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AWARD NUMBER DAMD17-95-C-5068

TITLE: Factors in Risk Prediction and Healing of Stress Fractures
and Fatigue Damage in the Female Skeleton

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REPORT DATE: November 1998

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY <i>(Leave blank)</i>	2. REPORT DATE November 1998	3. REPORT TYPE AND DATES COVERED Final (10 Jul 95 - 31 Dec 97)	
4. TITLE AND SUBTITLE Factors in Risk Prediction and Healing of Stress Fractures and Fatigue Damage in the Female Damage in the Female Skeleton		5. FUNDING NUMBERS DAMD17-95-C-5068	
6. AUTHOR(S) Donald B. Kimmel, DDS, Ph.D.			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Creighton University Omaha, Nebraska 68131		8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES			
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited		12b. DISTRIBUTION CODE	
13. ABSTRACT <i>(Maximum 200 words)</i>			
19990610136			
14. SUBJECT TERMS Defense Women's Health Research Program		15. NUMBER OF PAGES 25	
		16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited

Foreword

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Introduction

Stress fractures during Basic Training (BT) occur in 0.5-2% in men to 6-12% in women [1-4], in the US Army. Incidence is higher in Caucasians than in blacks [5], peaking during the second-third weeks, then occurring at a lower, but steady rate for the remainder of BT [6-7]. 75% of stress fractures are of the tibia and foot bones [8-10], producing morbidity requiring care, but usually cured by a few weeks restricted duty. Femur and pelvis fractures are less frequent [11-14], but tend to heal more slowly, and can result in medical discharge, occasionally incurring long-term Army obligation for service-related disability.

Identifying individuals at high risk of stress fracture during BT by a rapid, non-invasive, risk-free measurement, perhaps before their entry into the military (a "4-F skeleton" found at a MEPS), would benefit both female soldiers and the operation of the US Army. It would decrease the Army's: 1) risk of unintentionally injuring soldiers, 2) person-hours lost from duty, 3) commitment to training predictably unfit soldiers, and 4) resources expended caring for injured personnel. A successful stress fracture risk prediction program could lead not only to a less injury prone corps of Army women, but also to a male stress fracture risk prediction program and an understanding of risk factors that can be influenced by altered BT procedures.

A. Parallels of Stress Fracture and Osteoporotic Fracture

Osteoporotic and stress fractures share many characteristics (See Table 1). Thus, stress fracture risk prediction for the military and osteoporotic fracture risk evaluation in the elderly are likely to be similar tasks, meaning that

Osteoporotic Fx	Stress Fx
-bones too weak to sustain reasonable, intended uses	-bones too weak to sustain reasonable, <i>assigned</i> uses?
-occurs mainly in elderly women	-occurs in soldiers and athletes
-limited to a minority of elderly women	-limited to a minority of soldiers and athletes
-site-specific profile (hip, vertebrae, wrist,)	-site-specific profile (foot bones, tibia, femur, pubic ramus)
-sex preference (female)	-sex preference (female)

similar equipment might evaluate intrinsic risk in both. Each type of fracture occurs during activities that most participants complete *without* fracture, making the term "fragility" fractures appropriate for both. This simply implies the existence of a skeleton unable to endure loads tolerated well by a "normal" skeleton. Osteoporotic fracture risk has been successfully evaluated during the past decade [15-19]. It is reasonable to think

that applying similar screening instrumentation to military recruits would successfully evaluate their stress fracture risk.

1. Known Risk Factors for Stress Fracture

The risk for stress fracture during basic training is ~5X higher in women than men [1-4, 21]. Poor physical fitness, previous fracture [6, 8, 22], low femoral neck bone mineral density [23] and small bone size are known risk factors [24-26]. In women, osteoporosis risk factors, like amenorrhea, family history of osteoporosis, and smoking, seem to play a role [27]. Harsh training conditions have occasionally been implicated [28] and protective footwear can reduce stress fracture incidence [29]. Though modifying BT procedures to achieve comparable fitness with fewer stress fractures (delay of pre-identified "injurious" activities (e.g. lengthy road marches, heavy pack/weapon carrying) is reasonable, intrinsic risk factors of recruits need study [30].

2. Measurements of Risk

a. Densitometry

The most commonly measured intrinsic risk factor for osteoporotic persons is osteopenia, low bone mass. This is known because of the existence of excellent radiation-based densitometric techniques for measuring bone mineral density (BMD) *in vivo*. Lifestyle risk factor assessment adds little information to quantitative bone measurements. Since both osteoporotic and stress fracture populations suffer fragility fractures, one could reasonably expect lower bone mass in stress fracture subjects than in non-stress-fracture subjects. More importantly, 10-15 year prospective studies have proven that bone mass measurement predicts risk of not only osteoporotic fracture [15-19], but also *all* fractures [20]. For example, 39% of sixth decade women with normal spinal or wrist BMD eventually develop a fragility fracture. This percentage changes 5% for every .01 g/cm² of BMD (10% of population standard deviation). Thus, a 50yo woman with BMD .05 g/cm² *above* the population mean has only ~1/7 chance (14%) of developing osteoporotic fracture; a woman with BMD 0.1 g/cm² *below* the population mean has a 9/10 chance (89%). We will collect data to allow developing a similar risk prediction algorithm for young female Army recruits.

b. Other Bone Evaluation Techniques

Other bone evaluation techniques predict risk for osteoporosis as well as densitometry, but are better suited for screening, because they are quicker, more economical, and radiation-free. The best example is quantitative ultrasound (QUS) [31]. QUS may work because osteoporosis is characterized by low bone *strength*, a problem caused in practice not only by low BMD, but also by poor bone geometry [32]. For this

reason, osteoporosis investigators now implicate other factors, including poor trabecular structure [32] and fatigue damage [33-34], that seem to be measured by QUS [35]. Bone measurements involving ultrasound transmission and attenuation have thus evolved [36-40]. QUS, done quickly with portable instrumentation, prospectively and retrospectively identifies subjects at increased risk for osteoporotic fracture [41-47] with *no* radiation exposure. It is thus suitable for risk-free screening of large numbers of personnel.

C. Relationship of Fatigue Damage to Stress Fracture

Fatigue damage exists in repetitively-loaded bone, as it does in most materials subjected to repetitive loading [48-50]. It seems likely to predispose to fracture by decreasing bone stiffness [51]. Military BT may be a fatigue loading situation (e.g., 80-90 miles of marching and running in formation during eight weeks) that causes stress fractures. Certainly, association, if not causation, is implied.

The role of fatigue damage in stress fracture has been poorly studied, in equal part due to a lack of animal models and methodology for identifying fatigue damage. One purpose for bone remodeling in adult humans may be to repair fatigue damage [52-54]. Osteoporotic subjects often suffer from a low bone formation (remodeling) rate [55-58], suggesting that their skeletal fragility might be due to slow repair fatigue of damage. Little is known of remodeling rates in stress fracture soldiers, but similar mechanisms could exist. A low turnover animal model might enable better evaluation of the biologic mechanism by which the skeleton compensates for accumulation of fatigue damage. For example, if fatigue loading in a low turnover animal produces weakness at the usual rate, one must conclude that remodeling plays little role in the development of fatigue-related bone weakness. If fatigue loading in a low turnover animal produces no weakness, one could well conclude that remodeling, *not* microdamage, is the key in the development of fatigue-related bone weakness.

D. Animal Models

1. Externally Applied Loads

Animal models of controlled force application are the best hope for studying fatigue loading effects in the skeleton, because conditions (stress and strain distributions) can be specified, repetitive loading machines can be attached, and experimental conditions can be standardized among animals and experiments. Animal studies can compliment the clinical evaluation of risk by allowing both histomorphometric studies and bone strength evaluation.

Two types of *in vivo* mechanical loading models of external force application with the potential for controlled fatigue loading are currently available: through implants in large animals [59-62], or through muscle and overlying tissue in small animals [63-**Error! Bookmark not defined.**]. The pin implant models provide information, but two disadvantages, the requirement for major surgery likely to affect bone homeostasis [61, **Error! Bookmark not defined.**] and the permanent disruption of gait and normal daily loading conditions, limit them. Non-surgical external force application works through pads overlying soft tissues [63-68]. The advantages of noninvasive force application are: 1) lack of surgical intervention and periosteal disruption, 2) presence of normal cage activity with measured loads superimposed upon normal daily loading patterns, and 3) use of the rat, a mammalian model with a long history of extensive metabolic, endocrine, and skeletal research.

E. Gaps in Knowledge

Both osteoporotic fracture and stress fracture during military BT are fragility fractures. Considerable expertise for evaluating osteoporotic fracture risk exists that can be applied to stress fracture risk prediction. The prospective evaluation of fragility fracture can be done with quantitative ultrasound (QUS). Stress fracture risk prediction actually seems easy when compared to what has been done for osteoporotic fracture risk prediction. Prospective osteoporotic fracture risk evaluation studies for vertebrae involve 10^{4+} person-years of observation. Hip fracture with its lower incidence requires $\sim 5 \times 10^4$ person-years of observation. Prospective stress fracture risk studies should be limited to the eight week BT period. The logistics of recruiting free-living elderly populations and maintaining them on study (usually in the face of severe illness and death) are frightening. For this proposal, the logistics of recruiting a military pre-stress fracture population have been overcome by obtaining the approval of appropriate Army personnel at the Reception Station and Troop Medical Clinic at Fort Leonard Wood, MO, a BT facility for >5000 women/year.

The relationship of fatigue damage to stress fracture development remains unclear. Since stress fracture arises in fatigue loading situations, such studies can potentially assist in gaining a fundamental understanding of the induction of stress fracture. They can only be undertaken in animal models. Thus, this proposal combines the practical advance of proven risk prediction methodology in a clinical study, with controlled loading that can further the fundamental understanding of the interplay of skeletal conditions with fatigue loading, in an animal study.

Body of Report

A. Hypotheses

1. Clinical

A) Two subsets of female Army recruits with stress fracture during BT exist, one marked by low bone strength and the other by ineffective bone remodeling.

1) In one subset, prospective quantitative ultrasound (QUS) measurements of the calcaneus and medical history risk factors (e.g., amenorrhea, past fracture, poor fitness), individually, or in combination, predict stress fracture risk.

Directly as a result of this study, we expect that QUS measurements could become a method by which Army medical personnel can safely screen for new recruits at high risk for stress fracture.

2) In a second subset, QUS values are lower at the time of stress fracture than in time-matched non-fracturing controls.

This study may lead to other methods of prospectively screening for recruits at unacceptably high risk for stress fracture (bone biomarkers).

B) In female Army recruits without stress fractures, QUS values decline in the calcaneus during BT.

We expect this study to reveal a new non-invasive method for detecting bone changes after intense physical activity.

2. Basic

A) Both QUS and bone strength decline in tibiae of female rats exposed to *in vivo* fatigue loading. Histologic signs of fatigue damage are more frequent with increased fatigue loading.

We expect this experiment to reveal incremental fatigue damage in bone and validate our ability to detect it.

B) The healing response to fatigue damage is a combination of accelerated intracortical remodeling, accelerated periosteal bone deposition, and production of endocortical microcallus, that peaks at six weeks and ends by twelve weeks after last fatigue loading.

We expect this study to reveal the time-course of fatigue damage repair. While the results may not apply to the human skeleton, because of basic differences in intracortical remodeling of the rat and human skeletons, the results will be unique and may lead to building new paradigms.

C) Common metabolic conditions of the female skeleton, like estrogen depletion, low turnover, and high turnover influence the usual skeletal healing response after fatigue damage. Estrogen depletion and low turnover slow the response, while high turnover speeds it.

We expect this study to reveal new knowledge about the influence of pre-existing skeletal turnover levels on the response to fatigue damage.

D. Research Design- Clinical Phase I

Clinical Phase I consists of two stages (See Table 2).

We established a Bone Measurement Center in the Reception Station at Fort Leonard Wood,

Stage	Timing	Procedures	Location
I	Before BT	QUS & Questionnaire	Reception Station
IIA	At Time of SFx	QUS	Troop Medical Clinic
IIB	After BT	QUS	Troop Medical Clinic

BT- Basic Training; SFx- Stress Fracture

MO on 25 August 1995. It was equipped with three QUS devices (UBA575+; Hologic Corporation; Waltham, MA) and three full-time staff, a coordinator and two assistants. Two UBA575+'s were in the Reception Station; the third was in the Troop Medical Clinic. We trained the three staff members in the proper use of UBA575+ at the Bone Measurement Center. The team measured a maximum of 50-60 female soldiers per day during weeks of peak flow (1 June-15 August). The coordinator interfaced with Major Mary Laurin, MPT, who diagnosed stress fracture using a stepwise decision tree agreed to by several BT facilities (See *Stress Fracture Diagnosis* Below). The data were analyzed using logistic regression with stress fracture as dependent variable and QUS values and other risk factors as independent variables.

Measurements

Stage I

First, as the female recruits finished blood draws and other administrative duties pursuant to joining the Army during their two days at the Reception Station, we measured QUS in the non-dominant calcaneus in 93% of female recruits presenting during an eleven month period (N=~4350). We also administered a bone risk factors questionnaire. This portion of the project went ~50% faster than projected, because the flow of female soldiers was ~6000/yr, rather than the 4500/yr we had expected. The missed 7% were either on days of extremely high flow (>60/d) or unavailable during the two day measuring window. QUS evaluation caused minimal disruption to the flow of recruits through the Reception Station, due mainly to the cooperation of Colonel Mark Collins and his staff. As possible, we recorded fitness as the total points scored on the fitness test given to the recruits at the beginning of BT.

Stage IIA

At the time of stress fracture, we obtained repeat QUS values in each fracturing recruit and two members of her company that best matched her for age and baseline QUS values.

Stage IIB

At the close of BT, we performed repeat QUS on the calcaneus of 175 randomly chosen recruits who completed BT without stress fracture.

Stress Fracture Diagnosis

This algorithm was followed routinely during the first year of the study when Major Laurin was the chief Physical Therapist. She left Fort Leonard Wood in June, 1996. After that time, because of financial considerations, the diagnosis of stress fracture was done less rigorously.

Stress fracture in soldiers reporting to the Troop Medical Clinic (TMC) with a chief complaint of lower extremity pain, was diagnosed with uniformly-applied clinical/ radiographic criteria generally in use at all Army BT facilities. At the initial visit, "point tenderness" in the lower extremity or pelvis was established through examination of the painful area by an experienced clinician. Trainees with only generalized soreness returned to active BT with no diagnosis. Symptomatic soldiers were placed on limited duty. After seven days, symptom-free recruits returned to active BT with no diagnosis, while symptomatic soldiers remained on limited duty and returned to the TMC after an additional fourteen days. Soldiers symptom-free at the third visit returned to active BT with no diagnosis. All symptomatic soldiers remained on limited duty; lateral and AP radiographs of the painful region were taken. If stress fracture was visualized (by a healing response), a diagnosis of stress fracture was made. Symptomatic soldiers with normal radiographs returned after seven more days, for more radiographs. If stress fracture was present, the diagnosis was made. If the second set of radiographs was normal, a ⁹⁹Tm scan of the affected region was made. If the scan was focally active, a diagnosis of stress fracture was made. If the scan was not focally active, no diagnosis was made. Each soldier symptomatic at the fourth visit was followed clinically, returning to active BT at a time consistent with her rate of recovery.

Statistical Approach

Finally, we used logistic regression and Cox proportional hazards testing to calculate risk of stress fracture, using SOS (speed of sound), BUA (broadband ultrasound attenuation), and clinical history.

D. Research Results

1. Clinical Phase I

Baseline characteristics of the women measured in Phase I are in Table 3. They represent a typical cross-section of 1995-96 female Army recruits. Of this group, 327 (8.7%) suffered one or more stress fractures at an average of 22±6 days into BT.

The principal risk factors for stress fracture for these women were age and QUS measurement. Though SOS (speed of sound) and BUA (broadband ultrasound attenuation) were correlated ($r^2=0.37$; $P<0.0001$), SOS yielded somewhat higher risk coefficients than did BUA. Testing SOS and BUA in the same model showed that they were not independent fracture risk predictors, indicating that they measure similar bone properties underlying fracture risk. Greater age was a minor, independent predictor of fracture risk. Black soldiers were less than half as likely to have stress fracture as whites and Hispanics (4.7% vs. 10.2%). A positive smoking history was also an independent risk factor in this group, suggesting that it was a marker rather than a physiologic cause. When adjusted for race and age, the relative risks accorded to SOS and BUA are in Table 4.

Number	4234 women
Age	20.9±3.6 yrs
Interval	25 August 1995-15 July 1996
Height	164.1±6.8 cm
Weight	61.1±8.9 kg
BMI	22.7±2.8 kg/m ²
Race	50% White 9% Hispanic 35% Black 6% Other
SOS	1515.2±9.0 m/sec
BUA	100.1±19.0 dB/MHz

Variable	unadjusted		adjusted for age and race	
	Relative Risk	Confidence Interval	Relative Risk	Confidence Interval
SOS (m/sec)	1.99*	1.76-2.26	1.80*	1.57-2.07
BUA (dB/MHz)	1.73*	1.54-1.94	1.62*	1.39-1.81
PT Score	2.48	1.56-3.94	-	-
Age (yrs)	1.09+	1.06-1.12	-	-

*per standard deviation; +per year of age

Subgroup Analysis

Poor physical fitness, as determined by the PT (Physical Training) Score at entrance into BT, is a predictor of stress fracture. We collected PT Scores on 791 young women from the above group. We then did backward logistic regression, with all of the above factors plus PT Score. PT Score had a higher relative risk, but it was significantly different from the other two significant factors, smoking history and SOS (Table 5). More importantly, when placed in the same

Factor	Relative Risk	Confidence Interval
PT Score	2.60*	1.65-4.10
SOS	1.90+	1.23-4.67
Smoking	2.39	1.28-2.82
adjusted for race and age		
*increase in risk per SD decrease below mean		

model, both PT Score and SOS were significant predictors of stress fracture, indicating a need to screen for both.

Summary

Like osteoporotic fracture, stress fracture is a fragility fracture whose risk can be assessed prospectively by a simple non-radiation-based bone measurement. Furthermore, the risk assessed by bone measurement is independent of the Army's previous best prospective indicator of stress fracture risk, physical fitness assessment at the start of BT. A low bone QUS value is thus unrelated to poor physical fitness.

From a practical standpoint, however, QUS measurement fails to provide a way of pinpointing individual soldiers with such a high risk of stress fracture that they can be immediately excluded from the Army as is often done for asthma or poor eyesight that creates a certain risk of periodic physical incapacitation and risk to fellow soldiers.

2. Clinical Phase II

In completing Clinical Phase I, the first female phase of screening, we satisfied the work proposed in our grant application. However, with about one year remaining in our time period, we asked for and were granted permission to proceed to Clinical Phase II, the male screening phase. Assuming the same predictive ability for QUS seen in Phase I (RR= \sim 1.8) with a stress fracture incidence of 1%, we calculated we would need to measure \sim 4500 male soldiers [72]. That seemed possible before the 1 August 1997 termination of the grant. As the male recruits were processed, we measured QUS in the non-dominant calcaneus between 15 July 1996 and 1 May 1997. As possible (N=1454), we again recorded fitness as initial PT Score.

Baseline characteristics of the males (N=4711) are in Table 5. Of this group, 11 (0.23%) suffered a stress fracture, an insufficient number to analyze for relative risk of fracture *for any factor*. It is of note that BUA in these 11 was 87.2 ± 16.5 dB/MHz (vs. 96.8 ± 7.9 dB/MHz [Table 5]) and SOS was 1516.3 ± 19.9 m/sec (vs. 1519.7 ± 11.4 m/sec [Table 5]). This degree of difference in QUS values of fracturing vs. non-fracturing soldiers (Table 5) was similar to that in females, suggesting that QUS values differed in fracturing males and non-fracturing males, but that our study undersampled, creating a Type II error.

Number	4711 men
Age	21.2 \pm 3.2 yrs
Interval	15 July 1996-1 May 1997
Height	178.0 \pm 7.3 cm
Weight	76.8 \pm 12.6 kg
Race	63% White 12% Hispanic 19% Black 6% Other
SOS	1519.7 \pm 11.4 m/sec
PTScore	135.7 \pm 51.8
BUA	96.8 \pm 7.9 dB/MHz

Three significant changes in BT occurred at Ft Leonard Wood around 1 July 1996. First, the directors of BT at began to introduce strenuous activities at more gradual pace during BT. Second, Major Laurin left Fort Leonard Wood for advanced schooling at Fort Leavenworth, KS. Third, since all soldiers with stress reaction type injury are treated similarly, the formal diagnosis of stress fracture makes little difference. Thus, optimal resource management at the TMC dictated that fewer radiographs be taken of symptomatic soliders who may have had stress fractures.

It can be readily appreciated how each change reduces the chance of diagnosing stress fracture. The change in BT strategy seems likely to decrease stress fracture by reducing demands on recruits, making it partially responsible for the lower than expected incidence of stress fractures in our male soldiers. The departure of Major Laurin and the execution of fewer radiographs at the TMC, combined with the practical focus on simply returning soldiers to training, rather than making a diagnosis, also would tend to reduce the number of stress fractures diagnosed.

3. Clinical Phase III

We completed Clinical Phase II on about May 1, 1997. Army personnel at Ft Leonard Wood wondered if their changes in BT routines had reduced stress fracture incidence, due to the more gradual conditioning that was possible with the more gradual ramp-up to longer marches with heavy packs and weapons. They felt that if this were true, conditioning might no longer be a risk factor, but that QUS values would continue to be a risk factor. We asked for permission to extend our work to include measuring another 2100 female recruits.

To test this, we reequipped our Bone Measurement Center in the Reception Station with a new QUS device (Sahara; Hologic Corporation; Waltham, MA). The new device was six times faster than the previous UBA575+'s. We calculated with a relative risk of 1.8, and a stress fracture incidence of 3%, we would need to measure around 2100 female recruits [72]. We measured 2401 female recruits

between 15 June 1997 and 15 October 1997. Only 35 were diagnosed with stress fracture (1.4%), about one-sixth the number from the study done in 1995-1996. However, ~325 developed stress reactions requiring a visit to the TMC. Baseline characteristics are

Number	2401 women
Age	19.8±3.1 yrs
Interval	15 June 1997-15 October 1997
Height	163.8±7.7 cm
Weight	60.3±8.7 kg
Race	56% White 10% Hispanic 28% Black 6% Other
SOS	1577±48 m/sec
PTScore	84.3±60.6 (N=1524)
BUA	83.1±17.0 dB/MHz

given in Table 8. Note the different SOS and BUA values that are derived from the new instrument. Studies showed that the Sahara had about a correlation of about 0.7 for SOS and BUA, but with substantially different y-intercepts. The data were analyzed using logistic regression with stress fracture as dependent variable and QUS values as independent variables.

Variable	unadjusted		adjusted for fitness	
	Relative Risk	Confidence Interval	Relative Risk	Confidence Interval
SOS (m/sec)	1.47*	1.02-2.13	1.35	0.86-2.12
BUA (dB/MHz)	1.49*	1.02-2.18	1.38	0.98-2.15
Fitness	1.81*	1.13-2.88	-	-
Age (yrs)	1.08+	1.05-1.11	-	-

*per standard deviation; +per year of age

As with the 1995-1996 soldiers, QUS value remained a significant risk factor for stress fracture. However, a number of background circumstances changed in two years. The BT regime had been adjusted to be less demanding. The diagnosis of stress fracture was now more likely to be missed because of fewer radiographs and Major Laurin, who had paid close attention to diagnoses, was gone. We also collected PT Scores on about twice as many female recruits as previously, so chances are better that we have a better idea of the relationship of QUS and PT Score. Only 35 recruits were diagnosed with stress fracture (1.4%), about one-sixth the incidence from the 1995-1996 study. However, ~325 developed stress reactions requiring a visit to the TMC. The relative risks for all factors were less than previously. Though one cannot be sure whether BT adjustments or decreased ascertainment were the cause, it is reasonable to believe that stress fracture incidence has declined.

1. Basic Phase

a. *In Vivo* Loading Model and Experimental Design

The purpose here was to test skeletal responses to fatigue loading in low, normal, and high turnover skeletons. First, 83 6 month old intact female Sprague-Dawley rats were randomly divided into six groups for necropsy at -4, 0, 3, 6, 9, and 12 weeks from the last session of fatigue loading. The lower right leg was externally loaded *in vivo* in a four-point bending device on Monday, Wednesday, and Friday of each week for four consecutive weeks, a total of twelve loading sessions per rat (24,000 cycles). Fatigue loading in four point bending (2000 cycles/day, 2Hz @41N (2200-2500 μ E at periosteal

and $1800\mu\epsilon$ at endocortical surface by finite element modeling). The left leg was not externally loaded, serving as a control. During each loading session, rats were maintained under light ether anesthesia. Between loading sessions, rats returned to normal cage activity. The lower pads (loading points) of the device are 23mm apart and contact the medial leg surface, while the upper pads are 11mm apart and contact the lateral leg surface. Uniform strain is created at the lateral bone surface between the upper pads (Figure 1). This creates a region of the tibia 3.5-14mm proximal to the distalmost aspect of the tibia-fibular junction (TFJ), that is the region of concentrated fatigue loading. Force is applied from the lateral side of the leg by a motor driven, programmable, four-point bending apparatus, at 45-50N@2Hz, for 2000 cycles/d.

Intraperitoneal (IP) injections of calcein (8 mg/kg; Sigma, St. Louis, MO) were given on a schedule of 1d ON, 6d OFF, 1d ON, and 2d OFF (1-6-1-2) before sacrifice. At the time of tissue collection, all rats were anesthetized by IP injection of Ketamine (50 mg/kg body wt) and Xylazine (10 mg/kg). Death was induced by intracardiac injection of .25cc of Fatal Plus (Vortech Pharmaceuticals, Dearborn, MI). Right and left tibiae from each group were processed for histomorphometric analysis and *ex vivo* ultrasound evaluation.

b. Fatigue Damage Detection

1. Quantitative Ultrasound (QUS)

To quantitate fatigue damage, speed of sound (transmission mode) was measured by quantitative ultrasound (QUS) in the longitudinal axis of the right tibia using 1Mhz transducers (Panametrics, Waltham, MA). Comparison between right (loaded) and left (unloaded) tibiae was made with the Kruskal-Wallis test ($P<0.05$) and intergroup testing by Student Neuman-Keuls ($P<.05$).

2. Basic Fuchsin Block Staining

The loaded region of each tibia at 1-1.5cm proximal to the TFJ was immersed in 1% basic fuchsin [69] and allowed to dry. The bone was embedded as above and sectioned at $70\mu\text{m}$, then examined for the presence of stained and unstained cracks in the tissue. Stained cracks are presumed to have been present before the sectioning procedure.

c. Histomorphometry

All bones were cleaned of non-adherent muscle and soft tissue, and placed in 70% EtOH. For histomorphometry, the region of the tibial diaphysis extending from the TFJ to 1cm proximal to the TFJ was cut and placed in Villanueva stain [70] for 72hrs and then returned to 70% EtOH. Over the next 14 days, the specimens were dehydrated in

graded ethanols, defatted in acetone, and embedded in modified methyl methacrylate [71]. The samples were cross-sectioned at 70 μ m with a 0.6mm inter-section distance on a saw-microtome (Model 1600, Leitz Instruments; Deerfield, IL USA).

Two sections in the region 5.5-9.0 mm proximal to the TFJ were mounted on slides, given a random number, and analyzed semi-automatically with a light/epifluorescent microscope and a camera projecting an image onto a screen connected to a Windows NT computer with appropriate software (BIOQUANT True Color for Windows (R&M Biometrics, Nashville, TN)). For counting, the cross section was divided into lateral tibia periosteum,

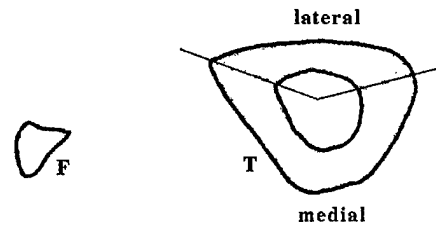


Figure 2
Cross-Section through Loaded Region
F-fibula; T-tibia; lateral and medial periosteum regions are indicated. The endosteum was considered as one unit.

medial tibia periosteum, tibial endosteum, and fibula periosteum (Figure 2). The following data were collected at the specified magnifications in each region: at 20X- cortical bone and medullary area; at 40X- bone surface (Ps.BS and Es.BS); at 100X- double and single-labeled calcein surface, double-labeled calcein surface, and woven bone surface (no surfaces fell into more than one category); and at 400X- interlabel width (IrL.Wi) at sites of double labeling. The following calculations are made for each region: periosteal woven bone area (PWo.Ar (%)), periosteal new lamellar bone area (PNLmB.Ar), endocortical new lamellar bone area (ENLmB.Ar), periosteal mineralizing surface (PMS/BS), and endocortical mineralizing surface (EMS/BS).

	-4 Wk	0 Wk	3 Wk	6 Wk	9 Wk	12 Wk
PWo.Ar*, %	0.0 \pm 0.0	2.78 ^a \pm 2.5	11.4 ^a \pm 17.6	0.0 ^b \pm 0.0	0.0 ^b \pm 0.0	0.0 ^b \pm 0.0
PNLmB.Ar, %	0.0 \pm 0.0	1.59 \pm 1.38	1.89 \pm 1.99	4.44 \pm 5.41	2.94 \pm 8.10	3.20 \pm 2.86
ENLmB.Ar*, %	0.0 \pm 0.0	0.39 \pm 0.68	2.98 \pm 2.70	7.10 ^{ab} \pm 1.21	5.89 ^{ab} \pm 5.23	0.0 \pm 0.0
PMS/BS, %	0.3 \pm 0.2	4.1 \pm 11.1	4.1 \pm 6.6	5.7 \pm 3.2	9.1 \pm 7.0	35.5 \pm 51.8
EMS/BS*, %	4.2 \pm 2.3	21.9 ^a \pm 15.4	35.5 ^a \pm 10.0	33.4 ^a \pm 15.2	10.2 ^a \pm 11.9	5.1 ^b \pm 1.1
QUS*, m/sec	3805 \pm 114	3738 \pm 171	3734 \pm 119	3790 \pm 84	3845 ^b \pm 67	3781 \pm 75

mean \pm SD
* Kruskal-Wallis (P<.05)
^a difference due to fatigue loading compared to -4wk P< .05
^b difference due to fatigue loading compared to -0 wk P< .05

In intact rats, no fractures occurred. No differences in total bone area existed. All histologic variables (Table 9) were obtained by subtracting left leg values from right leg

(loaded) values. Periosteal, but not endocortical, woven bone was present only at the end of the fatigue loading period and three weeks later. Significant endocortical accumulation of new lamellar bone was present at six and nine weeks after the end of fatigue loading, but was no longer apparent at twelve weeks. This change was mirrored by the significant trends in endocortical mineralizing surface. QUS was lower at Weeks 0-3 than at baseline, then returned to baseline by Week 6. Basic fuchsin staining revealed no quantitative or qualitative differences among the groups.

b. *In Vivo* Loading in Ovariectomized Rats

Ovariectomy induces high turnover in the skeleton, particularly accelerating formation at periosteal surfaces. 50 6 month old female Sprague-Dawley rats were ovariectomized from a dorsal approach. Ten weeks later, they were randomized into five groups for necropsy at -4, 0, 4, 8, and 12 weeks from the last session of fatigue loading. Loading as above was completed during the next four weeks. At autopsy both tibiae were collected for histomorphometric evaluation of periosteal formation surface (PFS), mineral apposition rate (MAR) and woven bone surface (WoBS).

Table 10a Bone Response To Fatigue Loading In Ovariectomized Rats							
Endpoint	Leg	-4 Wk	0 Wk	4 Wk	8 Wk	12 Wk	P ^a
pFS, %	Right	36±6	70 ^b ±18	27 ^b ±4	16±3	13±3	.0001
	Left	37±5	21.0±25	14±4	7±3	7±3	.0001
pMAR, µm/d	Right	1.3±0.1	1.9 ^b ±0.4	1.6 ^b ±0.2	1.1 ^b ±0.2	0.7±0.1	.0001
	Left	1.3±0.1	0.8±0.2	0.9±0.1	0.8±0.1	0.8±0.1	.007
pWoBS, %	Right	0.0±0.0	56 ^b ±16	6.7 ^b ±6	1.3±4	0.1±0.4	.0001
Table 10b Bone Response To Fatigue Loading during Estrogen Treatment							
pFS, %	Right	-	50 ^b ±6	14±3	5±2	13 ^b ±0.4	.0001
	Left	-	28±5	9±4	1.1±0.4	3.4±0.2	.0001
pMAR, µm/d	Right	-	1.6 ^b ±0.2	2.2 ^b ±0.1	0.9±0.1	1.0±0.1	.NS
	Left	-	1.1±0.2	1.5±0.1	0.8±0.1	0.7±0.1	NS
pWoBS %	Right	-	58 ^b ±12	11±4	0±0	2.6±1.2	.0001
TotAr, mm ²	Right	-	6.3±0.2	5.5±0.3	5.4±0.3	5.7 ^b ±0.3	NS
	Left	-	5.9±0.1	6.0±0.2	5.9±0.2	6.2±0.3	NS
NBAR, mm ²	Right	-	.035 ^b ±.012	.09 ^b ±.03	.064 ^b ±.015	0.13±.012	.02
	Left	-	.001±.001	.013±.012	0±0	0±0	NS
mean±SEM, ^b difference within each group (Left [C] vs Right [L] leg) P< .05							

Periosteal mineral apposition rate was transiently increased by fatigue loading up to 8 weeks after cessation of loading, but then returned to values seen on the control side. Similarly, formation surface was much higher on the loaded leg than on the

control side up to four weeks after cessation of loading. The loading response appears to be of similar timing, but more marked in ovariectomized rats than in intact rats above (compare to Table 9).

c. *In Vivo* Loading in Estrogen-Treated Intact Rats

Estrogen treatment suppresses bone turnover, particularly at periosteal surfaces. It is a reasonable substitute for antiresorptives like bisphosphonates. 40 6 month old female Sprague-Dawley rats were treated with 17- β -estradiol (10 μ g/kg in corn oil 2X/wk) for two weeks, a dose that blocks bone loss in newly ovariectomized rats. Then, as treatment continued, they were fatigue-loaded as above for four weeks, and killed at 0, 4, 8, and 12 weeks from the last session of fatigue loading. At autopsy, both tibiae were collected for histomorphometric evaluation of periosteal formation surface (PFS), mineral apposition rate (MAR) and woven bone surface (WoBS).

No fractures were observed. There were no differences in total bone area among groups. Periosteal formation surface was significantly greater in the loaded legs of the Week 0 group than at any other time (Table 10b). Periosteal mineral apposition rate (pMAR) was significantly greater due to loading at Weeks 0-4. Periosteal, but not endocortical, woven bone in the right loaded leg was present only at 0-4 weeks after the end of the fatigue loading period. All periosteal woven bone had finished forming by Week 8. New bone area (NBAR) was significantly greater in loaded (right) legs at 0-8 weeks post fatigue loading. Consistent with greater periosteal than endosteal strains in vivo fatigue loading causes a greater periosteal than endocortical response.

Summary

In vivo fatigue loading causes a robust, but transient periosteal and endocortical response. The endocortical response is more marked and long lasting, producing new lamellar bone that is incorporated into the existing bone in an indistinguishable fashion by 12 weeks after cessation of fatigue loading. The endocortical surface response of bone formation is as expected considering the bone accumulation pattern. The periosteal response adds only woven bone that disappears by six weeks after cessation of fatigue loading. The results in ovariectomized and estrogen-treated rats suggest that the skeletal response to fatigue loading is somewhat greater in rats with a higher turnover rate.

The trend in QUS, a measurement that can detect fatigue damage in many materials, is compatible with a burden of fatigue damage that increases then declines. The basic fuchsin staining studies failed to find any trend that would support the QUS

trends. We regard this dichotomy as methodologic and suggest the fatigue damage is really present.

While it was definitely possible to perform fatigue loading in these rats, it is clear that the findings are far from definitive. Though we detected no fractures, the periosteal and endosteal woven bone responses are rather similar to what is seen during the healing of human stress fractures, including raised periosteum and localized transient woven bone. It is likely that, in the absence of detectable fracture, the response in the loaded region is a typical periosteal stress reaction. We are relatively certain that we did not induce fatigue fracture in this experiment, and suggest that either longer-term loading or mildly more intense loading would be necessary. Our pilot work showed that 2000 cycles per loading session (@2Hz) was our maximum because of damage to the overlying soft tissue. It is possible that other types of apparatus that can deliver loading cycles at 10-30Hz might be more successful in inducing fatigue damage in vivo. We never saw Haversian remodeling induced in the cortical bone of rats in response to fatigue loading.

Conclusions

Clinical

The most important new conclusion of this report is that Army recruits with weak bones are more likely to have stress fractures during Basic Training. Recruits with weak bones can be identified prospectively by the same instrumentation used to quantitate osteoporotic fracture risk. We confirmed that less physically fit soldiers are more likely to have stress fracture. Recruits with poorer fitness tend to have weak bones. There are usually elements of self-selection by stronger persons for a lifestyle that results in better conditioning involved in this. We also confirmed that sex, age, race, and past smoking history are measurable risk factors. It seems likely that the Army could very efficiently: 1) measure fitness, 2) measure QUS, 3) record age, 4) query on smoking history. If they could stratify companies of persons with fitness and QUS one standard deviation below normal and over age 25 with a history of smoking, and give such persons a twelve week BT with a very gradual increase in activities, the Army would see reductions in stress fracture.

Basic

The most marked response to measured fatigue loading in small animals is woven bone formation at the periosteal surface. The excess woven bone subsides within four weeks of stopping fatigue loading. No fatigue damage could be detected and

confirmed with certainty. It appeared that skeletons of higher turnover mounted a somewhat more vigorous response to fatigue loading.

References

1. Protzman RR, Griffis CG. 1977 Stress fractures in men and women undergoing military training. *J Bone Jt Surg* 59A:825.
2. Black JR. 1982 Stress fractures of the foot in female soldiers: a two-year survey. *Mil Med* 147:861-62.
3. Pester S, Smith PC. 1992 Stress fractures in the lower extremities of soldiers in basic training. *Orthop Rev* 21:297-303.
4. Reinker KA, Ozburne S. 1979 A comparison of male and female orthopaedic pathology in basic training. *Mil Med* 144:532-536.
5. Finestone A, Shlamkovitch N, Eldad A, Wosk J, Laor A, Danon YL, Milgrom C. 1991 Risk factors for stress fractures among Israeli infantry recruits. *Mil Med* 156:528-30.
6. Garcia JE, Grabhorn LL, Franklin KJ. 1987 Factors associated with stress fractures in military recruits. *Mil Med* 152:45-8.
7. Ross J. 1993 A review of lower limb overuse injuries during basic military training. Part 1: Types of overuse injuries. *Mil Med* 158:410-5.
8. Jones BH, Bovee MW, Harris JM, Cowan DN. 1993 Intrinsic risk factors for exercise-related injuries among male and female army trainees. *Am J Sports Med* 21:705-10.
9. Greaney RB, Gerber FH, Laughlin RL, Kmet JP, Metz CD, Kilcheski TS, Rao BR, Silverman ED. 1983 Distribution and natural history of stress fractures in US Marine recruits. *Radiology* 146:339-346.
10. Anderson EG. 1990 Fatigue fractures of the foot. *Injury* 21:275-9.
11. Sjolín SU, Eriksen C. 1989 Stress fracture of the femoral neck in military recruits. *Injury* 20:304-5.
12. Lund L, Ganderup C. 1990 Stress fracture of the femoral neck in soldiers: a report of two cases. *Mil Med* 155:357.
13. Provost RA, Morris JM. 1969 Fatigue fracture of the femoral shaft. *J Bone Jt Surg* 51A:487-498.
14. Long MM, Stetts DM. 1985 Stress fractures of the femoral neck. *Orthop Nurs* 4:69-71.
15. Hui SL, Slemenda CW, Johnston Jr CC. 1989 Baseline measurement of bone mass predicts fracture in white women. *Ann Int Med* 111:355-361.
16. Black DM, Cummings SR, Genant HK, Nevitt MC, Palermo L, Browner W. 1992 Axial and appendicular bone density predict fractures in older women. *J Bone Min Res* 7:633-638.
17. Ross PD, Wasnich RD, Vogel JM. 1988 Detection of prefracture spinal osteoporosis using bone mineral absorptiometry. *J Bone Min Res* 3:1-11.
18. Gärdsell P, Johnsell O, Nilsson BE. 1991 The predictive value of bone loss for fragility fractures in women: a longitudinal study over fifteen years. *Calc Tiss Int* 49:90-94.
19. Cummings SR, Black DM, Nevitt MC, Browner W, Cauley J, Ensrud K, Genant HK, Palermo L, Scott J, Vogt TM. 1993 Bone density at various sites for prediction of hip fractures. *Lancet* 341:72-75.
20. 3825. Sowers MR, Galuska DA. 1993 Epidemiology of bone mass in premenopausal women. *Epidem Rvws* 15:374-398.
21. Brudvig TJ, Gudger TD, Obermeyer L. 1983 Stress fractures in 295 trainees: a one year study of incidence related to age, sex, and race. *Mil Med* 148:666-667.
22. Taimela S, Kufala UM. 1990 Stress injury proneness: a prospective study during a physical training program. *Int J Sports Med* 11:162-5.
23. Pouilles JM, Bernard J, Tremollieres F, Louvet JP, Ribot C. 1989 Femoral bone density in young male adults with stress fractures. *Bone* 10:105-8.
24. Giladi M, Milgrom C, Simkin A, Danon Y. 1991 Stress fractures. Identifiable risk factors. *Am J Sports Med* 19:647-52.
25. Milgrom C, Giladi M, Simkin A, Rand N, Kedem R, Kashtan H, Stein M, Gomori M. 1989 The area moment of inertia of the tibia: a risk factor for stress fractures. *J Biomech* 22:1243-8.

26. Giladi M, Milgrom C, Simkin A, Stein M, Kashtan H, Margulies J, Rand N, Chisin R, Steinberg R, Aharonson Z. 1987 Stress fractures and tibial bone width. A risk factor. *J Bone Joint Surg* 69B:326-9.
27. Friedl KE, Nuovo JA, Patience TH, Dettori JR. 1992 Factors associated with stress fracture in young army women: indications for further research. *Mil Med* 157:334-8.
28. Zahger K, Abramovitz A, Zelikovsky L, Israel O, Israel P. 1988 Stress fractures in female soldiers: an epidemiological investigation of an outbreak. *Mil Med* 153:448-50.
29. Schweltnus MP, Jordaan G, Noakes TD. 1990 Prevention of common overuse injuries by the use of shock absorbing insoles. A prospective study. *Am J Sports Med* 18:636-41.
30. Giladi M, Milgrom C, Danon Y, Aharonson Z. 1985 The correlation between cumulative march training and stress fractures in soldiers. *Mil Med* 150:600-1.
31. 0950. Baran DT, Kelly AM, Karellas A, Gionet M, Price M, Leahey D, Steuterman S, McSherry B, Roche HJ. 1988 Ultrasound attenuation of the os calcis in women with osteoporosis and hip fractures. *Calc Tiss Int* 43:138-142.
32. Kleerekoper M, Villanueva AR, Stanciu J, Rao DS, Parfitt AM. 1985 The role of three dimensional trabecular microstructure in the pathogenesis of vertebral compression fractures. *Calc Tiss Int* 37:594-597.
33. Avallone EA, Baumeister T III. *Mark's standard handbook for mechanical engineers*. McGraw-Hill: New York, 1987.
34. Strong AB. *Fundamentals of composites manufacturing: materials, methods, and applications*. Soc. Mech. Eng: Dearborn, MI, 1989.
35. 4170. Hans D, Arlot ME, Schott AM, Roux JP, Kotzki PO, Meunier PJ. 1995 Do ultrasound measurements on the os calcis reflect more the bone microarchitecture than the bone mass?: a two-dimension histomorphometric study. *Bone* 16:295-300.
36. Waud CE, Lew R, Baran DT. 1992 The relationship between ultrasound and densitometric measurements of bone mass at the calcaneus in women. *Calc Tiss Int* 51:415-418.
37. Damilakis JE, Dretakis E, Gourtsoyiannis NC. 1992 Ultrasound attenuation of the calcaneus in the female population: normative data. *Calc Tiss Int* 51:180-183.
38. Baran DT, McCarthy CK, Leahey D, Lew R 1991 Broadband ultrasound attenuation of the calcaneus predicts lumbar and femoral neck density in Caucasian women: a preliminary study. *Osteo Int* 1:110-113.
39. Agren M, Karellas A, Leahey D, Marks S, Baran D 1991 Ultrasound attenuation of the calcaneus: a sensitive and specific discriminator of osteopenia in postmenopausal women. *Calc Tiss Int* 48:240-244.
40. McCloskey EV, Murray SA, Miller C, Charlesworth D, Tindale W, O'Doherty DP, Bickerstaff DR, Hamdy NAT, Kanis J. 1990 Broadband ultrasound attenuation in the os calcis: relationship to bone mineral at other skeletal sites. *Clin Sci* 78:227-233.
41. Stegman MR, Heaney RP, Recker RR, Travers-Gustafson D, Leist J. 1994 Velocity of ultrasound and its association with fracture history in a rural population. *Am J Epidem* 139:1027-1034.
42. Travers-Gustafson D, Stegman MR, Heaney RP, Recker RR. 1994 The Saunders County Bone Quality Study: ultrasound, densitometry and other fracture risk factors. *Am J Epidem* 139:1027-1034.
43. Heaney RP, Stegman MR, Chesnut CH, Avioli LV, Brandenburger GH. 1995 Ultrasound velocity through bone predicts incident vertebral deformity. *J Bone Min Res* 10:341-345.
44. 6990. Bauer DC, Gluer CC, Genant HK, Stone K. 1995 Quantitative ultrasound and vertebral fracture in postmenopausal women Fracture Intervention Trial Research Group. *J Bone Min Res* 10:353-358.
45. 4163. Turner CH, Peacock M, Timmerman L, Neal JM, Johnston CC. 1995 Calcaneal ultrasonic measurements discriminate hip fracture independently of bone mass. *Osteo Int* 5:130-135.

46. 4167. Ross P, Huang C, Davis J, Imose K, Yates J, Vogel J, Wasnich R. 1995 Predicting vertebral deformity using bone densitometry at various skeletal sites and calcaneus ultrasound. *Bone* 16:325-332.
47. Heaney RP, Avioli LV, Chesnut CH, Lappe J, Recker RR, Brandenburger GH 1989 Osteoporotic bone fragility: detection by ultrasound transmission velocity. *J Am Med Assn* 261:2986-2990
48. Carter DR, Hayes WC. Fatigue life of compact bone-I effects of stress amplitude, temperature and density. *J Biomech* 9:27-34, 1976.
49. Carter DR, Hayes WC. Fatigue life of compact bone-I effects of microstructure and density. *J Biomechanics* 9:211-218, 1976.
50. Carter DR, Hayes WC. Compact bone fatigue damage- I residual strength and stiffness. *J Biomechanics* 10:325-337, 1977.
51. Carter DR, Caler WE. 1985 A cumulative damage model for bone fracture. *J Orth Res* 3:84-90.
52. Burr DB, Martin RB, Schaffler MB, Radin EL. 1985 Bone remodeling in response to *in vivo* fatigue microdamage. *J Biomech* 18:189-200.
53. Burr DB, Milgrom C, Boyd RD, Higgins WL, Robin G, Radin EL. 1990 Experimental stress fractures of the tibia. *J Bone & Joint Sur* 72B:370-5.
54. Frost H. 1991 Some ABC's of skeletal pathophysiology. 5. microdamage physiology. *Calc Tiss Int* 49:229-231.
55. Kimmel DB, Recker RR, Gallagher JC, Vaswani A, and Aloia JL. 1990 A comparison of iliac bone histomorphometry in post-menopausal osteoporotic and normal subjects. *Bone and Mineral* 11:217-235
56. Carasco MG, deVernejoul MC, Sterkers Y, Morieux C, Kuntz D, Miravet L. 1989 Decreased bone formation in osteoporotic patients compared with age-matched controls. *Calc Tiss Int* 44:173-175.
57. Foldes J, Parfitt AM, Shih MS, Rao DS, Kleerekoper M. 1991 Structural and geometric changes in iliac bone: relationship to normal aging and osteoporosis. *J Bone Min Res* 6:759-766
58. Meunier PJ, Edouard C, Alexandre C. 1979 Bone histomorphometry in osteoporotic states. in Barzel US, ed. *Osteoporosis II*, New York: Grune and Stratton; 27-47.
59. Goodship AE, Lanyon LE, McFie H. 1979 Functional adaptation of bone to increased stress. *J Bone Jt Surg* 61B:539-546.
60. Lanyon LE, Goodship AE, Pye CJ, MacFie JH. 1982 Mechanically adaptive bone remodelling. *J Biomech* 15:141-154.
61. Churches AE, Howlett CR. 1982 Functional adaptation of bone in response to sinusoidally varying controlled compressive loading of the ovine metacarpus. *Clin Orth Rel Res* 168:265-280.
62. O'Connor JA, Lanyon LE, MacFie H. 1982 The influence of strain rate on adaptive bone remodelling. *J Biomech* 15:767-781.
63. Turner CH, Akhter MP, Raab DM, Kimmel DB, Recker RR. 1991 A non-invasive, *in vivo* model for studying strain adaptive bone modeling. *Bone* 12:73-79.
64. Akhter MP, Raab DM, Turner CH, Kimmel DB, Recker RR. 1992 Characterization of *in vivo* strain in the rat tibia during external application of a four-point bending load. *J Biomech* 25:1241-1246.
65. Raab-Cullen D, Akhter M, Kimmel D, Recker R. 1994 Bone response to alternate day mechanical loading of the rat tibia. *J Bone Min Res* 9:203-211.
66. Raab-Cullen DM, Akhter MP, Kimmel DB, Recker RR. 1994 Periosteal bone formation is stimulated by externally-induced bending strains. *J Bone Min Res* 9:1143-1152.
67. Seireg A, Kempke W. 1969 Behavior of *in vivo* bone under cyclic loading. *ASME Pub*; BHF-8:2-7.

68. Torrance A, Mosley J, Suswillo R, Lanyon L. 1994 Noninvasive loading of the rat ulna *in vivo* induces a strain-related modeling response uncomplicated by trauma or periosteal pressure. *Calc Tiss Int* 54:241-247.
69. Burr DB, Stafford T. 1990 Validity of the bulk-staining technique to separate artifactual from *in vivo* bone microdamage. *Clin Orth Rel Res* 260:305-308.
70. Villanueva AR, Lundin KD. 1989 A versatile new mineralized bone stain for simultaneous assessment of tetracycline and osteoid seams. *Stain Techn* 64:129-138.
71. Baron R, Vignery A, Neff L, Silverglate A, Santa Maria A. 1983 Processing of undecalcified bone specimens for bone histomorphometry. in: *Bone Histomorphometry: Techniques and Interpretation* (ed, RR Recker), (CRC: Boca Raton, Florida):13-35.
72. Schlesselman JJ. 1974 Sample size requirements in cohort and case-control studies of disease. *Am J Epidemiol* 99:3381-384.