S. S. PATIL

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Genetics An Intrinsic Risk Factor For Stress Fractures
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Sheetal Shivaji Patil

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Christopher J. Vonach
Major Professor's Signature

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GENETICS AN INTRINSIC RISK FACTOR FOR STRESS FRACTURES

By

Sheetal Shivaji Patil

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ABSTRACT

GENETICS AN INTRINSIC RISK FACTOR FOR STRESS FRACTURES

By

Sheetal Shivaji Patil

Stress fractures are an overuse injury to bone resulting from accumulated repetitive load cycles. There is a mounting body of indirect evidence that genetic factors could play a role in stress fracture predisposition. Identifying genetic variants which increase the susceptibility to stress fractures would improve identification of athletes predisposed to stress fractures and could lead to a greater understanding of the patho-physiology and treatment of stress fractures. PURPOSE: To determine the influence of T to C transition within exon 2 of the vitamin D receptor (VDR) gene defined by endonuclease Fok 1 on the risk of stress fractures in competitive athletes. METHODS: Twenty-seven competitive athletes, 12 with stress fractures and 15 without stress fractures volunteered for the study. DNA analysis was done by restricted fragment length polymorphism. Medical and sports history was obtained through a questionnaire. RESULTS: Group comparisons indicated that the groups were equivalent in their body composition and stature. Each athlete with a stress fracture was matched with one control on the basis of age, gender, race and sport. Chi-square done on 24 subjects revealed no significant difference in the genotype distribution between the stress fracture and the control groups ($p = 0.65$). CONCLUSION: The VDR gene polymorphism is not involved in the pathogenesis of stress fractures.

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Chapter 1

INTRODUCTION

Stress fractures are overuse injuries to bones resulting from accumulated repetitive load cycles. Unlike a typical bone fracture, stress fractures occur due to repeated, low stress loads rather than a single, high-stress load cycle. Tiny cracks in bones can develop when muscles become fatigued and can no longer absorb the shock of recurring impacts. When this happens, muscles transfer the stress to the bones, creating a fracture (Bennell - 1997). Early stress fracture incidence studies in army recruits suggest the rate of stress fractures among female military trainees during basic training was higher (11.5%) than their male peers (7.5%) (Shaffer –1997). By the mid-1900's, the condition was reported in non-military populations with increasing frequency (Beck –2000 and Frisch, R. E – 1981).

Only a fraction of athletes and military recruits undergoing similar vigorous training sustain fracture, suggesting the existence of pre-existing risk factors for this injury. In addition to factors such as nutrition, demographics and hormone levels, stress fractures are associated with several bone-strength determinants including bone mineral density, bone geometry, micro architecture, bone turnover, and material properties such as collagen cross linking (Chesnut –2001). Decreased bone density may be due to a variety of factors, including mutations within genes involved in bone formation, remodeling, or bone matrix formation (Finestone –1991). Therefore, when a genetically susceptible individual participates in strenuous exercise, this environmental stress exposes the otherwise clinically understated mutation and results in stress fracture (Finestone –1991).

A stress fracture in an athlete affects the person physically, emotionally and financially. Much sport time is lost, and a financial burden is placed on the individual due to surgery and other costs. Identifying genetic variants which may increase susceptibility to stress fractures would improve identification of athletes predisposed to stress fractures. This could lead to a greater understanding of the patho-physiology and treatment of stress fractures, resulting in lower incidence of stress fractures in susceptible athletes.

Despite the previously mentioned potential benefit, little is currently known about the influence of genetics on stress fracture risk. However, genetic variants influencing bone mineral density would be likely candidates. Several polymorphisms of the vitamin D receptor (VDR) gene have been identified and correlated with bone mineral density (Fleet–1995, Morrison et al –1992, Zmuda–2000, Langdahl–2000), including one involving a T to C transition (ATG to ACG at the first potential start site) (Sajio -1991, Sturzenbecker–1994, Arai–1997). This polymorphism creates an alternative translation start codon that results in a shorter isoform of the VDR gene (Cusack –2003).

The Fok 1 polymorphism in the VDR gene has been associated with a 13% lower lumbar spine bone mineral density and a greater rate of bone loss at the hip (4.7 vs 0.5% for ff vs FF genotypes. 'F' for the absence of the endonuclease Fok1 restriction site and 'f' for its presence) in post menopausal Mexican-American women (Gross–1996). Furthermore, children with the FF genotype absorb more calcium and have an 82% greater bone density than children with the ff genotype (Ames -1999). These previous results suggest a substantial relationship between the VDR gene and bone health at one or more levels.

Nakamura et al (2002) reported that bone mineral density in young male athletes with different impact loading depended on the VDR genotypes. They suggested the FF genotype might respond more sensitively to differences in impact loading in regulating bone mineral density, rather than merely being a predictor of high BMD. Nakamura et al (2002) VDR genotypes, and bone phenotypes of the lumbar spine and femoral neck in 44 highly trained young male athletes and 44 age-matched non-athletic controls. On average, the athletes had a significantly higher bone mineral content than controls, resulting from a combination of increased volume and density at both sites. When the athletes were compared with the controls within each VDR genotype, however, increased spinal volume was found only in athletes with the FF but not in those with the Ff genotype. Differences in bone mineral content in the lumbar spine and femoral neck between the controls and the athletes were greater in subjects with FF than those with Ff. These findings suggest the adaptive response of bone to exercise in athletes may depend in some part on inter individual allelic variance of the VDR gene at the translation initiation site. If this genetic marker is associated with susceptibility for stress fractures in competitive athletes is not known.

The purpose of the study was to determine the influence of T to C transition within exon 2 of the VDR gene defined by endonuclease Fok 1 on the risk of stress fractures in competitive athletes. We hypothesized the possession of f –allele will occur more frequently in athletes with a history of stress fractures compared to athletes without stress fractures who are similar in activity, age, gender and.

Chapter 2

LITERATURE REVIEW

Breithaupt first described stress fractures as the "syndrome of painful swollen feet associated with marching" among Prussian soldiers in 1855 (Bijur –1997). For many years following his description, stress fractures were almost solely described in military populations. However, in the last 15-20 years, incidence of stress fractures has been increasingly recorded in non-military populations. As a larger segment of the population adopts a lifestyle that includes vigorous weight bearing activities such as running, soccer or gymnastics, stress fractures become a growing concern for clinicians and athletic trainers.

Most reports from US military recruit populations find the incidence of lower extremity stress fractures to be 0.2-4.0% in men (Almeida -1999, Beck 1996 and Jones–1993 a) and 1.0-7.0% in women (Kelly-2000 and Knapik-1997). In non-military athletic population the highest incidence is reported in members of track and field teams with rates varying from 10-31% (Johnson –1994 and Beck –2000). Other activities with high rates of stress fractures are lacrosse, figure skating, gymnastics, ballet, basketball, soccer and aerobic dance (Johnson-1994).

Pathogenesis

Over the years the theories proposed to explain the etiology of stress fractures included spasticity and spasm of the interosseous muscles (Deutschlander -1921), impaired circulation (Solane –1936) and inflammatory causes such as non-suppurative osteomyelitis (Roberts –1939). Detlefsen (Detlefsen –1937) and Hartley (Hartley -1942, 1943) both hypothesized that stress fractures are related to bone exhaustion, similar to

fatigue fractures. Stress fracture development represents the end product of bone fatigue that results from repetitive loading. The processes of micro damage accumulation and bone remodeling play an important function in stress fracture pathogenesis.

Skeletal turnover is the result of bone remodeling and is achieved through the activation of microscopic bone remodeling units called basic multi cellular units (BMU) consisting of osteoclasts and osteoblasts respectively resorbing and laying down new bone (Peters -1993). Osteonal remodeling primarily serves to remove and replace fatigue damaged regions of compact bone. During the repair of matrix micro damage, osteoclasts tunnel into bone and remove damaged regions. Osteoblasts then concentrically fill in the resorption space, forming a completed osteon (Bentolila -1998, Martin -1995, Mori – 1994 and Parfitt -1996). Increased intracortical remodeling results from increased cyclic loading (Burr-1989 and Mori -1993). In the first phase of remodeling, osteoclasts resorb pre-existing bone, resulting in more and larger porosity within the cortex. The resorption phase is estimated to last for about 6-7 weeks. Thus, increased intracortical remodeling results in increased bone porosity, which lasts several months after onset. As a consequence of the increase in remodeling space, porosity volume in bone expands at the expanse of bone tissue volume (Eriksen -1995 and Jee -1989). Schaffler et al (1990) proposed increases in intracortical porosity would have a dramatic effect on decreasing the stiffness of cortical bone. Continued loading of this focally osteoporotic bone would increase local stresses and strains, accelerate bone micro damage accumulation, cause periosteal hypertrophy and ultimately result in stress fracture (Schaffler -1990).

Two possible theories, not necessarily mutually exclusive, may explain the development of a stress fracture. These theories are primary micro damage and primary remodeling.

Micro-Damage

Bone strain from repetitive loading, initiates the production of micro damage at sites under high levels of stress. A remodeling response is stimulated at the damaged site in order to affect repair. In physiological situations, a balance exists between these two processes and the micro damage is adequately repaired (Frost –1991). The development of a stress fracture is thought to occur when micro damage production exceeds repair (Schaffler –1989).

Primary remodeling

A local or generalized accelerated bone remodeling may be an initiating stimulus to stress fracture development. Various factors may result in accelerated bone remodeling including genetics, bone strain, systemic or reproductive hormones and dietary intake. Since osteoclastic resorption precedes formation in the remodeling process, there is a time lag in which the bone is in a deformed state. Micro damage may occur at these focal areas of weakness leading to a stress fracture if loading continues. The difference between these two theories lies in whether the process of remodeling precedes or follows micro damage production (Bennell, K L –1996 b).

Population at Risk for Stress Fractures

Certain demographic populations appear to be more susceptible to stress fractures. Childhood and adolescence are the most critical periods of skeletal mineralization. More than 90% of peak skeletal mass is present by age 18 years (Slemenda –1994). Important

increments in skeletal mass result from physical activity during childhood (Slemenda – 1991). In a study by Bailey et al (2000), the ages of peak calcium accumulation rates, 14 years in boys and 12.5 years in girls, lagged behind the peak height velocity by 0.6 years (boys) and 0.7 years (girls). These time lags can have important implications. If linear growth in height represents the growth of skeletal volume, the lag implies bone mineralization does not keep up with the rapidly expanding skeletal volume. Intense exercise during childhood and adolescence may also result in primary amenorrhea and low peak bone mineral density (Lavienja –2003). These factors could result in low bone density and stress fractures in adolescents whose bones have not fully matured.

In a study by Goldberg et al (1994) on athletic populations, the highest incidence of stress fractures was observed in freshman athletes. Of the 58 stress fractures diagnosed during 3 years of the study period, 39 fractures were in freshman (67%), 10 in sophomores (17%), 5 in juniors (9%) and 4 in seniors (7%). This could be due to sudden increase in the intensity and/or quantity of training as well as introduction of a new training activity. Also, highest incidence of stress fractures in the athletic population was found to be in individuals participating in high impact activities such as cross-country and track events (Bennell –1996a and Goldberg -1994). Female endurance athletes carry a number of risk factors for bone loss that are closely related: large training volumes, high intensity training, high incidence of amenorrhea, low body mass index, low body fat content and comparatively low energy and calcium intake. Furthermore, intense training can contribute to delayed menarche in girls. In a study by Frisch et al (1981), menarche was delayed 0.4 years (5months) for each year of training before menarche (age of menarche =13.32+/- 0.162 years, $p<0.005$, $r=0.527$). Females with later menarche may

have lower bone density (Armamento-Villareal –1992 and Lu -1994). The combination of estrogen deficiency with any of these other factors could increase the risk of bone loss and subsequent stress fracture (Lavienja –2003).

Risk Factors

Several risk factors for stress fractures can act individually or synergistically to predispose an individual to stress fractures.

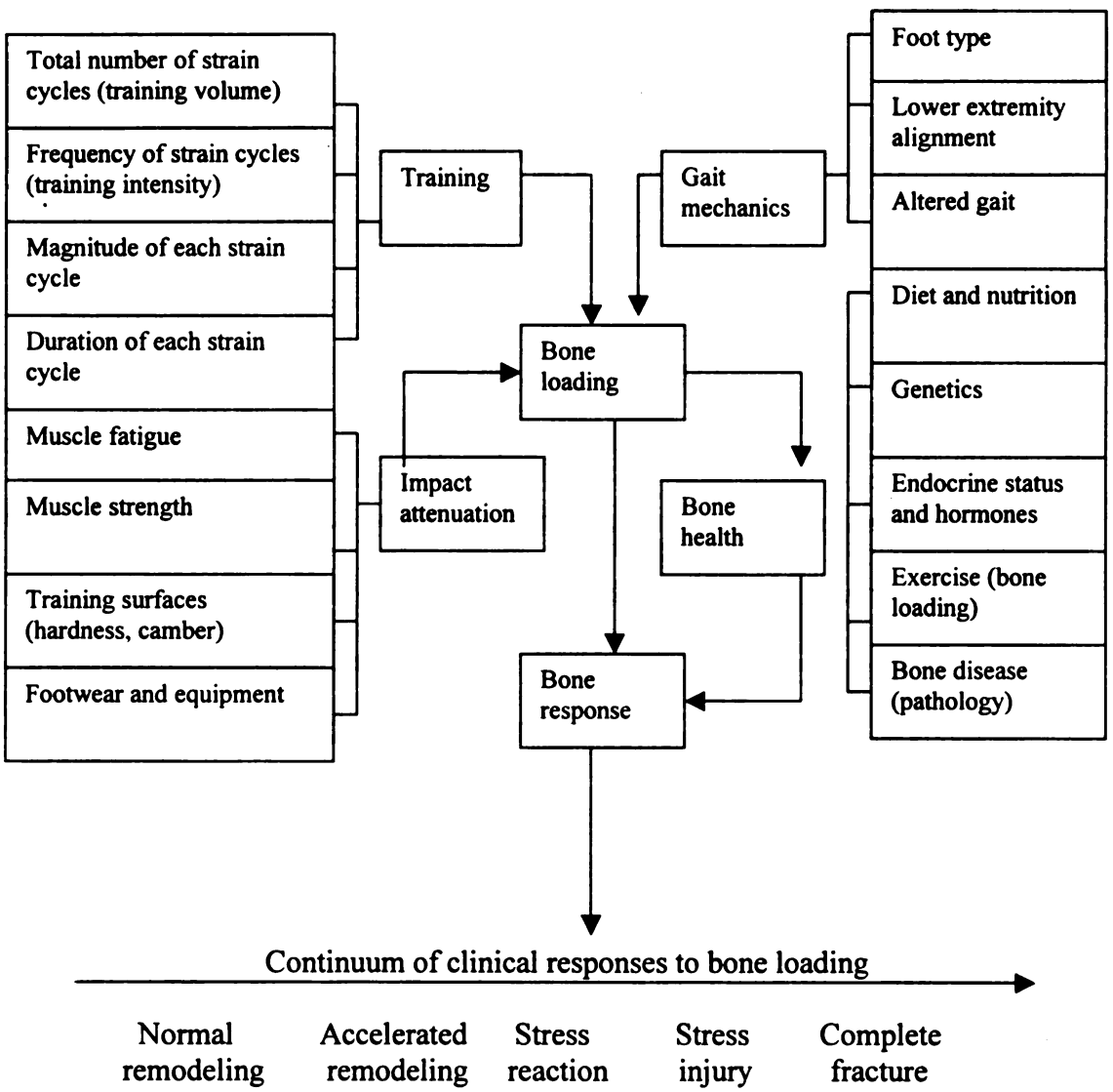

Figure 1.1: Interrelationship Among Various Risks Factors (Bennell -1999 b).

Demographic factors

a. Gender

Macleod et al (1999) studied the bone scans of all recruits of an army training regiment who were referred with suspected trauma. A gamma camera was used to take static bone scans of the pelvis and lower limbs three hours after an injection of 555 MBq technetium-99m oxidronate. Scans were classified as abnormal if stress fractures or shin splints were present. The referral rate for bone scanning was 4.2% (143/3367) for men and 14.2% (121/855) for women. The difference in the percentage of male and female recruits with abnormal bone scan results [3% (101) men and 10.9% (93) women] was found to be significant (x^2 =94.7, $p< 0.001$). The authors concluded women are more likely to suffer from stress fractures and shin splints during army basic training. In a study by Brunet et al (1990) data was obtained from 1505 questionnaires completed by recreational and competitive runners (1130 males and 375 females). The questions focused upon training, injuries sustained, medical care, and anatomical imbalances. The rate of stress fractures in women [28% (n= 83)] increased with increasing mileage (>30miles/week) as compared to men [11.7% (n=299)]. A retrospective review conducted by Hame et al (2004) showed female Division I athletes have higher incidence of stress fractures as compared to male athletes. Division I athletes who sustained a fracture while enrolled at the university were identified through sports injury monitoring system and athletic medical files. Non-stress fractures and stress fractures were identified separately. The incidence rate was based on the athletes who sustained primary (first time) fracture during the study period. Of the 349 total primary fractures, 269 (77.1%) were fractures and 80 (22.9%) were stress fractures. The overall incidence of total

fractures for males and females was similar but females had a higher incidence of stress fractures (95% CI, 2.11-5.38, p =0.001). Brudvig et al (1983) collected data on 339 stress fractures occurring in 295 trainees. 178 stress fractures were radiologically diagnosed in 151 female recruits and 161 stress fractures in 144 male recruits during a 12-month period. Female recruits had a higher rate of incidence of stress fractures in comparison with their male counterparts (3.41% compared to 0.90%). This discrepancy in stress fracture rates in males and females may not be attributable to gender per se, but rather to gender-related factors such as hormonal levels, nutrition, body size, bone size, geometry, or density.

A study by Jarvinen et al (2003), evaluated potential gender-related differences in skeletal responsiveness to mechanical loading between both growing (experiment 1) and mature (experiment 2) female and male rats. A third experiment assessed whether the skeletal effects of estrogen would be coupled with the mechanical loading-induced regulation of bone integrity by comparing the skeletal responsiveness of sham-operated and ovariectomized female rats. In experiment 1, using peripheral quantitative computed tomography (pQCT) and mechanical testing of the femoral neck; female rats were observed to exhibit lower responsiveness to external loading than male rats [3.0% vs. 25% in cross-sectional area ($p<0.003$), 4.2% vs. 27% in bone mineral content ($p<0.001$), -0.6% vs. 10% in volumetric bone mineral density ($p< 0.08$) and 4.7% vs. 28% in fracture strength of the femoral neck ($p< 0.06$)]. Also, relative to the mechanical demands placed on the skeleton, bones of the young female rats were consistently denser (>50%) than those of the males. In experiment 2 virtually identical exercise-induced benefits were seen [2.1% vs. 10% in cross-sectional area ($p< 0.3$), 3.4% vs. 18% in bone mineral

content ($p<0.5$), 2.5% vs. 23% in volumetric bone mineral density ($p<0.002$) and –1.1% vs. 27% in fracture strength of the femoral neck ($p< 0.041$) in female vs. male rats respectively]. In experiment 3, pQCT and mechanical testing analysis showed reduced bone density in the ovariectomized rats but also better responsiveness to mechanical loading in the estrogen depleted rats [-3.5% vs. 9.1% in cross-sectional area ($p< 0.07$), -0.4% vs. 12 % in bone mineral content ($p<0.036$), 4.4% vs. 9.6% in volumetric bone mineral density ($p<0.439$) and –4.2% vs. 16% in fracture strength of the femoral neck ($p< 0.031$) in ovariectomized vs. sham respectively). The results of these 3 experiments suggest estrogen has little primary effect on the sensitivity of female bone to respond to external loading, but rather deposits extra stock of mineral into female bones in puberty. This estrogen-driven extra condensation of the female skeleton seems to persist into adulthood, simultaneously damping the responsiveness of the female skeleton to mechanical loading.

Nieves et al (2005) examined gender-related differences in axial and appendicular bone mass, size, geometry, and biomechanical competence and their relationships with body composition in 18-year-old male ($n = 36$) and female ($n = 36$) army recruits matched for height and weight. Each female cadet was pair-matched with a male cadet for height within 1 in and weight within 5 lb. Males had a higher lean mass (92%) compared with females (79%). No gender differences were observed for vertebral bone mineral content (BMC) or vertebral height, although males had greater width and thus bone area (BA) at the spine. Males had greater BMC and BA at the femoral neck and total femur ($p < 0.02$). Geometric variables of the hip including neck diameter and neck-axis length were also greater in males ($p < 0.02$). There was greater cross-sectional moment of inertia, safety

factor, and fall index in males (all $p < 0.02$). Males had greater tibial BMC, volumetric bone mineral density, and cortical area and thickness compared with females ($p < 0.01$), with both greater periosteal circumference ($p = 0.011$) and smaller endosteal circumference ($p = 0.058$). Statistically controlling for lean mass reduced gender differences, but males still had 8% higher hip BMD ($p = 0.24$) and 5.3% higher total tibial BMD ($p = 0.05$). The authors concluded that differences in bone mass and geometry confer greater skeletal integrity in males. This may contribute to the lower incidence of stress and osteoporotic fractures in males.

b. Age

Several military studies have examined the association of older age with risk of stress fracture. Among more than 3,000 male marine recruits (age 18+ years), the cumulative incidence of stress fracture was found to be 1.7 (1.06-3.21) times higher during 12 weeks of basic training among men over the age of 21 years. With only 37 recruits above age 25, the 21+ age group mainly represented the ages 21-25. The stress fracture incidence rate between age group 18-20 was 1.01 and that of 21+ was 1.82 (Gardner –1988). Lappe et al (2001) conducted a prospective study on 3,758 female recruits undergoing army basic training for 8 weeks over a period of 10 months. A total of 504 stress fractures occurred in 319 soldiers. The authors observed women with stress fractures were older than women without stress fractures even after adjusting for race and bone density [RR =1.07(1.05-1.1)]. In a study by Brudvig et al (1983) on 295 military recruits, age categories were arbitrarily defined as 17-22 years, 23-28 years and 29-34 years. In both the male and female recruits, the rate of incidence of stress fractures increased with each age group, 1.27, 2.32 and 5.01 respectively. In a prospective study, Milogram et al (1994)

followed 796 male elite infantry recruits (17-26 years) during 14 weeks of basic training. 190 recruits were diagnosed with stress fractures, but none were observed in subjects over 19 years of age. The risk of stress fracture was inversely proportional to age on both univariate and multivariate analysis. Each year of increase of age above 17 years reduced the risk of stress fracture by 28%. These studies suggest age is a risk factor for stress fracture but the causal link between age and stress fracture is hard to determine.

c. Race

In a study by Brudvig et al (1983) data was collected on 339 stress fractures occurring in 295 trainees during a 12-month period. The rate of incidence in the recruits was determined in relation to the race of the recruits. The rate of incidence was highest in the Caucasian training population (1.92) compared to Blacks (0.56) and others (0.62), suggesting higher susceptibility of stress fractures in Caucasian population compared to other racial groups. A survey of 1,630 female recruits found the lifetime prevalence of self reported stress fractures among white or Asian women to be 1.6 times higher [RR= 1.6 (1.2-2.1)] than for Black women (Friedl -1992). In a prospective study conducted by Lappe et al (2001) on 3,758 female recruits undergoing army basic training for 8 weeks over a period of 10 months, whites [RR =1.18 (1.07-1.31)] and Hispanics had the highest rate of stress fractures, while Blacks had the lowest [RR =1.00]. These rates were adjusted for bone density and age. This difference in stress fracture rates between different racial groups could be due to differences in bone mineral density. Bachrach, et al (1999) studied racial differences in bone mineral acquisition in a longitudinal study of 423 Asian, Black, Hispanic, and white males and females (age: 9-25years). Bone mass of the spine, femoral neck, total hip and whole body was measured annually for up to 4

years by dual energy x-ray absorptiometry (DEXA). Age-adjusted mean bone mineral curves for areal bone mineral density and volumetric bone mineral density were compared for the 4 ethnic groups. Consistent differences in areal and volumetric bone density were observed only in Black and non-Black subjects. Among females, African-Americans had greater mean levels of areal bone mineral density and volumetric bone mineral density at all skeletal sites. Like females, African-Americans males had consistently greater mean values than non-Blacks for all bone mineral density and volumetric bone mineral density measurements. Therefore, differences in bone mineral densities could be the reason for racial differences in stress fracture predisposition.

Anatomic factors

A number of military and civilian studies have examined the relation between bone characteristics (geometry and density) and the occurrence of stress fractures.

a. Bone density

Clinically, bone density measurements are used to predict the likelihood of fracture (Clark –1988 and Melton –1993). It is feasible that the level of bone density required by physically active individuals to resist repetitive strains without developing fatigue fractures may be greater than the less active population who subject their bones to much lower forces.

In a prospective study by Beck et al (1996), 626 male marine recruits were followed for 12-weeks during basic training. The recruits underwent anthropometric measurements and DEXA scans of the femoral mid-shaft and the distal third of the tibia at baseline. Conventionally obtained frontal plane DEXA scan data was used to measure the bone mineral density (BMD). During training, 23 recruits (3.7%) presented with a total of 27

radiologically confirmed stress fractures in various locations in the lower extremity. BMD measurements were compared between fracture cases and controls. BMD in the fracture cases was significantly smaller as compared to the controls ($p<0.03$). In a study by Pouilles et al (1989), femoral BMD was measured by DEXA in 41 young military recruits who had one or several stress fractures. The stress fracture group had lower bone mineral density than a control group, consisting of 48 young military recruits matched for age, height and weight. For femoral stress fractures, BMD was significantly lower than in controls for the femoral neck ($p<0.05$) and Ward's triangle ($p< 0.01$). For calcaneus fractures BMD was significantly lower only in the trochanter ($p<0.05$). Ward's triangle best reflects strength of the proximal femur in vitro (Vose –1965) and the most significant difference between the stress fracture and the control group was found at this site.

In a case control study, Myburg et al (1990) compared 25 athletes with scintigraphically confirmed stress fractures with 25 control subjects matched for age, weight, height, sex, and exercise history. BMD measured by DEXA was significantly lower in athletes with stress fractures than in controls. In the spine, BMD was 1.01 +/- 0.14 g/cm^2 in athletes with fractures and 1.11 +/- 0.13 g/cm^2 in control subjects ($p< 0.02$). In the femoral neck, it was 0.84 +/-0.09 g/cm^2 in athletes with fractures and 0.90+/- 0.11 g/cm^2 in control subjects ($p<0.005$). BMD was also significantly lower in the Ward's triangle ($p<0.01$) and the greater trochanter ($p<0.01$). In a prospective study, Valimaki et al (2005) evaluated risk factors for symptomatic stress fractures among 179 Finnish male military recruits, aged 18-20 years. Bone mineral content and BMD were measured by DEXA at the lumbar spine and at the hip. After adjusting for age, weight, height,

exercise, smoking, alcohol and calcium intake, femoral neck bone mineral content ($p<0.02$) and BMD ($p<0.03$) as well as total pelvis bone mineral content ($p<0.02$) and BMD ($p<0.04$) were significantly lower in the stress fracture group (n =15) than in the controls (n =164). The results of these studies indicate decreased BMD can predispose individuals to stress fractures.

b. Bone geometry

Bone strength is also related to bone geometry. The structural properties of long bones vary with age and gender and are largely dependent on body size (Miller –1980), although there is great variation in bone geometry even among individuals of similar age and build. In addition, within any long bone, the geometry is complex and changes continuously along its length. There is a greater variation in structural geometry than in bone material properties, including BMD (Martens –1981). Thus, differences in bone geometry might partly explain differences in stress fracture predisposition. The asymmetry of balance and unequal torsion of bones as well as the increased muscular activity in the asymmetrical extremities in prolonged or repeated physical stress situations may affect the outcome and the location of the stress fracture (Morscher – 1977). In a study by Friberg (1982), 130 Finnish army recruits suffering with stress fractures were studied for leg length discrepancy. Leg length was measured by a standing X-ray method developed by the author. The majority of the patients with a unilateral stress fracture had a leg length asymmetry of 5mm or more. Unilateral stress fractures of tibia, metatarsals and femur occurred in 73% of the cases in the longer leg and only 16% of the cases in the shorter leg. This study demonstrated leg length asymmetry could be a risk factor for stress fractures. As demonstrated by electromyography, a leg length

discrepancy of less than 10 mm can lead to remarkable increase in the activity of several muscle groups and make it impossible to maintain a complete resting position (Morscher –1977). Prolonged pronation has also been related to stress fractures. Prolonged pronation flattens the medial longitudinal arch, which consists of the plantar fascia and the tarsals and metatarsals. A flattened arch puts increased stress on the tarsals and metatarsals and the plantar fascia, which can initiate bone remodeling and possibly a stress fracture (Tauton –1981). In a study by Milogram et al (1988) the biomechanical mechanism of tibial diaphyseal stress fractures was studied prospectively in a group of 286 Israeli military recruits. Before training each recruit had roentgenograms taken of his tibiae. Measurements of total tibial and cortical widths in the anteroposterior (AP) and mediolateral planes were made on these roentgenograms at two levels: at the point of the narrowest tibial width on AP roentgenograms (Level 1) and at the point of the narrowest width on lateral roentgenograms (Level 2). The tibial cross section was idealized as an eccentric ellipse within an ellipse, and on the basis of measurements taken from the roentgenograms, the cross-sectional area (compression strength), area moments of inertia about AP and mediolateral axes of bending (bending strength), and the area polar moment of inertia (torsional strength) were calculated for each cross section. During the course of 14 weeks of training, 20% of the recruits sustained tibial diaphyseal stress fractures, all of which were along the medial cortex. Using stepwise logistic regression analysis the tibia's bending strength along an AP axis of bedding at Level 2 was found to be the most significant factor determining whether or not a recruit would develop a tibial stress fracture.

In a cross-sectional study, Crossley et al (1999) determined whether runners with a history of tibial stress fractures (TSF) differed in tibial bone geometry from those who never sustained a stress fracture (NSF). Forty-six male runners (23 TSF; 23 NSF) ranging in age from 18 to 42 years were recruited for the study. Tibial bone geometry (cross-sectional dimensions and area) was calculated from a computerized tomography (CT) scan at the junction of the middle and distal thirds. DEXA provided measurements of tibial bone area. Stress fractures were diagnosed clinically as well as with triple-phase isotope bone scan. The TSF group had significantly smaller tibial cross-sectional area ($P = 0.02$) and DEXA tibial bone area ($P = 0.02$), after adjusting for height and weight, than the NSF group. This finding supports the contention that bone geometry plays a role in stress fracture development and male athletes with smaller bones in relation to body size are at greater risk for this bony injury. These studies demonstrate an association between anatomical structure and stress fracture incidence.

c. Bone cell dynamics

Changes in bone cell dynamics may influence the risk of stress fracture. Stress fractures develop if micro damage cannot be successfully repaired by the remodeling process and accumulates to form symptomatic macro cracks in bone (Li –1985). Depressed bone remodeling may not allow normal skeletal repair of naturally occurring micro damage. Accelerated remodeling resulting from excessive bone strain or from the influence of systemic factors may also weaken bone, because bone resorption occurs before new bone is formed. This could promote the initiation of micro damage at remodeling sites (Johnson –1963). Since direct assessment of bone remodeling in humans is invasive and impractical, measurements of biochemical markers of bone turnover may

provide information about the role of bone remodeling in stress fracture development (Bennell –1998). Products of bone breakdown include calcium and bone matrix degradation products such as hydroxylysine glycosides, collagen pyridinium cross-links, cross-linked telopeptides, and hydroxyproline (Watts-1999). Hydroxyproline is a modified amino acid derived from proline by a post-translation hydroxylation occurring within the peptide chain. Hydroxyproline is found mainly in collagens, comprising about 13% of the amino acid content of these proteins (Christenson –1997, Clemens –1997 and Prockop –1987). Because free hydroxyproline liberated from the breakdown of collagen is not reutilized for collagen biosynthesis, most of the endogenous hydroxyproline present in biological fluids is derived from the degradation of various forms of collagen (Prockop –1987 and 1979). Givon et al (2000) conducted a case-control study on 2,591 Israeli soldiers to profile individuals prone to stress fractures. Of the 2,591 soldiers, 318 were diagnosed with clinically and scintigraphically proven stress fractures, 237 soldiers with symptoms but with normal scintigraphy and 2,036 soldiers with no symptoms. A subset of 40 soldiers with stress fractures and 40 symptomatic control subjects were chosen randomly and underwent an evaluation of bone turnover and metabolism parameters. This included radioimmunoassay of serum parathyroid hormone; osteocalcin, bone specific alkaline phosphatase and 25-hydroxy vitamin D. Mean bone specific alkaline phosphatase (37.6 versus 26.2 units/ L., $p< 0.0001$) and osteocalcin levels (10.8 versus 8.8 ng/ml, $p< 0.00003$) were significantly higher in the stress fracture group than in the control subjects. Mean serum levels of 25-hydroxy vitamin D were significantly lower in subjects with stress fractures (25.3ng/ml) than in the control subjects (29.8ng/ml, $p<0.033$). However, the observed differences between the two groups could be because

the measurements were made at the time of fracture repair. In a study on male and female athletes, Bennell et al (1998) were unable to demonstrate that single or multiple measurements of bone turnover markers can predict stress fractures. A 12-month prospective study was conducted to evaluate bone turnover in 46 female and 49 male athletes, 20 of who developed a stress fracture. Baseline levels of bone turnover were evaluated in all athletes and monthly in a subset consisting of 20 athletes with stress fractures and a matched control group. Bone formation was assessed using serum osteocalcin measured by human immunoradiometric assay and bone resorption by urinary excretion of pyridinium cross-links. Athletes who developed stress fractures had similar baseline levels of bone turnover compared with their non-stress fracture counterparts ($p<0.10$). Results of serial measurements showed no differences in average levels of osteocalcin and pyridinium cross-links in those who developed stress fractures ($p<0.10$) compared with the control group. In the athletes with stress fractures there was also no difference in bone turnover levels prior to or following the onset of stress fractures (Bennell –1998). The investigators inability to demonstrate that single or multiple measurements of bone turnover can predict stress fractures may be due, in part to their high biological variability. Short-term day-to-day variations of 26% have been reported for 24-hour urinary excretion of pyridinium cross-links (Colwell –1993). Therefore, if bone turnover levels are different in athletes who develop stress fractures, larger sample sizes and/or multiple baseline measurements might be required to detect these differences.

III. Physical fitness

a. Aerobic fitness

Studies of US military recruits have consistently shown significant associations between low aerobic fitness levels and higher risk of stress fracture during basic training. Shaffer et al (1999), in a prospective study, established the rates and risk factors for stress fractures in recruit's under-going rigorous physical training in order to develop an algorithm to identify those entering training with a higher probability of stress fractures. 1, 286 males (age: 17-28 years) were randomly selected for the study. Baseline physical fitness was measured by a standardized field fitness test, the Initial Strength test (IST), which included a 1.5-mile maximal effort run. A 39-item questionnaire including age, race, and history of prior musculoskeletal injury, recent physical activity and exercise patterns was administered within the first 3 days after arrival at training. Stress fractures were diagnosed clinically and by a confirmatory radiograph and/or triple-radiograph. Of the 1,286 subjects, 52 were diagnosed with a total of 56 lower extremity stress fractures. Slower IST time was associated with an increased risk of stress fracture during training [RR =2.00 (1.03-3.90), $p<0.04$]. These data suggest risk of stress fracture during rigorous physical training is increased by poor physical fitness. Montgomery et al (1989) studied 505 SEAL trainees to identify pre-training factors, which predispose recruits to overuse injuries. A week before training, questionnaires were administered to determine pre-training physical activity level. Only 19.8% of the trainees reported regular running of 25 or more miles per week prior to entering training. Thirty -two trainees suffered a stress fracture (6.3% incidence rate). The occurrence of stress fractures by pre-training running history was determined. The percentage of stress fractures for those running less than 4 miles per week was significantly ($p<0.027$) greater than those running 25 or more miles per week. Thus, running may have a sparing effect on the subsequent development of

stress fractures. These studies show the initial fitness level is a critical factor in placing individuals at risk for stress fractures. Dramatic changes in bone's mechanical environment may precipitate changes in cellular dynamics that could lead to development of a stress fracture. Poor physical conditioning does seem to increase the risk of stress fractures in military recruits, probably because the mechanical loading experienced by unfit individuals during intensive training represents a remarkable change in skeletal load. Israeli defense forces (IDF) studies have reported no significant association between aerobic fitness and risk of stress fracture (Giladi –1991 and Swissa –1989). Giladi et al (1991) studied 289 male Israeli recruits during 14 weeks of basic training to identify risk factors for stress fractures. Diagnosis of stress fractures was made on the basis of either positive radiographs or a positive scintigram. Ninety-one recruits were found to have 184 stress fractures. Aerobic fitness was assessed in 270 recruits by calculating maximal oxygen consumption (VO_2 max) indirectly using the Astrand nomogram of heart rate. A total of 89 recruits suffered from stress fractures. No difference was observed in the VO_2 max value between the recruits with (42.8+/-8.0 mlO_2/kg/min) and without stress fractures (43.1+/-8.0 mlO_2/kg/min). In a study by Swissa et al (1989), 279 male recruits between the ages of 18-20 were evaluated during 14 weeks of basic training. Each recruit was questioned about his pre-training participation in sports activities. Aerobic fitness was assessed by calculating VO_2 max according to the technique of Astrand nomogram of heart rate. A total of 84 recruits were diagnosed with stress fractures. Sixty-one of the 279 recruits (22%) did not participate in any sport activity prior to basic training. One hundred and sixty recruits (57%) participated in running and jogging and the remaining 58 recruits (21%) participated in various sports. No correlation was found between pre-

training sport activity and stress fractures. The frequency of stress fractures was almost identical (31%) among those who participated or did not participate in sport activities. No correlation was found between aerobic physical fitness and the incidence of stress fractures. Aerobic fitness is a highly modifiable characteristic. Extensive training of recruits prior to basic training can increase their level of fitness. The difference between these findings and those of other studies may also be partially explained by the use of different modalities used to test aerobic fitness.

b. Muscle strength and endurance

Muscles can act dynamically to cause stress fractures by increasing bone strain at sites of muscle attachments (Stanitski –1978 and Meyer- 1993). However, under normal circumstances muscles exert a protective effect by contracting to reduce bending strains on cortical bone surfaces (Scott -1990). Sudden and large increases in training activity can cause muscular tissue damage (Dressendorfer –1991 and Hikida –1983). During initiation of a new exercise or training regime, muscles may adapt faster than bones. This may result in larger muscular forces and higher bone strains. When muscular force production is compromised by muscular fatigue, bones may be required to absorb more energy than they are accustomed to. Excessive muscular force and muscular fatigue may cause bones to bend and strain to a higher degree than normal. This unaccustomed straining may result in the damage that causes stress fractures. Like fatigue, damaged muscular tissue may reduce the force-generating capacity of muscles (Donahue -2001). In a study by Beck et al (2000) anthropometry and DEXA scans of the mid-thigh and distal third of the lower leg were done prior to 12-week training. Using DEXA data, bone structural geometry and cortical dimensions were derived at scan locations and muscle

cross-sectional area was computed at the mid-thigh. A total of 75 (37 females and 38 males) cases of stress fractures were identified. Measurements were compared within gender between pooled fracture cases and controls. In both genders, fracture cases had smaller thigh muscle cross sectional area as compared to controls (men: $p< 0.003$, women: $p<0.047$). After correction for height and weight, section moduli (Z) and bone strength indices (Z/bone length) of the femur (men: $p<0.0018$, women: $p<0.0060$) and tibia (men: $p<0.037$, women: $p<0.029$) were significantly smaller in fracture cases of both genders. The maximum strain than can be generated in a given bone is proportional to the strength of the muscles acting on the bone. Individuals vary in muscle strength, and if muscle forces are indeed the osteogeneic stimulus for adaptation, those with weaker muscles should also have weaker bones (Beaupre -1990 and Meulen -1993). In the study by Beck et al (2000), thigh muscle cross-sectional area was positively correlated with femur and tibia section moduli, explaining 41% and 27% of the variability (R^2) in femur section modulus and 33% and 22% of variability in tibia section modulus for men and women respectively. Smaller muscle cross-sectional areas in fracture cases would generate lower peak forces (osteogenic stimuli), consistent with their smaller bone geometries. Those beginning training with weaker muscles and smaller bone geometries would thus be more susceptible to bone failure during training.

c. Flexibility

The role of flexibility is difficult to evaluate as flexibility encompasses a number of characteristics including active joint mobility, ligamentous laxity and muscle length. Giladi et al (1987) studied 289 male Israeli recruits during 14 weeks of basic training to identify risk factors for stress fractures. Diagnosis of stress fractures was made on the

basis of either positive radiographs or a positive scintigram. Orthopedic examination including foot and tibial radiographs and tibial bone width was done prior to training. Eighty-nine recruits were diagnosed with stress fractures. Recruits with stress fractures at all anatomical sites ($p<0.016$) and tibial stress fractures ($p<0.034$) had a significantly greater hip external rotation than the recruits without stress fractures. A high degree of external rotation of the hip results in increased femoral anteversion in a closed kinetic chain, combined with external proximal tibial torsion. This may result in compensatory hyperpronation of the foot (Korpelainen –2001). Kaufman et al (1999), in a prospective study observed an association between foot structure and the development of overuse injuries in 449 navy SEAL trainees. Foot and ankle ranges of motion were measured by a registered physical therapist using a handheld goniometer. Diagnosis of stress fractures required clinical presentation and a positive radiograph, nuclear bone scan or both at a site consistent with clinical presentation. The amount of foot inversion had a differential effect on the location of the stress fracture. Subjects with restricted hind foot inversion had more femoral stress fractures, while subjects with increased hind foot inversion had more tarsal/metatarsal stress fractures. Many overuse injuries are associated with excessive pronation of the foot. O'Connor (2004) determined that greater pronation of the foot leads to greater energy absorption in the foot invertor muscles and tendons. While the results of the study by O'Connor (2001) were not conclusive, the EMG data suggested that perturbation of the foot might not elicit adaptations in activation patterns of the foot invertor muscles. Passive properties may lead to greater tissue loads about the ankle. This could have elicited the stress fractures observed in the studies by Korpelainen –2001 and Kaufman et al (1999). Montgomery et al (1989) studied 505 SEAL trainees to

identify pre-training factors, which may predispose recruits to overuse injuries. A week before training, orthopedic examination were conducted. The orthopedic examination consisted of evaluating the hips, knees, ankles and feet bilaterally for flexibility and static measures. Thirty-two trainees suffered a stress fracture (6.3% incidence rate). No significant differences between those with and without stress fractures were noted for any of the variables measured, but subjects with stress fractures tended to have less ankle flexibility and a slightly higher knee varus/valgus value. The difficulty in assessing the role of muscle and joint flexibility in stress fractures may relate to a number of factors, including the relatively imprecise methods of measurement, the heterogeneity of these variables and the fact that both increased and decreased flexibility may be contributory. In the study by Montgomery et al (1989), the true number of stress fractures was probably underestimated, as the clinical diagnosis of stress fractures may have prohibited the identification of specific predictive alignment characteristics. Clinical diagnosis has lower sensitivity compared with radiography or scintigraphy.

d. Body composition and stature

Anthropometric characteristics, such as height and weight, and soft tissue composition, such as lean mass and fat mass, could theoretically affect stress fracture risk directly by influencing the forces applied to bones or indirectly via effects on bone density and menstrual function. Body size and soft tissue composition could directly affect stress fracture risk by influencing the forces applied to bones. Both lean and fat mass plays independent roles in determining skeletal integrity. Lean mass is postulated to be a determinant of bone mass because of the structural and functional relationships between muscle and bone (Binder –2000). Fat mass could exert protective effects on

bone by increasing the mechanical loading forces acting on the skeleton during weight bearing conditions and also through the conversion of steroids to estrogen that occurs in adipose tissues (Simpson –1989). In a prospective study by Beck et al (1996), 626 male marine recruits were followed for 12-weeks during basic training. The recruits underwent anthropometric measurements and DEXA scans of the femoral mid-shaft and the distal third of the tibia at baseline. During the training, 23 recruits (3.7%) presented with a total of 27 radiologically confirmed stress fractures in various locations in the lower extremity. The average difference in body weight between the stress fracture and control group was almost 11% ($p< 0.0006$). Fracture cases were also relatively lighter for their height as indicated by a significantly lower body mass index ($p<0.01$). In general, stress fracture subjects were smaller in body size compared with controls. Body size may be a risk factor in military recruits, where size variations are likely to be greater than in athletes. Common training requires similar weight packs and other equipment regardless of recruit body weight. In contrast to previous finding, Valimaki et al (2005), found tall stature a risk factor for stress fractures. This study evaluated risk factors for symptomatic stress fractures among 179 Finnish male military recruits, aged 18-20 years. Fifteen men incurred a stress fracture during military service. Those who experienced a fracture were taller than those who did not ($p< 0.047$). The author's attributed tall stature to be a risk factor because of the greater magnitude of the bending moment during force application in longer bones. Consequently, the long bones of the lower extremity are subjected to high bending moments and hence high tensile and compressive stresses. Previous studies of athletes have not reported differences in height, weight or body mass index between those who sustained a stress fracture and those without (Bennell –1996 c and Barrow –

1988). Lack of association in athletes may be because athletes of a particular sport tend to be relatively homogenous in terms of somatotype and body composition.

Oral contraceptives

Oral contraceptive pill (OCP) is usually prescribed in the form of a combined estrogen and progesterone tablet. The combined pill can be presented as a monophasic, biphasic or a triphasic pill. Oral contraceptive use is associated with reduction of endogenous sex steroids (Bemben –1992). By reducing bone turnover rates, oral contraceptives may be able to reduce the risk of stress fracture development (Bennell – 1999a). However, oral contraceptive use in pubertal girls seems to suppress endogenous sex hormone production long before skeletal maturity has been reached (Hartard –2004). Oral contraceptive use could therefore interfere with normal acquisition of peak bone mass in young women. In a retrospective analysis of 69 endurance athletes, OCP use for more than three years in women younger than 22 years or OCP use for more than 50% of the time after menarche in women aged 22-35 years was associated with 7.9% lower spine bone mineral density and a 8.8% lower proximal femur bone mineral density ($p < 0.01$ for both sites) (Hartard –2004). A stepwise model of multiple regression analysis was done using oral contraceptive years, age at OCP initiation, BMI and menarche as independent variables to further analyze the relationship between bone mineral density of the spine and OCP use. Age at first OCP use was found to be the best predictor of vertebral bone mineral density (Hartard –2004). A prospective study conducted by Polatti et al (1995) reported a monophasic pill prevented a 7.8% increase in bone mineral density of the lumbar vertebrae observed in the untreated control subjects within the study period. Two epidemiological studies in Great Britain suggested an increased

relative risk for fractures in pre menopausal women who had used oral contraceptives compared with those who had never used oral contraceptives (Cooper –1993 and Vessey –1998). In the study by Cooper et al (1993), 46,000 women (25-65 years old) enrolled in the Royal college of general practitioners oral contraception study were followed for 482,023 person-years. Of these, 284,882 (59%) were among users of the oral contraceptive pill and 197,201 had not used any OCP. The mean duration of pill use among OCP users was 3.7 years. Incidence rates for all fractures were calculated using person-years of observation as the denominator in each age group. After adjusting for age, smoking, parity and social class, the risk of all fractures among women who had used oral contraceptives was significantly ($p<0.05$) increased when compared with women who had never used the pill (RR =1.20., 95% CI 1.08-1.34). In the study by Vessey et al (1998), 17,032 women were recruited to examine the relationship between OCP use and fractures occurring at various sites. The study included 310,000 women-years of observation, 123,000 in those never using OCP and 187,000 in OCP users. To examine the possible association between fractures and OCP use, two measures of pill exposure were used: total duration of use and interval since last use. When all fractures were combined, there was a modest but significantly ($p<0.001$) increased risk with total duration of OCP use. There was also a significant heterogeneity ($p<0.01$) when overall fracture rates were examined in relation to recovery of OCP use. In the studies by Cooper and Vessey, the subject population above 50 years was minimal. Accordingly, it might be too early to expect to show any beneficial effect of OCP use on fracture risk. In a study by Weaver et al (2001), the effect of quantified resistance and high impact exercise training on bone mass as modified by age and OCP use was studied in 179 women (18-31

years). Total and regional bone mineral density, bone mineral content and biochemical markers of bone turnover were assessed. OCP users had lower bone turnover at baseline but there was a decrease in total bone mineral content from baseline compared with non-OCP users at 24 months. Spine bone mineral content and bone mineral density decreased in the exercise and OCP group at 6 months and remained significantly below non-exercisers who used OCP at 2 years ($p< 0.02$). In a study by Barrow et al (1988) women who had used oral contraceptives for at least one-year (n =26) had significantly fewer stress fractures than non-users (n =126) (12% versus 29%). A cross-sectional study found that female athletes who had been amenorrheic for less than three years and who had taken the oral contraceptive pill had similar spinal bone density to those with regular menses since menarche (Cann –1988). In a cross-sectional study in 524 Canadian women with a mean age of 36.3 years, bone mineral density of the spine and of the proximal femur was significantly greater in women using oral contraceptives for less than 3 months compared to those with greater than 3 months of oral contraceptive use (Prior – 2001). These conflicting results in the various studies could be due to differences between studies including pill dosage and formulation, menstrual history, duration of pill use, age at which subjects were exposed to the oral contraceptive and outcome measures. More prospective double blind randomized trials using proper control groups need to be conducted.

Amenorrhea

The detrimental effects of athletic amenorrhea on bone mass were first identified in the 1980s (Linnell –1984 and Marcus –1985). In athletes, exercise- induced osteogenic benefits are decreased when training is associated with menstrual dysfunction (Morris –

1999). The main mechanisms responsible for the deleterious effects of menstrual disturbances on bone density are thought to be low circulating estrogen (Jilka –1992) and under- nutrition (Zanker –1998). In the presence of low estrogen and progesterone concentrations, the response of bone to mechanical loading is altered, resulting in increased bone resorption and suppression of bone formation (Notelovitz –1991). This altered bone metabolism is believed to be in response to a direct hormone action on the bone metabolic cells (Hoshimo -1995). Also, an indirect influence affect the concentration and sensitivities to the bone related factors such as cytokines, growth hormone, insulin-like growth factor I and the responsiveness of bone to parathyroid hormone (Pacifici -1996). Progesterone levels may have a direct influence on bone formation through the stimulation of osteoblastic cell activity and bone formation (Demarest -1991) and an indirect influence by decreasing the glucocorticoid inhibition of bone formation (Chen -1977). Hence, the primary response to low estrogen and progesterone concentrations is accelerated bone turnover, specifically increased bone resorption in response to estrogen deficiency and decreased formation in response to progesterone deficiency (Hughes -1995).

Recent evidence suggests hormonal imbalances can also occur with regular menses. Specifically, anovulation and reduced lutenizing hormone pulse frequencies have been observed in trained runners with regular normal length cycles. These disturbances, which are similar but more marked in amenorrheic athletes, suggest bone health of athletes with regular normal length cycles may also be at risk (Loucks – 1989, Pirke –1990 and Prior – 1990). In a study by Zanker et al (1998), 33 women distance runners (mean age 27.2 years) were studied to explore the relationship between biochemical markers of bone

turnover, indices of nutritional status and serum estradiol concentration. Amenorrheic women had a lower body mass index and a negative energy balance than oligomenorrheic ($p< 0.001$) and eumenorheic women ($p<0.001$). Eumenorheic women had a higher serum estradiol concentration than the oligomenorrheic ($p<0.001$) or amenorrheic women. Serum levels of osteocalcin and bone alkaline phosphatase (BAP) were higher in the eumenorheic than the oligomenorrheic ($p<0.001$, osteocalcin and BAP) and amenorrheic women ($P< 0.001$, osteocalcin and BAP). Serum levels of osteocalcin and BAP correlated with body mass index ($r =0.9$, $p< 0.001$ and $r =0.88$, $p< 0.001$ respectively) and serum estradiol concentration ($r =0.85$, $p<0.001$ and $r =0.83$, $p< 0.001$) in amenorrheic women. These results suggest that a low body mass index and estrogen deficiency are associated with disruption of bone formation in amenorrheic women distance runners.

Gremion et al (2001) in a prospective longitudinal study determined the role of menstrual status on BMD changes. Long distance runners with and without regular menses (age: 19-37 years) were studied. Changes in areal BMD were measured at 1-year interval. Among 10 eumenorrheic, 11 oligo-amenorrheic and 9 oral contraceptive users, there was no difference in energy, calcium or protein intakes. Eumenorrheic athletes were defined by at least 10 menstrual cycles per year, for at the last 3 years. Oligo-amenorrheic athletes were defined by less than 5 menstrual cycles per year, for at least the 2 previous years and users of oral contraceptives for at least 4 years. Baseline BMD levels were significantly lower in the oligo-amenorrheic group than in the other 2 groups at the lumbar spine ($p< 0.005$). Over a 1-year interval, during which the three groups did not differ in terms of running distances and dietary intakes, oligo-amenorrheic women

displayed a significant decrease in lumbar spine BMD in lateral view ($p<0.005$) as compared to both the other groups. Thus, oligo-amenorrhea in long distance runners with adequate dietary intakes could be deleterious for the axial skeleton. One study found that while amenorrheic and eumenorrheic groups reported a similar prevalence of single stress fractures, 50% of the amenorrheic runners reported multiple stress fractures compared with only 9% of those regularly menstruating (Canham –1998). Barrow et al (1988) also found that lifetime menstrual history affected the risk of stress fracture incidence in college track athletes. Data were collected from 241 runners through a questionnaire. The data received from each runner were assigned to one of three groups according to the runner's menstrual history since menarche: very irregular (0-5 menses/year), irregular (6 to 9 menses/year) and regular (10-13 menses/year). Regular runners had a significantly younger age of menarche than either the irregular or very irregular groups ($p<0.05$). The incidence of stress fractures in the regular group 29% (35/120) was much lower than both the irregular 39% (20/51) and very irregular group 49% (34/69) ($p<0.05$). This increase in stress fracture frequency associated with increasing menstrual irregularity suggests that relatively small changes in menstrual regularity affect the maintenance of bone composition through a decrease in estrogen and thus increasing predisposition to stress fractures.

Nutrition

Several dietary factors such as calcium, phosphorus, fluoride, zinc, copper, magnesium, vitamin K and phytoestrogens have an influence on bone mass (Reid –1997) and bone turnover (Robins –1997). Dietary deficiencies, in particular dietary calcium may contribute to the development of stress fractures by influencing bone density and

bone remodeling. However, it is difficult to clarify the role of diet as (i) accurate assessment of habitual dietary intake is problematic, (ii) nutrients may exert their effects on bone over a number of years and measurement of current intake may not represent lifetime status, (iii) calcium balance is negatively influenced by other dietary factors and (iv) calcium operates as a threshold nutrient, whereby intake above a certain level produces no additional effects on bone. Myburgh et al (1990) in a case-control study on 50 female athletes determined differences in nutrient and dairy intake in athletes with (n=25) and without (n=25) stress fractures. Seven-day diet records indicated similar energy and nutrient intakes, except athletes with stress fractures had lower calcium intake (697+/-242 mg/day compared with 832+/-309 mg/day, $p< 0.02$). The estimated intake of dairy products per week was significantly lower for injured subjects compared with the control subjects at the time of study ($p<0.05$) and since leaving school ($p<0.05$). Also, a weak but a significant positive correlation was observed between calcium intake and bone mineral density in the weight bearing bones, particularly the femoral neck ($r =0.34$, $P<0.05$) and total proximal femur (0.33, $p<0.05$) suggesting the importance of adequate dietary nutrients in maintenance of bone health. Alcohol excess may also have deleterious effects on bone homeostasis through increased excretion of calcium and magnesium, gastro-intestinal and pancreatic malfunction and inhibition of intestinal calcium absorption (Seaman et al -1983, Lalor et al -1985 and Lalor et al -1982). Studies of chronic alcohol consumption in growing male and female rats have indicated that bone growth is suppressed, leading to a failure to acquire a normal peak bone mass (Turner-1987). Bone loss in adult rats fed ad libitum a liquid diet containing increasing concentrations of ethanol resulted in a dose-dependent decrease in trabecular thickness,

bone turnover and bone formation rate (Turner -2001). When equated to humans, the doses used in the adult rat experiments ranged from the low-end moderate (3% of caloric intake) to high alcoholic levels (35% of caloric intake) (Turner -1987 and 2001). These findings in rats suggest even moderate levels of alcoholic beverage consumption in humans may have the potential to reduce bone turnover and possibly have deleterious effects on the skeleton. In a 10 month prospective study on 3,758 female recruits, Lappe et al (2001) observed that compared to their non-stress fractures counterparts, female recruits who developed stress fractures were more likely to report current or past smoking [RR =1.05 (1.02-1.08)].

Genes

There is a mounting body of indirect evidence that genetic factors could play a role in stress fracture predisposition. Physical response to a particular environmental stimulus, such as exercise may be mediated by individual genetic variability. Twin studies and parent-offspring studies have shown that genetic predisposition explains a significant amount of the variance in peak bone mass, broad band ultra-sound attenuation, and hip axis length; all of which have a strong genetic component independent of bone mineral density (Kelly –1995, Jones –2000 and Arden –1996). Some gene polymorphisms may be associated with peak bone mass and others with bone loss. Singer et al (1990) described multiple stress fractures in monozygotic twins. Both affected individuals, who served in the same military unit, sustained stress fractures at the same anatomical sites and the onset of symptoms was traced to the sixth week of basic training in both. Recurrence of stress fractures at different anatomical loci in the same individual may imply an inherent bone structure abnormality that could be genetically predetermined.

The secosteroid hormone vitamin D, its receptor (VDR) and the metabolizing enzymes involved in the formation of the biologically active form of the hormone together are major players in the vitamin D endocrine system. This system plays an important role in skeletal metabolism, including intestinal calcium absorption. Also, vitamin D receptor (VDR) mediates the biological actions of 1, 25 – dihydroxycholecalciferol (Cusack–2003). Large inter- individual differences exist in the vitamin D endocrine system (Utitterlinden –2004). One approach to understand these inter individual differences is to study the influence of variations in the DNA sequence of important proteins of this system. As a result, the VDR gene has been targeted in the research on the genetic determinant influencing bone status (Utitterlinden –2004, Gross–1996 and Ames -1999). A number of polymorphisms of the VDR gene have been identified and correlated with bone mineral density (Fleet –1995, Morrison –1992, Zmuda –2000 and Langdahl –2000). Gross et al (1996) studied a novel polymorphism at the translation initiation site in exon 2 of the VDR gene. This is the only known protein polymorphism in the VDR gene. This polymorphism results in a T → C transition (ATG to ACG at the first potential start site), recognized by the Fok 1 restriction enzyme by restriction fragment length polymorphism (RFLP) (Sajio-1991, Sturzenbecker –1994 and Arai –1997). It creates an alternative translation start codon that results in a shorter isoform of the VDR gene (Cusack–2003). This polymorphism is also referred to as the start codon polymorphism (SCP). Two protein variants can exist corresponding to the two available start sites: a long version of the VDR protein (the T allele or the f allele; and also referred to as the M1 form, i.e., methionine at first position) and a protein

shortened by three amino acids (the C allele or F allele; also referred to as the M4 form, i.e., methionine at forth position) (Uitterlinden–2004).

The Fok 1 RFLP can be considered an independent marker in the VDR gene since there is no linkage disequilibrium (LD) with any of the other VDR polymorphisms and the LD area surrounding this polymorphism is very small (Uitterlinden–2004 and Vidal-2003). Therefore, LD with another polymorphism is not a likely explanation for the association observed with this polymorphism. So functional studies should be focused on the polymorphism itself.

In a study by Gross et al (1996) on a group of 100 postmenopausal Mexican-American Caucasian women found subjects with the ff genotype (15% of the study population) to have a12.8% lower BMD at the lumbar spine than FF subjects (37% of the population) ($p = 0.01$). Over a 2-year follow-up period, a decrease in BMD at the femoral neck was greater in ff compared with FF subjects (-4.7% vs. -0.5%, $p = 0.005$). In a study in children aged 7-12 years (Ames-1999), the Fok 1 polymorphism at the VDR translation initiation site was associated with bone mineral density and calcium absorption. The Fok1 polymorphism at the VDR translation initiation site was significantly associated with BMD ($p = 0.02$) and calcium absorption ($p = 0.04$). Children who were FF homozygotes had a mean calcium absorption that was 41.5% greater than those who were ff homozygotes and 17% greater absorption than Ff heterozygotes. BMD was 8.2% greater in the FF genotype than the ff genotype and 4.8% higher than the Ff genotype. Tajima et al (2000) suggest bone metabolic response to exercise is mediated differently between carriers and non-carriers of the f allele of the VDR gene, suggesting functional differences between the genotypes, given gene-environment interaction. These

results suggest a substantial relationship between the VDR gene and bone health at one or more levels.

Nakamura et al (2002) reported that BMD in young male athletes with different impact loading depended on the VDR genotypes, implying that the FF genotype may respond more sensitively to differences in impact loading in regulating BMD, rather than merely being a predictor of high BMD. The VDR genotypes, as detected by endonuclease Fok 1, and bone phenotypes of the lumbar spine and femoral neck were examined in 44 highly trained young male athletes and 44 age-matched non-athletic controls (age: 18-21 years). As a whole, the athletes had a significantly higher bone mineral content resulting from a combination of increased volume and density at both sites than the controls. When the athletes were compared with the controls within each VDR genotype, however, the increase spinal volume was found only in athletes with the FF (2.92 + 1.14) but not in those with the Ff (1.98 + 1.10) genotype ($p<0.01$). Differences in bone mineral content in the lumbar spine and femoral neck between the controls and the athletes were greater in subjects with FF than those with Ff. These findings suggest the adaptive response of bone to exercise in athletes may depend in some part on inter individual allelic variance of the VDR gene at the translation initiation site. It is not known if this genetic marker is associated with susceptibility for stress fractures in competitive athletes.

A stress fracture in an athlete affects the person physically, emotionally and financially. There is a great amount of time lost from the sport, and a financial burden is placed on the individual due to surgery and other costs. Identifying genetic variants which increase the susceptibility to stress fractures would improve identification of athletes predisposed to stress fractures. Understanding the relationship between stress

fractures and genetic markers could potentially lead to a greater understanding of the patho-physiology and treatment of stress fractures. This could ultimately lead to advances that would result in lower incidence of stress fractures in susceptible athletes.

Therefore, the purpose of this study was to determine the influence of T →C transition within exon 2 of the VDR gene defined by endonuclease Fok 1 on the risk of stress fractures in competitive athletes. We hypothesized the possession of f -allele will occur more frequently in athletes with a history of stress fractures compared to activity, age, gender and race matched controls without a history of stress fractures.

Chapter 3

METHODS

Subjects

Thirty-two competitive male and female athletes from Michigan State University volunteered to participate in this research study. Fifteen athletes had previous history of stress fractures (height = 168.98 $\pm$ 9.89 Cm, Weight = 61.69 $\pm$ 9.40 Kg) and 17 athletes without stress fracture (height = 171.48 $\pm$ 8.27 Cm, Weight = 65.87 $\pm$ 10.62 Kg) were considered as controls. Stress fracture history was self-reported. Athletes with stress fracture were matched with controls on the basis of age, gender, race and sport.

Potential subjects were provided information regarding the study. Upon agreement of participation, subjects were asked to read and sign an informed consent form, which was approved by the Michigan Sate University Committee on Research Involving Human Subjects (see Appendix). The following data were collected from the subjects after signed consent.

Anthropometrics

Subjects standing weight (kg) and height (cm) were measured with a calibrated beam balance and a stadiometer respectively. Skin folds were measured with a Lange caliper using standard procedures. Body fat percentage was estimated according to Jackson and Pollock (1985) for male athletes and Warner et al (2004) for female athletes.

Questionnaires

All subjects completed two questionnaires; one regarding their sports history and family history of stress fractures and a social habits questionnaire (Paffenbarger –1993) (see Appendix).

Blood sample

5 ml of whole blood obtained form an anticubital vein was collected into a tube. Blood was stored at -80 degree Celsius utill DNA could be extracted from this blood sample using commercially available kits (Qiagen Inc.). Extracted DNA was stored at -20 degree Celsius utill further analysis.

DNA analysis

Stored DNA was analyzed to determine the influence of T to C transition within exon 2 of the VDR gene defined by endonuclease Fok 1 on the risk of stress fractures in competitive athletes. The VDR genotype was determined by polymerase chain reaction (PCR) as described by Nakamura et al (2002) with slight modifications in the thermo cycling conditions (94 C for 5 minutes, 94 C for 30 seconds, 56 C for 30 seconds, 72 C for 1.5 minutes and 72 C for 2 minutes for 35 cycles), followed by restriction fragment length polymorphism analysis using the endonuclease Fok 1, which recognizes ATG as part of its restriction site. The sequence of primers used was, left primer: agggcgaatcatgtatgagg and right primer: tcaaagtctccagggtcagg. PCR products were purified using Qiagen PCR clean up kit. 1ug PCR product was digested with 1U FokI enzyme in a total reaction volume of 20ul. 8% polyacrylamide gels were run after staining with etidium bromide (0.5ug/ml in 1* TBE). The gels were visualized in Bio-Rad Gel Doc 2000 using Quantity-One software. The presence and absence of the Fok 1 site were denoted as f and F, respectively and individuals were categorized as homozygotes for ff or FF, and Ff heterozygotes.

Statistical analysis

Comparison between athletes with and without stress fracture for the anthropometric data was performed with a t-test. The relationship between FF, Ff and ff alleles and occurrence of stress fractures was tested with Chi square test. Association between oral contraceptive use, alcohol consumption and menstrual history with stress fracture was analyzed with a Chi square test. All tests were performed as two-sided, with a priori significance level selected as $P<0.05$.

Chapter 4

RESULTS

Of the 32 athletes, three athletes with stress fractures did not have any controls for sport and race and were therefore excluded from all the analyses. Genotype analysis on two athletes was unsuccessful. Nutrition and body composition information was unavailable for 6 athletes (3 controls and 3stress fracture). Athletes with stress fracture were matched with one control on the basis of age, gender, race and sport.

Group Comparisons

A t-test on 22 subjects (9 stress fracture and 13 controls) revealed no significant differences in fat mass (FM), fat free mass (FFM), height and weight between the stress fracture and control group. This indicates that the groups were equivalent in their body composition and stature (Table 4.1).

Genotype

Of the 12 stress fracture athletes, 8 (66%) were heterozygous (Ff) and 4 (33%) were homozygous (FF). In the 15 control subjects, 10 athletes (66%) were heterozygous (Ff) and 5 (33%) were homozygous (FF). Chi-square revealed no significant difference in the genotype distribution between the stress fracture and the control groups ($p = 0.65$). In the genotype analysis, each athlete with a stress fracture was matched with one control on the basis of age, gender, race and sport.

Menstrual History and Oral Contraceptive Use

Of the 19 female athletes, 26.31% were oligomenorrheic/amenorrheic and 73.68% were eumenorrheic. Of the 9 athletes with stress fracture, 44.44% were oligomenorrheic/ amenorrheic and 55.55% were eumenorrheic. Out of 10 athletes without stress fractures,

10% were oligomenorrheic/ amenorrheic and 90% were eumenorrheic. There was a trend (p =0.10) for a higher proportion of oligomenorrheic/amenorrheic athletes in the stress fracture group. The percentage of OCP use in this group of athletes was 57%. Of the 9 athletes with stress fracture, 66% were OCP users and of the 10 controls, 55% were OCP users. There was no significant difference in stress fracture incidence between the two groups based on OCP use (p = 0.62).

Alcohol Intake

Data on alcohol intake were available for 22 subjects. 54% of athletes consumed alcohol on a regular basis and 46% consumed no alcohol. Out of 9 stress fracture athletes, 66.66% were alcohol consumers and 33.33% were non-drinkers. In the control group, out of 13 athletes, 38.46% regularly consumed alcohol and 61.54% did not. Chi-square revealed no significant differences between the two groups (p = 0.19) for alcohol intake.

Table 4.1: Chi-square and t-test Results

Variables	Stress Fracture	Controls
Height (Cm)	170.18 ± 10.47 (9/15)	171.58 ± 8.60 (13/17)
Weight (Kg)	63.27 ± 9.45 (9/15)	65.10 ± 10.63 (13/17)
Fat Free Mass (Kg)	51.33 ± 8.55 (9/15)	52.95 ± 9.72 (13/17)
Fat Mass (Kg)	11.64 ± 2.61 (9/15)	12.19 ± 4.26 (13/17)
Percentage with f allele (p =0.65)	66% (12/15)	66% (12/17)
Percentage alcohol consumers(P =0.19)	66% (n =9/15)	38.46% (1317)
Percentage with amenorrhea (P =0.1)	44% (9/15)	10% (10/17)
Percentage of OCP users (P =0.62)	66% (9/15)	55% (10/17)

Chapter 5

DISCUSSION

Previous research has evaluated trends in possible risk factors for stress fractures in athletes. The purpose of this research study was to determine the influence of T →C transition within exon 2 of the VDR gene defined by endonuclease Fok 1 on the risk of stress fractures in competitive athletes. As the frequency of genetic polymorphisms can differ markedly in populations, misleading associations can arise from poor matching of cases and control subjects. No differences between the stress fracture and the control group were observed in regards to their body composition and stature, indicating equivalence between the groups in this case-control study. The subjects were also matched for age, race, gender and sport.

Genotype

Results of this study do not support the hypothesis that the possession of f -allele occurs more frequently in athletes with a history of stress fracture compared to activity, age, gender and race matched controls without a history of stress fracture. The distribution of the F and f alleles of the VDR gene was similar in both groups, suggesting that this polymorphism is not a primary cause of stress fractures in competitive athletes. The subject sample was primarily Caucasian in this study. Genotype distribution of the translation initiation codon polymorphism (T → C) in this study was comparable with genotype distribution in other Caucasian populations (Gross –1996, Ferrari –1998 and Eccleshall –1998).

As many factors are involved in the control of bone formation and resorption, predisposition of stress fracture is likely multi-factorial involving the interactions of both

environmental and genetic influences. Environmental influences may be more important in the predisposition of stress fractures in competitive athletes.

It is important to analyze all known VDR polymorphisms and their interrelationships since they interact to determine VDR expression and activity (Whitfield –2001). In a normal active cell, certain promoter polymorphisms collaborate with certain 3' untranslated region of the gene (UTR) polymorphisms in regulating the amount of VDR mRNA being available in a certain target cell. Together, they determine the expression of the known FokI variants, F and f (Uitterlinden –2004). However, the polymorphism studied can be considered an independent marker in the VDR gene since there is no linkage disequilibrium (LD) with any of the other VDR polymorphisms and the LD area surrounding this polymorphism is very small (Uitterlinden–2004 and Vidal-2003). Therefore, LD with another polymorphism is not a likely explanation for the association observed with this polymorphism.

Gene-gene interactions may also be responsible for the lack of allelic difference between the two study groups. Bone mineral density, which is a risk factor for stress fractures, is influenced by a number of different genes (Mirandola –2002, Hosoi –1999, Mizunuma -1997 and Murray –1997). Thus, the accumulated effects from many genes may lead to the observed phenotype for stress fracture. Uitterlinden et al (2001) reported an interlocus interaction between the VDR gene and the ColIA1 gene, and concluded that interactions between the two loci are responsible for an increased fracture risk in female's aged 55 and above. These observations show that the influence of the VDR genotype on stress fracture may depend upon the presence or the absence of allelic variations in other genes.

Furthermore, an association between stress fracture and VDR genotype may be present with a much larger sample size. However, our results suggest that any association is not significantly robust to detect in a screening of 32 competitive athletes. Therefore, it appears there is little potential in utilizing VDR genotype as a screening device for stress fractures in athletes.

Menstrual History and Oral Contraceptive Use

The female reproductive system is sensitive to physiological stress and female athletes are at a risk of developing disorders such as amenorrhea. Skeletal problems such as stress fractures and osteoporosis associated with menstrual abnormalities could develop in female athletes. The rate of bone mineral loss in amenorrheic athletes has been observed to be equal to menopausal women (Warren –2003).

This research study showed a trend towards high incidence of stress fracture in amenorrheic athletes. Studies with small sample of runners have noted a high incidence of stress fractures in amenorrheic runners when compared with eumenorrheic runners (Lindberg –1984 and Marcus –1985). A low subject number in this study, led to a lower statistical power, which could be a reason we did not observe a statistical significance. However, this trend does support previous research and suggests that screening for stress fracture risk using the VDR genotype would, at best, be less effective than obtaining a menstrual history for the female athletes.

These data show no association between oral contraceptive pill (OCP) use and stress fractures. There have been a number of studies relating to the use of OCP by female athletes (Hartard –2004, Barrow –1998, Cann –1998, Cooper –1993 and Vessey –1998) that have yielded conflicting results. This could be due to differences among studies in

pill dosage and formulation, menstrual history, duration of pill use, age at which subjects were exposed to the oral contraceptive and outcome measures.

Alcohol Intake

Alcoholism is associated with a number of factors known to increase risk of osteoporotic fractures, such as, poor nutrition, liver disease, malabsorption of nutrients, and vitamin D deficiency (Seeman –1996). Diamond et al (1989) suggested that excessive alcohol consumption decreases bone formation, leading to defective mineralization. In a study by Lappe et al (2001), excessive alcohol intake, defined as a self-report of 10 or more alcoholic drinks a week, was a risk factor for stress fracture, even after controlling for age, bone density and race. Long-term excessive alcohol intake was associated with low bone mass in both genders (Daimond –1989 and Seeman – 1996).

In this study, no significant association was reported between alcohol consumption and stress fractures. This could be due to low alcohol consumption observed in this group. The athletes in this study can be classified as moderate drinkers as predominantly most athletes (54%) did not consume alcohol more than 2-3 times a month.

Clinical Implications and Future Studies

This study found no allelic difference in the stress fracture and the control group. Stress fracture depends not only on the intrinsic properties of the skeleton, but also on several factors independent of bone and related to failure load. These factors have their own heritable and non-heritable components and are difficult to control. Isolated genetic markers, which contribute to a small amount in the total variance of bone strength, may be insufficient to detect significant differences in incidental stress fractures in most cases.

Large-scale, carefully controlled studies looking at multiple haplotypes influencing a well-defined biological pathway, such as osteoblast-osteoclast activity, calcium homeostasis and hormonal imbalances may be a more realistic approach in identifying important genetic markers predisposing athletes to stress fractures.

Limitations

The small sample size contributes to low power of the study. Of the 32 subjects tested, 3 stress fracture subjects had to be excluded from the analyses due to lack of controls for sport and race. Stress fracture and the control groups started out with 15 and 17 subjects respectively, but 3 subjects from each group did not complete all the study procedures. A large sample size would have been ideal, but due to time constraints and subject availability, it was not feasible.

Another study limitation was the inability to measure bone mineral density due to cost constraints. The translation initiation codon polymorphism (T $\rightarrow$ C) in the VDR gene adversely influenced bone mineral density in several studies. It would have been interesting to see if there were differences in bone mineral density between athletes with and without stress fractures. However, even if the VDR polymorphism influenced bone mineral density, our data suggest that the influence was not sufficient to put athletes at increased risk for stress fracture.

In conclusion, data from the present study confirm the association between oligomenorrheic/amenorrhea and increased risk of stress fracture in female athletes and suggest that screening for VDR genotype is not an effective strategy for determining division 1 athletes at risk for stress fractures.

Appendix A

INFORMED CONSENT FORM

GENETICS AN INTRINSIC RISK FACTOR FOR STRESS FRACTURES

Primary Investigators:

Dr. Christopher Womack

Dr. John Powell

Dr. Jeffrey Kovan

Sheetal Patil

Summary of research protocol:

You have been asked to participate in a study investigating the incidence of the genes COL2A1, COL9A3 and TGF beta-1 in athletes with and without stress fractures. Your participation in this study will help us understand how specific genes may predispose athletes to stress fractures.

You will be asked to fill two questionnaires. A blood sample will be taken for extraction of DNA. Peripheral bone density testing will be done using a bone sonometer. Your height, weight, abdominal and thigh skinfolds will also be measured.

Estimate of subject's time:

The study will take not more than 25 minutes.

Experimental procedures:

Height and Weight- Your standing weight (kg) and height (cm) will be measured with a calibrated beam balance and stadiometer.

Questionnaires- One questionnaire will provide information about your medical & sports history along with familial history of stress fractures. The other questionnaire will be a

record of their dietary and social habits.

Blood Sample- About 10ml of blood (2 tablespoons) will be collected into a tube. The blood will then be stored and DNA extracted from this blood sample. Your DNA will be kept in the freezer in Dr. Womack's laboratory for a period of 5 years.

Peripheral Bone Density- Peripheral bone density testing will be done using a bone sonometer. It is a non-invasive device and measures bone mineral density in less than a minute by passing sound waves through a finger.

Skinfolds- Abdominal and thigh skinfolds will be measured with a Lange caliper using standard procedures.

Risk/Discomforts:

You were chosen for this study because you are a competitive athlete, who has either had a stress fracture or participates in a sport where stress fractures commonly occur. Sterile procedures will be used while taking the blood sample. Risks of blood sample can include bruising and swelling. You may experience some mild discomfort during the blood sample.

Payment:

You will not be paid for this study.

Voluntary participation:

Your participation in this study is completely voluntary and dependent upon your consent. You may choose to withdraw from this study at any time. Furthermore, any results of the testing that you complete will be available to you. In addition, you may request at any time that your stored DNA be disposed of and made unavailable for further analysis.

Confidentiality and anonymity:

Your privacy will be protected to the maximum extent allowable by law. Presentation or publication of your results will in no way identify you personally. The records and results obtained will only be available to the investigators of this study (and to yourself if you wish) and will not be shared with others, unless you give prior consent with approval.

Contact person

If you have any questions and/or concerns about your participation in this study, please contact the investigator, Dr. Christopher Womack at (517) 353-5222, or by e-mail at cwomack@msu.edu. If your questions or concerns regarding your rights as a study participant, or are dissatisfied at any time with any aspect of this study, you may contact - anonymously, if you wish- Peter Vasilenko, Ph.D., Chair of the University Committee on Research Involving Human Subjects (UCRIHS) by phone: (517) 355-2180, fax: (517) 432-4503, e-mail: uchrihs@msu.edu , or regular mail: 202 Olds Hall, East Lansing, MI 48824.

Injury:

If you are injured as a result of your participation in this research project, Michigan State University will assist you in obtaining emergency care, if necessary, for your research related injuries. If you have insurance for medical care, your insurance carrier will be billed in the ordinary manner. As with any medical insurance, any costs that are not covered or in excess of what are paid by your insurance, including deductibles, will be your responsibility. The University does not provide financial compensation for lost wages, disability, pain or discomfort. This does not mean that you are giving up any legal rights you may have. You may contact Chris Womack at (517) 353-5222 with any questions or to report an injury.

If you agree to participate in this study, please sign your name below. By completing and returning the form, you indicate your voluntary agreement to participate.

Signature of participant

Signature of investigator

Date

UCRIHS APPROVAL FOR THIS
PROJECT EXPIRES: JUL 2 6 2005

SUBMIT RENEWAL APPLICATION
ONE MONTH PRIOR TO
ABOVE DATE TO CONTINUE

Appendix B

MEDICAL HISTORY QUESTIONNAIRE

Identification Number:

DOB:

Height: Weight:

1. Have you ever been diagnosed with stress fracture?

   Yes ( ) No ( )

2. Approximate date of diagnosis (day/month/year):

3. Have you had any other general fracture?

   Yes ( ) No ( )

4. Do any of your parents have a history of fracture?

   Yes ( ) No ( )

5. Which sport do you play for?

6. How long have you been competitive in your sport?

7. Have you been competitive in any other sport?

   Yes ( ) No ( )

8. List the other sports you have been competitive in.

9. How long have you been competitive in these other sports?

10. Are you on birth control pills or do you use any birth control patch?

    Year started: Years used:

11. Age at menarche:

12. Frequency of menses:

    Every month: Every 2 months: Every 3 months: Once/twice a year:

Appendix C

SOCIAL HABITS QUESTIONNAIRE

(Paffenbarger –1993)

How many servings of the following foods do you eat? (Please respond to each food)

	Almost Never	1-3/Month	1-2/Week	3-6/Week	1-2/Day	3-5/Day	6+/Day
Eggs							
Whole milk							
Low fat milk							
Cream							
Yogurt							
Cheese							
Ice cream							
Butter							
Margarine							
Poultry							
Fish							
Beef, Pork, Lamb							
Vegetables							
Green salads							
Breads and cereals							
Fruits							
Fruits juices							

	Almost Never	1-3/Month	1-2/Week	3-6/Week	1-2/Day	3-5/Day	6+/Day
Sweet desserts							
Candy							
Salty Snacks							
Tea							
Coffee							
Wine, sherry							
Beer, ale							
Whiskey, gin							

1. How often do you eat in "fast food places" (hamburger, fried chicken, taco, deep-fried food cafes)? Number of times per month:

2. How often do you eat "TV dinners"? Number of times per month:

3. How often are you dieting (eating less than you would like)? (Please check one)

a. Never b. Rarely c. Sometimes d. Often e. Always

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