Bone Turnover Markers Do Not Predict Stress Fracture in Elite Combat Recruits

Ran Yanovich PhD, Rachel K. Evans PhD,
Eitan Friedman MD, PhD, Daniel S. Moran PhD

Received: 4 May 2012 / Accepted: 20 November 2012 / Published online: 13 December 2012
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Abstract

Background With bone resorption rates greater than formation, stress fracture pathogenesis plausibly involves bone remodeling imbalance. If this is the case, one would anticipate serum levels of bone turnover markers would be higher in patients with stress fractures than in those without.

Questions/purposes We therefore asked whether: (1) bone turnover markers differ between soldiers who will or will not have stress fractures during basic training; (2) bone turnover markers change during basic training;

The institution of one of the authors (DSM) has received, during the study period, funding of USD 10,000–USD 100,000 from the United States Army.

All ICMJE Conflict of Interest Forms for authors and *Clinical Orthopaedics and Related Research* editors and board members are on file with the publication and can be viewed on request.

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Each author certifies that his or her institution approved the human protocol for this investigation, all investigations were conducted in conformity with ethical principles for research, and informed consent for participation in the study was obtained.

This work was performed at the Heller Institute of Medical Research, Sheba Medical Center, Tel-Hashomer, Israel.

and (3) serial bone formation or bone resorption markers differ between subjects with and without stress fractures during basic training?

Methods We performed serial determinations of serum bone formation (bone alkaline phosphatase [BAP] and procollagen type I amino-terminal propeptide [PINP]), and resorption (tartrate-resistant acid phosphatase [TRAP5b] and cross-linked collagen telopeptide [CTX]) biomarkers, measured at 2- to 4-week intervals (during 18 weeks) in 69 male soldiers in the Israeli Defense Forces during elite basic training. Twenty-two soldiers (32%) were diagnosed with stress fractures. The mean training week at diagnosis was 8.0 ± 2.0 weeks.

Results We observed no differences in bone turnover markers between soldiers with and without stress fractures. During basic training, the mean values of all subjects for bone turnover markers (BAP, PINP, and CTX) changed in comparison to their mean levels at induction (43.9 versus 37.3 $\mu\text{g/L}$, 110.4 versus 78.0 $\mu\text{g/L}$, 1.4 versus 1.1 ng/mL , respectively). We found no changes in bone formation and resorption markers between subjects with and without stress fractures.

Conclusions These specific bone turnover markers cannot be considered as either diagnostic or predictive tools for stress fracture detection in young male military recruits.

R. Yanovich, D. S. Moran
Institute of Military Physiology, IDF Medical Corps,
Tel-Hashomer, Israel

R. Yanovich, D. S. Moran (✉)
Heller Institute of Medical Research, Sheba Medical Center,
Tel-Hashomer 52621, Israel
e-mail: dmoran@sheba.health.gov.il

R. K. Evans
US Army Research Institute of Environmental Medicine,
Natick, MA, USA

E. Friedman
Susanne Levy Gertner Oncogenetics Unit,
Sheba Medical Center, Tel-Hashomer, Israel

E. Friedman
Sackler School of Medicine,
Tel Aviv University, Tel Aviv, Israel

D. S. Moran
Ariel University Center of Samaria, Ariel, Israel

Level of Evidence Level II prognostic study. See the Guidelines for Authors for a complete description of levels of evidence.

Introduction

Stress fractures are common among individuals who engage in repetitive, high-volume physical activity. Athletes and combat soldiers are particularly susceptible, especially during basic recruit training [3, 17]. Although the exact mechanisms underlying the stress fracture remain elusive [19], these injuries may reflect an imbalance between bone formation and bone resorption as a result of repeated cyclic weightbearing activity [4]. This imbalance lasts a few weeks to a few months, depending on various contributing variables (eg, activity history, training intensity, fitness, and nutrition). If this view is correct, it seems plausible that such an imbalance would be reflected in measurable biochemical parameters that are associated with bone formation and/or resorption.

Although the bone scan is considered the most sensitive method for detecting stress fractures [21], it involves ionizing radiation and its use for monitoring stress fracture healing was discouraged by Moran et al. [18]. An additional limitation of this and other imaging tools used for stress fracture diagnosis (eg, MRI, CT, or conventional bone radiography) is that they may not be readily available in the field, particularly for soldiers in training presenting with symptoms compatible with stress fractures. Thus, identifying novel, diagnostic tools for stress fractures that do not involve ionizing radiation exposure and that can be performed on the training grounds is important to the military setup.

Biochemical markers of bone turnover metabolism are easily measured in the serum and are useful for monitoring bone formation and/or resorption [23]. These markers are used clinically for assessing the dynamics of metabolic bone imbalance in various pathological bone disorders [14]. Accelerated, abnormal bone remodeling (the hallmark of Paget's disease of the bone [6]) has been reflected in changes in bone formation and resorption markers, such as bone alkaline phosphatase (BAP), procollagen type I aminoterminal propeptide (PINP), cross-linked collagen telopeptide (CTx), and tartrate-resistant acid phosphatase (TRAP5b) [5, 25]. Thus, if abnormal adaptation with a resultant imbalance of bone remodeling is a primary event in stress fracture pathogenesis, it seems plausible and testable that bone turnover markers may show distinct patterns in individuals in the acute phase of stress fractures, compared with nonstress fracture controls. Several studies [9, 12, 15] have assessed, with inconsistent results, the levels of bone turnover markers as a function of activity levels in athletes and soldiers. A study conducted on

Swedish premier league soccer players found that a reduction in physical activity led to an increase in bone resorption levels [12]. In contrast, the study of Luria et al. [15] found a decrease ($p < 0.001$) in bone resorption marker (CTx and TRAP5b) levels and in PINP levels, a bone formation marker, after a prolonged submersion concomitant with a substantial reduction in physical activity among submariners [15]. Additionally, increased physical activity was associated with an increase in bone formation and resorption among Israeli combatants [9] although it has been associated with a decrease in bone resorption marker level among soccer players [12].

We therefore asked whether (1) bone turnover markers differ between soldiers who will or will not have stress fractures during basic training; (2) bone turnover markers change during basic training; and (3) serial bone formation or bone resorption markers differ between subjects with and without stress fractures during basic training?

Patients and Methods

We recruited all 85 male combat soldiers from an elite Israeli Defense Forces (IDF) combat unit between August and November 2008. All soldiers who participated in this study succeeded in a 5-day sorting course performed 2 months before basic training. All subjects were enrolled in the same unit at the two times mentioned, and as such were asked to volunteer for this study. Sixty-nine soldiers (81%) successfully completed the 18-week surveillance during their basic training while 16 did not. The reasons for the dropout of 16 soldiers from this study were various illnesses (eg, influenza), the subjects' wiliness, and lack of suitability in subjects who were moved to other combat units.

The basic training course during the 18-week period included prolonged marches (up to 55 km) carrying stretchers, physical education classes (twice a day), weight-carrying exercises (for 30–60 minutes a day), and basic combat maneuvers including short marches (eg, 10–15 km, carrying the required equipment [average weight, 25 kg/trainee]). The 69 eligible participants were divided into two subsets: individuals with a clinical diagnosis of stress fracture confirmed by bone scintigraphy (stress fracture group, $n = 22$), and those who had no clinical or scintigraphic evidence of stress fractures (no stress fracture group, $n = 47$). A total of 22 subjects with stress fractures were diagnosed during the 18-week basic training course. Forty-six percent (10 subjects) of these injuries occurred during Weeks 1 through 6, 32% (seven subjects) of the injuries occurred during Weeks 7 through 12, and 23% (five subjects) occurred during the final 6 weeks (Weeks 13–18) of basic training (Fig. 1). All participants

Fig. 1A–B The changes (mean $\pm$ SE) in markers of bone formation for (A) bone alkaline phosphatase (BAP) and (B) procollagen type I aminoterminal propeptide (PINP) in both study groups are shown along with the number of stress fractures in the group with stress fractures during the 18-week basic training period. Differences are marked with their p values over each horizontal line.

gave written informed consent before the beginning of the study, which was approved by the Human Use Review Committees of the IDF Medical Corps, the Sheba Medical Center, and the US Army Research Institute of Environmental Medicine.

Anthropometric measurements included body height and weight, body fat percentage, BMI, and lean body mass. Height (cm) was measured using a stadiometer (with an accuracy of ± 1 cm) and body weight (kg) was determined by a metric scale (with an accuracy of ± 0.1 kg). To avoid errors, the same researcher completed all anthropometric measurements at each data collection point. Body fat percentage was calculated according to a previously published method [24] from four sites of skin-fold thickness (biceps, triceps, subscapula, and suprailiac) using the Lange Skin-fold Caliper (Lange Skinfold Caliper, Beta Technology, Santa Cruz, CA, USA). None of the anthropometric measures differed substantially between the two study groups (Table 1).

Injury surveillance, anthropometric data, and blood collections were conducted by the unit's physicians at

Table 1. Anthropometric characteristics (mean $\pm$ SD) of the study groups

Parameters	Group		95% CI
	Stress fracture (n = 22)	No stress fracture (n = 47)	
Age (years)	18.8 $\pm$ 0.4	18.6 $\pm$ 0.4	18.4–18.9
Height (cm)	177.4 $\pm$ 6.5	177.2 $\pm$ 6.0	174.6–180.2
Weight (kg)	70.1 $\pm$ 5.2	71.6 $\pm$ 6.6	67.8–72.3
BMI (kg/m^2)	22.3 $\pm$ 1.4	22.8 $\pm$ 2.0	21.7–22.9
Body fat (%)	16.6 $\pm$ 2.8	16.9 $\pm$ 3.0	15.4–17.8
Lean body mass (kg)	58.4 $\pm$ 4.9	59.4 $\pm$ 4.6	56.3–60.5

recruitment and subsequently every 2 to 4 weeks. Two experienced orthopaedic surgeons (CM, AF) examined all participants periodically (every 2–4 weeks), specifically focusing on stress fracture-related symptoms and signs. Stress fractures were diagnosed (confirmed by bone scintigraphy) and treated according to the IDF protocol [11]. Blood samples were collected from each participant at baseline (Week 0) and every 2 weeks (10 times) during the 18-week basic training period. Before blood sample collection, subjects were requested to fast for 10 hours, and blood was drawn within 1 hour of rising (between 05:00–09:00 AM, depending on the unit's schedule). Each blood draw consisted of four 7.0-mL tubes (BD Vacutainer[®] Blood Collection Tubes, Becton, Dickinson & Company, Franklin Lakes, NJ, USA), centrifuged within 2 hours at 4°C at a speed of 2000 rpm for 15 minutes, after which the serum was aliquoted and stored at -70°C until sample analysis was performed.

BAP was assayed by ELISA (Metra[®] BAP EIA kit, QUIDEL[®] Corporation, San Diego, CA, USA). The intra-assay coefficient of variation (CV) was 4.1%; the interassay CV was 5.4%. The reference value for healthy men was 23.2 $\mu\text{g/L}$, with a reference range of 15.0 to 41.3 $\mu\text{g/L}$. Amino-terminal PINP was measured by the UniQ[®] RIA (UniQ[®] PINP RIA, Orion Diagnostica, Espoo, Finland). The intraassay CV was 4.6% and the interassay CV was 9.6%. The reference value for healthy males was 45 $\mu\text{g/L}$ with a reference range of 22 to 87 $\mu\text{g/L}$ [9].

TRAP5b was measured by ELISA, using the Metra[®]-TRAP5b assay (QUIDEL[®] Corporation). The intraassay variation was 5.9% and interassay CV was 10.0%. The reference value for young healthy men was 4.0 ± 1.4 U/L. C-terminal telopeptides of CTx (Serum CrossLaps[®]) were analyzed by ELISA kits (Nordic Bioscience Diagnostics, Herlev, Denmark). The intraassay CV was 7% and interassay CV was 10.4%. The reference value for healthy men was 0.12 ± 0.75 ng/mL, with a reference range of 0.01 ± 0.71 ng/mL.

To answer our questions we used generalization of ANOVA which is provided by the SAS GENMOD procedure with the GEE method for repetitions [27]. To take into consideration the repeated measurements for each participant in each group (stress fracture versus no stress fracture), we used this method which correctly takes into consideration the repeated measures with time for each participant in each group [27]. The generalized ANOVA provides F-tests to assess the differences between groups (stress fracture versus no stress fracture) and the significance of changes with time. We adjusted for multiple comparisons where needed by using the Tukey-Kramer method, with the exact p values reported. This generalized ANOVA provides significance levels for all comparisons with time and between groups and 95% CIs for these differences if required. This type of ANOVA is necessary for designs with repeated measurements for the same participant at different times.

Results

During basic training, starting at induction, none of the bone turnover markers differed between subjects who subsequently did and did not have stress fractures. Bone formation (BAP and PINP) and resorption (TRAP5b and CTx) markers' mean levels were similar among the two study groups (Table 2).

In general, the mean values of BAP, PINP, and CTx for all participants were higher than the published reference levels for the same age group [9]. During basic training, the mean values for bone turnover markers changed ($p < 0.001$) in comparison to their mean levels at induction (Table 3). For CTx, we noted a difference ($p = 0.004$) between the change with time (from baseline to Week 18) between the stress fracture group to the no stress fracture group (Table 3). The difference between induction day to Week 18 was smaller for the participants with stress fractures than for those without stress fractures. The mean values of BAP, PINP, and TRAP5b did not change in a different manner in the group with stress fractures compared with those without stress fractures throughout basic training (Table 3).

Table 3. Statistical analysis with p values

Marker	Week effect (DF/ χ^2)	Group effect (DF/ χ^2)	Week times group interaction (DF/ χ^2)
BAP ($\mu\text{g/L}$)	< .001 (9/95.64)	0.236 (1/1.40)	0.078 (9/15.51)
PINP ($\mu\text{g/L}$)	< .001 (9/173.46)	0.561 (1/0.34)	0.124 (9/13.94)
TRAP5b (U/L)	< .001 (9/72.59)	0.284 (1/1.15)	0.133 (9/13.71)
CTx (ng/mL)	< .001 (9/173.29)	0.135 (1/2.24)	0.004 (9/24.00)

DF = degrees of freedom; χ^2 = chi-square; BAP = bone alkaline phosphatase; PINP = procollagen type I amino-terminal propeptide; TRAP5b = tartrate-resistance acid phosphatase; CTx = cross-linked collagen telopeptide.

Table 2. Comparison of mean values (mean $\pm$ SE) of BAP, PINP, TRAP5b, and CTx

Category	Marker	Group	Week			
			0	6	12	18
Bone formation	BAP (μg/L)	SF	45.2 ± 3.3	46.4 ± 3.3	45.3 ± 3.9	39.2 ± 2.4
		(p value)		(1.00)	(1.00)	(< 0.001)
		NSF	43.2 ± 2.8	43.9 ± 2.8	39.4 ± 2.0	34.7 ± 1.5
		(p value)		(1.00)	(0.63)	(< 0.001)
	PINP (μg/L)	SF	107.6 ± 6.3	104.5 ± 6.9	90.2 ± 5.5	83.6 ± 6.5
		(p value)		(1.00)	(0.01)	(< 0.001)
		NSF	112.4 ± 10.5	100.1 ± 6.9	89.8 ± 6.7	66.9 ± 4.3
		(p value)		(0.96)	(0.02)	(< 0.001)
Bone resorption	TRAP5b (U/L)	SF	3.9 ± 0.2	3.8 ± 0.2	4.1 ± 0.3	3.8 ± 0.2
		(p value)		(0.89)	(0.41)	(0.89)
		NSF	4.2 ± 0.2	4.4 ± 0.3	4.3 ± 0.2	4.2 ± 0.2
		(p value)		(0.78)	(0.90)	(1)
	CTx (ng/mL)	SF	1.3 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1
		(p value)		(0.02)	(0.02)	(0.02)
		NSF	1.5 ± 0.1	1.2 ± 0.1	1.4 ± 0.1	1.2 ± 0.1
		(p value)		(< 0.001)	(0.07)	(< 0.001)

BAP = bone alkaline phosphatase; PINP = procollagen type I amino-terminal propeptide; TRAP5b = tartrate-resistance acid phosphatase; CTx = cross-linked collagen telopeptide; SF = stress fracture; NSF = no stress fracture.

Table 4. P values and percentage of change in the differences between means of the bone metabolism markers

Category	Marker	Stress fracture (n = 22)			No stress fracture (n = 47)		
		Week 0 versus 6	Week 0 versus 12	Week 0 versus 18	Week 0 versus 6	Week 0 versus 12	Week 0 versus 18
Bone formation	BAP ($\mu\text{g/L}$)	1 (+2.7%)	1 (+0.2%)	0.01 (−13.3%)	1 (+1.6%)	0.63 (−8.8%)	< .001 (−19.7%)
	PINP ($\mu\text{g/L}$)	1 (−2.9%)	0.01 (−16.2%)	< .001 (−22.3%)	0.96 (−10.9%)	0.02 (−20.1%)	< .001 (−40.5%)
Bone resorption	TRAP5b (U/L)	0.89 (−2.6%)	0.41 (+5.1%)	0.89 (−2.6%)	0.78 (+4.8%)	0.9 (+2.4%)	1 0%
	CTx (ng/mL)	0.02 (−17.9%)	0.02 (−13.4%)	0.02 (−19.4%)	< .001 (−16.8%)	0.07 (−6.7%)	< .001 (−21.5%)

BAP = bone alkaline phosphatase; PINP = procollagen type I amino-terminal propeptide; TRAP5b = tartrate-resistance acid phosphatase; CTx = cross-linked collagen telopeptide.

During basic training we observed no differences between bone formation markers between subjects from both study groups. For BAP, the mean values of both study groups had alterations that were of similar direction and magnitude (Fig. 1A), starting with 3% and 1% increases (with and without stress fractures, respectively) during the first 6 weeks, followed by decreases of 13% for the group with stress fractures ($p = 0.01$) and 20% for the group without stress fractures ($p < 0.001$) at the end of basic training (Table 4). For PINP, during basic training the mean values decreased in both study groups throughout the study period (Fig. 1B), reaching decreases of 22% for the group with stress fractures and 41% for the group without stress fractures ($p < 0.001$) at Week 18, compared with the levels on induction day (Table 2).

The bone resorption markers of the two study groups had similar alterations in direction and magnitudes during the 18-week surveillance period. Mean serum levels of TRAP5b did not change appreciably during the 18 weeks of basic training in both study groups (Fig. 2A). In comparison with levels measured on induction day, the mean CTx levels (Fig. 2B) decreased during the first 6 weeks by 18% in the group with stress fractures ($p = 0.02$) and 17% in the group without stress fractures ($p < 0.001$). Additionally, during the entire 18 weeks of basic training, the mean levels of these bone resorption markers decreased by 19% in the group with stress fractures ($p = 0.02$) and 22% in the group without stress fractures ($p < 0.001$) (Table 4).

Discussion

Stress fractures are thought to reflect an imbalance in bone remodeling whereby the bone resorption rate exceeds the bone formation rate. With that in mind, we investigated

whether bone turnover markers measured in sera during basic training could be used to differentiate between soldiers who had stress fractures from soldiers who did not. Specifically we asked whether: (1) bone turnover markers differ between soldiers who will or will not have stress fractures during basic training; (2) bone turnover markers change during basic training; and (3) serial bone formation or bone resorption markers differ between subjects with and without stress fractures during basic training?

Our study has four major limitations. First, this is a relatively small study with a limited number of subjects and controls of a defined ethnic subgroup with restricted age and gender distributions. Because our study focused on elite combat soldiers from a specific unit, we could recruit only the above-mentioned population, which is a small and unique group and thus may not be representative of the population of soldiers with stress fractures in the Israeli army or elsewhere. Given the study scope and its location in Israel this limitation seems to be irresolvable presently, and can be addressed only by future studies in other groups of soldiers in Israeli or other military units from other countries. Second, bias may have been introduced as we included only soldiers who successfully completed the 18 weeks of basic training. Although we had 100% compliance in recruitment to this study, only 81% successfully completed the 18-week surveillance period (Patients and Methods section). This is an acceptable rate of unsuccessful completion for studies and in all likelihood did not introduce a major bias in the results. Third, because soldiers were diagnosed with stress fractures at different times during basic training this represents an inherent weakness of the study that cannot be corrected using the current study design. Fourth, the bone turnover markers are the ones commonly used in similar studies aimed at assessing bone turnover parameters [9, 15]. However it is possible that an

Fig. 2A–B The change (mean $\pm$ SE) in markers of bone resorption for (A) tartrate-resistance acid phosphatase (TRAP5b) and (B) cross-linked collagen telopeptide (CTx) in the groups with stress fractures and no stress fractures, and the number of stress fractures in the group with stress fractures during the 18 weeks of basic training are shown. Differences are marked with their p values over each horizontal line.

additional yet unmeasured marker may differ between the patients and control subjects or a change with time as a function of the existence or lack of stress fracture. However, limited funds that were available for this study prohibited use of a more comprehensive approach (eg, proteomics) as a way to explore novel protein markers for bone turnover rates. Nevertheless, this study is unique by the unselected manner of its participants and by the serial, prospective, and unselected manner in which the data and serum measurements were collected.

We found neither bone formation (BAP and PINP) nor bone resorption (TRAP5b and CTx) markers could distinguish between soldiers who had stress fractures and those who did not. Our findings are in line with previously published results of a 12-month prospective study [2] that focused on 95 track and field athletes, aged 17 to 26 years (49 males, 10 with stress fractures; and 46 females, 10 with stress fractures). In that study, baseline levels of bone turnover markers (osteocalcin and N-telopeptides of type 1 collagen) did not differ between subjects who subsequently had stress fractures and those who did not. These findings led Bennell

et al. [2] to conclude that single or even multiple sequential measurements of bone turnovers are not clinically useful in predicting the likelihood of stress fractures that affect athletes. Similarly, bone turnover markers, sex hormones, and sex hormone-binding globulin levels did not statistically differ in Finnish soldiers with ($n = 15$) and without ($n = 164$) stress fractures [26]. Furthermore, animal models of fracture and callus formation suggest bone turnover markers are poor predictors of bone healing [13, 22]. The possible predictive value of bone turnover markers in fracture risk has been reported for individuals affected by medical conditions that are characterized by accelerated bone turnover such as hyperthyroidism [10], osteoporosis [7, 20], and Paget disease of the bone [1]. Except for the relatively rare metabolic bone diseases (eg, Paget disease), in these large prospective studies, serum markers reflecting bone turnover were not predictive for evaluating osteoporotic fracture risk in postmenopausal women [7, 10]. Most studies, as reviewed by Looker et al. [14], concluded that biochemical markers of bone remodeling are neither surrogates nor substitutes for measurement of bone mineral density in predicting fracture risk. Thus it seems that these conventional bone remodeling serum markers are poor predictors of stress fracture risk and therefore have no clinical utility.

We found alterations in mean levels of the measured bone turnover markers do occur during basic training in all participants, regardless of their stress fracture status. Moreover, the mean levels of all markers tested were in the same direction and of similar magnitude in individuals who had stress fractures develop and those who did not. Notably the elevated levels detected at baseline in our study population probably reflect the intensive physical activity undergone by recruits to the special IDF combat unit before their formal induction, as many of these individuals train heavily to qualify for service in these elite units.

Our results are consistent with those of other studies that investigated bone turnover markers' change with time during military basic training [8, 9, 16]. In a study conducted on 91 US female recruits the researchers observed similar trends in bone formation (BAP and PINP) and resorption (TRAP and CTx) biomarkers. All mean values of bone turnover markers increased during a 10-week US Army basic combat training indicating an increased bone turnover in soldiers during military training [16]. Similar increases in bone turnover markers (BAP, PINP, TRAP5b, and CTx) were reported in a study conducted on 199 Israeli military recruits from a gender-integrated combat unit during a 4-month period, whereas bone formation (osteocalcin, BALP) and resorption (TRAP) markers were observed to decrease during 10 weeks of military training among 40 British male recruits [8]. In all these studies, the mean values of the bone turnover markers changed with

time during military training. In our study, during 18 weeks of basic training, serum bone turnover markers (BAP, PINP, TRAP5b, and CTx) in soldiers in an elite IDF unit were altered from baseline. However, these changes were of similar magnitude and in the same direction in soldiers who were diagnosed with stress fractures and in those without stress fractures. These findings are in line with the results of Moran et al. [19] who did not find any differences in mean values of serum bone markers (TRAP, BAP, CTx, IL-1B, IL-6, PTH, and TNF- α) between soldiers who did or did not have stress fractures during a 4-month basic training. Moreover, the lack of difference was observed 1 year after the end of the basic training.

The results of our study suggest that serum bone turnover markers (BAP, PINP, TRAP5b, and CTx) cannot be considered clinically relevant diagnostic or predictive tools for stress fracture evaluation in soldiers during basic training. Moreover, our data may be consistent with the notion that the molecular mechanisms involved in stress fracture pathogenesis are complex, and that a simple imbalance in bone remodeling processes may not be the primary event in stress fracture pathogenesis, however this remains speculative.

Acknowledgments We thank the subjects for volunteering for this study. We also acknowledge the expert laboratory assistance of Jeffrey Staab MSc, United States Army Research Institute of Environmental Medicine, and the staff of Pennington Research Laboratories in Baton Rouge, LA, USA. We also thank the staff at the Institute of Military Physiology at the Heller Institute for Medical Research for their research support, Arie Laor MD for the statistical analysis, Tomer Erlich MD, the Heller Institute physician, Charles Milgrom MD and Aharon Finestone MD, the orthopaedic surgeons, and the staff at the Central Orthopaedic Clinic of the IDF for facilitating this study. In particular, we thank Leonardo Trejo MD, a nuclear medicine specialist, and Israel Cohen MSc, the bone scan technician.

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