)	208	11.2%	10.4%	13.0%
(control legs)	75	11.4%	11.3%	15.6%
4.5 months	61	10.7%	11.8%	6.7%
9 months	57	10.6%	11.6%	7.9%
21 months	40	12.5%	11.3%	13.3%
Between-trial				
Control legs	27	36.5%	34.6%	27.6%

Discussion

Past MRTA systems have demonstrated between-trial coefficients of variation on the order of 5% for human ulnar measurements and 12% for human tibial measurements with an MRTA configuration that provides rigid support at both the proximal and distal ends. Additionally, EI measurements with MRTA have been validated with trials of *in vivo/ex vivo* bending stiffness of monkey tibiae, again in a situation where the bones were rigidly supported. The present MRTA system does not rigidly support the ends of the tibia but utilizes mathematical models to account for free vibration within the proximal and distal joints. Rigid support of the proximal and distal tibia requires an experienced technician to achieve the reported reliability of 12% and the day-to-day changes in establishing this support may be partially responsible for the between-trial variation in bending stiffness measurement.

The current MRTA system that does not rigidly support the tibia has been the subject of three prior investigations involving measurement reliability. Thorne (2000) and Wootten (2001) have evaluated between-trial reliability of tibial measurements but were limited to use of earlier versions of the analysis algorithms and subsequently have found CVs on the order of 30%. Recently, Miller (2003) has studied between-trial

reliability and was able to achieve a CV of 19% with an experienced technician and a homogeneous subject pool. The studies conducted by Thorne and Miller involved data collection over time periods of less than one week and Wootten's research spanned six weeks, while the current study spanned 21 months.

The within-trial results of the present study are encouraging with bending stiffness coefficients of variation as low as 10.6% with the use of the present MRTA hardware. These results demonstrate that the unsupported tibia can be measured with reliability similar to MRTA systems that rigidly support the tibia. Between-trial coefficient of variation for bending stiffness in control legs remains problematic with a CV of 36.5%, indicating that improvements are required to bring this system up to the level of performance found in systems with rigid tibial support structures.

Further research is necessary to identify the sources of variation that are influencing the present MRTA configuration. The present study involved a homogenous population involved in exercise training of the opposite limb over a time period when bone growth and development are expected. To understand the potential influence of anthropometric characteristics on variability, a heterogeneous population should be studied, and to reduce the influence of bone growth and development, repeated measurements should be taken over the course of one week.

Past attempts to standardize foot and thigh position across measurement sessions have been unsuccessful in improving between-trial reliability (Miller 2003) indicating that either greater precision is required for true standardization or that additional factors are influencing measurement. One possibility for standardization of measurement conditions involves the optimization of the contact condition between the probe and the tibia. Minor changes in probe placement and contact pressure potentially have significant influence on measurement reliability, but these variables are difficult to quantify with the present system, so a form of feedback relating these quantities or their result should be explored. One method of feedback that may be useful is a real-time display of skin stiffness at one frequency. A large value for skin stiffness allows for a clear

differentiation between skin stiffness (k_s) and bone stiffness (k_b) (see Figure 3a.3), so as skin stiffness is maximized the quality of probe contact is theoretically maximized. A real-time display will allow the technician to modify the position of the probe and the contact angle with the tibia until a maximum skin stiffness for the subject is reached. The real-time display will also allow the technician to monitor the quality of contact across a series of measurements so that any minor change in subject position will be detected, thus allowing for repositioning to maintain the within-trial quality of measurement. Other methods of standardizing measurement conditions may prove useful in improving reliability, but they require additions to the current hardware, such as pressure transducers to quantify the amount of weight supported by the foot or to quantify the amount of pressure between the probe and the bone.

Bone mineral quantity and macroarchitectural variables can be measured with current technology but *in vivo* assessment of bone quality remains elusive, thus, bone strength and fracture risk cannot be fully predicted. MRTA addresses the issue of bone quality, thereby, complementing systems that quantify bone mineral and holds promise for improving the prediction of fracture risk. To fully realize the potential of MRTA, the past success in ulnar measurement has to be carried forth to other bones, such as the tibia, to address the heterogeneous nature of the skeleton. Reliability of tibial measurement with MRTA is currently in question but the advances in computational algorithms noted in this paper indicate that the ability of the system to fit the raw data is quite accurate, and the variability in mechanical positioning may be the main source of error. Past improvements to positioning of the tibia have been limited to operator experience and rigid fixation hardware, so a real-time objective feedback mechanism holds promise for a novel improvement to the system.

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JOURNAL MANUSCRIPT II

An examination of variability in mechanical response tissue analysis of the human tibia

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Abstract

Mechanical response tissue analysis (MRTA) is a method of predicting the cross-sectional bending stiffness (EI) of long bones through interpretation of the impedance response to low frequency (70-500Hz) vibration. The purpose of this study was to examine variability of tibial measurements associated with MRTA and, using these findings, develop a definitive plan to guide future enhancements to the system. MRTA has demonstrated good reliability with reported coefficients of variation of 3 to 5 percent in laboratory settings for measurement of the human ulna and monkey tibia, but due to the more complex geometry of the human tibia and contributions from a range of unresolved technical considerations, *in vivo* measurement reliability for the human tibia has been problematic, with between-trial coefficients of variation ranging from 12 to 40 percent. Previous MRTA research on the human tibia revealed additional resonances that have been associated with soft tissue effects between the bone and distal fixation hardware, leading to the development of algorithms that take free vibration at the proximal and distal ends into account. The current MRTA system takes advantage of the new algorithms and in an attempt to eliminate the variation associated with the rigid fixation of the tibia removes the fixation hardware. The system supports the foot and the thigh, allowing for measurement of the tibia while both proximal and distal ends vibrate within the joints without mechanical restriction. To evaluate the performance of the system, tibial MRTA measurements of physically active male (n=9) and female (n=6) subjects were taken on three separate days over the course of one week. Mean EI (male: 273±103 Nm^2; female: 155±70 Nm^2) and sufficiency (S) (male: 20±7; female: 21±7) values are comparable to those reported in the literature, but the coefficient of variation (CV) for within-trial (EI: 35-42%; S: 33-37%) and between-trial (EI: 48%; S: 34%) measurements are unacceptable. To improve measurement reliability, additional feedback mechanisms are required to standardize subject positioning and to identify acceptable measurements.

Introduction

Research has shown that the cross-sectional bending stiffness (EI) of a long bone is predictive (r^2>0.9) of the maximum strength of the bone (Roberts *et al.* 1996), thus accurate *in vivo* measurement of EI can be used to assess bone quality. Mechanical response tissue analysis (MRTA) is a non-invasive tool for evaluating EI of long bones through decomposition of the impedance response to low frequency vibration. The components of EI are Young's modulus of elasticity (E), an intrinsic material property, and the cross-sectional moment of inertia (I), an extrinsic geometric property. Other methods of estimating bone strength generally rely on extrinsic characteristics such as the quantity of bone mineral in a region or the geometric properties of cross-sectional diameter and cortical thickness and are thus limited by the fact that they do not account for intrinsic material variability (Watts 2002). Intrinsic material properties are based upon microarchitectural parameters, such as the alignment of collagen fibers and hydroxyapatite crystals (Currey 1969; Martin and Ishida 1989), and are therefore difficult to quantify *in vivo*. The strength of bone is dependent upon both intrinsic and extrinsic properties, so the ability of MRTA to quantify the combination of these properties avoids the problem of the *in vivo* measurement of intrinsic material properties. The quantification of bone mineral is widely used in the prediction of bone strength but is of limited use in the prediction of fracture risk (Marshall *et al.* 1996), and underestimates changes in bone strength resulting from exercise interventions (Jarvinen *et al.* 1999).

Validation trials of MRTA demonstrate a strong relationship (r^2 = 0.95) between bending stiffness as measured *in vivo* by MRTA and as measured *ex vivo* by 3-point mechanical loading (Roberts *et al.* 1996). Initial validation was determined by testing the MRTA system on cylindrical aluminum bars of varying cross-sections with a rubber pad was placed between the MRTA probe and the bar to simulate soft tissue. The value of Young's modulus (E) was known for the bars so theoretical values of EI were calculated based upon the radius of each bar. $EI_{(MRTA)}$ was highly predictive (r^2=0.99) of $EI_{(theoretical)}$ for the aluminum test bars and $EI_{(MRTA)}$ values were within 5% of the calculated values. Subsequently, on a population of 12 monkeys, *in vivo* tibial MRTA measurements were

conducted for comparison with *ex vivo* 3-point mechanical loading tests. This study showed that in addition to the strong relationship between $EI_{(MRTA)}$ and $EI_{(3\text{-point bending})}$, there is also a strong relationship ($r^2 = 0.92$) with the maximum load and $EI_{(MRTA)}$.

Human ulnar measurements were analyzed with a 7-parameter model for curve fitting and produce good reliability demonstrating average variations of 5.3% and 7.8% with repositioning between trials (Steele *et al.* 1988). Ulnar measurements on a version of the MRTA system that used an additional pad for limb restraint yielded precision errors of 2.9% (McCabe *et al.* 1991). Additional studies with limb repositioning resulted in variability of less than 5% for ulnar measurements, while tibial measures range in variability from 5% to 12% (Arnaud 1991). The ulna has a fairly uniform structure, which approximates a long cylindrical tube and allows for more reliable measurement. The human tibia is not radially uniform in cross section, the orientation of the cross section varies longitudinally, and the bone is slightly curved. The difference in uniformity of the tibia versus the ulna is one potential source of measurement variation.

Preliminary studies of tibial bending stiffness with the present MRTA hardware configuration (no proximal or distal restraints) have shown poor between-trial reliability with high coefficients of variation (CV), on the order of 30% (Thorne 2000; Wootten 2001; Miller 2003). However, the high CVs were associated with earlier versions of the MRTA software employing a 7-parameter model, as well as a combination of 6-, 9-, and 12-parameter models. Subsequent enhancements to the analysis software have achieved a short-term between-trial CV of 19% using the most recent version of the 12-parameter model (Callaghan 2003; Miller 2003), though these results were not reproduced in a long-term study of between-trial reliability (Callaghan 2003). Thus, the purpose of the present study was to evaluate the reliability and performance of the MRTA hardware and data analysis algorithms in a heterogeneous population over a short time frame to enhance our understanding of the components of variation and to develop a definitive plan to guide future enhancements to the system.

Methods

Subjects

Subjects for this study were recruited locally and completed an informed consent as approved by the Institutional Review Board of Virginia Polytechnic Institute and State University. Subject descriptive characteristics are presented in Table 3b.1 as mean±SD. Prior reliability studies of this version of MRTA have focused on homogeneous groups of college age females, so a heterogeneous group of subjects consisting of males and females was recruited for this study. All subjects were currently active and have been active for at least three years, participating in a variety of activities from high school sports to yoga and martial arts.

Table 3b.1. Subject characteristics (mean±SD).

	Male (n = 9)	Female (n = 6)
Age (yr)	30.7 ± 9.4	25.2 ± 2.9
Height (cm)	180.0 ± 5.7	162.4 ± 6.3
Weight (kg)	86.5 ± 13.4	59.3 ± 9.4
BMI ($kg \cdot m^{-2}$)	26.7 ± 4.0	22.4 ± 2.3

Equipment

The basic components of the MRTA system are the mechanical shaker, the impedance head, the contact probe, the system control and measurement software, and the analysis software. The mechanical shaker, impedance head, and contact probe are connected in series (Figure 3b.1) with the shaker physically driving the impedance head-probe with a randomly generated frequency from 0 to 1600Hz, as specified by the controlling software. The impedance head transmits both force and acceleration data to the measurement software, and the probe (<1 cm^2) provides transcutaneous contact with the bone at the mid-point of the tibia. This equipment is based upon the vertical test stand system with a single axis self-aligning probe as described by Steele (1988). MRTA has evolved from early versions of equipment that involved various means of fixation of the long bones and a manual frequency sweep to generate an impedance curve (Young *et al.* 1976; Peterson 1977; Young *et al.* 1979) to more recent versions that incorporate

computer controlled frequency generation and additional long bone support structures (Steele *et al.* 1988; Roberts *et al.* 1996; Hutchinson *et al.* 2001). The current version of the MRTA hardware supports the foot but does not provide rigid support of the tibia at either the proximal or distal end.

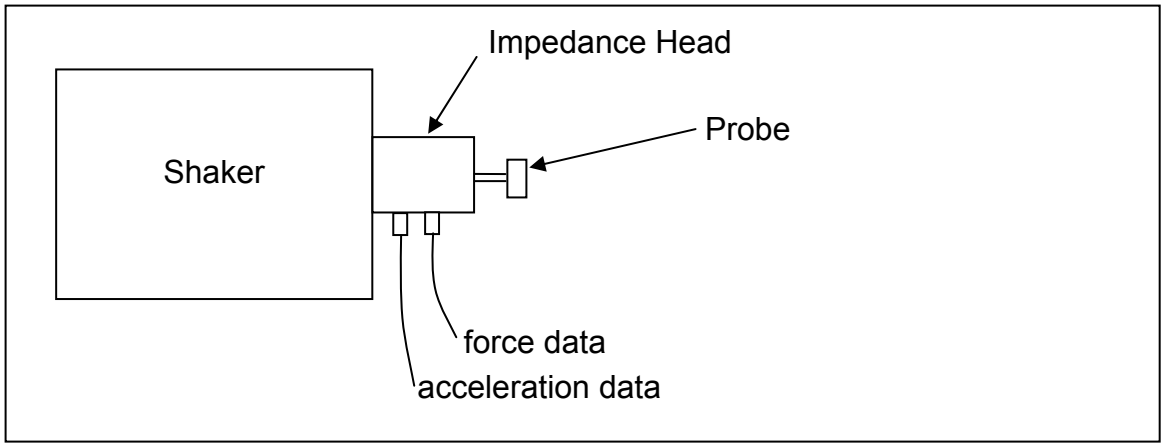

Figure 3b.1. MRTA measurement hardware.

Measurement Procedure

MRTA measurements were taken on both legs on three separate days over the course of one week. On the first day, tibial length was measured as the distance from the proximal edge of the medial condyle to the distal edge of the medial malleolus. To eliminate variability due to variation in tibial length measurement, the initial tibial measurement was used for each subsequent testing session. MRTA probe placement was determined as half of the tibial length and was marked on the anterior tibial crest as measured down from the medial condyle with the subject in a seated position. Subjects were positioned so that the MRTA probe made contact at the midpoint of the tibia on the anterior crest. Good transcutaneous contact with the bone was verified by palpating the proximal and distal tibia to confirm that vibration was being transmitted along the bone. After the subject was positioned, nine measurements were taken with each measurement requiring approximately 5-10 seconds of data collection. The probe remained in contact with the subject's leg throughout the series of measurements, which lasts approximately 5 minutes per leg. The raw data is displayed on a frequency graph after each measurement

for evaluation by the technician. If the raw data displays excessive noise as seen in Figure 3b.2, the subject's position is adjusted to improve contact with the probe.

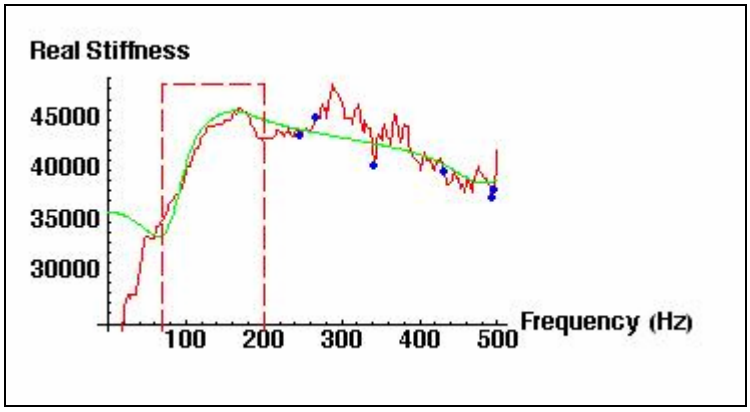

Figure 3b.2. Example of raw data with excessive noise indicating that the technician should reject this measurement.

Raw Data Analysis

Real-time force and acceleration data were converted with Fourier transforms from the time domain to the frequency domain for analysis. The analysis software decomposes the response data into real and imaginary components of stiffness and compliance, from which further computation attempts to match a mathematical model to the raw physical data. The frequency data was analyzed with the 12-parameter model of tissue behavior (Figure 3b.3) across the range of 70 to 500Hz. The curve fitting procedure involves an automated search for local maximums in the stiffness data (F/x = force/displacement) for both the real and imaginary components, and minimums in the real and imaginary components of the compliance (x/F = displacement/force) data (see Figure 3b.4). These maximum and minimum values correspond to the poles and zeros of

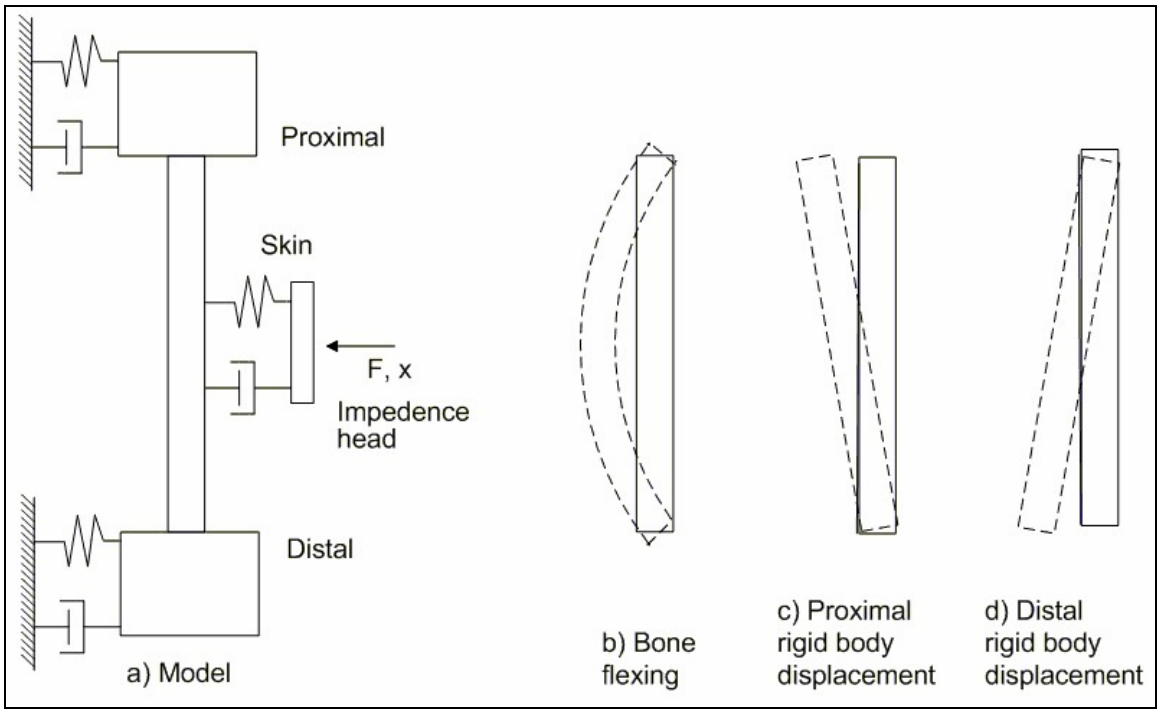

Figure 3b.3. 12-parameter model of tissue behavior (reproduced with permission: Steele, 2001), which accounts for vibration within both the proximal and distal joints. The 9-parameter model involves pinning either (c) the distal or (d) the proximal end.

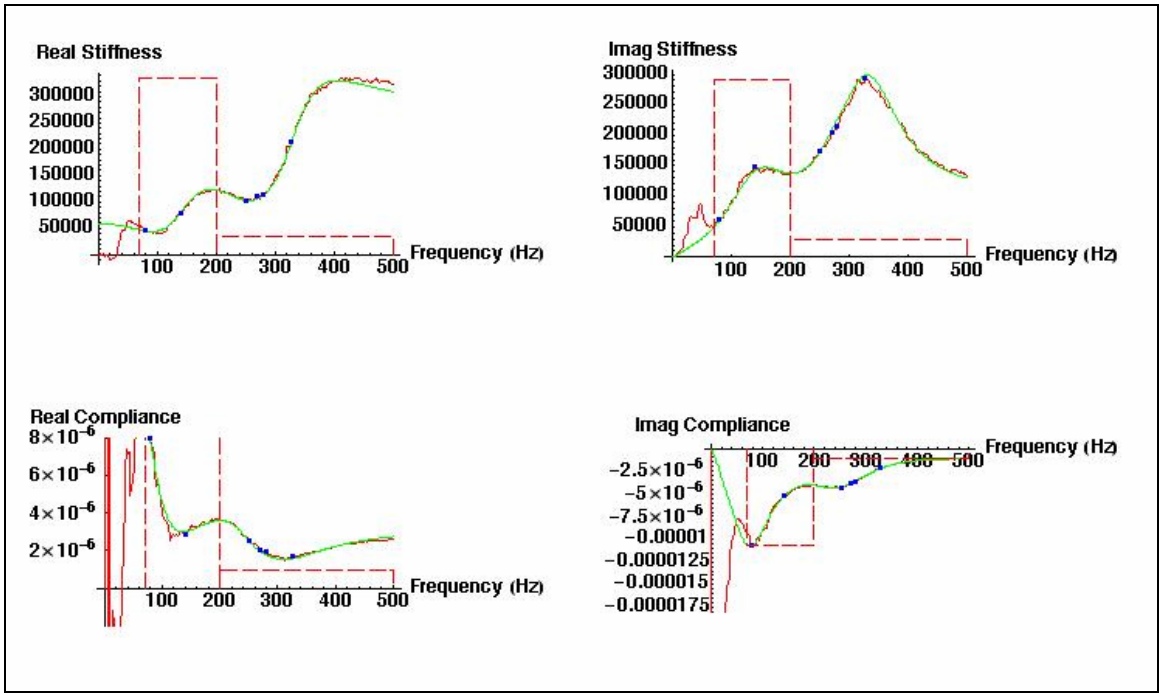

Figure 3b.4. 12-parameter model frequency plots for the real and imaginary components of stiffness and compliance from one MRTA measurement.

the impedance response which represent the system resonances. Proper identification of the true maximum and minimum values can fail due to noise in the raw data which can lead the iterative curve fitting process astray. To improve on the identification of maximum and minimum values, the curve fitting procedure performs an initial analysis of all the measurements taken on a bone within one trial and selects the measurement with the best curve fit on the basis of the lowest root mean square (RMS) error between the raw data and the curve fit. The maximum and minimum values from this measurement are then used as the starting point, or reseeding value, for the iterative curve fitting of all other measurements on the bone for this trial. Goodness of fit with the raw data is specified by the RMS error, and the requirement for an acceptable fit is an RMS error of less than 10 percent. The reseeding procedure has improved the automated curve fitting process from a prior yield of 70% acceptable curve fits to 87% in this study.

After the raw data has been fit with a prediction curve, an estimate of the bone stiffness is calculated (see Figure 3b.5). From bone stiffness (k_b) and tibial length (L) the cross-sectional bone bending stiffness (EI), the axial load capacity (Euler buckling load, P_{cr}), and the bone sufficiency (S) (ratio of P_{cr} to body weight) are calculated.

$$EI = k_b \cdot L^3/48 \qquad \text{(Eqn 3b.1)}$$

$$P_{cr} = EI \cdot (\pi/L)^2 \qquad \text{(Eqn 3b.2)}$$

$$S = P_{cr} / (\text{body weight}) \qquad \text{(Eqn 3b.3)}$$

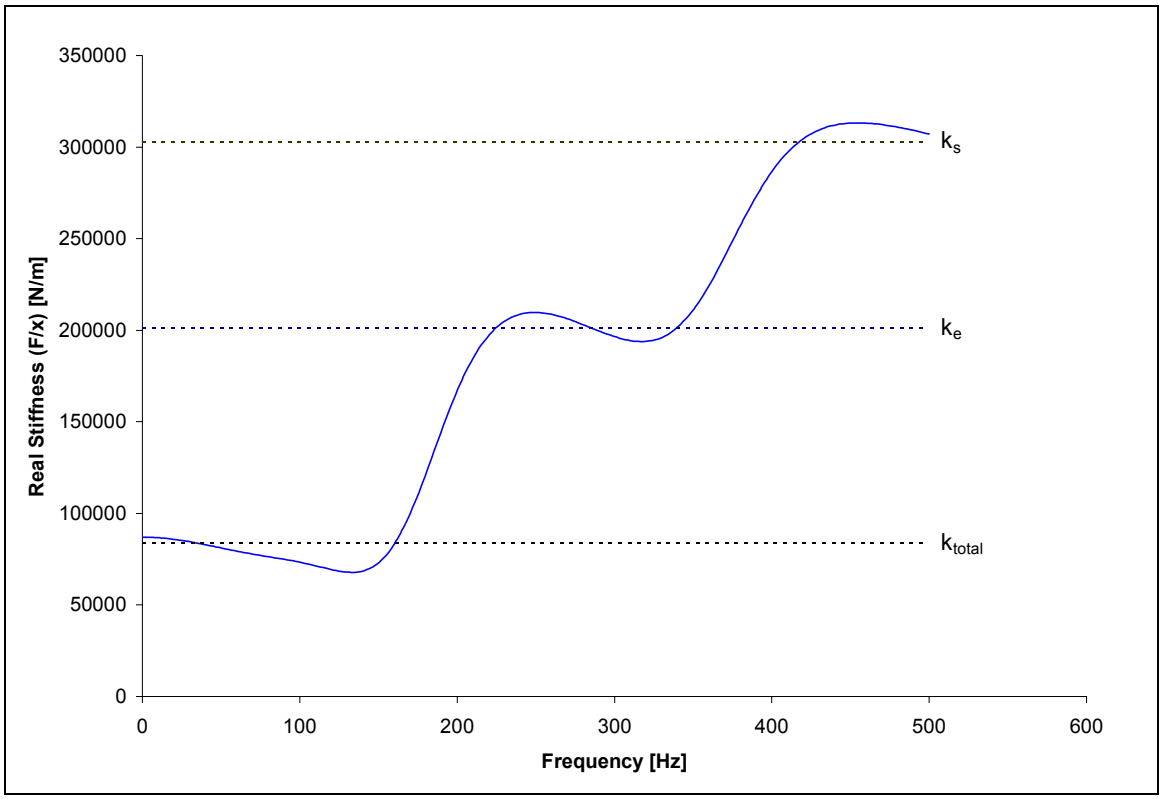

Figure 3b.5. Impedance curve with simplified approximations. The plateaus give roughly the skin stiffness [k_s] for high frequency, the skin plus bone [$k_e = (k_s^{-1} + k_b^{-1})^{-1}$] for the intermediate frequency, and the total [$k_{total} = (k_s^{-1} + k_b^{-1} + k_{end}^{-1})^{-1}$] for the low frequency, where k_{end} is the effective stiffness of the end support. Generally, however, there is strong coupling, so this approximation is not used in the curve fit algorithm.

Statistical Analysis

Initial analysis of the data involved calculation of the coefficient of variation (CV) for within-trial and between-trial measurements. The data collection and data analysis procedures generally result in the rejection of some measurements, therefore, the number of measurements per session is unequal across subjects. CV is typically calculated as the standard deviation divided by the mean, and for a group of subjects the average of the individual CVs is an estimate of the group CV. However, taking the average of individual CVs may underestimate the actual CV by up to 25% (Gluer *et al.* 1995). To control for a non-normal distribution of individual CVs and unequal numbers of measurements per subject a one-way ANOVA is applied to compute the mean square

error term (MS_E), with subject+bone (each measured tibia) as the factor. The standard error of measurement (SEM) is the square root of MS_E and the coefficient of variation is computed as the SEM divided by the mean of all measurements: CV% = (SEM/mean)·100. For consistency this method was used to calculate CV for both within-trial and between-trial comparisons, where within-trial CV evaluates nine measurements taken in a row on each tibia and the between-trial CV evaluates the mean measurement value for each tibia from each of the three days of testing.

To check for heteroscedasticity, bending stiffness residuals were plotted against a range of variables from body weight and height to EI and skin stiffness. To evaluate the normality of the data, histogram plots were generated for within-trail measurements.

Results

Descriptive statistics (mean±SD) for MRTA measurements of cross-sectional bending stiffness (EI), sufficiency (S), and damping index (DI) are presented for the entire group (Table 3b.2), for the nine male subjects (Table 3b.3), and for the six female subjects (Table 3b.4). The mean S for male subjects and the mean EI for female subjects are both comparable to means reported by Arnaud *et al* (1996a; 1996b), with S ranging from 20.3±5 to 23.4±6 for the male subjects and EI of 150±71Nm^2 for the female subjects. The mean EIs reported by Arnaud for male subjects were lower than the present finding, ranging from 195±48 to 224±69Nm^2, but the subject groups were also lighter, 78.9±7.9kg and 80±10kg, so the comparison of S values, which take subject weight into account, is more appropriate. S values were not reported for the female group, but they were of similar weight, 61±10kg, to females in the present study, so the comparison of EI is appropriate.

Table 3b.2. All subjects: MRTA output descriptive statistics (mean±SD) for all tibiae with acceptable measurements at each measurement session.

	N	EI [$N \cdot m^2$]	Sufficiency	Damping Index [$kg \cdot m \cdot s^{-1}$]
Session 1	30	213.1 ± 94.5	18.8 ± 6.3	20.6 ± 7.8
Session 2	28	230.0 ± 129.3	19.8 ± 7.0	21.8 ± 8.0
Session 3	30	236.0 ± 101.5	21.3 ± 7.0	23.2 ± 7.3
Total	88	226.3 ± 108.1	20.0 ± 6.8	21.9 ± 7.7

Table 3b.3. Male Subjects: MRTA output descriptive statistics (mean±SD) for all tibiae with acceptable measurements at each measurement session.

	N	EI [$N \cdot m^2$]	Sufficiency	Damping Index [$kg \cdot m \cdot s^{-1}$]
Session 1	18	258.8 ± 79.7	18.6 ± 6.2	23.6 ± 7.6
Session 2	17	282.2 ± 129.7	20.2 ± 7.3	26.0 ± 6.4
Session 3	18	279.4 ± 101.1	19.9 ± 7.2	26.3 ± 6.6
Total	53	273.3 ± 103.5	19.6 ± 6.8	25.3 ± 6.9

Table 3b.4. Female Subjects: MRTA output descriptive statistics (mean±SD) for all tibiae with acceptable measurements at each measurement session.

	N	EI [$N \cdot m^2$]	Sufficiency	Damping Index [$kg \cdot m \cdot s^{-1}$]
Session 1	12	144.4 ± 71.7	19.1 ± 6.7	16.1 ± 5.8
Session 2	11	149.3 ± 80.0	19.2 ± 6.8	15.4 ± 5.5
Session 3	12	171.0 ± 60.9	23.3 ± 6.4	18.6 ± 6.0
Total	35	155.1 ± 69.9	20.6 ± 6.7	16.7 ± 5.8

Coefficients of variation are presented in Table 3b.5 for within-trial measurements and between-trial measurements. Within-trial CV is based upon the acceptable measurements (those that are below the RMS criteria of 0.1) from the series of nine measurements taken for each tibia within each session. For each session, average values for EI, S, and DI are calculated for each tibia and these values are compared for the computation of the between-trial CV. The CVs from this study clearly demonstrate that while mean EI and S values are comparable to other reports, there are significant problems with measurement variation both within and between trials. DI provides another measure of bone strength, and in this study is outperforming both EI and S for within-trial variation, but the between-trial CV is no better than the CV for S, so its utility is questionable.

Table 3b.5. CV percentage for within-trial and between-trial measurements.

	EI	Sufficiency	Damping Index
Within-trial			
Session 1	34.7%	34.2%	18.4%
Session 2	42.3%	37.0%	16.7%
Session 3	36.0%	33.1%	16.5%
Between-trial	48.1%	33.8%	35.2%

Figure 3b.6 is an example representing the highest within-trial CV for an individual tibia found in the present study and demonstrates the unacceptable levels of variation within one series of nine measurements. An examination of the curve fits established for subjects with high within-trial variation indicates that while the impedance response curves display fairly regular features with distinct resonances, the amplitude can vary from measure to measure and greatly influence predicted EI. Each of the response curves seen in Figure 3b.6 displays distinct resonances and appears reasonable when viewed individually, but when they are superimposed they clearly demonstrate variability.

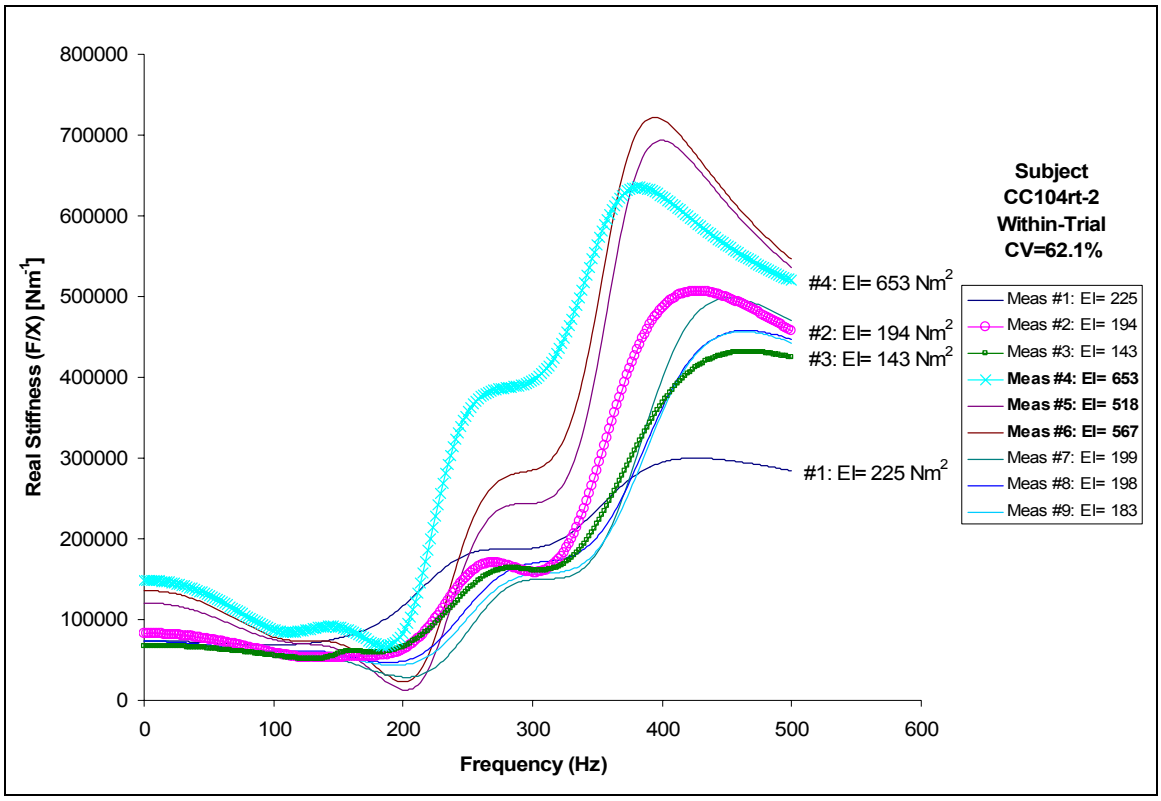

Figure 3b.6. Impedance response curves for nine measurements taken within one testing session. Note that EI varies from 143 Nm^2 to 653 Nm^2 and the within-trial CV = 62.1%.

When visually evaluating the between-trial impedance response curves in Figure 3b.7 on an individual basis, it is clear that distinct resonances lead one to believe that each curve is a valid measurement. When the three response curves are evaluated as a group they are clearly different and result in wide range of EI values, yielding a between-trial CV% of 83.5%. The variation found in both the within- and between-trial impedance response curves is problematic because the current subject positioning system is based upon a visual inspection of the raw data. If the raw data displays acceptably low levels of noise (compare Figures 3b.2 and 3b.4 for examples of high and low noise) and displays a common response with multiple resonances, the technician assumes that good positioning has been achieved and begins accepting measurements. Figures 3b.6 and

3b.7 clearly demonstrate that these visual criteria do not provide adequate feedback to the technician about the quality of the measurement.

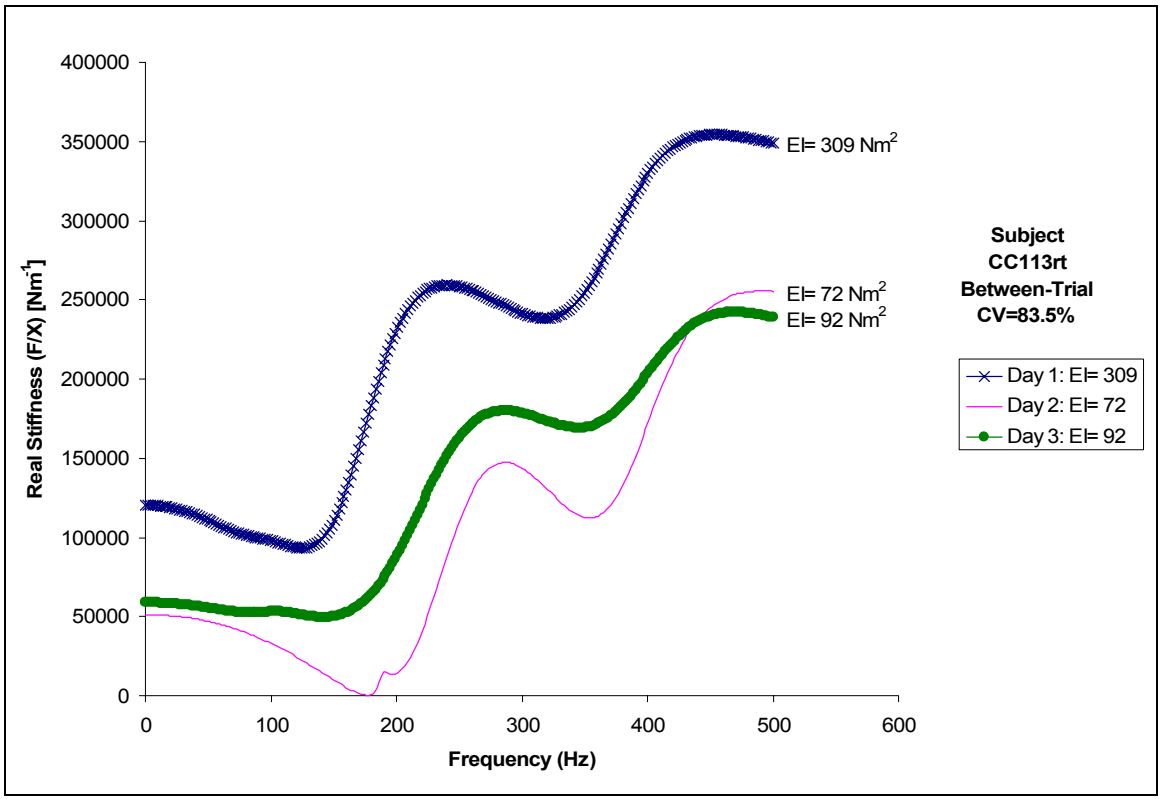

Figure 3b.7. Between-trial impedance response curves for measurements taken on three separate days. Note that EI varies from 72 Nm^2 to 309 Nm^2 and the between-trial CV = 83.5%.

The two current methods of determining EI and S from a series of measurements are to average all accepted measurements (Average EI, Average S) or to take the measurement with the lowest effective RMS error (Best EI, Best S). These EI and S values are used in the calculation of between-trial CV, with the "Average" values slightly outperforming the "Best" values. Average EI yields between-trial CVs for individual tibiae ranging from 2 to 66%, while Best EI CVs range from 11 to 84%. Figure 3b.8 demonstrates that with the current curve fitting algorithm the majority of raw data curves are fit with RMS < 0.1, bringing the amount of retained data to 87% which is a substantial improvement over the 70% data retention of previous algorithms.

Additionally, it can be seen that CV is relatively insensitive to a wide range of RMS acceptance levels. With the improvement to the curve fitting algorithm, most measures have low RMS levels and this criteria no longer appears to discriminate between accurate and inaccurate data. Figure 3b.9 demonstrates that the within-trial measurement data may not necessarily follow a normal distribution, in which case taking the average value to represent the data will be erroneous.

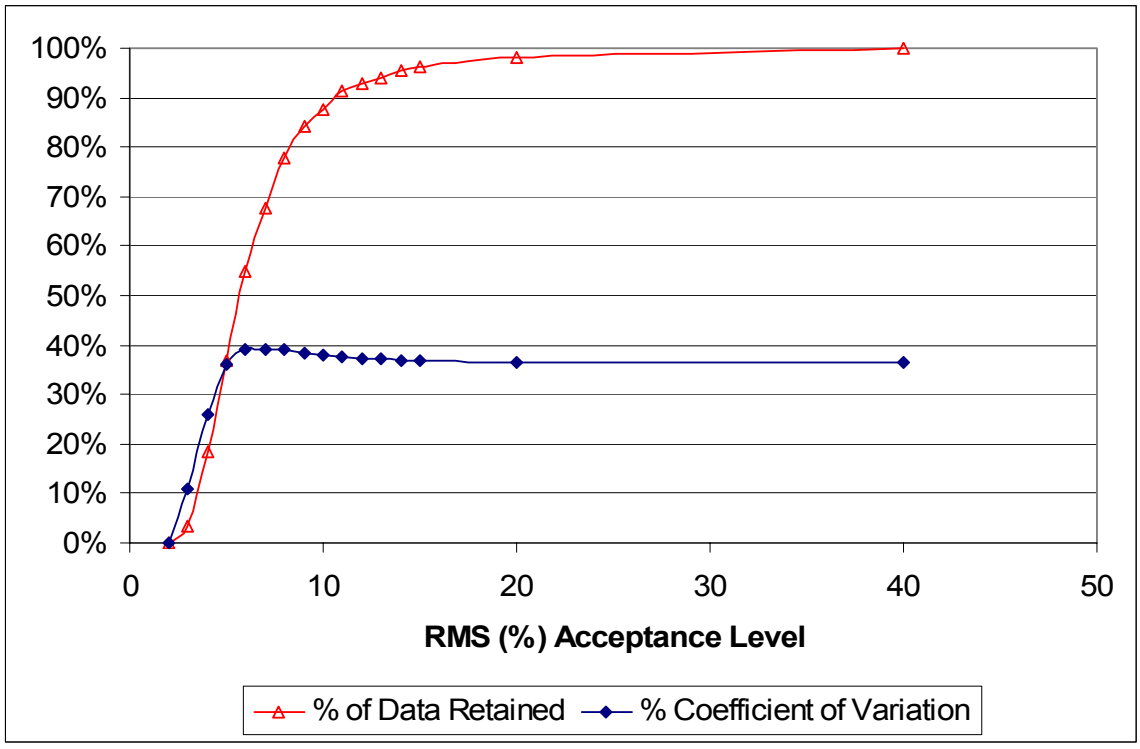

Figure 3b.8. RMS acceptance level versus the percent of measurement data retained and the within-trial percent coefficient of variation for combination of data across all three measurement sessions.

The result is that these current methods of determining EI are unreliable when used in conjunction with the current hardware.

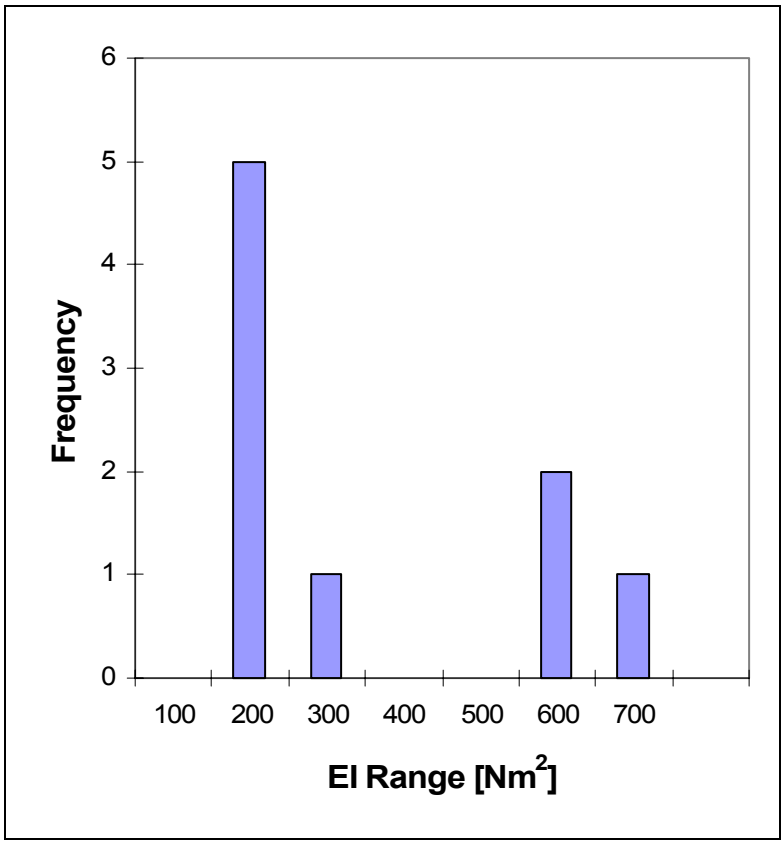

Figure 3b.9. Within-trial bimodal distribution of nine measurements.

To identify sources of variation, residual plots of EI were generated for a range of variables and demonstrate a general increase in within-trial variation as body weight, height, tibial length, and EI increase. These variables are all related to EI and display a typical pattern of heteroscedasticity, where the variability of measurement increases as the variable being measured increases in magnitude. Figure 3b.10 is representative of the findings in the graphs of weight, height, and tibial length.

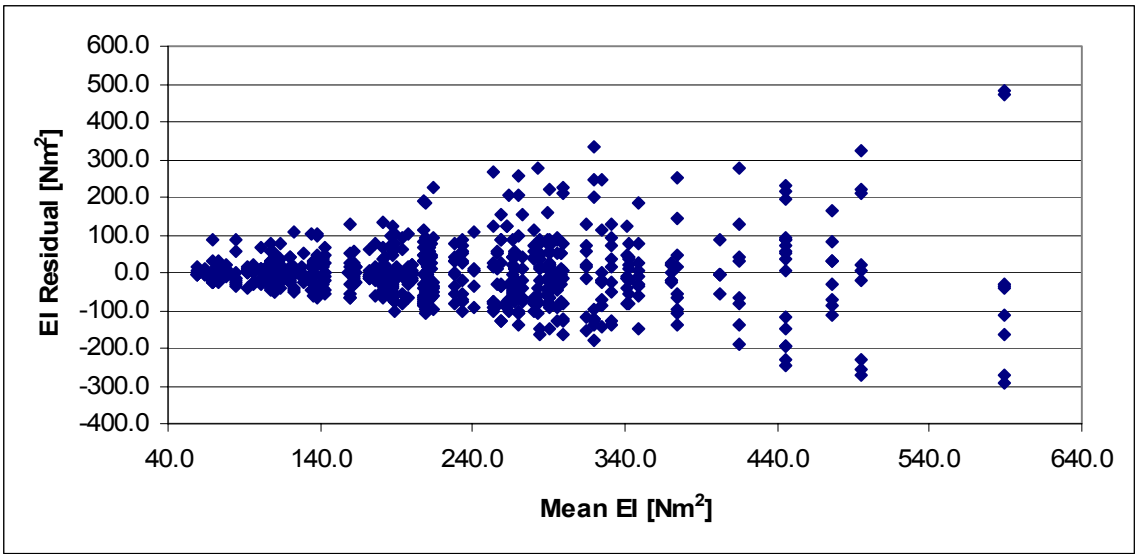

Figure 3b.10. EI residual versus mean EI. Demonstration of the heteroscedasticity found in this data set, where measurement variation increases with increasing EI. The CV statistic is proportional to the magnitude of the Mean EI, so it is an appropriate measure for heteroscedastic data.

Discussion

Evaluation of the human tibia presents additional challenges because it has a more complex geometry than the ulna. The ulna has a fairly uniform structure, which approximates a long cylindrical tube, while the human tibia is not radially uniform in cross section, the orientation of the cross section varies longitudinally, and the bone is slightly curved. Rigid fixation of the tibia is more difficult than the ulna and additional resonances appear on the impedance response curve indicating free vibration at the distal and/or the proximal ends. To account for these additional resonances new tissues analysis models were developed. A 9-parameter model was developed to account for conditions in which one end (the ankle) is free to vibrate, requiring the addition of three parameters (the mass, stiffness, and damping at the ankle joint) to the 6-parameter model. Further development has since led to a 12-parameter model, taking into account the condition of both ends (the ankle and knee) being free to vibrate, thus three additional parameters (the mass, stiffness, and damping at the knee joint) have been added (see Figure 3b.3.). The development of the 12-parameter computational model allows for

both ends of the bone to be free, eliminating the need for supporting hardware. In theory the elimination of supporting hardware will simplify measurement and reduce error introduced by the level of technician training and experience. Refinements to the 12-parameter model over the last year have improved it to the point of being the model of choice, though there is evidence that occasionally the 6 or 9-parameter models will outperform the 12-parameter model (Steele 2001). The performance of the models may depend upon anatomical variations in the joints of subjects.

Previous research achieved a within-trial coefficient of variation (CV) of 11.2% for EI and a between-trial (four measurements spanning 21 months) CV of 36.5% for EI (Callaghan 2003) in a homogeneous subject pool. The purpose of this study was to examine variability of tibial measurements associated with MRTA and, using these findings, specify a definitive plan to guide future enhancements to the system leading to CV levels and instrument performance that is characteristic of other precise clinical tools used to assess bone features in the clinical and research setting. For this study a heterogeneous group of subjects was recruited to provide insight into the variability associated with anthropometric characteristics, and repeated measurements were collected over the course of one week to control for the variation associated with mid to long-term bone development.

Comparison of the within-trial CVs from this study, on the order of 35%, to the previous study (Callaghan 2003), CVs on the order of 11%, indicates that technician experience is a key factor in controlling variability. The technician in the present study had less experience than the previous technician but during data collection the experienced technician observed some of the data collection and commented that the raw data curves looked acceptable. This fact supports the notion that simple visual inspection of raw data is insufficient to determine if the measurement is acceptable, as seen in Figures 3b.6 and 3b.7. The key areas of influence that the technician has in data collection are the physical positioning of the subject and the visual inspection of the raw data for acceptance or rejection. If visual inspection of the data is insufficient for discrimination between acceptable and unacceptable measurements, then the primary

influence that the technician has is on subject positioning. Precise quantification of the various subject positioning parameters with real time feedback will allow a novice technician to match or improve upon the positioning of an experienced technician. To develop a system in which a consistent or optimum subject position is identified, certain measurement conditions should be quantified and standardized as follow (see Figures 3b.11 and 3b.12):

1) amount of weight supported by the foot (F_{foot})
2) amount of preload at the probe-limb interface ($F_{preload}$)
3) amount of weight supported by the thigh (F_{thigh})
4) angle between the long axis of the limb and the probe (θ)

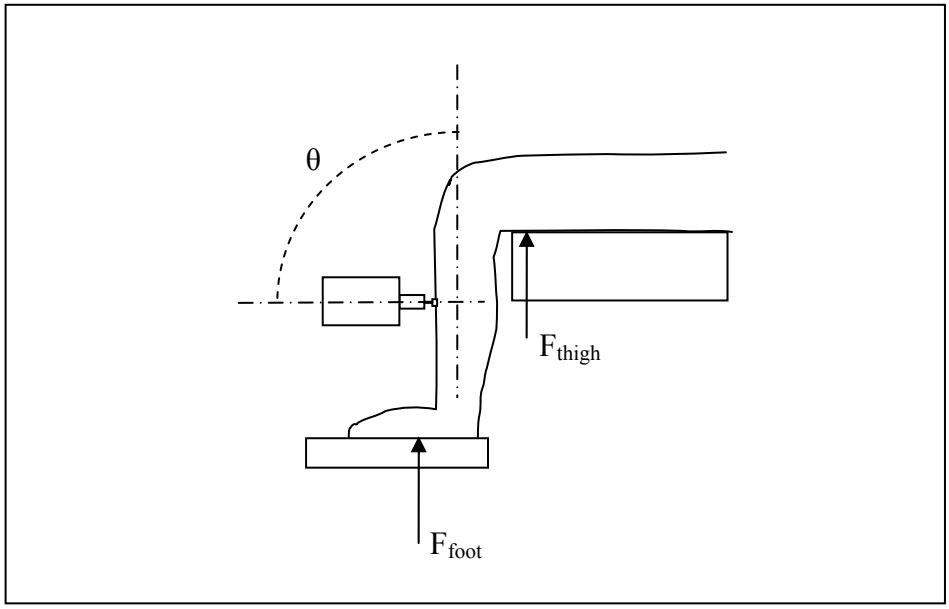

Figure 3b.11. Subject positioning and associated forces.

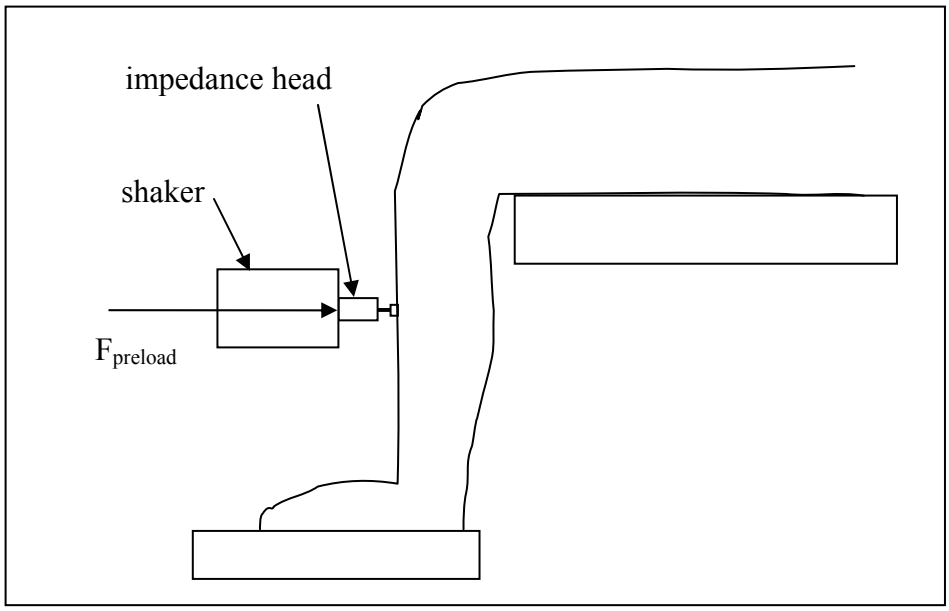

Figure 3b.12. Preload between the probe and the limb.

The amount of weight supported by the foot is subjectively quantified by the technician, but a pressure transducer on the foot support could eliminate this subjective step thus eliminating one source of variability. The system presently has a gross level of preload set by a spring system which holds the shaker, and a fine level of preload between the impedance head and the shaker. During data collection, some subjects found that the pressure of the probe against their leg varied from day-to-day and occasionally subjects would mention that the pressure varied within one session. Subjects' legs were not constrained during measurement, and it was found that for some subjects their legs would drift away or to the side resulting in the loss of appropriate contact with the probe. When contact with the probe was lost, the subject would be repositioned, which may explain a significant portion of the within-trial variability. The bimodal distribution seen in Figure 3b.9 may be related to repositioning within one trial. Two potential mechanisms for quantifying preload are either to incorporate a pressure transducer in line with the probe, or constructing a simple gauge that indicates the depth to which the probe is displaced toward the shaker. An instrument that would allow the technician to easily monitor preload, or the ability to record preload during a measurement would be ideal.

Recording preload during each measurement would allow researchers to evaluate the effect of preload on data variability and EI estimation.

To address the issue of leg drift during measurement, a simple modification to MRTA subject positioning will incorporate an adjustable support for the distal thigh. Presently, the thigh of the limb being measured has no lateral restraint, allowing the limb to move over time, thus the probe contact point may vary across the series of measurements within one session. It appears that a support that gently constrains the thigh will allow the subject to relax and will prevent positioning drift during the measurement series. This support will not involve clamping of the tibia, so the 12-parameter model should remain valid, and it should be straightforward, so that both the technician and the subject will understand its application and function with a simple explanation.

The male subjects in the present study were generally taller than the female subjects measured in previous reliability studies of this MRTA system and it was found that the measurement chair could not be adjusted high enough to unweight the feet of tall subjects. A chair with a larger vertical range of motion will give the technician more control for standardizing the amount of weight that is supported by the subject's foot. As the foot becomes unweighted, gravity will tend to bring the tibia into a vertical position resulting in a standard 90 degree angle with the probe, though there should be an objective measurement of this angle.

In addition to the hardware modifications, there are two potential software modifications that have potential from improving reliability.

1) feedback for the operator to indicate the quality of contact with the bone

2) algorithms to identify acceptable measurements

A system of on-screen feedback that allows the technician to evaluate the quality of contact with the bone will allow for improved positioning. Presently, the technician has to take a measurement to evaluate the quality of contact, but a continuous display of

force and acceleration at one frequency level may allow the technician to adjust the subject's position until an optimal response is achieved. Additionally, a continuous feedback system would alert the technician to slight changes in the subject's position during the testing.

Presently, a series of measurements may have a wide range of EI values from which an average is taken, so a method of identifying false EI measurements could narrow the range and improve the system reliability. Simply rejecting measurements that are not in the physiological range is a starting point for this type of analysis.

The reliability of human tibial measurement with mechanical response tissue analysis is dependent upon several variables that are subjectively controlled, placing considerable variability into the hands of the technician. It is proposed that these variables should be objectively measured and controlled to study the effects of each on the variability of measurement.

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CHAPTER 4

Summary of Study

The purpose of this study was to examine the within-trial and between-trial reliability of human tibial measurement with MRTA and, based upon the findings of this study, develop a plan to improve system reliability. The current MRTA hardware configuration does not provide rigid support of either the proximal or distal tibia, and to date has not been able to reproduce the reliability of 5-12% between-trial CVs as reported for versions of tibial MRTA that have rigid proximal and distal supports. This study reports the best within-trial reliability (CV: 10.6 to 12.5%) for EI measurement on a system that does not rigidly support the tibia. These results were obtained with experienced technicians measuring a homogeneous population of college women. Subsequent experiments with a non-homogeneous population and a new technician resulted in poor within-trial reliability (CV: 35 to 42%), but served the purpose of identifying a major source of within-trial measurement error, namely, experience level of the technician. Anthropometric subject characteristics were not predictive of relative measurement error in this data set but were potentially overshadowed by the error introduced by the technician.

Evaluation of the within-trial results revealed that the criteria that have been utilized in the past as indicators of an acceptable measurement are insensitive to a major source of error. Smooth raw data curves that display one or more distinct resonances were originally thought to represent an acceptable measurement but this study has found that raw data fitting this profile can represent within-trial CVs on the order of 60% for a series of nine measurements. Thus, another feedback mechanism is required to improve the technician's ability to discriminate between acceptable and unacceptable measurements. Interpretation of the raw data display does not seem to differentiate between experienced and novice technicians, so the physical positioning of the subject is most likely the source of error.

Between-trial reliability was poor for both the experienced and novice technicians with CVs for EI measurement above 30% and 45% respectively. These poor data were instructive as they revealed problems with the data analysis; namely, the selection of an EI value for one trial. It turns out that the within-trial measurements do not necessarily follow a normal distribution, therefore selection of EI based upon an average of the within-trial measurements will generally be erroneous. The second method of EI selection from one set of measurements is to identify the measurement that has the lowest RMS for the curve fit to the raw data. With the current performance of the curve fitting algorithms most raw data is accurately fit with a prediction curve, but as mentioned earlier, a smooth raw data curve can follow an acceptable impedance pattern, but not necessarily represent an acceptable measurement, so a low RMS does not necessarily indicate a good measurement.

Recommendations for Further Study

From this analysis there are two pathways to pursue, one that improves the feedback to the technician, so that an objective measurement of subject position can be obtained, and the second is the development of a method to discriminate between acceptable and unacceptable measurements after the data has been collected. To address the issue of subject positioning, there are two points to consider, one is that there should be a real-time feedback display, so that the technician can monitor whether or not the subject is changing position during the series of measurements, and the second and more important point is that the subject's position be standardized and optimized so that measurements will reflect a consistent reading of the bone. The ability to maintain a consistent measurement position throughout a trial is secondary to the ability to return to a consistent point between trials, as seen in the reasonable within-trial CV that is coupled with a poor between-trial CV obtained with an experienced technician. To develop a system in which a consistent or optimum subject position is identified, certain measurement conditions should be quantified and standardized as follow (see Figures 4.1 and 4.2):

1) amount of weight supported by the foot (F_{foot})

2) amount of preload at the probe-limb interface ($F_{preload}$)

3) amount of weight supported by the thigh (F_{thigh})

4) angle between the long axis of the limb and the probe (θ)

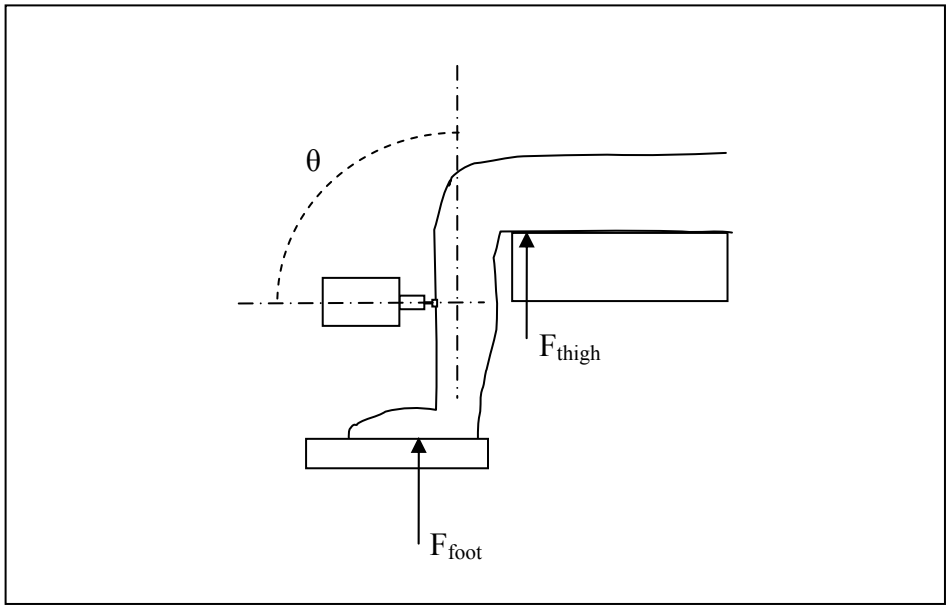

Figure 4.1. Subject positioning and associated forces.

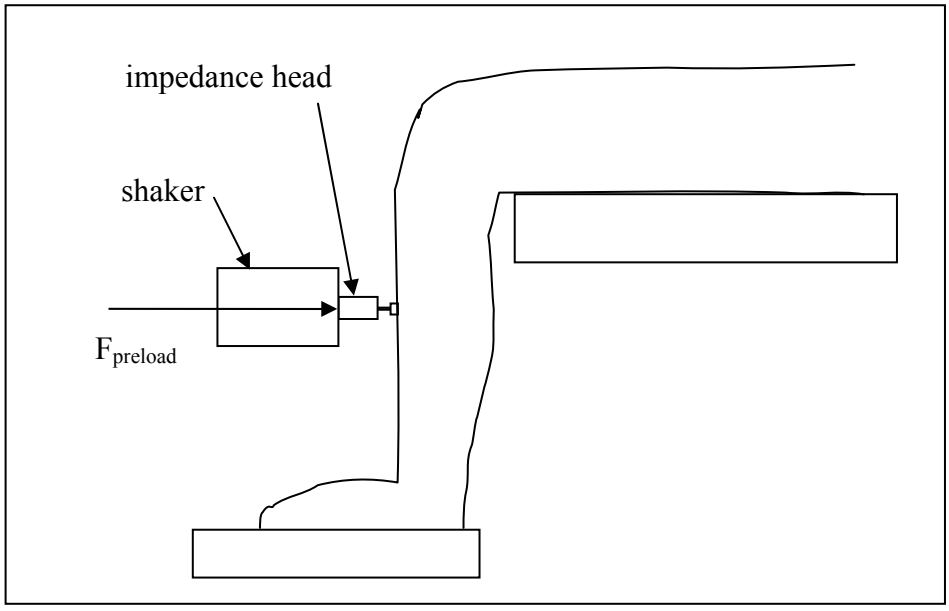

Figure 4.2. Preload between the probe and the limb.

The amount of weight supported by the foot is subjectively quantified by the technician, but a pressure transducer on the foot support platform (Figure 4.1) could

eliminate this subjective step thus eliminating one source of variability. The system presently has a gross level of preload set by a spring system which holds the shaker, and a fine level of preload (Figure 4.2) between the impedance head and the shaker. During data collection, some subjects found that the pressure of the probe against their leg varied from day-to-day and occasionally subjects would mention that the pressure varied within one session. Subjects' legs were not constrained during measurement, and it was found that for some subjects their legs would drift away or to the side (Figure 4.3) resulting in the loss of appropriate contact with the probe. When contact with the probe was lost, the subject would be repositioned, which may explain a significant portion of the within-trial variability. The bimodal distribution seen in Figure 3b.9 may be related to repositioning within one trial. Two potential mechanisms for quantifying preload are either to incorporate a pressure transducer in line with the probe, or constructing a simple gauge that indicates the depth to which the probe is displaced toward the shaker. An instrument that would allow the technician to easily monitor preload, or the ability to record preload during a measurement would be ideal. Recording preload during each measurement would allow researchers to evaluate the effect of preload on data variability and EI estimation.

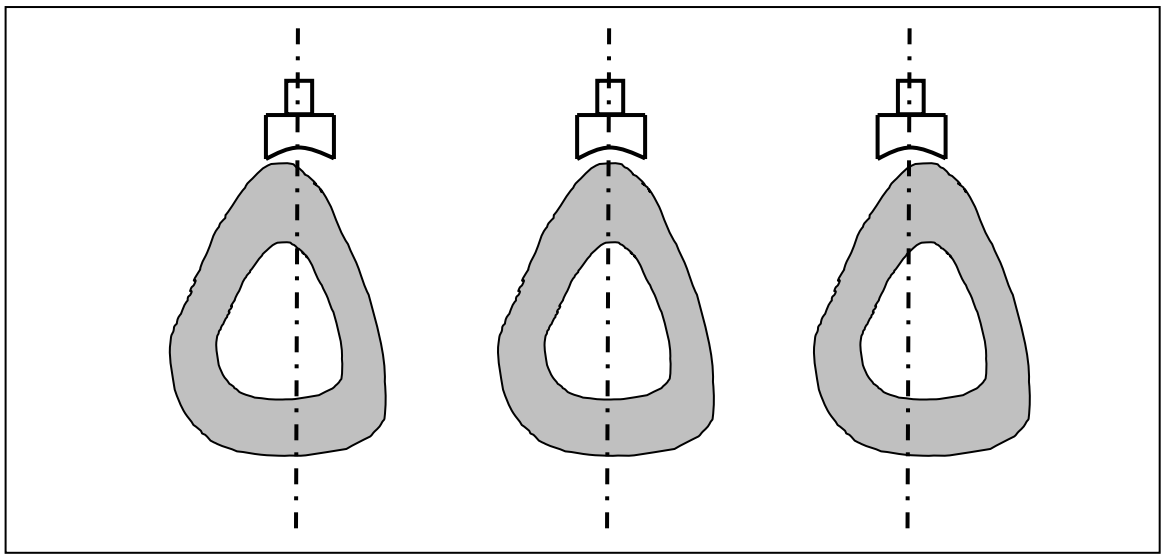

Figure 4.3. Lateral shift of tibia in relation to the probe.

To address the issue of leg drift during measurement, a simple modification to MRTA subject positioning will incorporate an adjustable support for the distal thigh.

Presently, the thigh of the limb being measured has no lateral restraint, allowing the limb to move over time, thus the probe contact point may vary across the series of measurements within one session. It appears that a support that gently constrains the thigh will allow the subject to relax and will prevent positioning drift during the measurement series. This support will not involve clamping of the tibia, so the 12-parameter model should remain valid, and it should be straightforward, so that both the technician and the subject will understand its application and function with a simple explanation.

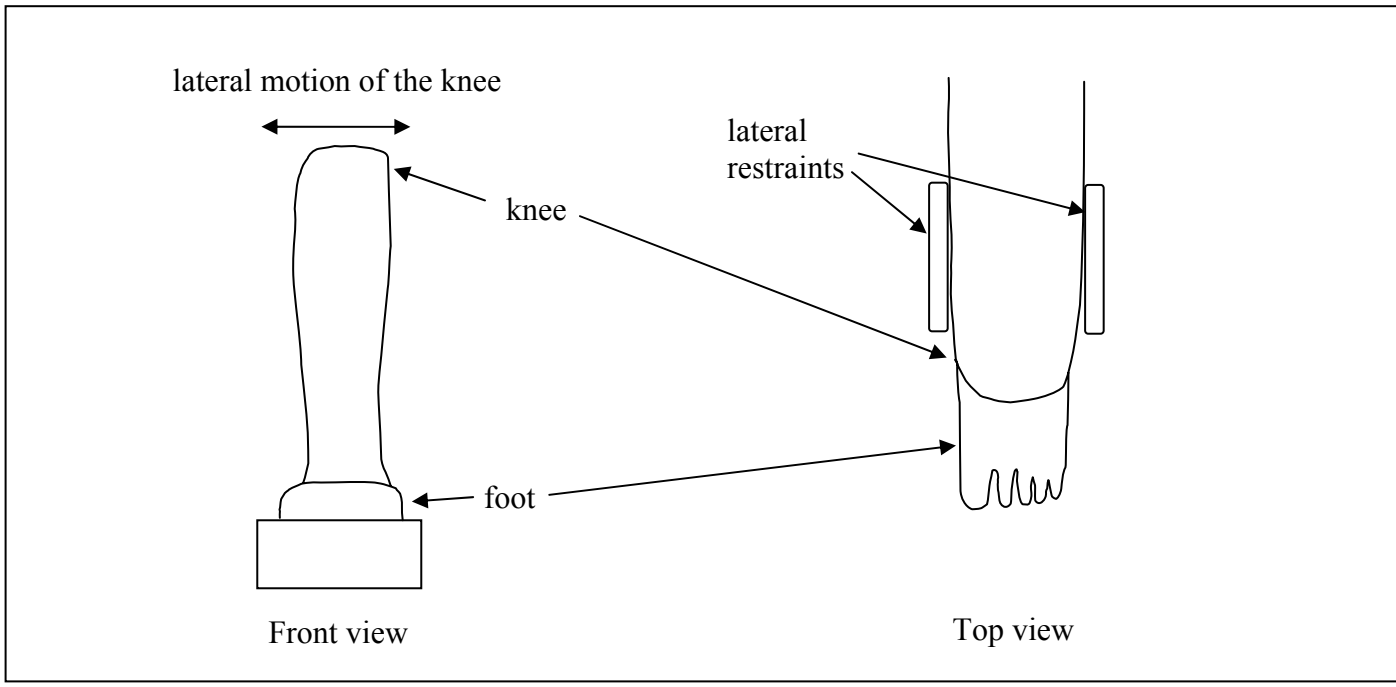

Figure 4.4. Lateral motion and restraint of thigh during MRTA measurement.

The male subjects in the present study were generally taller than the female subjects measured in previous reliability studies of this MRTA system and it was found that the measurement chair could not be adjusted high enough to unweight the feet of tall subjects. A chair with a larger vertical range of motion will give the technician more control for standardizing the amount of weight that is supported by the subject's foot. As the foot becomes unweighted, gravity will tend to bring the tibia into a vertical position resulting in a standard 90 degree angle with the probe, though there should be an objective measurement of this angle.

In addition to the hardware modifications, there are two potential software modifications that have potential from improving reliability.

1) feedback for the operator to indicate the quality of contact with the bone
2) algorithms to identify acceptable measurements

A system of on-screen feedback that allows the technician to evaluate the quality of contact with the bone will allow for improved positioning. Figure 4.5 demonstrates the potential for the probe to be in contact with the bone at various angles which may be difficult to quantify but will potentially influence the measurement. Presently, the technician has to take a measurement to evaluate the quality of contact, but a continuous display of force and acceleration at one frequency level may allow the technician to adjust the subject's position until an optimal response is achieved. Additionally, a continuous feedback system would alert the technician to slight changes in the subject's position during the testing.

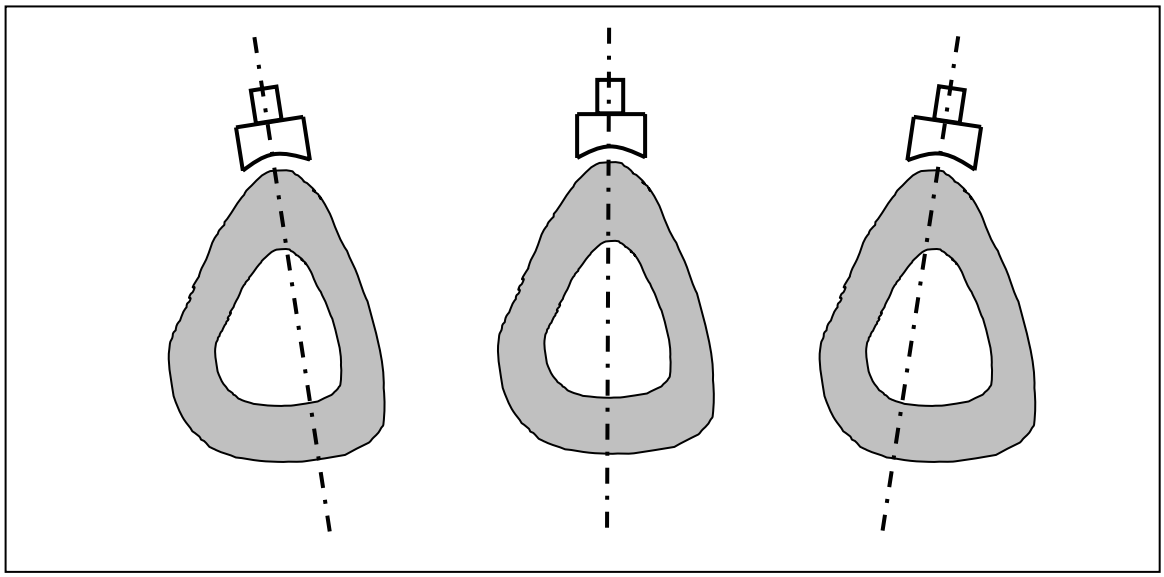

Figure 4.5. Angle of probe in relation to tibial cross section.

Presently, a series of measurements may have a wide range of EI values from which an average is taken, so a method of identifying false EI measurements could narrow the range and improve the system reliability. Simply rejecting measurements that are not in the physiological range is a starting point for this type of analysis. Clearly,

there is still work to be done on technical issues related to measurement variability, but progress has been made with analysis algorithms and a series of experiments with mechanical adaptations may further improve reliability.

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APPENDIX A - MRTA Testing Procedures

Pre-test Instructions for Subjects

The subject should be instructed to follow these pre-test guidelines before MRTA testing: (1) do not engage in vigorous activity the day before or the day of testing, (2) wear shorts and a short-sleeve shirt.

MRTA Setup Instructions

This sequence must be followed exactly when turning on the MRTA: (1) turn on the power to the computer, (2) set gain to10-11 o'clock, (3) turn on both signal conditioners. The MRTA must first be calibrated before any measurements can be performed. We are looking for the peaks and zeros to range from 7 to 9 grams (the shaker weighs 8 grams). If more than 1 peak or zero in the 70-200 mHz range is outside of this range, or 3 peaks or zeros in the 70-500mHz range, this value will be deemed as invalid and calibration will be performed again.

Anatomical Measurement and Landmarks

The tibia will be measured with an anthropometric ruler to determine bone length and the midshaft level. The anatomical landmarks for the tibia are the medial condyle proximally and the medial malleolus distally. These landmarks will be marked are the length measured. Make sure that the ruler is parallel to the tibia when measuring the tibia. Record the bone length to the nearest 0.1 cm. Perform 2 trials. If the tibial length of the 2 trials is equal to or less than 0.4 cm of one another, take the mean of these measures as the tibial length. If the tibial length of the 2 trials is greater than 0.4 cm apart, take a third measure; then, take the mean of the 2 closest measures as the tibial length. The midshaft measurement may be obtained by taking half of the tibial length and measuring up from the medial malleolus. The midshaft level should be marked. Skinfold measures will be taken directly over the midshaft point of the tibial crest. Two trials will be performed with each limb. If the skinfold values of the 2 measures are equal to or less than 1.0 mm, take the mean of these measures as the tibial skinfold value. If the skinfold values of the 2 measures are greater than 1.0 mm, take a third measure; then, take the mean of the 2 closest measures as the tibial skinfold value.

Subject Positioning

Instruct the subject to remove their shoes and socks. Position the subject so the measured leg is directly in front of the shaker. Adjust the chair so that the thigh is parallel to the ground. Then, move the chair back or forth in order to obtain a 90 degree angle between the femur and tibia. Confirm with a goniometer. Continue to make adjustments until a 90 degree angle is obtained. Adjust the foot support so the foot in completely supported. Adjust the vertical shaker positioning so it is parallel to the midshaft marking. Adjust the shaker in a horizontal plane and/or rotate the subject's lower leg until the shaker is directly over the tibial crest. Retract the shaker, pull the lower leg up within ~ 1 cm of the shaker, and release the shaker directly onto the tibial crest. Leave the shaker in contact with the skin for ~ 5 seconds. Release the shaker and check for a good indentation in the subject's skin. If good contact was made, release the shaker and wait 2 minutes before obtaining the first measure. If the shaker made poor contact, readjust until good contact is made, confirm, then wait 2 minutes before obtaining the first measure. Palpate the calcaneous and the anterior tibia to confirm adequate tibial vibration. Palpate the subject's quadriceps to check for undue muscular tension. Ask the subject if the pressure/sensation of the shaker in comfortable/tolerable.

Testing Instructions for Subjects

- Please remain quiet during the measurement period
- Avoid movement of the limb being measured
- Try to relax the limb being tested as much as possible
- Please communicate any discomfort to the investigator on hand

MRTA Measurement Procedures

After a 2 minute period, begin measurement of the tibia. Perform 9 consecutive measurements. When the frequency curve is displayed after each measure, scrutinize the "quality" of the line. If the curve is relatively smooth, it may be assumed that the positioning of the shaker is good and measurements may be continued. If there is a saw-toothed appearance to the curve, especially in the 70-200 mHz range, reposition the shaker and begin a new series of measures. THIS IS VERY IMPORTANT!

Data Analysis

In the multi-file analysis window, the following settings should be set as follows before analysis begins:

- Weight amplitude for the lower frequency range 1.00
- Weight amplitude for the higher frequency range 0.01
- Lower frequency range 70-200 mHz
- Higher frequency range 200-500 mHz

Initially, choose the 7-parameter model. Set the iteration mode to 1. All maximum iterations should be set to 40. The weighting for flexibility for the iterations and for pole-zero fit should be set to 0.1000. Finally, set the "save iteration progress" to no.
Next, select the files to be analyzed and run the application. Scroll down to the last file in the output and click the file labeled "sumDF". In the batch analysis results, scroll down to the bone indices and error indices portion. Select the files for analysis using the following parameters:

1) Select the 5 files with the lowest cumulative RMS differences for stiffness and compliance.
2) Duplicate the file with the lowest cumulative RMS differences for stiffness and compliance. Insert a "0" at the front of the file number as a means to seed the file upon reiteration.
3) Repeat the analysis with identical settings as described previously except the iteration mode must be changed to 2 instead of 1.

In the batch analysis results, scroll down to the bone indices and error indices portion. Record the following values: 1) EI current – average and variation, 2) RMS difference stiffness, and 3) RMS difference compliance.
Repeat the entire sequence for the 9- and 12-parameter models. Accept the value that returns the lowest cumulative RMS difference for stiffness and compliance.

APPENDIX B - Subject Forms and Recruitment

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Informed Consent for Participants in Research Projects Involving Human Subjects

Title of Study: **Reliability of Mechanical Response Tissue Analysis**

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

Principal Investigators: Christopher Callaghan, M.S., William Herbert, Ph.D.

I. Purpose of this Research

I am invited to participate in a study to assess short term measurement reliability of mechanical response tissue analysis (MRTA) for the estimation of bone bending stiffness. MRTA is an evolving technology, so as changes are made to the system, measurement reliability must be reestablished.

The MRTA system consists of a desktop computer and a three foot tall metal stand that holds a sensor and a small metal contact probe. The probe is about half the size of a standard key on a computer keyboard and is pressed against the skin to make contact with bones of either the forearm or lower leg. The probe transmits a vibration to the bone; this vibration feels similar to the vibration of a pager or mobile phone. The probe transmits the bone's response to the vibration back to the sensor which transmits this information to the desktop computer.

The information collected with the MRTA system allows for the estimation of bone bending stiffness, which is a measure of bone strength. The goals for this technology are to be able to identify people at risk for stress fractures, which can occur during intense exercise such as running, and to identify people at risk for fractures related to osteoporosis, a condition where the bones have become weak.

II. Procedures

I agree to participate in the study for a period of three days.

Day 1: (~10 minutes) Informed consent.
(~10 minutes) General health and exercise history questionnaire.
(~5 minutes) Measurement of height, weight, tibial lengths and skinfold thickness of the lower leg..
(~5 minutes) Orientation to MRTA measurement procedures.
(~15 minutes) MRTA measurements

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Day 2: (~5 minutes) Measurement of weight and tibial lengths.
(~15 minutes) MRTA measurements.

Day 3: (~5 minutes) Measurement of weight and tibial lengths.
(~15 minutes) MRTA measurements.

The length of my lower leg bone, the tibia, will be measured from the distal medial malleolus (ankle) to the medial condyle (knee) with an anthropometer (ruler for measurement of the body). Skinfold thicknesses of my lower leg will be measured by a standard procedure of grasping a fold of skin on my lower leg and measuring its thickness with calipers. Access to my lower leg is necessary for measurements, so I will either wear exercise shorts or loose pants that can be rolled above knee height.

For MRTA measurements, I will sit in a chair with my hip and knee flexed at 90-degree angles, and one foot resting on a platform. A technician will place a metal probe on the midpoint of my tibia that will produce a vibratory sensation through my leg, similar to the vibration of a pager or mobile phone. I will relax the muscles of the leg being tested. These procedures will be performed on both of my legs with the probe in contact with each leg for less than 5 minutes.

III. Risks

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 pressure on the skin at the point of probe contact.

IV. Benefits

The benefit of participating in this research project is that I will be contributing to the development of a technology that may advance our understanding of bone strength. Further improvement with this work may contribute to studying ways to identify people who are more likely to develop stress fractures of the lower leg brought about by demanding jumping and running exercise or those more likely to develop osteoporosis.

I understand that this technology is still in a developmental stage and the data collected may or may not accurately represent the strength of my leg bone. Upon request, I will be provided with a summary of the research results at the conclusion of the data analysis.

No promise or guarantee of benefits has been made to encourage my participation.

V. Anonymity and Confidentiality

All information collected during the course of my participation in this study that is personally identifiable with me will be kept confidential. The information gathered in this study may be used in presentations and written reports, but will have my name and identity replaced with a subject number.

VI. Compensation

There will be no compensation for participation in this research project.

VII. 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.

VIII. Subject's Responsibilities

I voluntarily agree to participate in this study and I know of no reason why I should not participate. I have the following responsibilities:

1. I will report any pain or condition that indicates that I should withdraw from the testing.
2. I will arrive in a timely fashion to each scheduled testing session.
3. I will not exercise strenuously on the day before or the days of MRTA testing.

IX. 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, Foods and Exercise have granted approval to conduct this research protocol.

X. Subject's Permission

I have read and understand the Informed Consent and conditions of this project. I have had all my questions about participating in this study answered. I hereby acknowledge the above and give my voluntary consent:

_____Date_____
Subject signature

_____ Date _____
Witness

Should I have any pertinent questions about this research or its conduct, and research subjects' rights, and whom to contact in the event of a research-related injury to the subject, I may contact:

Christopher E. Callaghan
(703) 598-0843
chcallag@vt.edu
Principal Investigator

William G. Herbert, Ph.D.
(540) 231-6565
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Faculty Advisor / Departmental Reviewer
Human Nutrition, Foods & Exercise

David M. Moore, DVM
(540) 231-4991
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Chair, Virginia Tech Institutional Review Board for the Protection of Human Subjects
Office of Research Compliance – CVM Phase II (0442)
Research Division

This Informed Consent is valid from 7/15/03 to 7/15/04.

[NOTE: Subjects must be given a complete copy (or duplicate original) of the signed Informed Consent.]

Reliability of Mechanical Response Tissue Analysis
General Health and Exercise History Questionnaire

Name: _____ Date of Birth: _____

Phone #: _____ E-mail address: _____

Have you ever injured a leg, hip, knee, ankle or foot severely enough that you required medical attention? Please circle (Yes or No) and give details below:

injury (broken/fractured, sprained, …)
treatment (surgery, cast, pins, aircast, knee immobilizer, brace, crutches, wheelchair, …)
your age at time of injury and approximate length of treatment/recovery

Are you currently participating in either a structured or unstructured exercise program? Please circle (Yes or No) and give details below:

(sports, physical labor, general daily activity (walking, biking,…),…)
(approximate hours per week or distance traveled per week)

Have you participated in either structured or unstructured exercise in the past? Please circle (Yes or No) and give approx. months-(ages)-intensity(low, moderate, high):

(sports, physical labor, general daily activity (walking, biking,…),…)
For instance: soccer-4 months/year (10-13 years old)-moderate intensity
soccer-4 months/year (14-17 years old)-high intensity

Participants needed for research on bone strength measurement.

We are conducting a series of non-invasive bone strength measurements to establish the reliability of "mechanical response tissue analysis" (MRTA). This technology utilizes low frequency vibration to estimate the bending strength of bones in the lower legs and forearms.

Participation involves three days of testing in 231 War Memorial Hall (second floor of the Gym):

Day 1: ~45 minutes
Day 2: ~20 minutes
Day 3: ~20 minutes

There is no compensation for participation. Your only benefit will be the satisfaction of contributing to the development of a technology that may advance our understanding of bone strength, which may contribute to the prediction of stress fractures or fractures related to osteoporosis.

If you are interested in participating, please contact Chris Callaghan (chcallag@vt.edu) 703-598-0843.

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bone strength testing Chris (chcallag@vt.edu)

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APPENDIX C - MRTA Data Processing Procedure

- sort ~12,000 measurements into folders for batch processing
    - use AppleScript "CEC- replace…" to fix file/folder names
    - Folder names in format "Subject#001"
- generate "file_list" for 18 subsets of data
    - at present the "file_list" should be limited to 1000 measurements
    - this is a preparation for analysis with any parameter model
    - use MPW or BBEdit to generate list of all files in "Measurements" folder, then edit this list to remove lines that do not point to data files
        - be sure to remove ".DS_Store" lines from "file_list"
        - Mathematica looks for "#./...../Subject#301/" preceding each set of measurement files so that it can organize graphs and select the Best Fit (lowest rmsEffective) for generation of JPEG files.
    - create "file_list"s for both 7-parameter and 12-parameter models
        - the 7-p model should be run first as a test to see if the "file_list" is valid
        - the 7-p model analyzes ~10 files per second
        - the 12-p model analyzes 1 file per ~10 seconds
        - save copies of "file_list" with the following naming conventions for reference
        - file_list-ASnnn-nnn-xxxxxx-mmp

AS =	measurements from the Army Bone Health Study
nnn-nnn =	subject number range
001-040 =	subjects 1 to 40
xxxxxx =	describes bone surface measured and whether files are analyzed before/after the "adjustFile" changes
pAF =	pre-adjustFile, so measurements are analyzed without adjusting the files (each measurement is analyzed independently on the 1st pass and each bone/session is analyzed independently on the 2nd pass)
AF =	after adjustFile changes were made, so files have been changed to have the subject numbers for each subject match across all of the measurement sessions (330AS, 330AS2, 330AS3 will now all be 330AS) - this allows the analysis to select the best measurement for a bone (across all time periods for all measurements of that bone) to serve as the seeding measurement

- the 2nd pass results represent the analysis with seeding across all trials for that bone

TC =	Tibial Crest measurements
U =	Ulnar measurements
TAM =	Tibial Anterio-Medial Surface measurements

mmp =	model number
7p =	7 parameter model
12p =	12 parameter model

- analyze MRTA data with 12p model
    - each "file_list" represents a unique set of data to be processed
    - convert raw data files to Unix format (with TextToMac)
    - select appropriate "file_list-…" and rename to "file_list"
    - convert "file_list" to Unix format
    - run "MRTALoop" with option 4 (reseeding)
        - this requires ~10 seconds per measurement
        - "output_summary" file is generated
            - each measurement individually analyzed
                - 1st pass: output at top of file including first "summary table"
            - each measure analyzed with reseeding within trial
                - 2nd pass: output below first "summary table"
            - rename "output_summary" and "output_sumDF"
                - (see naming conventions listed above)
            - delete "scratch" file
        - each measurement subdirectory will have an "output_12p" file
            - these files contain data to be used by "Mathematica" for the generation of graphs for visual inspection
    - copy raw data to a new location
        - this will preserve the "output_12p" files
            - potentially use AppleScript to rename "output_12p" files; a unique Script may have to be written; additionally, check into possible interference with "Mathematica"
        - also will preserve original measurement files from changes that will be made to subject number by "AdjustFile"
    - run "AdjustFile"
        - "AdjustFile" uses the current "file_list" to search through each "Subject#__" folder and modify all files in that folder to have the same subject # listed in the header info.
            - converts 1AS, 1AS2, 1AS3,… to 1AS, 1AS, 1AS,…
            - this allows for reseeding across all trials

                - actually modifies the data files, so be sure to save a copy of original files
        - run "MRTALoop" with option 4 (reseeding)
            - output_summary file is generated
                - each measurement individually analyzed
                    - 1st pass: output at top of file including first "summary table"
                - each measure analyzed with reseeding across all trials
                    - 2nd pass: output below first "summary table"
- convert data analysis output into spreadsheet format
    - use **Excel macro (MRTA_Clean)** for conversion process
- process "summary table" data (Best EI and Best Sufficiency)
    - recalculated EI and Sufficiency based upon original bone lengths
        - use **Excel macro (Extract_bone_lengths)** to extract bone lengths for calculations
    - code data for appropriate training group (Concentric, Eccentric)
    - code data for limb trained (non-dominate = trained)
    - convert to SPSS file for statistical analysis
- generate descriptive statistics for "Best" tibial crest and ulnar measurements
    - 12p-individual analysis
    - 12p-reseeding within trial analysis
    - 12p-reseeding across trials analysis
- review measurement outliers
    - prepare graphs and visually inspect raw data vs. prediction curve

APPENDIX D - Data Analysis Output

Within-trial MRTA CV analysis (Short-term: 3 days over 1 week)

Day 1 N = 247 **91.1%** = meet RMSeff < 0.1
total n = 271

Source	Dependent Variable	Type III Sum of Squares	Df	Mean Square	Standard Deviation	Weighted Mean	Coefficient of Variation %
Error	GOOD_EI	1161421	217	5352.17	73.16	210.53	**34.7%**
	GOOD_SUFNCY	9175	217	42.28	6.50	19.04	**34.2%**
	GOOD_DI	3178	217	14.64	3.83	20.84	**18.4%**

Day 2 N = 227 **85.7%** = meet RMSeff < 0.1
total n = 265

Source	Dependent Variable	Type III Sum of Squares	Df	Mean Square	Standard Deviation	Weighted Mean	Coefficient of Variation %
Error	GOOD_EI	1907794	199	9586.90	97.91	231.64	**42.3%**
	GOOD_SUFNCY	10694	199	53.74	7.33	19.80	**37.0%**
	GOOD_DI	2614	199	13.14	3.62	21.69	**16.7%**

Day 3 N = 225 **85.9%** = meet RMSeff < 0.1
total n = 262

Source	Dependent Variable	Type III Sum of Squares	Df	Mean Square	Standard Deviation	Weighted Mean	Coefficient of Variation %
Error	GOOD_EI	1485428	195	7617.58	87.28	242.38	**36.0%**
	GOOD_SUFNCY	9788	195	50.19	7.08	21.38	**33.1%**
	GOOD_DI	2989	195	15.33	3.92	23.77	**16.5%**

MRTA data for between-trial reliability (short-term: 3 days over 1 week)

Subject #	bone	Session	Sex	Mean EI	Mean Suff	Mean DI
100	lt	1	Male	159.8	16.4	20.7
100	lt	2	Male	253.7	26.1	19.9
100	lt	3	Male	292.0	30.0	20.7
100	rt	1	Male	187.5	19.6	16.6
100	rt	2	Male	188.9	19.7	23.5
100	rt	3	male	181.3	18.9	26.7
101	lt	1	female	73.3	15.1	13.2
101	lt	2	female	59.5	12.2	12.6
101	lt	3	female	137.6	28.3	17.5
101	rt	1	female	69.7	14.5	10.6
101	rt	2	female	104.7	21.8	8.5
101	rt	3	female	109.6	22.8	14.0
103	lt	1	female	84.3	14.2	11.0
103	lt	2	female	no acceptable measurements		
103	lt	3	female	163.8	27.5	10.9
103	rt	1	female	107.6	18.1	12.7
103	rt	2	female	128.8	21.6	12.4
103	rt	3	female	210.1	35.3	13.5
104	lt	1	male	270.3	21.1	23.9
104	lt	2	male	283.9	22.2	23.6
104	lt	3	male	186.8	14.6	20.3
104	rt	1	male	272.4	21.0	29.5
104	rt	2	male	320.0	24.6	22.7
104	rt	3	male	401.8	30.9	22.4
105	lt	1	male	267.3	16.2	17.1
105	lt	2	male	no acceptable measurements		
105	lt	3	male	197.3	11.9	29.6
105	rt	1	male	127.3	7.9	4.9
105	rt	2	male	414.5	25.7	26.2
105	rt	3	male	495.0	30.7	36.6
106	lt	1	male	325.1	23.0	25.6
106	lt	2	male	445.1	31.5	31.3
106	lt	3	male	331.0	23.4	24.5
106	rt	1	male	374.0	26.4	35.7
106	rt	2	male	161.4	11.4	23.1
106	rt	3	male	445.8	31.5	35.1

Subject #	bone	Session	Sex	Mean EI	Mean Suff	Mean DI
107	lt	1	female	262.4	27.0	25.7
107	lt	2	female	213.0	21.9	21.0
107	lt	3	female	298.3	30.7	26.3
107	rt	1	female	207.8	21.7	21.5
107	rt	2	female	279.7	29.2	21.3
107	rt	3	female	228.7	23.9	23.8
108	lt	1	male	342.4	17.2	20.2
108	lt	2	male	589.3	29.6	35.1
108	lt	3	male	233.4	11.7	20.2
108	rt	1	male	172.8	8.3	12.2
108	rt	2	male	101.3	4.9	12.0
108	rt	3	male	263.5	12.7	27.3
109	lt	1	male	258.7	23.5	27.2
109	lt	2	male	187.0	17.0	25.3
109	lt	3	male	189.6	17.3	19.7
109	rt	1	male	371.0	33.8	29.4
109	rt	2	male	207.8	18.9	24.4
109	rt	3	male	158.0	14.4	19.4
110	lt	1	female	183.0	19.5	14.9
110	lt	2	female	283.3	30.2	20.3
110	lt	3	female	213.9	23.2	24.5
110	rt	1	female	134.3	14.1	23.7
110	rt	2	female	194.3	20.4	20.9
110	rt	3	female	211.8	22.6	28.8
111	lt	1	male	341.1	20.6	29.7
111	lt	2	male	476.0	28.8	39.4
111	lt	3	male	348.6	21.1	41.5
111	rt	1	male	290.9	17.4	18.6
111	rt	2	male	176.1	10.6	32.0
111	rt	3	male	289.1	17.3	32.7
112	lt	1	male	122.1	10.1	26.1
112	lt	2	male	255.7	21.1	27.5
112	lt	3	male	120.5	10.0	19.9
112	rt	1	male	209.0	16.8	28.8
112	rt	2	male	241.2	19.4	29.1
112	rt	3	male	314.8	25.3	24.3

Subject #	bone	Session	Sex	Mean EI	Mean Suff	Mean DI
113	lt	1	female	64.8	8.4	6.6
113	lt	2	female	92.1	11.9	8.0
113	lt	3	female	141.4	18.3	13.2
113	rt	1	female	266.8	33.3	18.5
113	rt	2	female	77.8	9.7	8.5
113	rt	3	female	113.3	14.1	13.6
114	lt	1	male	268.1	17.3	28.6
114	lt	2	male	295.4	19.1	28.2
114	lt	3	male	290.2	18.8	27.3
114	rt	1	male	299.3	18.8	30.7
114	rt	2	male	200.7	12.6	18.9
114	rt	3	male	290.6	18.3	25.4
116	lt	1	female	135.2	21.1	15.6
116	lt	2	female	113.1	17.6	18.9
116	lt	3	female	117.6	17.4	20.4
116	rt	1	female	143.5	22.1	19.5
116	rt	2	female	96.5	14.9	16.7
116	rt	3	female	106.2	15.5	16.6

APPENDIX E - Curriculum Vita

CURRICULUM VITA

Christopher E. Callaghan
102 North Ithaca Road
Sterling, VA 20164
(703) 430-3568

EDUCATION:

2003 Ph.D. CLINICAL EXERCISE PHYSIOLOGY
Virginia Polytechnic Institute and State University, Blacksburg, Virginia
(Dissertation: *Reliability of Tibial Measurement with Mechanical Response Tissue Analysis*)

1996 M.S. EXERCISE PHYSIOLOGY
Virginia Polytechnic Institute and State University, Blacksburg, Virginia
(Thesis: *Cardiopulmonary Analysis of Habituation to Simulated Kayak Ergometry*)

1993 MASTER OF BUSINESS ADMINISTRATION
The R.B. Pamplin College of Business
Virginia Polytechnic Institute and State University, Blacksburg, Virginia

1991 B.S. AEROSPACE ENGINEERING, Mathematics Minor
Virginia Polytechnic Institute and State University, Blacksburg, Virginia

RESEARCH INTERESTS:

Development of bioengineering technologies in bone strength assessment.
Delivery of cardiac rehabilitation education and services across the Internet.
The influence of t'ai chi chuan on various aspects of health.

EMPLOYMENT:

1997-98 Clinical Coordinator, Cardiac Therapy & Intervention Center, Virginia Tech, Blacksburg, VA

1996-97 Laboratory Coordinator, Cardiac Therapy & Intervention Center, Virginia Tech, Blacksburg, VA

1994-98 Graduate Teaching Assistant, Department of Human Nutrition, Foods, & Exercise, Virginia Tech, Blacksburg, VA

1985-93 Programmer/Consultant (part time), locations in DE, PA, and VA

ADDITIONAL EXPERIENCE:

2000	Physical Therapy Aide, National Rehabilitation Hospital, Suburban Regional Rehab, Bethesda, MD
1998-99	Internet Site Administrator, http://www.bev.net/health/cardiac/, Blacksburg Electronic Village, Blacksburg, VA
1994-95	Data Management Consultant, Cardiac Therapy & Intervention Center, Virginia Tech, Blacksburg, VA
1995	Graduate Student Intern, Montgomery Regional Hospital, Cardiac Rehabilitation, Blacksburg, VA
1993-95	Graduate Student Intern, Cardiac Therapy & Intervention Center, Virginia Tech, Blacksburg, VA
1994-95	Volunteer Tutor, Christiansburg, VA

COMPUTER EXPERIENCE:

FORTRAN, LabVIEW, BASIC, Pascal,
SAS, SPSS, dBase, Delphi, HTML

PROFESSIONAL ORGANIZATIONS:

1995	American College of Sports Medicine

CERTIFICATIONS:

1997	ACSM Exercise Specialist
2000	ACSM Registered Clinical Exercise Physiologist

RESEARCH &
PRESENTATIONS:

CE Callaghan, L Pierson, J Cook, WG Herbert, Differences Between Male and Female Correlations of Various Health Outcome Measures, *Med Sci Sports Exerc*, abstract; vol 30(5 Suppl), 1998, American College of Sports Medicine 45th annual meeting, Orlando, FL, June 1998

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Blevins JS, Callaghan CE, Herbert WG. Need for sleep quality measures in cardiac rehabilitation. *J Cardiopulm Rehab*. 1997;17:S331.

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Davis SE, Garvin E, Callaghan CE, Craft L, Davis R, Herbert WG, Ocel, JV. Validity and test-retest reliability of the Stairmaster Crossrobics 2650UE. *Med Sci Sports Exerc*. 1996;28:S208.

W.G. Herbert, C.E. Callaghan, D.R. Southard, B.H. Southard, Management by Data, American Association of Cardiovascular and Pulmonary Rehabilitation Ninth Annual Meeting, Portland, OR, October 1994

S.E. Davis, C.E. Callaghan, Database Management in Community Based Cardiac Rehabilitation, Workshop in Preventative and Therapeutic Exercise, Blacksburg, VA, June 1994

C.E. Callaghan, Shuttle to Support NASA Split/Sprint Mission to Mars, University Space Research Association National Conference, Coco Beach, FL, June 1991