

# Stress Fracture and Military Medical Readiness: Bridging Basic and Applied Research

KARL E. FRIEDL<sup>1</sup>, RACHEL K. EVANS<sup>2</sup>, and DANIEL S. MORAN<sup>3</sup>

<sup>1</sup>Telemedicine and Advanced Technology Research Center, Fort Detrick, MD; <sup>2</sup>US Army Research Institute of Environmental Medicine, Natick, MA; and <sup>3</sup>Heller Institute of Medical Research, Sheba Medical Center, Tel Hashomer, ISRAEL

## ABSTRACT

FRIEDL, K. E., R. K. EVANS, and D. S. MORAN. Stress Fracture and Military Medical Readiness: Bridging Basic and Applied Research. *Med. Sci. Sports Exerc.*, Vol. 40, No. 11S, pp. S609–S622, 2008. **Purpose:** Military recruits and distance runners share a special risk of stress fracture injury. Recent efforts by US and Israeli military-sponsored researchers have uncovered important mechanisms and practical low-cost interventions. This article summarizes key findings relevant to prevention of stress fracture, including simple strategies to identify and to mitigate risk. **Methods:** Published research supported through the Bone Health and Military Medical Readiness research program and related military bone research was analyzed for contributions to preventing stress fracture in military recruits and optimizing bone health. **Results:** Thousands of military recruits helped test hypotheses about predictors of risk, safer exercise regimens, and rest, nutrition, gait training, and technology interventions to reduce stress fracture risk. Concurrent cellular, animal, and human laboratory studies were used to systematically investigate mechanisms of mechanical forces acting on bone and interactions through muscle, hormonal and genetic influences, and metabolism. The iterative and sometimes simultaneous process of basic discovery and field testing produced new knowledge that will provide safer science-based physical training. **Discussion:** Human training studies evaluating effects on bone require special commitment from investigators and funders due to volunteer compliance and attrition challenges. The findings from multiple studies indicate that measures of bone elasticity, fragility, and geometry are as important as bone mineral density in predicting fracture risk, with applications for new measurement technologies. Risk may be reduced by high intakes of calcium, vitamin D, and possibly protein (e.g., milk products). Prostaglandin E2, insulin-like growth factor 1, and estrogens are important mediators of osteogenesis, indicating reasons to limit the use of certain drugs (e.g., ibuprofen), to avoid excessive food restriction, and to treat hypogonadism. Abnormal gait may be a correctable risk factor. Brief daily vibration may stimulate bone mineral accretion similar to weight-bearing exercise. Genetic factors contribute importantly to bone quality, affecting fracture susceptibility and providing new insights into fracture healing and tissue reengineering. **Key Words:** BONE BIOLOGY, GROUND REACTION FORCES, MECHANOTRANSDUCTION, MILITARY PERSONNEL, TRAINING STUDIES, GENOMICS

Soldiers and athletes are the two main groups that have motivated research in musculoskeletal research due to the risk of training injuries. For the US and the Israeli armies, a key focus of musculoskeletal research in recent years has been stress fracture. Stress fracture is somewhat specific to military training or at least occurs with greater regularity and frequency in the military compared with most athletic training programs, except distance running. Women are more susceptible than men, and the continuing expansion of female participation in the military makes this a relevant priority. Some of the initial high rates of stress fracture came from subjecting women to training programs that had been

successful for training men but which now produced much higher rates of musculoskeletal injury; 50% of women have one or more injuries by the end of basic training, including stress fractures. Weight-bearing exercise stimulates bone mineral accretion, but new exercise also stimulates bone and muscle remodeling and increases risk for stress fracture in some individuals. This carries enormous costs to the Department of Defense in terms of lost duty time, medical costs, and attrition of new recruits. No other agency deals with stress fracture issues of this magnitude. Thus, we targeted stress fracture in initial-entry training programs as a high-priority objective in military medical readiness research. This built on previous research by the US Army and Navy and related efforts by the Israeli Defense Forces (IDF), some of which will be described as background in this review. Stress fracture injury is currently being addressed through a concerted effort that leverages resources and expertise between Israeli and US researchers and that includes opportunistic funding and collaborations with academic centers. The focus of the research is to understand biomechanical influences on bone in the lower extremities, including interactions with genetics,

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Address for correspondence: Karl E. Friedl, Ph.D., Telemedicine and Advanced Technology Research Center (TATRC), Building 1054, Fort Detrick, MD 21702-5012; E-mail: karl.friedl@us.army.mil.

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diet, and other health habits, and to translate findings into practical solutions that will prevent stress fracture injury.

In 1994, special funding was provided by the US Congress to address military women's health issues. This provided an opportunity to address the high injury rates of women in training and to specifically address stress fracture (35). US and Israeli military researchers and extramural academic investigators working on stress fracture and related problems were funded through this program (4,5,15,30,45,67,68,75,77). In the following year, another funding opportunity was created through a congressional special interest program requested by the National Osteoporosis Foundation and Bone Coalition. Their intent was to expand funding opportunities in bone research through yet another federal agency, augmenting strong grant programs that already existed at the National Institute of Arthritis and Musculoskeletal and Skin Diseases and other organizations. Instead of duplicating programs in osteoporosis, army managers tailored this effort to the influence of exercise on bone biology in healthy young men and women, addressing the military stress fracture problem. It was apparent that many of the same risk factors for stress fracture were predictive of osteoporosis risk later in life, suggesting that interventions to prevent stress fracture might also benefit quality of life for military veterans later in life (36). The military program's focus on an actual fracture outcome also offered uniquely important contributions to osteoporosis and other bone disease research, where fractures are rarer and occur later in life (16). This program has continued intermittently for over a decade with support from the bone community and has now exceeded an investment of \$30M. Identified as the Bone Health and Military Medical Readiness (BHMMR) research program, the focus has been entirely on solving the problem of stress fracture. The purpose of this review is to summarize key findings from this BHMMR program, including some current studies that have been publicly reported but not yet published. These new findings cannot be meaningfully presented without the context of some of the previous and current related military research studies, particularly previous studies sponsored by the US Army and Navy and the IDF.

### Scope of the Problem

Initial epidemiological studies indicated high rates in both the US military (54) and the Israeli Defense Forces (IDF) (79). The data also indicated higher rates in women compared with men (54). Premilitary exercise activity was the single strongest predictor of risk for stress fracture (and other musculoskeletal injuries) in basic training for both men and women (56,109,110). Rates of injury are still high both in Israel and in the United States, especially for women. Even after a variety of training interventions that have reduced the incidence by more than half in the past decade, 6.6% of female recruits at the Marine Corps Recruit Depot encountered stress fractures in 12 wk of training (or 1.0 stress fractures per 1000 training days of exposure) (95). This

represents a significant impact to military readiness in terms of medical costs, lost duty days, and increased likelihood that the recruit will leave the military before completion of their first term of enlistment (119). These individuals are also much more likely to be reinjured with a second stress fracture (82). The most prevalent site of fracture is generally the tibia (79,80,95) but this varies widely between studies. For women in the military, the type of stress fracture may be more severe, including femoral and pelvic stress fractures (61,67,110), and these injuries have a longer recovery.

### Empirical Training Interventions

The uniqueness of stress fracture incidence in military training immediately suggests a solution—change the training. Athletes are generally not incurring these high rates yet successfully accomplish their training objectives. In earlier research, it was noted that stress fracture rates peaked in the third week of military training (92), and this was interpreted as a period of special susceptibility to injury during training-induced bone remodeling. It was hypothesized that a break in physical training activities at week 3 would allow bone remodeling to catch up and injury rates could be attenuated. The army surgeon general directed that this hypothesis be tested in a large study of recruit training at Fort Bliss, Texas. Unfortunately, the data from this study did not support the hypothesis. Rates of stress fracture/reaction ranged widely between 2% and 8% of the men within training companies, with no detectable differences between companies receiving the rest intervention and controls (92). Hypotheses regarding critical rest periods during remodeling may be more productively tested in the future with the inclusion of women and other experimental designs. Biochemical markers of bone remodeling responses could help pinpoint appropriate timing of a rest intervention. For example, a BHMMR study examined urinary markers of bone resorption every 3 d during 11 wk of marine basic training. Only the female marines had a large peak in deoxypyridinoline early in the training (by the second week), but the marker also peaked near the end of the training for both males and females at the same time that the volume of road marching in boots increased (111). This suggests that biochemical markers could be used to pinpoint group or individual periods of intense bone remodeling and reexamine the hypothesis that critical periods of rest will prevent the occurrence of stress fracture. A proven biomarker of injury risk could provide a simple intervention technology, especially in a convenient form such as a urinary semiquantitative test strip.

Some of the earliest investigations of stress fracture interventions focused on footwear, and the US Army changed policies from running in combat boots to running shoes in basic training. Israeli studies demonstrated substantial reductions in stress fracture rates using soft biomechanical orthoses (34). Although a simple sorbothane insole was not beneficial in a US study of 3000 marine recruits, there was

a trend to higher rates of stress fracture for individuals with the oldest running shoes (>6 months) (39), highlighting the importance of foot cushioning in prevention of stress fracture. The role of foot structure was also examined in a collaborative project with the Nike shoe company. Army recruits at Fort Benning, Georgia, demonstrated increasing musculoskeletal injury rates with increasing arch height, with the quintile of soldiers exhibiting the highest arches encountering nearly double the lower extremity injury rates of the quintile of soldiers with the flattest feet (26). A subsequent study of marine recruits observed that stress fracture injuries were higher with either low or high arches, with average arch heights at lowest risk (57). This is consistent with earlier findings in military recruits in Israel where flat feet were protective against femoral and tibial stress fractures but increased risk for metatarsal fractures (115). These observational studies can now be verified and extended through efficient laboratory studies of foot biomechanics and shock absorption (84–86).

Another practical approach to training interventions was to determine how much running was too much. A running dose–response study was conducted by the Naval Health Research Center with over 3000 male marine recruits, with three successive groups progressively receiving lower amounts of organized running during 11 wk of training. These data demonstrated that reducing total running volume from 55 to 33 miles reduced stress fracture by more than half (from 3.7% to 1.7%) and still produced final 3-mile run test times within an acceptable range (average <7 min-mile<sup>-1</sup>) (55). Although there was relatively little change in the majority of low-risk individuals, those identified as “high risk” for stress fracture appeared to gain the greatest benefit from the reduced mileage in terms of reduced stress fracture rates. In the first group with the highest total mileage, 902 men were classified as low risk based on prior-service physical activity questions (109) and 2.4% suffered stress fractures; 234 men were classified as high risk based on low fitness and activity levels and 8.5% suffered one or more stress fractures (55). In the final (third) group with the lowest total mileage, stress fracture rates were reduced to 2% of 247 high-risk men, indicating that this subset was specifically protected by limiting the training volume. A comparable set of improvements in female marine basic training changed running training by adding striders, eliminating formation runs and running on pavement and increasing the time wearing running shoes instead of boots for the first 4 wk. These injury countermeasures improved average final run times as well as the proportion of women graduating (118). These lessons have been repeatedly rediscovered with “epidemics” of stress fracture occurring whenever the discovered knowledge is not effectively passed on (2,38). The army recently institutionalized limits on training volume based on these and other studies demonstrating the net benefits to health and performance outcomes in basic training (63). These changes have substantially reduced but not eliminated stress fracture injuries.

## Imaging Properties of Bone that Predict Stress Fracture and Its Severity

Diagnosis of stress fracture has been a major challenge, with definitions ranging from high bone metabolic activity detected as hot spots with bone scans (“stress reactions”) to obvious high-grade fractures visible on plain film radiographs (6,54,56). The pragmatic approach has included functional impairment, with exquisite pain over the site of the injury and difficulty in weight bearing on injured lower extremities. Beck et al. (13) compared standard imaging approaches in tibial stress fracture evaluation to predict severity and recovery time. The ability to hop on the affected leg without pain was the standard of recovery. Fifty tibial stress fracture cases were compared by ratings of severity, and none of the four methods (plain x-ray, technetium bone scans, magnetic resonance imaging, and computed tomography) were very satisfactory in predicting the time to recovery; bone scan was the best (13). Altered gait is also a marker of injury and possibly even a predictor of injury risk; however, this requires a skilled observer or sophisticated equipment (84–86). Currently under investigation, mobile biomechanical assessments involving wearable sensors combined with sophisticated computational algorithms may eventually provide this information in an objective fashion (51).

Military programs have explored the use of bone mineral properties to predict stress fracture susceptibility. Initially, studies focused on bone mineral density (BMD) measurement technologies developed for clinical assessments of osteopenia and osteoporosis. An early study of recruits in the French Foreign Legion indicated that femoral and calcaneal stress fracture cases had 10% lower BMD (measured by dual photon absorptiometry) than uninjured male recruits at the end of training (93). A case–control study of US female soldiers found similar associations for 27 stress fracture cases with femoral neck BMD (69). In studies of 625 male and 708 female marine recruits at Parris Island, South Carolina, BMD was only different for the 37 women and not the 38 men with stress fractures (14,15). This study and an earlier study in Israel revealed that more important distinctions were provided by estimates of bone geometry (80,81). Using dual-energy x-ray absorptiometry (DXA) scan data, investigators calculated section moduli (Z) and bone strength indices (Z/bone length) for the femur and the tibia (14,15). From these data, men with stress fractures were found to have narrower bones but similarly thick cortices compared with uninjured men, women had thinner bones, and women with stress fractures had thinner cortices (15). This indicated sex differences in normal bone geometry as well as geometry differences between cases and controls, even after adjustments for height and weight. Recent and more detailed studies of West Point cadets included measures of tibial bone architecture using peripheral quantitative computed tomography (90) and confirm the findings that show thinner bones and thinner cortices in

women compared with men, with the same average body weights for men and women. This population represents an exceptionally fit group of young men and women yet they still encounter a very high rate of stress fracture in their first year when they conduct the largest volume of physical training (126). Although the men were still physically developing and showed a larger increase in BMD over 4 yr than the women, the women still incur higher rates of fracture (126). Jepsen et al. (53) and Tommasini et al. (116) discovered an unexpected relationship between growth patterns of long bones in mice and humans and bone properties. Slender tibiae typically found in young women (slender relative to weight) appear to compensate for loading with high BMD, reducing ductility to increase fragility. This may imbue a special susceptibility of young female bones to cumulative damage under intense repetitive loading (117).

Ultrasound has been investigated as a practical measure for use in the field and as a measure of important bone properties that may not simply reflect BMD. Qualitative ultrasound measurement of the calcaneus was significantly associated with stress fracture, signaling a twofold higher risk in a prospective study of 3758 female recruits at Fort Leonard Wood, Missouri (68). These measurements could be efficiently collected during the fitting of boots as recruits were processed. The training exposures included marching a total of 225 km (140 miles) on gravel roads carrying weapons and packs and running 135 km (84 miles) on asphalt roads during 8 wk of training (67). Unfortunately, the predictive measure was still not superior to that obtained from the response to the simple question of prior fitness history (109) or several other factors such as family history of osteoporosis and episodes of amenorrhea that also produce a twofold odds ratio (41,55). However, in a reanalysis of a slightly expanded data set, white women with low premilitary physical activity habits, a history of cigarette smoking, and a low qualitative ultrasound score were at significantly higher risk for stress fracture, primarily calcaneal and metatarsal fractures detected in this study (67). The bone properties actually represented in ultrasound measurements are uncertain but appear to involve something different than simply BMD. A recent study by the US Army Research Institute of Environmental Medicine also found that the correlation between calcaneal ultrasound measures and whole body DXA was poor (89).

### **Biomechanical Influences on Bone**

**Bone loading trades between microfracture accumulation and vigorous remodeling.** Clearly, mechanical loading can improve bone qualities and resilience against injury. The hypothesis that loading could improve bone resilience to fatigue was borne out in rodent studies where a 5-wk program of loading the ulna of one forearm produced nearly twofold increases in structural properties (e.g., minimum second moment of area, BMD) but realized a 100-fold increase in fatigue resistance of the bone (128). This suggests that a scientifically based and

monitored program can be developed to strengthen bone against training injuries rather than produce injuries. This has yet to be demonstrated in careful human studies.

Stress fracture injuries could be occurring as a result of an accumulation of microcracks in the bone that lead to a fracture with continued weight-bearing exercise or as a result of an exaggerated metabolic response in bone remodeling that occurs in response to the forces acting on the bone, including the accumulation of microdamage. Schaffler (105,106) proposed a testable hypothesis, suggesting that increased intracortical remodeling decreases the stiffness of cortical bone, permitting a rapid acceleration of microdamage during continued loading of the bone. Slowing the remodeling with an antiresorptive drug could prevent fractures, or it could accelerate fracture rates by slowing the repair of damaged bone. He tested this using a rabbit model of stress fracture and a bisphosphonate to reduce resorption. Although the rabbit model was not consistent, there may be some benefit from preventing resorption during the high impact, with a 50% reduction in bone scan activity and a trend toward reduced microdamage accumulation (107). However, bisphosphonate treatment was not beneficial in a study that attempted to prevent stress fracture in a randomized controlled trial with 324 Israeli recruits. The soldiers were administered residronate throughout 14 wk of basic training and were examined biweekly for tibial and femoral stress fracture, with no difference in stress fracture rates compared with the placebo-treated soldiers (78). A new imaging method has been developed that will permit exploration of the sequence of events in bone remodeling that accompanies new exercise demands, and it may help explain these observations with residronate. Microdamage in bones can now be detected *in vivo* after mechanical loading using an [<sup>18</sup>F]NaF tracer in positron emission tomography (72). Another technique being used in a BHMMR study (8) may also be useful in the further investigation of antiresorptive drug effects on stress fracture susceptibility. Ultrasound critical-angle reflectometry is a novel method developed in the laboratory of Peter Antich that allows characterization of elastic properties of bone. Osteoporotic patients treated with bisphosphonates demonstrated a reduction in bone elasticity using this technique, suggesting impairment of bone material properties with respect to fracture risk (96).

### **Fluid flow mechanisms of mechanotransduction.**

The need to understand bone remodeling under the influence of new exercise demands was apparent in attempts to describe the pathogenesis of stress fracture. The army program supported a series of fundamental studies on the transmission of mechanical signals to bone cells. External loading is transmitted locally in bone through a variety of forces including fluid flow, stretch, and magnetic fields. Substrate deformation (strain) appeared not to be as important as fluid flow effects on osteoblasts, primarily involving pressure and shear (136). Oscillating fluid flow, representing the physiological state that occurs within loaded bones, produced

immediate changes in intracellular calcium mobilization, peak activation of two mitogen-activated protein kinases within 30–60 min, and fourfold increase in osteopontin mRNA expression within 24 h (105,132,135). Through a series of such elegant studies with “exercised” cell preparations, it was shown that cell–cell communications are important to the bone cell responses, including prostaglandin E2 (PGE2) and nitric oxide secretion, certain kinases, and patterns of elaboration of a variety of bone matrix proteins (21,22,41,104,105,132,133,135). The effects on bone matrix proteins mediated through tight junctions was different than the cytosolic calcium response also stimulated by shear (132,135). Different types of fluid flow (e.g., reversing/oscillatory vs steady/pulsing) also produced different sets of responses, demonstrating a range of mechanisms involved in stimulating bone responses appropriate to specific demands placed on the bone (133,135). This type of understanding of the sequence of responses and the interplay of the range of mechanisms will provide a strong science basis for specific physical training regimens and refine empirical observations on the exercise programs that may prevent stress fracture (83). As an example, the elucidation of a refractory period in the intracellular calcium response to continued fluid flow stimulation (greater than 15 min) leads to a logical and testable hypothesis that dividing daily workouts into several discrete loading bouts might produce greater benefit to bone mineral accretion than a single bout (10,28,29). The important role of PGE2 in mechanotransduction (28,135) suggests another practical and important test regarding the use of some nonsteroidal anti-inflammatory drugs (NSAID) on interference with bone benefits of exercise (the hypothesis was tested in this program). More complicated problems such as bone loss in a weightless environment or in injury disuse may also be deciphered through these cell biology studies. For example, if load-sensitive connexin genes are not expressed in spaceflight, detected loads cannot be communicated to neighboring cells to stimulate the emerging cascade of events that appear to involve PGE2 stimulation of resorption and subsequent normal triggering of bone formation activities (138). Another important application of these fluid flow studies is in the refinement of tissue level observations made with high-frequency loading, as discussed in the next section.

**Vibration.** An early army investment in stress fracture physiology involved repetitive loading of the turkey ulna as a model of bone remodeling (99). The understanding of repetitive loading and fluid flow that came from this model contributed to the development of a simple nonpharmacological method to build bone in osteopenic individuals using a controlled mechanical stimulus. Low-magnitude, high-frequency loading of the bone could suitably replace or augment the osteogenic benefits of weight-bearing exercise (100,101). This could also be useful for soldiers at high risk for stress fracture. This was shown to be effective in increasing bone density in a study of premilitary age boys and

girls with low bone density and a sports fracture. The intervention increased bone density over control patients with very brief daily exposures to vibration (30 Hz, 0.3g) over a 1-yr period (43,44). The change in bone density was based on computed tomography after discovery of discrepancies in DXA estimates of BMD in growing children (i.e., changes in skeletal area and body composition) (130). These studies in humans are being complemented by studies in mice to optimize the treatment regimen and to further understand the effects in growing bones, already demonstrating a specific increase (30%) in bone formation rates on endocortical surfaces without negative effects elsewhere (131). Optimal dosing is also being evaluated. Qin et al. (94) has found evidence that marrow blood supply can be reduced by repetitive long duration fluid pressure applied in bone. Bone formation was stimulated even at low fluid pressure when the bone is loaded at a high rate; at the other extreme, high levels of pressure may trigger pathological remodeling and reduce bone quality (91,94).

**Ground reaction forces and gait analyses.** Running style may be an important determinant of stress fracture based on studies of the ground reaction forces that include not only peak tibial shock (84) but also biomechanical factors that place forces on the bones in other ways. An early study with Israeli recruits intensively evaluated for stress fracture risk found that external rotation of the hip was an important risk factor (42). A recent analysis of female distance runners indicated a greater absolute peak free moment (torque on a vertical axis that represents resistance to toeing in or out) that distinguished runners with tibial stress fractures from uninjured runner and suggested a predisposition to spiral stress fracture (84). Thus, it may be possible to prevent re-injury by retraining running styles. Conceivably, gait studies could be used diagnostically to prevent stress fracture and other musculoskeletal injuries (86). Another study in the program is using a unique approach to imaging, ultrasound critical-angle reflectometry, to measure bone elasticity. The particular goals of the study are to use this imaging technique to chart changes in elastic properties of bone in response to changes in strike mechanics (e.g., trained forefoot running) and running incline. This ambitious project is combining the ultrasound measurements of tibial bone with magnetic resonance imaging studies of the patterns of muscle recruitment to describe the influence of mechanical loads induced by muscle on bone properties (8).

**Mode of exercise and bone properties.** The effect of exercise training on BMD was examined in the BHMMR program with a retrospective meta-analysis. This project also tested the statistical technique with a reanalysis of the individual study data points, revealing similar associations. Weight-bearing exercise, activities involving jumping, and resistance exercise of moderate intensity conducted several times per week increased BMD by 1–3% in young and older men and women (58–60). Results were almost certainly confounded by bone site specificity associated with the mode of exercise. Many of the studies in this analysis did

not focus on what appear to be the most affected sites associated with the particular exercise; thus, the benefits to bone may be greater than observed in the meta-analysis.

New studies are underway to examine the effect of supervised resistance and aerobic training programs on bone properties. A joint US-Israel military study found that 13 wk of recruit training with men and women increased biochemical markers of bone formation and had no effect on markers of bone resorption (7). A parallel but controlled study of supervised training in healthy female college students had similar findings of increased markers of bone formation but not resorption for groups involving resistance exercise, aerobic exercise, and combined exercise, with smaller changes in the aerobic-only group (71). Women assigned to aerobic exercise compared with controls demonstrated measurable increases in trabecular BMD in the distal tibia, assessed using peripheral quantitative computed tomography (33), but similar changes were not evident in groups receiving resistance exercise (70). These studies highlight the need to better understand the significance of the markers and measurements used in bone studies.

### Dietary Interventions

Both Israeli and Finnish recruits with stress fractures demonstrate lower average vitamin D (25-hydroxy vitamin D) levels than their noninjured peers (45,103). The actual importance of calcium and vitamin D in stress fracture susceptibility was unknown until a recently completed randomized controlled trial. Female recruits at the Great Lakes Naval Training Base in Chicago were supplemented with calcium ( $2 \text{ g}\cdot\text{d}^{-1}$ ) and vitamin D ( $800 \text{ IU}\cdot\text{d}^{-1}$ ) and compared with placebo treatment. The intervention was successful in reducing stress fracture rates by 27% in a test population of 3703 young female recruits (66). This substantial blanket supplementation (additional to normal dietary intakes) was still below safe upper limits ( $2.5 \text{ g}\cdot\text{d}^{-1}$  calcium,  $2000 \text{ IU}\cdot\text{d}^{-1}$  vitamin D) (1). This suggests a simple and safe nutritional intervention to stress fracture occurrence.

Inadequate adult intakes of calcium and vitamin D are associated with increased rates of osteoporosis and osteomalacia, respectively. Bone disease outcomes formed an important part of the basis of the recommendations for the revised Dietary Reference Intakes in 1997, funded in part by the BHMMR program. There was still insufficient experimental data on bone outcomes for either of these nutrients for the expert committee to recommend an estimated average requirement. Instead, an adequate intake was provided, with  $1000 \text{ mg}\cdot\text{d}^{-1}$  calcium and  $200 \text{ IU}\cdot\text{d}^{-1}$  vitamin D for the age range encompassing most soldiers. An adequate intake is experimentally determined based on intakes of a group of healthy people, leaving uncertainty about the proportion of the healthy population actually covered by this intake (1). The new recommendations were adopted in a revision of military nutrition standards

influencing the composition of field rations as well as master menus (3).

Calcium intake recommendations were challenging for the Institute of Medicine due to the limited data for any outcome measure, but there was even greater uncertainty about the variability in the population for vitamin D. Key research gaps included genetic differences in vitamin D metabolism, skin pigmentation effects on vitamin D metabolism in the sun, and geographical differences in sunlight exposure (1). An army request for proposals emphasized these gap areas identified by the expert panel for the Dietary Reference Intakes. These factors are currently being addressed in studies in the BHMMR program at Creighton University and Medical University of South Carolina. Preliminary data indicate inadequate vitamin D intakes during late winter in northern climates (49). This is a uniquely important topic for the military due to the special environments with extended sun deprivation affecting vitamin D levels and bone health such as submarines and Antarctic service (31,137).

Although vitamin D increases calcium uptake from the small intestine, exercise itself plays a known role in increasing calcium bioavailability (1). In a group that is already optimized for fitness and exercise habits, cadets at the US Military Academy, milk consumption was still one of the most important factors associated with BMD at multiple sites in 755 young men (102). Men, more than women, appear to still be in a significant skeletal growth phase at military recruit age, and vitamin D and calcium intakes may be particularly important to the prevention of stress fracture, although this has yet to be demonstrated. An unpublished study followed lumbar bone density 6 months in young 94 male and 84 female cadets demonstrating 4.7% and 2.8% increases in lumbar (L1-L4) BMD through 4 yr at the US Military Academy, generally corresponding to a period of observation between ages 18 and 22 (126). As a separate observation, the men from this study inexplicably showed a decline in BMD 6 yr later whereas BMD for the women remained stable (88).

Other nutrients of potential importance to bone health were investigated, including zinc (108) and protein-fortified supplements. A particularly novel concept for an osteogenic supplement (vitamin K, protein, and other specific components intended to enhance calcium bioavailability) in a specially formulated military food bar and taste-matched isocaloric food bar was tested with navy midshipmen at the US Naval Academy (19,20). Unfortunately, this 3-yr prospective study failed due to compliance and attrition problems, leaving an intriguing countermeasure yet to be proven (20). Another study investigated high protein intake effects on bone formation, testing the hypothesis that high protein intake stimulates insulin-like growth factor 1 (IGF-1) secretion rates. It was further hypothesized that exercise and protein might together synergistically stimulate IGF-1 responses, with measurable benefits to osteogenesis. The study demonstrated significant but small elevations

of circulating IGF-1 and urinary markers of bone formation with very high dietary protein ingestion ( $2.2 \text{ g}\cdot\text{kg}^{-1} \text{ body weight}\cdot\text{d}^{-1}$ ) and a 6-month training program compared with still relatively high protein intakes ( $1.1 \text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ ) and carbohydrate supplementation (9). Although only male data were analyzed in the most recent study of West Point cadets that found bone benefits of milk consumption in this population, the recent vitamin D and calcium supplement study at Great Lakes Naval Station (described above) demonstrates specific benefits to preventing stress fracture with supplementation of the female recruit diets. A conclusion from all of these nutrition studies in the BHMMR program is that whole food provided in the form of milk products with high protein, calcium, and vitamin D, if tolerated by the individual, is an optimal nutritional intervention with advantages to stress fracture prevention.

Negative effects of health behaviors on bone were examined, especially for interactions with exercise influences. Prostaglandins play an apparently important role in bone formation in response to biomechanical influences. Kohrt and Schwartz (65) tested this hypothesis with a randomized controlled study evaluating BMD responses over 9 months in female runners. A group using ibuprofen immediately before exercise was compared with women taking ibuprofen immediately after exercise and to placebo. Preliminary data analyses indicate that ibuprofen blocked the bone benefits of exercise (65). This practical finding is important to the military where nonsteroidal anti-inflammatory drug (NSAID) use is prevalent. It also confirms the importance of prostaglandin in the mechanism of exercise-induced bone mineral accretion. A separate animal study using female rats with loading-induced stress fractures indicated that a cyclooxygenase-2 enzyme inhibitor may delay healing (bone formation rate) (74). These two studies help to confirm the importance of the role of prostaglandins in normal bone mineral accretion in response to biomechanical stimuli and provide practical guidance for use of NSAID to avoid compromising exercise benefits and bone healing.

Important effects of chronic ethanol abuse were observed, and the results implicated mechanisms other than simple ethanol toxicity (76,123). These centered on reduced bone formation reversible by parathyroid hormone but not affected by exercise (121,122). This occurred at relatively low levels of ethanol administration to rodents, equivalent to relatively small daily consumption for humans, suggesting that ethanol ingestion may impair bone formation during exercise training and delay bone repair mechanisms after injury (121). This included ethanol-induced reduction of mRNA levels for IGF-1. This was important to the military due to the continued high prevalence of alcohol abuse by young men and women consistent with their civilian peers.

### **Sex Steroid Influences on Bone**

The critical role of estrogens in maintaining BMD and bone strength has long been recognized through the changes in susceptibility to osteoporosis that follow sharp reductions

in estrogen exposure in menopause. Less well known are the androgen influences on bone, although the typically more gradual reduction of testosterone during andropause in males is now also a recognized cause of male osteoporosis and treated with androgen replacement therapy. Both the principal male and female sex steroids, testosterone and estradiol, were investigated for their important effects on bone. It should be noted however that direct effects of sex steroids on bone are difficult to demonstrate unless there is a profound and sustained hypogonadism, as normal day-to-day and circadian variations in blood hormone levels will not adequately reflect the chronic hormonal effects on bone. This was confirmed in a West Point study where serum testosterone and estradiol were not significantly lower in men and women with stress fractures (even though stress fracture prevalence was high), although there were some associations with BMD and changes over time (126). A better indicator of chronic hypogonadal status is available for women in the form of menstrual cycles; several epidemiological surveys have connected amenorrhea and oligomenorrhea for periods of 6 months or longer to increased risk for stress fracture in military women (36,95).

An important series of studies by Anne Loucks at the University of Ohio demonstrated that exercise does not induce amenorrhea in healthy young women if expended calories are adequately replaced. In other words, exercise itself was not a cause of amenorrhea (75). Previous associations made between high volume running, amenorrhea, and bone loss were confounded by exercise as one of the common weight loss behaviors associated with disordered eating in anorexia. Furthermore, men were subject to the same influences of energy deficiency on sex steroid suppression, although men appear to be able to tolerate a large deficit before becoming hypogonadal. Within 5 d of inadequate energy intake by the women, biochemical markers of resorption increased and markers of bone formation decreased, demonstrating the rapid and important effects of sex steroid hormones on bone health (52). This occurred although they continued to exercise. In a separate multicenter study of female collegiate distance running athletes, disordered eating as measured by the eating disorder inventory was a risk factor for low BMD that appeared to be independent of amenorrhea (23). This highlights the importance of specific dietary components that affect bone in addition to overall energy balance.

Manipulation of hormone levels with exogenous administration was also examined as part of the BHMMR studies. One study evaluated estrogen replacement of female distance runners. The concept was that low-dose oral contraceptive pills would provide estrogen exposure sufficient to protect bone against stress fracture during intense training even if food intakes were inadequate or in the presence of underlying amenorrhea. A randomized trial evaluated the effects of low-dose estrogen administration on stress fracture occurrence and BMD in collegiate female distance runners over 2 yr (62). This study, involving recruitment of subjects from five different academic centers, provided

30  $\mu\text{g}$  ethinyl estradiol per day (and 0.3 mg norgestrel) to the treatment group. Although 150 women were baselined and randomized in the study, 127 returned for the 1-yr follow-up and 96 returned for the 2-yr follow-up; thus, the statistical power of the study was weakened by high attrition. There were also unexpected compliance challenges, where 33% of the continuing participants switched groups during the 2-yr period; this included a nonrandom change, with amenorrheic women being the least likely to use or continue with treatment. Nevertheless, in the eighth year of attempting to complete this very challenging study, preliminary analyses indicate a trend to improve BMD with treatment compared with no treatment. An important finding that emerged from this analysis is that amenorrheic/oligomenorrheic women may benefit from estrogen replacement if normal cycles cannot be restored (62).

Low-dose estrogen administration could theoretically increase risk of bone loss in women with normal ovarian cycles of estrogen secretion. This could occur by reducing the total estrogen exposure or by suppressing associated secretion of various androgens (such as testosterone and dehydroepiandrosterone). The preliminary results from the Kelsey study with distance runners administered low-dose estrogen suggest against a negative effect although the greatest benefits appeared to be for women with already suppressed ovulatory cycles (i.e., less than normal estrogen levels). The progestagen in the drug formulation could also provide weak androgenic activity. Gordon et al. (46–48) extensively evaluated the effects of weak androgens such as HEA on bone formation and other local responses including antiresorptive cytokines. Their studies focused on anorexic women deficient in sex steroid secretion and with weak bones. In a randomized trial, 61 young women with anorexia were administered either DHEA (50  $\text{mg}\cdot\text{d}^{-1}$ ) or hormone replacement therapy (ethinyl estradiol = 20  $\mu\text{g}$ ; levonorgestrel = 0.1 mg) for 1 yr. Improvements in hip and spine BMD were accounted for by weight gain that occurred in both groups, although both groups had decreases in some of the biochemical markers of resorption, and DHEA administration appeared to have a greater effect on circulating IGF-1 and markers of osteogenesis (47). Thus, DHEA administration appears to have provided benefits, but it is unclear if these occurred through direct actions on bone or were mediated through other factors.

Wren et al. (129) used an elegant model to trace the effect of androgen on bone *in vivo* by selectively increasing androgen receptor in immature and mature osteoblasts in male and female mice. The effect was to reduce overall bone turnover and to produce a lower quality bone with reduced stiffness, cortical area, and strength, although trabecular bone volume was increased and activity on the periosteal surface increased. Thus, the direct effects of this “superandrogenization” on bone do not explain the observed beneficial effects of androgen replacement in male osteoporosis that also includes many indirect mechanisms of androgen action (e.g., stimulation of IGF-1 secretion,

metabolism to estrogens, and nonspecific receptor binding). There has always been concern about the adverse effects of exogenous androgens to the growing skeleton, but these results suggest new reasons for concern. The interactions of androgens with key loading signalers such as IGF-1 and PGE2 is still poorly understood, but these may be preferable for pharmacological support in special circumstances where exercise loading may not be feasible such as a weightless environment in spaceflight (138).

## Genomic Studies

Most (70%) of the variability in human BMD has been attributed to genetics, but other aspects of bone properties such as fragility are not so well understood. Friedman investigated the potential role of type I collagen genes and stress fracture in IDF soldiers. Mutations in COL1A1 and COL1A2 have been implicated in the brittleness of bone in osteogenesis imperfecta (40,97). Ultimately, Friedman did not find a distinction in genetic profiles between men with stress fracture compared with those without (37,45), including no new association between vitamin D receptor gene and individuals with stress fracture or low bone density (32,37). Other studies (124), including some with results not yet reported, find similar negative results for associations between specific genetic markers commonly associated with bone and stress fracture or other measures of bone quality, suggesting that the relationship between these specific gene polymorphisms and bone properties may be more complicated (e.g., involving another nearby locus yet to be described).

As evidenced by many of these projects, bone integrity is modifiable by environmental factors. It may also be quite a bit more subtle than the differences observed in disease states affecting bone. Both of these factors make mouse models particularly useful in identifying genes that affect bone properties and in studying their epigenetic interactions (17). Blank (17) gives the example of one gene identified through quantitative trait loci (QTL) mapping, *Alox15*, that may be important in favoring differentiation of mesenchymal stem cells to adipocytes (instead becoming osteoblasts). This gene is important in metabolizing arachidonic and linoleic acids to peroxisome proliferator-activated receptor gamma agonists, possibly the basis of its role in femoral BMD in mice but, even more importantly, it may perform this same role through its occurrence in a human BMD QTL region (17). In the army program, Shi et al. (114) investigated the role of peroxisome proliferator-activated receptor and their laboratory discovered the importance of glucocorticoid-induced leucine zipper protein in moderating the rate of adipocyte differentiation, where fat cells in the marrow are increased at the expense of osteoblasts in response to some drugs such as glucocorticoids (113). This general line of studies opens the door to understanding the balance in nature that may limit human performance enhancement. For example, “supermice” transgenic strains with *wnt10b* protein genes that favor development of

osteoblasts over adipocytes are characterized by bones that are four times the normal density but this density may compromise elasticity, allowing great strength for load bearing but increasing fragility and injury risk. This is an example of how mouse genomics can systematically uncover previously unrecognized mechanisms and provide a scientific basis for sound interventions. The US Army supported a series of such mouse genomic studies of bone properties through a network of collaborating laboratories.

One study has already been reprinted in 2005 as a classic report by the *Journal of Bone and Mineral Research* (12), and this is cited as a model for the use of genomics to uncover determinants of bone properties (16). The study tested crosses of B6 and C3H mouse strains, corresponding to mice with low and high bone mass (producing larger mice with intermediate BMD), and identified key alleles that were additive in their effect on BMD (12). They also found QTL that mapped to both femoral and vertebral BMD and other QTL that mapped only to specific sites, consistent with clinical findings of disparate femoral and vertebral BMD. Overlapping QTL alleles for other phenotypes in related investigations, body weight and serum levels of IGF-1, suggest the possibility of more complicated interactions and indirect effects of the identified QTL on BMD (11,12). The C3H mice with high bone mass had 35% higher circulating levels of IGF-1 than the lower bone density B6 mice; the bone IGF-1 was also higher, possibly signifying important local autocrine roles (98).

IGF-1 effects on bone mineral accretion were also tested in other studies using new congenic strains and knockouts, again demonstrating the importance of this key hormone (18,87). More complicated interactions with exercise were also observed. Exercise (20 jumps per day, 5 d·wk<sup>-1</sup>, 20- to 30-cm heights, for 4 wk) increased serum IGF-1 in C3H mice only but increased tibial bone area and periosteal bone formation only in B6 mice (64). This suggests that IGF-1 receptors were saturated in the C3H mice (that already had

high bone density) and pointing to other mechanisms that must be important in the B6 mouse bone improvements.

A substantial family of projects funded through the BHMMR program have continued this work with systematic exploration of the genetic determinants of bone properties including stiffness (“Young’s modulus”) and fragility (53,120,125,134), the relationships to body composition and bone properties (with data populating the “phenome database”) (27), and other aspects of bone regional strength and microstructure (92,112,120), epistatic interactions between pairs of genes with comparisons of many strains (16,37) and many methodological improvements (24,25,73,74). The data from these studies are now being translated through other programs to human parallels in large data sets from the Anhui Province in China and from the Framingham study (50).

Wang et al. (127) studied fracture healing in mice to characterize the variety of genetically determined patterns of healing that recapitulate developmental processes, shedding light on how bone quality is determined as well as how fractures heal. By focusing on the transcription and the coordinated response of many expressed genes, they documented the different strategies followed by different strains of mice within phases of bone healing. These studies demonstrated a consistent sequence of proteoglycan removal followed by collagenous protein matrix dissolution before osteoclastic activity in early cartilage tissue formation. These and other studies funded in the BHMMR form a foundation for the development of new tissue engineered replacement and repair of articular cartilage that makes up load-bearing structures in new military research programs.

## CONCLUSIONS

The BHMMR research program has been successful in advancing knowledge to prevent stress fracture for several reasons. A full range of techniques, models, and

TABLE 1. Examples of key accomplishments of the Army’s Bone Health and Military Medical Readiness (BHMMR) research program, showing the interrelationship between basic discoveries and potential applications of those discoveries.

Basic Research Finding	Application to Test/Tested
Discovered that tight junctions communicating fluid flow signals to osteoblasts become refractory to continued stimulation within 15 min (10,28,29,83)	Physical training programs intended to provide maximum benefits to bone responses should be conducted in short and more frequent daily bouts
Discovered that repetitive loading of the rat ulna produces bone that is much more ( $\times 100$ ) resistant to bending stress than other commonly measured properties would indicate ( $\times 2$ improvement in bone quality) (128)	If loading can produce large benefits, this may explain why fitness history has such high predictive value for stress fracture in basic training. A bone training program (e.g., jumping) might be devised that produces much larger benefits to reducing fracture risk than previously imagined (83)
Characterized stimulation of osteogenic responses from high-frequency but low-level vibration in developing bone (131)	Vibration at high frequency was tested on this basis and shown to provide 1–3% increase in BMD in young men and women of premilitary age (43,44)
Explored the significance of IGF-1 in genetic models, finding repeatedly that BMD is importantly influenced by the expression of IGF-1 genes and by local and circulating levels of the hormone (11,17,98)	Chronic stimulation of IGF-1 levels through environmental influences such as high protein diet in addition to exercise might increase BMD (9)
Explored differences in gaits in runners recovered from stress fracture injuries that were associated with higher ground reaction forces in biomechanical studies (84–86)	Inappropriate gait may be also be the cause of stress fracture and might be prevented with retraining of running style
Discovered a regulatory mechanism, glucocorticoid-induced leucine zipper protein, through which corticosteroids moderate the balance between adipogenesis and new osteoblasts (113)	This discovery may provide new opportunities to moderate the effects of chronic stress on obesity and osteopenia

TABLE 2. Examples of key accomplishments of the Army's Bone Health and Military Medical Readiness (BHMMR) research program, showing results of tested applications that helped advance scientific understanding.

Tested Application	Contribution to Scientific Knowledge
<p>Ibuprofen and related drugs that reduce PGE2 production may block bone mineral accretion if taken just before weight-bearing exercise, as PGE2 appears to be important <i>in vitro</i> and in animal studies</p> <p>Antiresorptive drugs (e.g., bisphosphonates) should reduce the rate of remodeling and may reduce stress fracture risk during periods of intense training; data from a rabbit stress fracture model provided some support to this hypothesis</p> <p>High-volume exercise may produce amenorrhea only indirectly, when increased energy demands are not met with adequate energy intake</p> <p>Suppression of ovarian cycles (assessed by reduction in LH pulsatility) may produce a reduction in estrogen exposure that will cause adverse changes in bone metabolism</p>	<p>Found that there was significantly less gain in BMD in women taking ibuprofen just before running which indicates that prostaglandins are indeed important in mechanotransduction in bone (65)</p> <p>Found that there was no benefit (and possibly a higher rate of injury), which indicates that remodeling provides more resilient bone, possibly by eliminating brittle bone (78)</p>
<p>Oral contraceptive formulations (e.g., continuous low estrogen and progestagen combinations) may provide protection to bone during high-intensity training in female collegiate distance runners</p> <p>Calcium and vitamin D is important to bone health and, even acutely, dietary supplementation may provide benefits to reducing stress fracture incidence</p>	<p>Pinpointed energy balance, not exercise, as the important determinant of changes in LH pulsatility (75), suggesting a hypothalamic glucostat regulatory function</p> <p>Observed acute changes in markers of bone metabolism following measured changes in LH pulsatility (52), suggesting greater than expected sensitivity to hypoestrogenemia, or perhaps important direct effects of gonadotrophins on bone</p> <p>Identified a subset of women (those with menstrual dysfunction) who appeared to show benefit from estrogen "replacement" therapy (62), suggesting that the cyclic estrogen environment in normally menstruating women maintains bone</p> <p>Demonstrated a substantial reduction in stress fracture incidence (66), which suggests that calcium availability is important and may be rate limiting during remodeling in young bones during intense exercise</p>
<p>Genetic markers of bone disease that related to bone quality such as Col1A missense genes, estrogen receptor gene, and vitamin D receptor gene might be productive predictors of stress fracture susceptibility in new recruits</p>	<p>Found that important bone disease genes did not provide a common explanation for stress fracture injury, suggesting more complex factors and epigenetic interactions (37)</p>

multidisciplinary expertise of engineers and scientists was accessed to solve a specific problem. This problem-solving focus required a different approach than a more traditional process of distributing funds to independent investigator-initiated grants. This included extending difficult projects until key goals had been achieved, keeping specialized and productive teams together, convening exchanges and assessment of preliminary data and overall objectives, responsiveness to new findings with agile support to additional study aims and new collaborations, and supporting military medical collaborations studying relevant populations and conditions.

The results of these studies provided near-term interventions that, if implemented, could substantially reduce the risk of stress fracture in susceptible individuals. The basic research provided new insights into points of intervention, providing new science to apply in practical tests (Table 1). Studies in this program also provide a nice example of the thesis that well-designed applied studies nearly always contribute to improved understanding of basic mechanisms (Table 2). Simple science-based interventions include vitamin D and calcium supplementation and constraints on running volume. More complicated and costly interventions include identification of individuals at risk (based primarily on entry level fitness levels) with individual treatments to build bone density (e.g., vibration platform treatment), correct estrogen deficiency in women, and retrain abnormal running gaits. Longer-range interventions will likely include practical and accurate imaging and biomarkers of risk (e.g., automatic gait assessment, biochemical markers of bone activity) to determine who is at risk, when they

should temporarily curb further physical activity, and precisely what forms of exercise will most benefit the strengthening of their bones for the demands to be applied to those bones.

The interest that has been stimulated in the research community in the military's problem of stress fracture will continue to help generate new ideas and advance understanding in this area. There are many dual-use applications of these findings, with contributions to the understanding of osteoporosis as properties of bone and the role of remodeling becomes clearer. Similarly, investments in the understanding of other bone diseases contribute to solving the stress fracture problem by exploring the determinants of brittle bone properties in osteogenesis imperfecta and by understanding the normal and abnormal regulation of osteoclasts in Paget's disease that may help to explain greater susceptibility to stress fracture through suboptimal remodeling responses.

This program has been made possible by the individuals who thoughtfully conducted the research, including those who collaborated closely with the military to ensure that their work addressed relevant concerns. It has also been made possible by the management staff, notably Ms. Buffy Burdette, the excellent scientific reviewers from the bone research community who have participated in various levels of review of the program, and the proponents of this program, including Dr. Joan Goldberg (American Society for Bone and Mineral Research), Ms. Bente Cooney (National Osteoporosis Foundation), and Ms. Charlene Waldman (Paget's Foundation).

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