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A 12-Month Prospective Study of the Relationship Between Stress Fractures and Bone Turnover in Athletes

K. L. Bennell,[1] S. A. Malcolm,[2] P. D. Brukner,[3] R. M. Green,[4] J. L. Hopper,[4] J. D. Wark,[5] P. R. Ebeling[6]

[1]School of Physiotherapy, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, 200 Berkeley St, Carlton, Victoria 3053 Australia
[2]School of Human Biosciences, La Trobe University, Melbourne, Australia
[3]Olympic Park Sports Medicine Centre, Melbourne, Australia
[4]Department of Public Health and Community Medicine, The University of Melbourne, Australia
[5]The University of Melbourne, Department of Medicine and Bone and Mineral Service, The Royal Melbourne Hospital, Australia
[6]Bone and Mineral Service, Department of Diabetes and Endocrinology, The Royal Melbourne Hospital, Australia

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Abstract. Bone remodeling may be involved in the pathogenesis of stress fractures in athletes. We conducted a 12-month prospective study to evaluate bone turnover in 46 female and 49 male track and field athletes aged 17–26 years (mean age 20.3; SD 2.0) 20 of whom developed a stress fracture. Baseline levels of bone turnover were evaluated in all athletes and monthly bone turnover levels were evaluated in a subset consisting of the 20 athletes who sustained a stress fracture and a matched comparison group who did not sustain a stress fracture. Bone formation was assessed using serum osteocalcin (OC) measured by human immunoradiometric assay and bone resorption by urinary excretion of pyridinium cross-links (Pyr and D-Pyr); high performance liquid chromatography and N-telopeptides of type 1 collagen (NTx) using ELISA assay. Athletes who developed stress fractures had similar baseline levels of bone turnover compared with their nonstress fracture counterparts ($P > 0.10$). Results of serial measurements showed no differences in average levels of Pyr, D-Pyr, or OC 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 bony pain. Our results show that single and multiple measurements of bone turnover are not clinically useful in predicting the likelihood of stress fractures in athletes. Furthermore, there were no consistent temporal changes in bone turnover associated with stress fracture development. However, our results do not negate the possible pathogenetic role of local changes in bone remodeling at stress fracture sites, given the high biological variability of bone turnover markers and the fact that levels of bone turnover reflect the integration of all bone remodeling throughout the skeleton.

Key words: Bone turnover — Bone remodeling — Stress fractures — Exercise.

Stress fractures are partial or complete fractures of bone resulting from repetitive mechanical loading. They are common in athletes [1, 2] and in military recruits involved in basic training [3]. Prevention of this injury requires an understanding of its pathogenesis and of possible factors that place an individual at risk. There is a need to develop and evaluate screening procedures that may be useful in predicting the likelihood of stress fracture.

Bone strain arising from mechanical loading stimulates the remodeling process, normally resulting in a bone better suited to withstand the applied load [4]. However, mechanical loading is also associated with production of microdamage in bone. This has been shown in bone specimens [5], in animals following a period of forced exercise [6–8], and in human biopsy samples [9, 10]. The magnitude of bone strain required for microdamage initiation in humans appears within the range normally experienced by humans during physical activity. Stress fractures develop if microdamage cannot be successfully repaired and thus accumulates to form symptomatic 'macrocracks' in bone.

One of the mechanisms to prevent microdamage progression is repair via the remodeling process. Mori and Burr [8] noted a significant increase in new remodeling sites after bone microdamage was initiated in dogs. However, microdamage may also occur at preexisting sites of remodeling where osteoclastic resorption weakens an area of bone and subjects it to higher strains before the addition of new bone by osteoblasts. Li et al. [7] employed an exercising rabbit model to assess sequential pathological changes in the internal structure of the tibia over a 10-week period. Within the first week, osteoclastic resorption cavities appeared in the tibial cortex and interstitial lamellae followed in the second week by small cracks. By the third week, incomplete fracture of the tibial cortex was found in some specimens. Over the remaining 6 weeks, the resorption cavities gradually filled with bone and converted to haversian bone. One specimen developed a cortical fracture. Thus, most tibiae adapt successfully to changes in bone strain from repetitive loading through internal remodeling but fractures may appear if excessive stress continues in a tibia weakened by osteoclastic resorption.

It is apparent that bone remodeling plays a role in stress fracture pathogenesis and that perturbations in bone remodeling, either generalized or focal, may predispose to this

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Correspondence to: K. Bennell

Table 1. Means (SD) of subject characteristics and unadjusted baseline pyridinoline, deoxypyridinoline, N-telopeptide, and osteocalcin comparing athletes who developed stress fractures (SF) with those who did not (NSF) in both men and women

	Women		Men	
	SF n = 10	NSF n = 36	SF n = 10	NSF n = 39
Age	20.6(1.8)	20.4(2.3)	20.3(1.5)	20.2(2.0)
Height (cm)	164.4(4.8)	167.8(6.2)	177.9(5.5)	179.1(6.4)
Weight (kg)	57.1(4.9)	58.9(5.5)	68.0(4.1)	69.8(7.6)
Athletic training (hrs/wk)[a]	9.3(4.5)	12.9(5.8)	11.6(4.5)	12.6(3.7)
Running distance (km/wk)[a]	45.1(22.8)	40.2(30.9)	60.8(44.1)	56.8(39.9)
Pyr:Cr (nmol:mmol)	80.21(19.92)	78.48(25.63)	71.42(25.71)	91.62(39.39)
D-Pyr:Cr (nmol:mmol)	13.48(5.67)	13.18(5.45)	14.04(5.04)	16.56(9.58)
NTx:Cr (nmol BCE:mmol)	106.40(83.2)	88.17(52.3)	88.90(35.39)	117.26(63.97)
OC (ng/ml)	7.84(4.01)	6.46(3.11)	9.44(2.65)	10.36(4.26)

SF = stress fracture, NSF = nonstress fracture
[a] In year preceding study

injury. Accelerated bone remodeling resulting from excessive bone strain or from the influence of systemic factors may weaken bone due to an associated increase in the remodeling space. This could allow the accumulation of microdamage with repetitive mechanical loading. Conversely, depressed bone remodeling, in particular bone formation, may not allow normal skeletal repair of naturally occurring microdamage. It is conceivable that either sequence could lead to the development of a stress fracture in individuals training intensely.

Since direct assessment of bone remodeling in humans is invasive and impractical, measurement of biochemical markers of bone turnover may provide information about the role of bone remodeling in stress fracture development. A prospective study of 104 male military recruits found that plasma hydroxyproline (a nonspecific indicator of bone resorption), measured in the first week of a training program, was significantly higher in those who subsequently sustained stress fractures than in those who remained uninjured [11]. This supports the concept that elevated bone turnover may be a stimulus for stress fracture development and that biochemical markers may have a role in predicting individuals at risk for this bony injury. However, results from military recruits may not generalize to athletes as they represent different populations. In particular, athletes are accustomed to intense physical activity whereas recruits often commence a training program with low initial fitness levels.

A limited number of case-control studies have measured biochemical markers of bone turnover in small numbers of female athletes with and without a history of stress fracture [12–14]. However, these studies involved only single measurements at variable times after diagnosis. There are no prospective data that assess whether bone turnover differs in athletes who subsequently develop a stress fracture compared with those who do not. We postulated that biochemical markers of both bone resorption and bone formation may prove useful in a clinical setting to aid identification of athletes most at risk for this injury. In addition, assessment of temporal changes in bone turnover prior to and following a stress fracture may provide insight into the response of bone to excessive loading.

Therefore, the aims of our prospective study were first to assess the clinical usefulness of baseline measures of bone turnover in the detection of athletes at risk of sustaining a stress fracture over the next 12 months. Secondly, we related serial measurements of bone turnover markers to stress fracture status and compared measurements in the months preceding with those in the months following a stress fracture.

Methods

The experiment consisted of a 12-month prospective study with a nested case-control component.

Subjects

Eligible subjects consisted of male and female track and field athletes ranging in age from 17 to 26 years and registered with a Victorian athletics club. Subjects were excluded from participation if they reported past (within 12 months) or present use of anabolic steroids or human growth hormone, had a history of bone disease, or were currently taking any medication likely to influence bone density (with the exception of the oral contraceptive pill). Initially, 111 athletes (53 F, 58 M) entered the study. By the end of 12 months 16 athletes (14%: 7 F, 9 M) had withdrawn because of work commitments, retirement from athletics, or relocation. Only the 95 athletes (46 F, 49 M) who remained in the study are included in the analyses. Of the remaining athletes, 16 (5 F, 11 M) competed in sprints, 35 (17 F, 18 M) in middle-distance running, 19 (9 F, 10 M) in distance running, 11 (6 F, 5 M) in hurdles, 10 (5 F, 5 M) in jumps, and 4 (4 F, 0 M) in multi-events.

During the 12-month study period, 10 women (21.7%) and 10 men (20.4%) sustained at least one stress fracture as diagnosed by positive findings on clinical examination, triple phase isotope bone scan, and computed tomography (CT) [1]. One man and one woman developed stress fractures on more than one occasion. The overall stress fracture incidence rate of 21.1% found in this cohort is comparable with other studies of track and field athletes [2, 15]. Of the total 26 stress fractures sustained by the athletes, 45% occurred in the tibia, 15% in the navicular, 12% in the fibula, and 8% in the metatarsal bones.

There was no significant difference in age, height, weight, weekly training volume, or weekly running distance when comparing the group who sustained a stress fracture with the group who did not in either sex (Table 1). We have previously reported further details regarding the cohort characteristics and stress fracture distribution [1]. In addition, we have previously reported diet, total body bone mineral content, (BMC) regional BMD, training, anthropometry, soft tissue composition, biomechanical features,

and menstrual characteristics when comparing the stress fracture and nonstress fracture groups in this cohort [16].

Procedures

This research project was undertaken following approval from the Human Experimentation Ethics Committees of La Trobe University and The Royal Melbourne Hospital. Subjects gave written informed consent prior to participation.

Measurement of Bone Turnover. Blood and urine samples were requested from all athletes at baseline, and then at monthly intervals over the course of the study period. Seventy-two percent of females and 88% of males provided at least 10 samples over the 12 months. Single baseline samples from all 95 athletes remaining in the study were analyzed at its conclusion for bone turnover markers. Monthly samples, however, were analyzed for bone turnover markers only in a subset of athletes, consisting of those 20 athletes who sustained a stress fracture during the study and a comparison group who did not sustain a stress fracture (11 F, 15 M) and for whom at least 10 samples were obtained, frequency-matched for age, sex, athletic event, and menstrual characteristics. Eighty percent of stress fracture athletes provided at least 10 samples.

Blood and urine samples were collected on the same day between 0800 and 1100 hours following an overnight fast. For female subjects, samples were collected during the early follicular phase of the menstrual cycle, defined as within 7 days following the onset of menstrual bleeding. Subjects were requested not to train within 2 hours preceding sample collection to allow for any acute effects of exercise on markers of bone turnover. For the majority of samples, subjects reported not training for at least 12 hours prior to collection.

To measure osteocalcin, 15 ml of blood from an antecubital vein was collected into plain tubes. Serum osteocalcin was measured by a human immunoradiometric assay (Immutopics, Inc. San Clemente, CA). The intra- and interassay coefficients of variation (CV) were 7% and 9%, respectively. The normal reference range for serum osteocalcin in our laboratory is 2.8–8.8 ng/ml for men and women with a mean ± SD age of 34.4 ± 8.5 years.

To measure pyridinium cross-links [pyridinoline (Pyr) and deoxypyridinoline (D-Pyr)] and N-telopeptides of type 1 collagen (NTx), a second-void, early morning, 2-hour urine collection was made after an overnight fast. The concentration of total pyridinium cross-links in each urine sample was measured by high performance liquid chromatography (HPLC). Fluorescence was measured at 297 nm (ex) and 395 nm (em). Pyr and D-Pyr were quantitated using an external standard prepared from sheep cortical bone and calibrated against a highly purified pyridinoline standard kindly provided by Professor D. Eyre (University of Washington, Seattle, WA, USA). The intra- and interassay CVs for this assay are both less than 9%. Where possible, all analyses on a single subject were performed in a single assay run. Recoveries of samples for this assay are on average 99.6% (range 88.5–125.0%). Urinary Pyr and D-Pyr values were adjusted for urine creatinine (Cr) excretion. Any values lying outside two standard deviations (SDs) from the mean of a specimen run were repeated in duplicate assays. Urinary Cr concentration was measured by the Jaffe reaction using a Beckman Synchron CX7 Analyser (Beckman Instruments Inc., Palo Alto, CA) prior to hydrolysis. The CV for measurement of Cr concentration was less than 8%.

N-telopeptide Immunoassay cross-linked N-telopeptides of type 1 collagen were measured in duplicate ELISA assays of unextracted, nonacidified urine (Osteomark, Ostex Internation, Seattle, WA). Values were expressed as nmol bone collagen equivalents (BCE) per mmol creatine, measured as stated above. The intra- and interassay CVs were both less than 8%.

Measurement of Bone Mass. At baseline, total BMC was measured by dual energy X-ray absorptiometry (DXA) using a Hologic QDR 1000W densitometer (Hologic Inc., Waltham, MA). DXA short-term *in vivo* precision was evaluated from triplicate scans in 15 normal, healthy subjects. The CV was 0.6% for total body BMC.

Measurement of Menstrual Factors. Details pertaining to menstrual history were obtained by questionnaire. Menstrual status during the 12-month study, including number of menses and use of the oral contraceptive pill (OCP), was assessed prospectively from a diary maintained by all female subjects.

Statistical Analyses

Bone turnover measures were transformed to reduce skewness and to approximate normal distributions. The transformation function chosen was that which best achieved a normal distribution. Comparisons between groups were conducted using independent *t*-tests. Analyses of stress fracture status as a function of the four baseline measurements of bone turnover (log Pyr, log D-Pyr, log NTx, and log OC), total body BMC, and other covariates were carried out using logistic regression.

To assess whether serial monthly measurements of bone turnover differed in athletes who developed a stress fracture, the relationship of transformed measures of bone turnover (log Pyr, log D-Pyr, and Sqrt OC) to stress fracture status and to potential confounding variables such as age and sex was assessed by linear regression. NTx measurements were not obtained for serial samples. For each individual, all measurement time points were included regardless of their relationship to stress fracture onset. In order to take account of these repeated measurements of bone turnover in the same individual, the regression parameters were estimated using a multivariate normal model fitted by the software package FISHER [17, 18]. For a more detailed description of this modeling method, see Hopper et al. [19].

In those athletes who developed a stress fracture, the monthly Log Pyr, Log D-Pyr, and Sqrt OC measurements were expressed as a z-score by subtracting the mean and dividing by the SD. Each individual's monthly z-scores were organized according to their time location prior to or following stress fracture onset, designated as the first onset of pain. At each such time point, a median z-score across the group was calculated using individual z-scores. The number of individual z-scores at each time point varied depending on when athletes developed stress fractures during the study. Examination of any differences in bone turnover between the periods of pre- and poststress fracture were made by plotting the median z-scores at each such time point.

To evaluate whether bone turnover prior to stress fracture differed from that following fracture, athletes served as their own controls. For each athlete, monthly measurements of bone turnover were ranked from highest to lowest. The observed sum of the rankings prior to a stress fracture was compared with the expected sum, under the null hypothesis that there was no difference between values pre- and poststress fracture, using the normal approximation to the Wilcoxon distribution [20]. The group average of these individual statistics was then compared with zero using an unpaired *t*-test.

Mean bone turnover values pre- and poststress fracture were also compared using the multivariate autoregressive normal model. In athletes who developed a stress fracture, mean bone turnover values prior to the stress fracture were compared with those following, irrespective of their temporal distance from stress fracture onset. In order to further evaluate whether bone turnover values in close temporal proximity to stress fracture onset differed from those at more distant time points, mean values within 1, 2, or 3 months preceding, and for the same periods following the stress fracture, were also compared.

Results

Does a Baseline Measure of Bone Turnover Predict Which Athletes Develop a Stress Fracture?

The means (SD) of baseline unadjusted biochemical markers of bone turnover comparing athletes who did with those who did not develop a stress fracture are shown in Table 1.

Table 2. Multivariate autoregressive model showing regression coefficient estimates (β) and standard errors (SE) for the relationship of log pyridinoline, log deoxypyridinoline, and square root osteocalcin to age, sex, and stress fracture status

Bone turnover marker	Independent variables	β	SE	*P* value
Log Pyr				
	Sex[a]	0.0826	0.0260	⩽0.001
	Age	−0.2988	0.0343	⩽0.001
	Age^2	0.0062	0.0022	⩽0.001
	Stress fracture[b]	0.0442	0.0269	⩽0.10
Log D-Pyr				
	Sex[a]	0.0812	0.0305	⩽0.01
	Age	−0.0310	0.0065	⩽0.001
	Stress fracture[b]	0.0378	0.0311	⩾0.10
Sqrt OC				
	Sex[a]	0.4398	0.1130	⩽0.001
	Age	−0.0714	0.0224	⩽0.01
	Stress fracture[b]	0.1665	0.1150	⩾0.10

[a] 1 = men, 0 = women
[b] 1 = yes, 0 = no

There was no difference between stress fracture and non-stress fracture groups in log Pyr, log D-Pyr, log NTx, or log OC in either sex ($P > 0.10$). None of the baseline bone turnover markers predicted stress fracture occurrence, either before or after adjusting for total body BMC and sex ($P > 0.10$). The role of bone mass in predicting stress fracture risk in this cohort has been previously reported by Bennell et al. [16].

Are Integrated Serial Measurements of Bone Turnover Different in Athletes Who Develop a Stress Fracture?

Analysis of serial measurements indicated that bone turnover marker measurements at any time point in an individual athlete were moderately correlated with one another. Correlations between repeated Pyr and D-Pyr measurements were dependent upon the time between measurements, varying from 0.52 to 0.32 as the interval increased. However, for OC, these correlations were approximately 0.42 and independent of the time interval. The average within-subject, month-to-month variation of the bone turnover markers [measured as the CV(SD)] was 21.7 (12.0)% for Pyr, 28.6 (17.1)% for D-Pyr, and 21.8 (11.0)% for OC. Comparison of the mean bone turnover values across the seasons did not reveal evidence of seasonal variation for any of the bone turnover markers.

The 20 athletes who developed stress fractures and the frequency-matched group of 26 athletes who did not were well matched for age [SF: 20.4 (1.6); NSF: 20.8 (2.5) years], height [SF: 171.1 (8.6); NSF: 172.8 (9.5) cm], and weight [SF: 62.5 (7.1); NSF: 62.6 (8.2) kg]. The relationships of bone turnover markers to age, sex, and stress fracture status are shown in Table 2. There was no association between bone turnover markers and total body BMC. Age and sex were significantly associated with Pyr, D-Pyr, and OC. Mean levels were higher in males than females, and in younger compared with older subjects. After adjusting for age and sex, integrated monthly Pyr, D-Pyr, and OC values were 5%, 4%, and 35% higher, respectively, in those athletes who developed a stress fracture, although these were not significant. There was no interaction between the effects of stress fracture status and sex on the bone turnover markers.

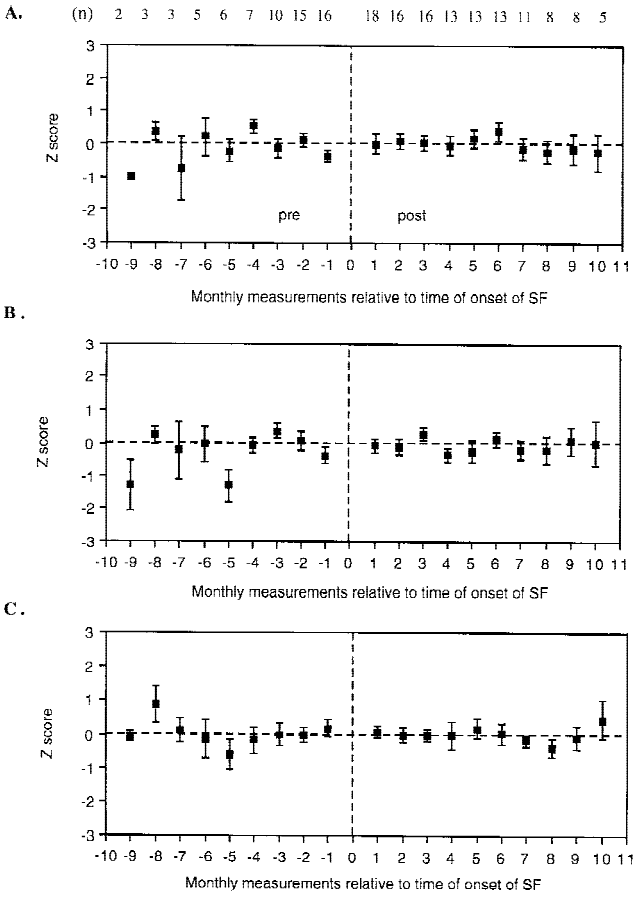

Fig. 1. Median z-score (SE) for each monthly measurement in athletes with stress fractures. The number of measurements at each time point is shown across the top. **(A)** Pyr, **(B)** D-Pyr, **(C)** OC.

Females were also analyzed separately to assess whether the effect of menstrual factors altered the relationship between bone turnover and stress fracture status. Years since menarche, total number of menses during the 12-month study, and use of the OCP during the study were associated with all three bone turnover markers in univariate analyses. However, after adjusting for age in an autoregressive multivariate model, the relationships between bone turnover markers and stress fracture status were unaltered by allowing for these menstrual factors.

Do Measures of Bone Turnover Differ Prior to and Following a Stress Fracture Episode?

In order to examine any temporal trends or patterns in bone turnover in athletes prior to and following their stress fracture, median z-scores at each time point were plotted (Fig. 1). The graphs reveal considerable variability in bone turnover during the 12 months both within and between individuals. No obvious patterns are apparent for Pyr, D-Pyr, or OC.

Using the Wilcoxon distribution statistics, there was no significant difference between bone turnover measurements

preceding and following a stress fracture in either men or women, or in both sexes combined. The results of multivariate autoregressive analyses comparing bone turnover values prior to those following a stress fracture confirmed the results of the nonparametric tests. After adjusting for sex, age, and total body BMC, there was no significant difference in Pyr, D-Pyr, or OC levels pre- and poststress fracture. Similarly, mean bone turnover values in close temporal proximity to stress fracture onset (i.e., within 1, 2, or 3 months of stress fracture) did not differ from mean values at time points more distant from the stress fracture.

Discussion

In vitro and *in vivo* findings implicate repetitive mechanical loading, bone remodeling, and microdamage accumulation in the pathogenesis of stress fracture [5, 7, 21]. Since it is not feasible to directly assess bone remodeling in athletes, we measured biochemical markers of bone turnover in an effort to improve understanding of the mechanisms underlying stress fractures.

For both male and female athletes, baseline levels of bone turnover did not differ between those who subsequently developed a stress fracture and those who did not. This supports the findings of previous cross-sectional studies in athletes [12–14] but is inconsistent with the results of a small prospective study where serum hydroxyproline concentrations were higher in just five male military recruits who developed stress fractures compared with 99 control recruits [11]. However, since hydroxyproline is not specific to bone, the elevated levels may have been derived from nonskeletal sources.

Our 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 [22]. The long-term variability of 2-hour urine collections is probably similar 24–27% [23]. In our study, monthly variation for Pyr, D-Pyr, and OC ranged from 21% to 29%. 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.

However, the possibility of differences in bone resorption or formation between stress fracture and nonstress fracture groups is not necessarily excluded by the results of our study. Although serum markers are less variable than urinary markers [23], a combination of bone formation markers is necessary to fully characterize osteoblastic activity [24–26]. In addition, if locally increased bone remodeling is associated with stress fracture development, it is possible that this would not influence levels of circulating bone turnover markers as these reflect the integration of all bone remodeling throughout the skeleton [27]. Furthermore, if trabecular bone, with its greater metabolic activity, contributes more to bone turnover levels than cortical bone, this may explain the relative insensitivity of bone turnover markers to stress fractures that are primarily cortical lesions.

Analysis of monthly samples revealed a moderate correlation between bone marker levels within athletes suggesting that an individually determined level of bone turnover may exist for each person. Variations around this level could occur depending on the influence of factors such as hormonal status, physical activity, and dietary intake. This is feasible given recent reports of genetic effects on bone turnover existing over a wide age range [28, 29]. Furthermore, in the female athletes in our study, menstrual indices were related to levels of bone turnover markers.

In conclusion, the results of this study imply that single measurements of bone turnover are not useful in predicting the likelihood of stress fracture in individual athletes. Analysis of serial samples also failed to detect a significant association between levels of bone turnover and stress fracture occurrence. These results do not necessarily negate the role of bone remodeling in the pathogenesis of stress fractures in athletes. However, they do indicate that bone turnover, as measured by the biochemical markers used in this study, is not associated with stress fracture development in this male and female athletic cohort. Direct assessment of osteoclastic and osteoblastic activity at the site of stress fracture would be very useful in evaluating the response of bone to intense, repetitive loading. Such a technical refinement could be performed using a suitable animal model of stress fracture.

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