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Trunk and Lower Extremity Movement Patterns, Stress Fracture Risk Factors, and Biomarkers of Bone Turnover in Military Trainees

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Context: Military service members commonly sustain lower extremity stress fractures (SFx). How SFx risk factors influence bone metabolism is unknown. Understanding how SFx risk factors influence bone metabolism may help to optimize risk-mitigation strategies.

Objective: To determine how SFx risk factors influence bone metabolism.

Design: Cross-sectional study.

Setting: Military service academy.

Patients or Other Participants: Forty-five men (age_{pre} = 18.56 ± 1.39 years, $height_{pre}$ = 176.95 ± 7.29 cm, $mass_{pre}$ = 77.20 ± 9.40 kg; body mass $index_{pre}$ = 24.68 ± 2.87) who completed Cadet Basic Training (CBT). Individuals with neurologic or metabolic disorders were excluded.

Intervention(s): We assessed SFx risk factors (independent variables) with (1) the Landing Error Scoring System (LESS), (2) self-reported injury and physical activity questionnaires, and (3) physical fitness tests. We assessed bone biomarkers (dependent variables; procollagen type I amino-terminal propeptide [PINP] and cross-linked collagen telopeptide [CTx-1]) via serum.

Main Outcome Measure(s): A markerless motion-capture system was used to analyze trunk and lower extremity biomechanics via the LESS. Serum samples were collected post-CBT; enzyme-linked immunosorbent assays determined PINP and CTx-1 concentrations, and PINP : CTx-1 ratios were calculated. Linear regression models demonstrated associations between SFx risk factors and PINP and CTx-1 concentrations and PINP : CTx-1 ratio. Biomarker concentration mean differences with 95% confidence intervals were calculated. Significance was set a priori using $\alpha \leq .10$ for simple and $\alpha \leq .05$ for multiple regression analyses.

Results: The multiple regression models incorporating LESS and SFx risk factor data predicted the PINP concentration ($R^2 = 0.47$, $P = .02$) and PINP : CTx-1 ratio ($R^2 = 0.66$, $P = .01$). The PINP concentration was increased by foot internal rotation, trunk flexion, CBT injury, sit-up score, and pre- to post-CBT mass changes. The CTx-1 concentration was increased by heel-to-toe landing and post-CBT mass. The PINP : CTx-1 ratio was increased by foot internal rotation, lower extremity sagittal-plane displacement (inversely), CBT injury, sit-up score, and pre- to post-CBT mass changes.

Conclusions: Stress fracture risk factors accounted for 66% of the PINP : CTx-1 ratio variability, a potential surrogate for bone health. Our findings provide insight into how SFx risk factors influence bone health. This information can help guide SFx risk-mitigation strategies.

Key Words: biomechanics, movement assessment, overuse injuries

Key Points

- Trunk and lower extremity biomechanical patterns may influence bone health during physical activity.
- Clinical movement assessments can identify biomechanical patterns that may affect bone health.
- Comprehensive assessments are required to identify all relevant risk factors for stress fracture.

Lower extremity stress fractures are common among military service members, affecting as many as 1 in 3 male service members.[1] These injuries result in significant lost duty time, increased medical costs, and attrition from military service.[1,2] Given the high prevalence and costs associated with musculoskeletal injuries among the military, it is critical to understand the factors that increase the musculoskeletal injury risk.[3] The risk of a stress fracture (SFx) during military training is influenced by a number of factors, including lower extremity movement quality,[4,5] physical activity and physical fitness levels,[6–8] history of musculoskeletal injury,[6] and anthropometric measures.[9,10] Yet how risk factors for SFx influence bone metabolism during military training remains unknown.

Characteristic	Time in Relation to Cadet Basic Training, Mean ± SD	
	Pre	Post
Age, y	18.56 ± 1.39	18.71 ± 1.39
Height, cm	176.95 ± 7.29	181.57 ± 5.70
Mass, kg	77.20 ± 9.40	76.59 ± 7.31
Body mass index, kg/m^2	24.68 ± 2.87	23.23 ± 1.89
Landing Error Scoring System score	NA	4.86 ± 2.15
Time to posttraining blood draw, d	NA	11.51 ± 2.85

Abbreviation: NA, not applicable.

The Landing Error Scoring System (LESS) is a clinical assessment used to identify trunk and lower extremity biomechanical (movement) patterns that are associated with musculoskeletal injuries.[5,11] The LESS can discriminate between individuals who are at increased risk for lower extremity injury, including SFxs, and those who are not.[5] The other SFx risk factors, including physical activity, history of musculoskeletal injury, and anthropometric measures, can be reliably assessed using standard military physical fitness assessments and self-report questionnaires.

Bone is a metabolically active tissue that undergoes continuous remodeling via bone resorption and formation (*turnover*).[12,13] Carboxy-terminal crosslinking telopeptide of type I collagen (CTx-1) is released during bone resorption,[12,13] and procollagen type I amino-terminal propeptide (PINP) is released during bone formation.[12] These particles enter the bloodstream, where their concentrations can be measured as surrogates for bone metabolism.[13] Bone turnover increases in response to physical activity, such as military training.[1,14] Bone turnover begins with osteoclastic activity that initially outpaces osteoblastic activity, resulting in greater bone resorption than formation and making the bone more susceptible to injury.[10,13] Thus, examining biomarkers of bone turnover can provide insight into the extent to which established lower extremity risk factors influence bone turnover during military training.[1,14] Bone turnover biomarkers can also be acutely influenced by protein-rich food consumption,[13] exercise,[13,15] diurnal variations,[16] and the menstrual cycle.[13] Thus, these factors should be controlled during data collection and analyses.

Understanding how risk factors for lower extremity SFxs influence biomarkers of bone metabolism during military training will allow for the development of targeted risk-mitigation strategies. The purpose of our study was to identify how trunk and lower extremity movement patterns and other known lower extremity SFx risk factors influence bone turnover biomarkers after military training. We hypothesized that aberrant movement patterns and established SFx risk factors would be associated with biomarker profiles indicative of high bone turnover.

METHODS

Participants

Forty-five male cadets from a US service academy participated in this study (Table 1). These cadets formed a convenience sample from a larger prospective study (Kenneth L. Cameron, PhD, MPH, ATC, unpublished data, 2020). Participants in the larger prospective study were eligible for the present study if they (1) were between 18 and 23 years old, (2) completed a self-report questionnaire at the beginning of Cadet Basic Training (CBT), and (3) completed a jump-landing movement assessment at the end of CBT as part of the larger prospective study. Volunteers were excluded from the present study if they (1) were unable to complete CBT or (2) had a history of a neurologic or metabolic disorder. All eligible male cadets (n = 800) were sent a standardized recruitment e-mail. Cadets who responded to the e-mail and volunteered to participate in this study provided written informed consent and were further screened to ensure they met the inclusion criteria. The local institutional review board approved all study procedures, recruitment materials, and informed consent documents.

Data Collection

Participant Demographics. Participant age (years), height (centimeters), and mass (kilograms) were recorded at the time of the pre-CBT Army Physical Fitness Test (APFT) and again at the post-CBT blood draw. These data were used to calculate the body mass index (BMI = mass [kilograms]/height [centimeters]^2) of each participant (Table 1).

Movement Assessment. Participants performed a jump-landing movement assessment in the penultimate week of CBT. They performed 3 trials of a jump-landing movement assessment from a 30-cm-tall box to a target area located 0.9 m in front of the box. Participants were instructed to complete a vertical jump for maximal height immediately after landing in the target area. Participants did not receive feedback or coaching concerning technique, other than being informed of what constituted a *successful trial*. A trial was deemed successful if the individual (1) jumped off the box with both feet leaving the box at the same time; (2) jumped forward, and not vertically, to reach the target area; (3) landed with both feet in the target area; and (4) completed the task in a fluid motion (Figure).[17]

We used the LESS, a validated 2-dimensional assessment of trunk and lower extremity movement patterns with good intrarater (intraclass correlation coefficient [2, k] = 0.84, SEM = 0.42) and interrater reliability (intraclass correlation coefficient [2,1] = 0.91, SEM = 0.71), to quantify the movement quality of the jump landings.[17] The original 17-item LESS scoring rubric has been expanded to a 22-item scoring rubric that identifies trunk and lower extremity movement patterns during a jump-landing assessment (Table 2). The LESS items are evaluated at initial ground contact and during the time interval between initial ground contact and peak knee flexion.[17,18] A larger LESS score indicates a more aberrant movement pattern.

Figure. Jump-landing movement assessment.

A markerless motion-capture system captured and analyzed all LESS data. The markerless motion-capture system allows for accurate real-time scoring of the LESS via a single Xbox Kinect camera (version 2; Microsoft Corp, Redmond, WA) positioned 3 m in front of the participant and a laptop running proprietary software (Physimax Technologies Ltd, Tel Aviv, Israel). This automated LESS testing platform has been validated against expert LESS raters ($\kappa_{avg} = 0.48 \pm 0.40$, prevalence- and bias-adjusted $\kappa_{avg} = 0.71 \pm 0.27$, percentage agreement $= 0.85 \pm 0.14$), with the majority of LESS items demonstrating near perfect agreement.[19]

Baseline Questionnaire. A self-reported questionnaire was administered to all participants at the start of CBT to assess previous and current physical activity levels, previous and current musculoskeletal injuries, and overall current physical wellbeing (see Supplemental File 1; available online at http://dx.doi.org/10.4085/1062-6050-134-19.S1). The Marx Activity Rating Scale was included in the baseline questionnaire.[20]

Table 2. Summary of Landing Error Scoring System Results

Item	Participants Displaying Error, No. (%)
Knee-flexion angle, IC	5 (11.63)
Hip-flexion angle, IC	3 (6.98)
Trunk-flexion angle, IC	8 (18.60)
Heel-to-toe landing	5 (11.63)
Asymmetric foot contact	4 (9.30)
Asymmetric foot contact timing	1 (2.33)
Asymmetric heel-toe/toe-heel landing	1 (2.33)
Lateral trunk-flexion angle, IC	7 (16.28)
Medial knee position, IC	5 (11.63)
Stance width: narrow	0 (0.00)
Stance width: wide	17 (39.53)
Foot internal rotation	2 (4.65)
Foot external rotation	8 (18.60)
Knee-flexion DSP	1 (2.33)
Hip-flexion DSP	4 (9.30)
Trunk-flexion DSP	15 (32.56)
Excessive trunk-flexion DSP	7 (16.28)
Maximum medial knee position	14 (32.56)
Asymmetric loading	13 (30.23)
Knee "wobble"	2 (4.65)
Sagittal-plane joint DSP	1 = 34 (79.07); 2 = 0 (0.00)
Overall impression[a]	1 = 33 (76.74); 2 = 8 (18.60)

Abbreviations: DSP, joint angle displacement from initial ground contact to maximum joint angle during the descent phase of the jump landing; IC, initial ground contact.

[a] *Overall impression* was the rater's subjective assessment of the participant's overall jump-landing movement quality.

Army Physical Fitness Test. The APFT is a valid measure for evaluating an individual's ability to complete soldiering tasks and consists of 2 minutes of push-ups (count), 2 minutes of sit-ups (count), and a timed 2-mi (3.2-km) run (minutes).[21,22] Individual event raw (counts and time) and scaled (0–100 points) scores and a cumulative scaled (summation of individual scaled scores, 0–300 points) score are recorded; both direct recording and soldier self-reporting of scores are acceptable means of data acquisition.[23] The APFT is completed during the first week of CBT as part of routine military training; as such, the study team did not intervene.

Post-CBT Blood Draw Food, Physical Activity, and Injury Log. Participants self-reported their food and beverage consumption and physical activity over the 12 hours preceding the post-CBT blood draw. They were asked to answer the following items: (1) "Please indicate what food and beverage you have consumed for each time point: Dinner (yesterday), Breakfast (today), Other (in the previous 12 hours)," and the time of each meal or snack was recorded; (2) "Please indicate what exercise you completed during each time period: 1800–2359 (yesterday), 0000–0559 (today), 0600–1159 (today)," and the duration (in minutes) of each activity was recorded. All responses for both questions were in the form of free text. Respondents also self-reported the frequency (days per week), duration (minutes), and types of physical activity they routinely participated in immediately preceding CBT. They were requested to report all physical activity in which they engaged, ranging from low-intensity walking or marching to high-impact varsity sports. Individuals were dichotomized as having consumed protein-rich (eg, eggs) or non–protein-rich (eg, soda) foods and coded as such in the database. The study team made decisions regarding which foods were protein rich or non–protein rich, based on foods previously identified in the literature as being protein rich and possibly influencing bone biomarker concentrations.[13] Physical activity was quantified as the average per-day duration (minutes) of activity and qualified as high, low, or no impact.

Participants self-reported musculoskeletal injuries they sustained during CBT. A *musculoskeletal injury* was defined as an injury to a muscle, bone, tendon, or ligament that resulted in the cadet reporting to the medical staff for evaluation or treatment. Injury data included the body region, injury type (eg, sprain, strain, fracture), number of days the cadet missed or was limited during CBT as a result of the injury, and if the cadet continued to have any signs or symptoms of the injury at the time of the post-CBT blood draw.

Post-CBT Blood Draw. Post-CBT blood draws were completed within 2 weeks of the end of CBT and between 6 and 8 AM. Blood was collected in a 5-mL red-top tube without additives and allowed to clot at room temperature for 30 to 60 minutes. Immediately after clotting, the samples were centrifuged at room temperature at 1300*g* for 10 minutes. The serum was extracted and aliquoted into cryotubes, which were stored at −80°C until they were analyzed.

Data Reduction

Movement Assessment: LESS. Jump-landing assessments were analyzed in real time using the Physimax motion-capture system. If a movement error was observed during at least 2 of the 3 trials, the error was recorded and counted in the total LESS score.[17] The Physimax system provided reports (total LESS scores and individual LESS item scores) for each participant. The data were consolidated in a common Excel spreadsheet (Microsoft Corp). Movement data were unavailable for 3 cadets. Therefore, our final sample size for statistical analysis was 42 cadets.

Biomarkers of Bone Turnover. Two commercially available enzyme-linked immunosorbent assays evaluated PINP (product HP0585; NeoScientific, Cambridge, MA; detection sensitivity = 1.0 μg/L) and CTx-1 (product HC0850; NeoScientific; detection sensitivity = 1.0 μg/L) serum concentrations. All enzyme-linked immunosorbent assay kits were from the same manufacturer and production batch. Serum samples were batch assayed in duplicate and standards were assayed in triplicate for each biomarker of interest. Our interassay coefficients of variation ranged from 0.06 to 0.21, for PINP and CTx-1, respectively.

Bone formation (PINP)-to-bone resorption (CTx-1) ratios were calculated (PINP/CTx-1 = PINP : CTx-1 ratios). This ratio indicates the amount of bone remodeling activity (ie, turnover).[13] The larger the ratio, the more likely the bone is positively remodeling and forming sufficient new bone. The smaller the ratio, the more likely the bone is negatively remodeling and is resorbing more bone tissue than it is forming.[13] Biomarker data were natural log transformed for analyses so that the data had a more normal distribution.

Statistical Analyses

We used PASW Statistics for Windows (version 21.0; IBM Corp, Armonk, NY) to analyze all data. Simple and multiple linear regression models indicated how qualitative measures of lower extremity movement patterns and other SFx risk factors predicted each post-CBT biomarker concentration (PINP, CTx-1) and the bone turnover ratio (PINP : CTx-1). Simple linear regression analyses demonstrated how the total LESS score, each individual LESS item, and each known SFx risk factor predicted biomarker concentrations and bone turnover ratios. The SFx risk factors were previous physical activity quantity and type, history of lower extremity injury or surgery, pre-CBT fitness, anthropometric measures (height, mass, BMI, and the change in each), and food consumption and physical activity in the 12 hours preceding the post-CBT blood draw. Individual LESS items and SFx risk factors that predicted 1 or more of the biomarkers or the bone turnover ratio ($P \leq .10$) were then included in multiple regression models to predict each biomarker and the bone turnover ratio. We selected a more liberal statistical significance level ($P \leq .10$) a priori for the simple regression models, as we wanted to include as many potentially meaningful variables in the multiple regression models as possible to create more robust models. Statistical significance for the multiple regression models was set a priori at $\alpha \leq .05$. Means and 95% confidence intervals (CIs) are reported as the original (untransformed) measures.

RESULTS

Regression Model Covariates

Eating breakfast before the post-CBT blood draw was associated with higher PINP : CTx-1 ratios (0.81; 95% CI = 0.66, 0.99; $P = .04$). Exercise within 12 hours of the post-CBT blood draw was associated with higher CTx-1 concentrations (1.34 μg/L; 95% CI = 1.11, 1.62; $P < .01$) and PINP : CTx-1 ratios (0.62; 95% CI = 0.45, 0.87; $P < .01$). Thus, both variables were included in the multiple regression models as covariates.

Trunk and Lower Extremity Movement Patterns: LESS

A summary of the observed LESS errors is provided in Table 2. Simple linear regression revealed a number of significant predictors for PINP and CTx-1 concentrations and the PINP : CTx-1 ratio. The presence of foot internal rotation was associated with higher PINP concentrations and higher PINP : CTx-1 ratios. Similarly, excessive trunk-flexion displacement was associated with higher PINP concentrations. More lower extremity sagittal-plane displacement (smaller individual LESS item score) was associated with higher PINP : CTx-1 ratios. Heel-to-toe landing was associated with higher CTx-1 concentrations; no other variables were significant predictors of CTx-1 concentrations. The total LESS score was not a significant predictor of any biomarker variable (Table 3).

Multiple linear regression analyses incorporating only movement data did not significantly predict PINP or CTx-1 concentrations or PINP : CTx-1 ratios. In the multiple regression models, foot internal rotation was associated with higher PINP concentrations and PINP : CTx-1 ratios. More lower extremity sagittal-plane displacement was also associated with higher PINP : CTx-1 ratios. Heel-to-toe landing was associated with higher CTx-1 concentrations. No other variables were significant predictors within the multiple regression models (Table 3).

Lower Extremity SFx Risk Factors

Simple linear regression revealed a number of significant predictors for PINP and CTx-1 concentrations and the PINP : CTx-1 ratio. An injury during CBT and the raw sit-up score were associated with higher PINP concentrations and PINP : CTx-1 ratios. Greater post-CBT mass was associated with higher CTx-1 concentrations, and larger pre- to post-CBT mass differences were associated with higher PINP concentrations and PINP : CTx-1 ratios (Table 4).

Lower Extremity SFx Risk Factors and Movement Quality

Multiple linear regression models incorporating both movement quality and other SFx risk factors predicted

Table 3. Predictability of the Landing Error Scoring System (LESS) Results on Biomarkers of Bone Turnover

			Simple Regression Models		Multiple Regression Model	
Biomarker	Overall Model	Predictors	Mean Change (95% CI)	*P* Value	Mean Change (95% CI)	*P* Value
PINP	$R^2 = 0.16$, $P = .15$	LESS total score	1.03 (0.93, 1.14)	.58	—	—
		Heel-to-toe landing	0.98 (0.49, 1.95)	.96	0.86 (0.44, 1.69)	.67
		Foot IR[b]	**0.40 (0.15, 1.11)**	**.09**	0.40 (0.15, 1.11)	.09
		Excessive TFD[b]	**1.72 (0.97, 3.05)**	**.07**	1.54 (0.85, 2.76)	.16
		Sagittal-plane joint DSP	1.36 (0.78, 2.37)	.29	1.34 (0.76, 2.36)	.32
CTx-1	$R^2 = 0.14$, $P = .23$	LESS total score	0.97 (0.92, 1.02)	.27	—	—
		Heel-to-toe landing[b]	**0.73 (0.52, 1.00)**	**.06**	0.74 (0.53, 1.04)	.09
		Foot IR	0.90 (0.53, 1.50)	.68	0.91 (0.55, 1.52)	.73
		Excessive TFD	1.13 (0.84, 1.51)	.42	1.17 (0.87, 1.58)	.29
		Sagittal-plane joint DSP	0.84 (0.64, 1.11)	.23	0.86 (0.64, 1.14)	.29
PINP : CTx-1	$R^2 = 0.22$, $P = .06$	LESS total score	1.06 (0.97, 1.16)	.20	—	—
		Heel-to-toe landing	1.35 (0.75, 2.43)	.32	1.16 (0.67, 2.03)	.60
		Foot IR[b]	**0.45 (0.19, 1.08)**	**.08**	0.44 (0.19, 1.03)	.07
		Excessive TFD	1.53 (0.93, 2.51)	.10	1.31 (0.80, 2.14)	.29
		Sagittal-plane joint DSP[c]	**1.61 (1.01, 2.56)**	**.05**	1.56 (0.97, 2.51)	.07

Abbreviations: CI, confidence interval; CTx-1, carboxy-terminal crosslinking telopeptide of type I collagen; DSP, joint angle displacement from initial ground contact to the maximum joint angle during the descent phase of the jump landing; IR, internal rotation; PINP, procollagen type I amino-terminal propeptide; TFD, trunk-flexion displacement.

[a] Bold type indicates the variable was a significant predictor for either the simple or multiple regression models or both.
[b] Indicates significance at $P \leq .10$.
[c] Indicates significance at $P \leq .05$.

PINP concentrations and PINP : CTx-1 ratios. Foot internal rotation, excessive trunk-flexion displacement, and injury during CBT were associated with higher PINP concentrations and PINP : CTx-1 ratios. Heel-to-toe landings were associated with higher CTx-1 concentrations. Greater changes in mass from pre- to post-CBT were associated with higher PINP and CTx-1 concentrations (Table 4).

DISCUSSION

Consistent with our hypothesis, qualitative movement analysis and other known SFx risk factors were capable of predicting bone turnover serum biomarker concentrations after military training. Our findings provide important insight into how previously identified lower extremity SFx

Table 4. Predictability of Stress Fracture Risk Factors and Movement Quality on Biomarkers of Bone Turnover

			Simple Regression Models		Multiple Regression Model	
Biomarker	Overall Model	Predictors	Mean Change (95% CI)	*P* Value	Mean Change (95% CI)	*P* Value
PINP[a]	$R^2 = 0.47$, $P = .02$	Heel-to-toe landing	0.98 (0.49, 1.95)	.96	0.79 (0.43, 1.43)	.44
		Foot IR[b]	0.40 (0.15, 1.11)	.09	0.45 (0.18, 1.19)	.10
		Excessive TFD[a]	1.72 (0.97, 3.05)	.07	1.68 (0.96, 2.96)	.08
		Sagittal-plane joint DSP	1.36 (0.78, 2.37)	.29	1.08 (0.64, 1.79)	.78
		CBT injury[b]	0.47 (0.23, 0.94)	.04	0.40 (0.21, 0.79)	.01
		Sit-ups raw score[a]	0.99 (0.97, 1.00)	.08	0.99 (0.97, 1.01)	.49
		Mass post-CBT	0.99 (0.97, 1.02)	.68	0.99 (0.96, 1.03)	.75
		Mass difference[b]	0.94 (0.89, 0.98)	.01	0.95 (0.90, 1.00)	.05
CTx-1	$R^2 = 0.39$, $P = .08$	Heel-to-toe landing[a]	0.73 (0.52, 1.00)	.06	0.74 (0.53, 1.03)	.09
		Foot IR	0.90 (0.53, 1.50)	.68	0.97 (0.58, 1.61)	.91
		Excessive TFD	1.13 (0.84, 1.51)	.42	1.16 (0.85, 1.59)	.36
		Sagittal-plane joint DSP	0.84 (0.64, 1.11)	.23	0.83 (0.63, 1.11)	.22
		CBT injury	0.89 (0.62, 1.27)	.52	0.87 (0.60, 1.26)	.48
		Sit-ups raw score	1.00 (0.99, 1.01)	.51	1.00 (0.99, 1.01)	.82
		Mass post-CBT[a]	1.01 (1.00, 1.03)	.07	1.01 (0.99, 1.03)	.38
		Mass difference	0.99 (0.96, 1.02)	.49	0.97 (0.95, 1.00)	.07
PINP : CTx-1[b]	$R^2 = 0.66$, $P < .01$	Heel-to-toe landing	1.35 (0.75, 2.43)	.32	1.06 (0.70, 1.61)	.77
		Foot IR[b]	0.45 (0.19, 1.08)	.08	0.46 (0.24, 0.87)	.02
		Excessive TFD[a]	1.53 (0.93, 2.51)	.10	1.45 (0.98, 2.14)	.07
		Sagittal-plane joint DSP[b]	1.61 (1.01, 2.56)	.05	1.29 (0.91, 1.83)	.17
		CBT injury[b]	0.53 (0.29, 0.97)	.05	0.46 (0.29, 0.73)	<.01
		Sit-ups raw score[b]	0.98 (0.97, 1.00)	.01	1.00 (0.98, 1.01)	.41
		Mass post-CBT	0.98 (0.96, 1.00)	.12	0.99 (0.96, 1.01)	.24
		Mass difference[b]	0.95 (0.91, 0.99)	.01	0.97 (0.93, 1.01)	.14

Abbreviations: CBT, Cadet Basic Training; CTx-1, carboxy-terminal crosslinking telopeptide of type I collagen; DSP, joint angle displacement from initial ground contact to the maximum joint angle during the descent phase of the jump landing; IR, internal rotation; PINP, procollagen type I amino-terminal propeptide; TFD, trunk-flexion displacement.

[a] Indicates significance at $P \leq .05$.
[b] Indicates significance at $P \leq .10$.

risk factors may influence bone health. Specifically, our results demonstrate how aberrant movement patterns (eg, foot internal rotation, heel-to-toe landing) assessed through a common clinical movement assessment (LESS) and known SFx risk factors (eg, musculoskeletal injury, participant mass) increase concentrations of bone turnover biomarkers (PINP, CTx-1) and bone turnover ratios (PINP : CTx-1), which are surrogates for bone health. This information can be used to develop and refine strategies to improve bone health and subsequently reduce the SFx risk.

Trunk and Lower Extremity Movement Patterns: LESS

Trunk and lower extremity movement patterns observed during a validated clinical movement assessment predicted post-CBT serum concentrations of bone turnover biomarkers.[17,18] Overall movement quality did not predict PINP or CTx-1 concentrations or PINP : CTx-1 ratios. We evaluated movement quality using 2 methods: (1) the total cumulative LESS score and (2) the "overall impression" as scored on the LESS. The overall impression LESS item is the rater's subjective assessment of the individual's overall jump-landing movement quality. The jump is scored as *excellent* (score of 0) if the individual displays a soft landing and no frontal-plane knee motion and *poor* (score of 2) if the individual displays a stiff landing, a large amount of frontal-plane knee motion, or both. All other landings are scored as *average* (score of 1).[17] The lack of association between overall movement quality and PINP or CTx-1 concentrations or PINP : CTx-1 ratios was surprising, as a higher LESS score indicates overall poor movement quality,[17] which theoretically results in more skeletal stress and thus more bone turnover.[13] Additionally, the total LESS score has been associated with the SFx risk.[5]

The LESS was developed to identify lower extremity movement patterns associated with anterior cruciate ligament injury risk factors.[17] The LESS can identify these risk factors,[17] as well as movement patterns associated with the lower extremity SFx risk.[5] Thus, the overall impression item scored on the LESS may reflect factors that are irrelevant to or even protective against SFx risk (eg, excessive trunk-flexion displacement). Similarly, when the total LESS score is calculated, the presence of some LESS items that increase anterior cruciate ligament injury risk may actually reduce the SFx risk; when these items are included in the total LESS score, the score is higher, but the net stresses on the skeletal system may actually be less than for an individual who displays fewer movement errors (smaller LESS score). For these reasons, individual LESS items may be better predictors of bone turnover biomarker concentrations and ratios than overall movement profiles.

Our individual LESS item findings agree with those of a previous study[5] in which the researchers examined LESS items and the lower extremity SFx risk. Cameron et al[5] observed a relationship between preinjury ankle plantar-flexion angle and SFx risk in a prospective cohort study of military cadets. Furthermore, relationships exist between ankle-dorsiflexion angles and vertical ground reaction forces during landings.[24] Minimal plantar flexion, as is the case with heel-to-toe landings, results in higher peak vertical ground reaction forces as compared with toe-to-heel landings.[24] Heel-to-toe landings also increase the vertical ground reaction loading rate, which is a known SFx risk factor.[25]

Heel-to-toe landings in our study were associated with higher post-CBT CTx-1 concentrations (0.73 μg/L; 95% CI = 0.52, 1.00; $P = .06$). Higher CTx-1 concentrations may indicate excessive bone resorption, accelerated bone remodeling, and compromised bone strength.[13] A heel-to-toe landing accounted for 20% of the post-CBT CTx-1 concentration (3.68 ± 1.53 μg/L), but a minimum change in CTx-1 concentration of 54% has been suggested as the threshold for clinical meaningfulness.[12] However, the proposed 54% threshold was observed in an older, osteoporotic female population, so the smaller percentage changes observed in the young and physically active men in our investigation should be further examined to determine their clinical meaningfulness.

Foot internal rotation increased PINP concentrations post-CBT. This indicates the bone is positively remodeling and increasing in strength. This was surprising, as torsion and bending stresses concentrate in the bone cortex and stimulate osteoclasts to begin the bone-remodeling process.[9,14] Furthermore, previous work[4] with military cadets showed that individuals who displayed knee internal rotation >5° during a jump-landing assessment were 2 to 4 times more likely to sustain a SFx than individuals who had a neutral or externally rotated knee. The potential exists that what is visually observed as foot internal rotation during a jump-landing assessment occurs at the time of initial ground contact, when individuals commonly have a plantar-flexed foot and ankle. Foot and ankle plantar flexion cause the tibia to externally rotate.[26] Thus, when the ground reaction forces are greatest, at initial ground contact, the tibia is in a safer externally rotated position while the feet appear to be internally rotated. Furthermore, foot and ankle plantar flexion at initial ground contact mitigates ground reaction forces and loading rates, which may protect against SFxs.[25]

Bone turnover is initiated by osteoclastic activity that outpaces osteoblastic activity, resulting in greater bone resorption than formation.[10,13] Bone resorption takes 7 to 10 days and formation takes 2 to 3 months.[13] The mean time from the end of CBT to the post-CBT blood draw was approximately 12 days; however, bone resorption likely commenced at the beginning of CBT, which was approximately 8 weeks before the mean post-CBT blood draw. Thus, the post-CBT blood samples were likely collected after the cadets had passed the initial bone-breakdown period and when bone formation was outpacing resorption. This may also explain why we did not observe many variables that predicted post-CBT CTx-1 concentrations.

A lack of trunk and lower extremity sagittal-plane displacement was associated with larger PINP : CTx-1 ratios. A larger PINP : CTx-1 ratio indicates more bone formation than resorption. Overall trunk and lower limb displacement can be scored as 0 (*no error, sufficient sagittal-plane displacement*), 1 (*some sagittal-plane displacement*), or 2 (*no or minimal sagittal-plane displacement*). Thus, individuals who scored 2 had the largest increases in their PINP : CTx-1 ratios. This finding was surprising, as previous researchers[25,27] have shown that stiffer landings (less sagittal-plane displacement) increased ground reaction forces and ground reaction force loading

rates, which can increase the SFx risk. This result warrants further investigation.

Excessive trunk-flexion displacement mitigates ground reaction forces during jump landings[28] and therefore may protect against lower extremity SFxs. Our data support this suggestion. Excessive trunk-flexion displacement was associated with higher post-CBT PINP concentrations and PINP : CTx-1 ratios, indicating that more bone formation than bone resorption was occurring.[14,29]

Lower Extremity SFx Risk Factors

Previously identified lower extremity SFx risk factors (Table 4) predicted post-CBT biomarker concentrations. The majority of previously identified SFx risk factors predicted bone biomarkers, as we hypothesized (eg, APFT sit-ups), but others did not (eg, injury during CBT). And some risk factors that we hypothesized would strongly influence post-CBT bone biomarker concentrations (eg, APFT run times) were not predictive at all.

Pre-CBT physical fitness influenced post-CBT bone biomarker concentrations. Each additional sit-up a cadet completed during the pre-CBT APFT was associated with a higher PINP concentration and PINP : CTx-1 ratio. Higher PINP concentrations and PINP : CTx-1 ratios reflect bone formation, which protects against SFxs. Our findings agree with those of researchers[6,7,9] who showed that better performance on the sit-up component of standardized military physical fitness assessments was associated with a reduced injury risk.

We anticipated that pre-CBT APFT run times would strongly influence post-CBT biomarker concentrations; however, this was not the case. Poor aerobic fitness increases musculoskeletal stress and injury risks.[6–8] The post-CBT blood-sample collection may have occurred late enough in the training regimen that any initial negative changes in bone biomarkers (ie, higher CTx-1 concentrations) had passed and the bones were beginning to rebuild.[13] Thus, no relationship was observed between pre-CBT APFT run times and post-CBT biomarker concentrations. The potential also exists that we excluded individuals who were significantly out of shape at the beginning of the study, became injured during CBT, and were unable to complete the training, a study exclusion criterion.

Sustaining a musculoskeletal injury during CBT increased PINP concentrations and PINP : CTx-1 ratios. This result contradicted our hypothesis because a previous injury increases the risk of future injury.[6] This relationship has been observed for SFxs among military cadets.[30] The potential exists that the acute response to injury had passed and the bones and other tissues containing type I collagen (eg, tendons) were rebuilding, leading to increases in PINP concentrations and PINP : CTx-1 ratios. A previous SFx history was proposed to be a strong predictor of post-CBT biomarker concentrations; however, no participants in the study had a history of a SFx or had sustained an acute lower extremity fracture in the 6 months preceding CBT. It is possible that no participants had a history of SFx because individuals with a previous history of this injury may have sustained a new SFx during CBT and, thus, been excluded from our study.

Overweight individuals have an increased risk of SFx.[31] We observed similar findings in our investigation. Post-CBT mass predicted CTx-1 concentrations. Greater mass was associated with higher post-CBT CTx-1 concentrations. Conversely, authors[9,10] have also shown that individuals with low body weight were also at greater risk for SFx. We noted that greater changes in pre- to post-CBT mass (ie, more weight loss) were associated with higher PINP concentrations and PINP : CTx-1 ratios but not higher CTx-1 concentrations. Our findings align with emerging research[32] in which the effects of low energy availability on hormonal changes and bone health in male athletes and military service members were explored. Specifically, males completing an Army Ranger training course had low or negative energy availability, and consequential reductions in bone mineral content were seen over the 8-week training period. Although it is not likely that CBT was as extreme or the low or negative energy availability as profound as during the Army Ranger course, the cadets included in our study likely did experience periods of low energy availability. These periods of low energy availability could contribute to hormonal changes that in turn might affect bone metabolism and health.[32,33] Collectively, these results suggest that military personnel should maintain sufficient energy stores and a healthy weight, within the normal BMI range, to minimize the risk of SFx.[9,10,31]

Lower Extremity SFx Risk Factors and Movement Quality

Trunk and lower extremity movement patterns and other SFx risk factors combined to predict PINP concentrations and PINP : CTx-1 ratios after CBT. This strongly supports the work of researchers[4–10] who demonstrated that SFx risks were multifactorial and that all aspects of health and wellness should be considered and monitored to identify individuals at greater risk.

Regression Model Covariates

Eating breakfast and exercising before the post-CBT blood draw increased CTx-1 concentrations. Protein-rich food (eg, meat, eggs, milk) consumption can alter the concentrations of collagen byproducts in serum, which may be incorrectly identified as bone-resorption byproducts.[13] Exercise can also lead to artificially elevated levels of bone biomarker serum concentrations.[13,15] Thus, we statistically controlled for both eating breakfast and exercise before the post-CBT blood draws.

Limitations

Our study was not without limitations that should be considered when interpreting the results. The first limitation was the cross-sectional design. Serum samples were only collected post-CBT, but understanding how bone biomarkers change across time during military training is also important and may be a better predictor of the risk of SFx.[1,14] Second, we were unable to obtain resting or fasting blood samples, which would have interfered with normal military training. However, military personnel eat a standardized diet, so the risk of sample contamination from food consumption was equally likely for all participants.[29]

We also controlled for food and exercise contamination in our statistical models. Third, only healthy male cadets were examined, as we aimed to limit the possible effects of confounding variables, such as sex. It is known that males and females display different movement patterns,[17] bone biomarkers can be influenced by the female menstrual cycle,[13] and bone biomarkers respond differently to military training in male and female populations.[1,14] Thus, our findings cannot be generalized to females or other vulnerable populations. Finally, we did not directly examine the risk of SFx; instead, we used biomarkers that are representative of bone health but not direct proxies for the SFx risk.

CONCLUSIONS

Lower extremity SFx risk factors can predict post-CBT bone turnover biomarker concentrations. Our study expands on previous research as it provides insight into how known SFx risk factors may affect bone health at the molecular level. This information serves as the basis for future authors to track bone turnover biomarkers throughout military training; this work may help us to identify when bones are most susceptible to SFx. Once these vulnerable periods are identified, health care providers can work with military leadership to alter training so that external stresses are reduced during these times. Altered training practices that reduce external stresses during vulnerable periods should reduce the risk of SFx and increase military medical readiness.

DISCLAIMERS

The views expressed in this paper are those of the authors and do not reflect the official policy of the Department of the Army/ Navy/Air Force, Department of Defense, the Uniformed Services University of the Health Sciences, or the US government.

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