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Bone, Biomarker, Body Composition, and Performance Responses to 8 Weeks of Reserve Officers' Training Corps Training

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Context: Military personnel engage in vigorous exercise, often resulting in higher bone mineral density; however, leg bone injuries occur frequently in this population. Predictors of change in tibial bone quality and strength need to be characterized in this high-risk population.

Objectives: To examine the effects of an 8-week military training intervention on total body and site-specific bone density and tibial bone quality, serum biomarkers (parathyroid hormone and sclerostin), body composition, and physical performance and to investigate which outcome variables (biomarkers, body composition, and physical performance) predict estimated tibial bone strength in college-aged Reserve Officers' Training Corps (ROTC) participants.

Design: Prospective cohort study.

Setting: University of Oklahoma.

Patients or Other Participants: The ROTC participants (14 males, 4 females) were matched for sex, age, and mass to physically active control participants (14 males, 4 females). The ROTC participants engaged in an 8-week training intervention, while the physically active control group made no changes to their exercise routines.

Main Outcome Measure(s): Preintervention general health questionnaires were completed. Pre-, mid-, and postintervention bone scans (dual-energy x-ray absorptiometry and peripheral quantitative computed tomography); serum blood draws (parathyroid hormone and sclerostin); and physical performance measures (muscle strength and aerobic capacity) were obtained.

Results: The ROTC participants exhibited increased hip bone density mineral and content (both P values $\leq .02$) after the 8-week intervention. Sclerostin, but not parathyroid hormone, was a positive correlate and predictor in all ROTC models for estimated bone strength at the fracture-prone 38% tibial site (ie, 38% of the tibial length proximal to the distal end of the tibia). Both groups displayed decreased total body and regional fat mass, and ROTC participants' aerobic capacity increased (all P values $\leq .05$).

Conclusions: All bone, body composition, and performance measures either improved or were maintained in response to ROTC training. Sclerostin should be further investigated as a potential early indicator of changes in estimated tibial bone strength in military cohorts.

Key Words: military, estimated bone strength, stress fracture, physical performance

Key Points

- Eight weeks of resistance and aerobic training improved or maintained total body, lumbar spine, hip, and tibial bone mineral density in college-aged Reserve Officers' Training Corps participants.
- Sclerostin was positively associated with estimated bone strength at the 38% tibial site in Reserve Officers' Training Corps participants, suggesting that it may provide practitioners with key information about skeletal activity in the leg.

Bone turnover is a continual dynamic process in which the skeletal tissue responds to stimuli to meet the demands of the body for structural integrity, protection, and minerals. Physical activity is often viewed as osteogenic because muscle contractions and vertical ground reaction forces load the bone, resulting in microdamage and signaling bone resorption, followed by bone formation. Bone injury can occur when vigorous and potentially damaging bouts of physical activity are repeated without allowing adequate time for bone formation. The disturbance of this reparative process may reduce the integrity of the bone and increase the bone injury risk.[1] One of the most common bone injuries is stress fracture, characterized by cumulative microdamage or trauma to the bone resulting in reduced bone strength.[2]

Both competitive and tactical athletes can present with reduced bone strength that may develop into a stress fracture. Tactical athletes, such as military personnel,[3] often have a high incidence of bone injuries,[4] indicating that their training may reduce bone strength in fracture-prone areas

such as the lower tibia. Dual-energy x-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT) are used to assess *bone strength*, which is defined as the ability of mineralized material to resist bending forces. It is often estimated based on measures of bone mineral density (BMD) and content (BMC; eg, areal or volumetric density) and bone morphology and geometric properties (eg, cortical diameter or bone area).[5,6] Using both DXA and pQCT, Beck et al[5] found that recruits who presented with fractures had poorer physical fitness and body composition, smaller midthigh muscle cross-sectional area (mCSA), and less estimated thigh and tibial bone strength than uninjured recruits. Among college-aged adults at the US Naval Academy, Armstrong et al[4] reported that low total body areal BMD (aBMD) was inversely correlated with fracture risk during 8 weeks of training. Authors of most studies in young-adult military cohorts have assessed differences between those who had already sustained a fracture and those who had not; less research has been conducted on predictors of estimated tibial bone strength using a prospective design.[6] Although DXA and pQCT provide valuable information regarding estimated bone strength, these imaging techniques cannot assess bone cell responses and are not commonly used for military health assessments, thereby reducing their utility.

Serum markers of bone resorption and formation have been used to understand skeletal responses to military training for >30 years, but to date, many of these results have been contradictory. Prospective studies following military personnel have yielded inconsistent findings, as bone turnover markers of resorption and formation were either strongly associated[7] with or not related[8,9] to estimated bone strength. Parathyroid hormone (PTH) has been investigated as an endocrine regulator of bone resorption in military cohorts. Chronically elevated PTH can signal osteoclast activity, increasing bone resorption, and subsequent loss of bone strength, resulting in an increased risk for bone injury. The PTH responses to military training have been inconsistent, with researchers reporting chronically elevated serum PTH levels after basic training in male[10] and female[11] military recruits, no change in elite male combat trainees,[9] and decreased levels in male and female recruits over a 16-week basic training period.[12] Given the variable response of PTH to military training, additional research is needed to fully explain its role in bone resorption in this cohort.

Sclerostin is another biomarker produced by *osteocytes,* the bone cells responsible for sensing mechanical stimuli imposed on the skeleton. Increased mechanical loading downregulates sclerostin expression, whereas unloading conditions upregulate sclerostin expression.[13] Serum sclerostin levels often increase during periods of active bone resorption and may serve as a sensitive bone mass regulator.[13] In male soldiers, serum sclerostin concentrations were greater in those with a tibial stress fracture than in injury-free soldiers[6]; however, in young-adult female military recruits, serum sclerostin decreased but did not predict bone changes.[14] Serum biomarkers; bone morphology, density, and geometry; and measures of physical performance need to be concurrently investigated to provide a more comprehensive assessment of estimated tibial bone strength predictors in this cohort.

The primary aim of our study was to examine the effects of an 8-week resistance and aerobic training intervention on bone, biomarkers (PTH and sclerostin), body composition, and performance in a college-aged Reserve Officers' Training Corps (ROTC) population compared with a matched control group. The secondary aim was to determine which variables were most predictive of post-intervention estimated bone strength at the fracture-prone 38% tibial site. We hypothesized that the ROTC population would exhibit greater changes in bone and biomarkers and have superior body composition and performance measures compared with active control individuals. Furthermore, we hypothesized that biomarkers and physical performance would be strong predictors of estimated tibial bone strength after the intervention period.

METHODS

Participants and Study Design

All participants provided written informed consent, and the study was approved by the University of Oklahoma Institutional Review Board (#8600). The ROTC group comprised men and women aged 18 to 30 years who were active members of either the US Army or US Navy ROTC program that serves to prepare college students for future service in the US military as officers. The control group comprised men and women who were physically active ≥3 times per week and completed training logs to characterize the frequency and type of their training. Control group participants were matched for sex, age (±2 years), and mass (±2.5 kg) with an enrolled ROTC participant. Volunteers were excluded from the ROTC and control groups if they reported a history of musculoskeletal disease, injury, or both; current or past history of smoking; metal implants; pregnancy; or menstrual irregularities. This study required 6 visits (Figure 1). During visit 1, participants completed all paperwork and were familiarized with the testing procedures. Visit 2 consisted of a fasted blood draw, DXA and pQCT scans, and 1-repetition maximum (1RM) leg press and bench press. Visit 3 involved an aerobic capacity treadmill test. During midintervention testing, a DXA scan and 1RM leg press and bench press were performed. Postintervention testing repeated the methods from visits 2 and 3. For each visit, control participants were tested ±11 days of their respective matched ROTC participants.

Exercise Intervention

All ROTC participants completed the same biweekly, 8-week structured training program designed, administered, and controlled for specificity and progression by ROTC training staff, with >95% attendance rates. This program was a shift from more traditional basic training protocols that included distance runs, rucking or marching, push-ups, pull-ups, and sit-ups, which have been associated with a high injury risk.[4,7,15] Exercises in the new training program were categorized as high-intensity interval, resistance, or aerobic training, and all 16 training sessions aimed to incorporate all 3 types of exercises. An example of a circuit that was completed twice and followed by a 3-mi (4.8-km) run is described in Figure 2. The research team did not interfere with either group's exercises, and the newly

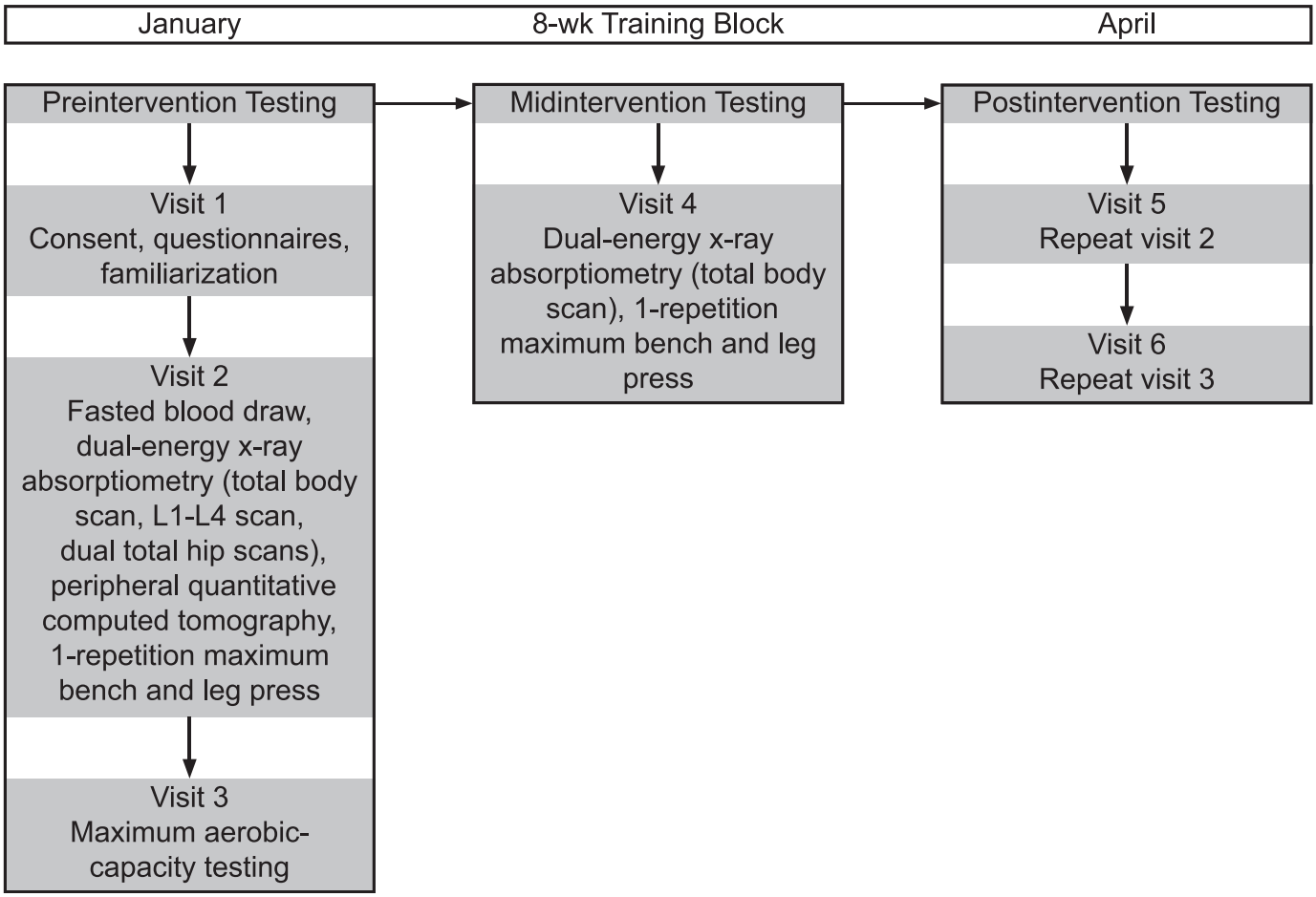

Figure 1. Study schedule of testing and description of testing measures.

designed intervention implemented by this ROTC program did not necessarily reflect ROTC training programs at other universities.

The DXA and pQCT Measures

Participants underwent DXA and pQCT scans for the assessment of body composition and bone measures as previously described.[16,17] We used DXA (Lunar Prodigy, enCORE 16; GE Healthcare) to measure whole-body total fat mass (FM; g), body fat percentage (BF%), bone-free lean body mass (BFLBM; g), and BMC (g). We measured aBMD for total-body, lumbar spine (L1–L4), and proximal femoral (total hip, femoral neck, and trochanter) scans. The in vivo coefficients of variation (CV%s) for aBMD and body composition variables ranged from 0.4% to 2.7%.

A pQCT scanner (XCT 3000 version 6.00; Stratec Medizintechnik GmbH) was used to measure nondominant tibial characteristics as previously described.[16,17] Tibial scans were obtained at 4%, 38%, and 66% of the tibial length proximal to the distal end of the bone. The 4% slice is composed primarily of trabecular bone, whereas the 38% and 66% sites reflect primarily cortical bone. The 38% site also provides key bone geometry data that are helpful for stress fracture research, as this area of the tibia commonly sustains the greatest loads.[5] The 66% site has the greatest mCSA. At the distal tibia (4%), measures consisted of total volumetric BMD (vBMD; mg/cm^3), total bone area (mm^2), trabecular vBMD (mg/cm^3), trabecular area (mm^2), and estimated bone strength index (BSI; mg/mm^4). At the 38% and 66% tibia sites, variables included total vBMD (mg/cm^3), total bone area (mm^2), cortical vBMD (mg/cm^3), cortical area (mm^2), cortical thickness (mm), polar moment of inertia (mm^4), and estimated bone strength, described as the torsional polar strength for strength-strain index (pSSI; mm^3). The mCSA (mm^2) was also calculated for the 66% tibia site. All scans were visually rated as <2, and the average pMovement was 46 mm^2, indicating little to no movement.[18] In our laboratory, the CV%s for all pQCT measurements range from 0.3% to 1.2%. The same qualified trained technician (B.S.B.) performed all quality-assurance tests, scans, and analyses for the DXA and pQCT measurements.

Serum Biomarkers

Participants were instructed to not exercise 24 hours before and to have fasted for at least 8 hours before blood draws. Approximately 10 mL of blood was collected via venipuncture between 8 AM and 9 AM. Clotted samples were centrifuged, aliquoted, and stored at −80°C. All samples were assayed in duplicate for PTH (EIA3645; DRG International Inc) and sclerostin (category no. TE1023-HS; TECOmedical, Quidel Corp). For all assays, the intra-

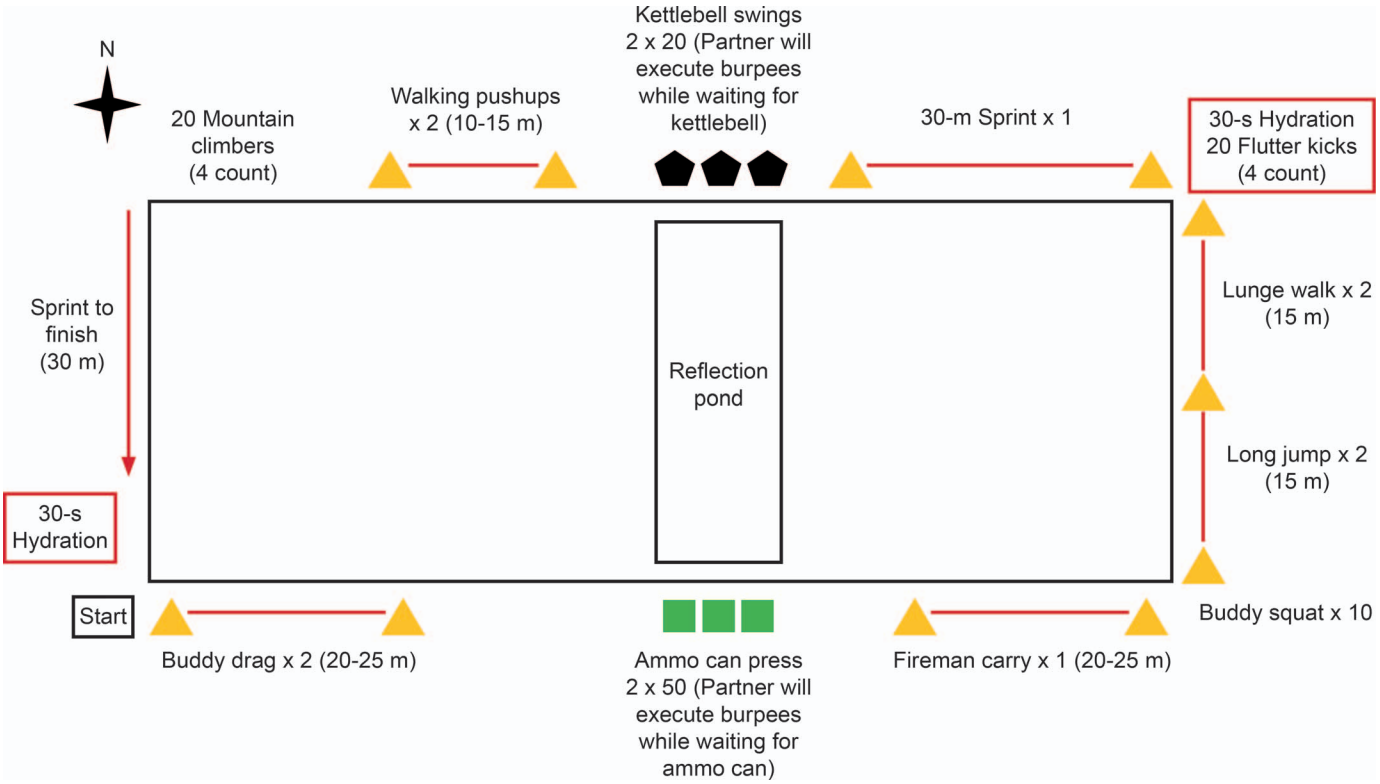

Figure 2. Sample Reserve Officers' Training Corps schematic for a high-intensity circuit training session. This circuit was completed 2 times and followed by a 3-mi (4.8-km) run. Abbreviation: Ammo, ammunition.

and interassay CV%s ranged from 0.2% to 9.4% and 4.7% to 8.5%, respectively.

Physical Performance Testing

Participants completed maximal muscle strength and aerobic capacity testing. The 1RM protocols were used to test decline leg-press (Body Solid) and flat bench-press (Cybex) strength, and intraclass correlation coefficients were between 0.997 and 0.999. Maximal aerobic capacity was measured using a modified Balke treadmill protocol with open-circuit spirometry (ParvoMedics) and continuous heart rate monitoring (model T31; Polar). At the end of each stage, participants reported their rating of perceived exertion (RPE), and peak oxygen consumption (VO_2 peak) was calculated as the average of the 2 highest consecutive 30-second VO_2 measurements. The average respiratory exchange ratio was 1.14, maximum heart rate was 196 beats per minute, and RPE was 19. After a visual inspection of VO_2 kinetics, we observed that 56 of the 64 exercise tests demonstrated a clear plateau in oxygen consumption, and 6 of the remaining 8 tests reached all other criteria for VO_2 peak.[19]

Questionnaires

Participants completed multiple questionnaires. The in-house general health questionnaire detailed their medical history, medication use, and smoking and physical activity habits. Physical activity and training logs addressed the frequency, intensity, duration, and time of exercise. The ROTC participants also answered questions regarding auxiliary physical activity outside the ROTC-mandated training sessions and injury history, such as medial tibial stress syndrome or stress fractures. The validated 7-day calcium intake questionnaire[20] was used to estimate daily calcium intake (mg/d). The validated bone-specific physical activity questionnaire (BPAQ)[21] was used to quantify past, current, and total osteogenic loads associated with the reported forms of exercise. Lastly, we instructed female participants to complete an in-house menstrual history questionnaire to obtain information about contraceptive use, age at menarche, and symptoms of menstrual cycle and hormonal disturbances in the 12 months before the study.

Statistical Analysis

Most dependent variables were normally distributed based on the Shapiro-Wilk test and are reported as unadjusted means ± SDs or means ± SEs. Calcium intake was non-normally distributed, so a Mann-Whitney *U* test was calculated to analyze baseline group differences. Two-way repeated-measures analyses of variance were used to determine group-by-time interactions and main effects for group and time; interaction effects were decomposed using paired *t* tests. Pearson product moment correlation coefficients were computed for postintervention biomarkers and postintervention 38% pSSI. Stepwise linear regression was used to identify predictors of postintervention 38% SSI (estimated tibial bone strength), such as postintervention PTH, sclerostin, BFLBM, BF%, VO_2 peak, and 1RM bench and leg press, as these values have been associated with tibial bone injuries in military individuals.[4,22] We considered 2 variable models because of the sample size.

An a priori power analysis using G*Power (version 3.1)[23,24] from 2 previously published studies using group-

Table 1. Baseline Participant Characteristics, Mean ± SD

Characteristic	Group	
	Reserve Officers' Training Corps (n = 18)	Control (n = 18)
Age, y	20.4 ± 2.4	21.2 ± 1.8
Height, m	1.76 ± 9.1	1.78 ± 6.7
Mass, kg	73.4 ± 10.9	74.5 ± 10.7
Calcium intake, mg/d	1165 ± 571	1072 ± 725
Bone-specific physical activity questionnaire		
Past	46.9 ± 27.8	53.2 ± 36.1
Current	6.2 ± 4.2	6.8 ± 5.5
Total	26.6 ± 14.6	29.9 ± 19.0
Physical activity, d/wk	4.9 ± 1.4	4.9 ± 1.0
Resistance training, d/wk	4.1 ± 1.6	3.5 ± 1.8
Aerobic training, d/wk	3.5 ± 1.7	2.4 ± 1.8

by-time interaction designs was performed to determine the necessary sample size. Gaffney-Stomberg et al[25] investigated biomarker changes in military personnel during separate times of the year. Effect sizes for summer PTH and sclerostin ranged from 0.26 to 0.61, suggesting a sample size between 8 and 32 was needed. Evans et al[12] investigated aerobic performance and body composition in military recruits over 4 months. Effect sizes for VO_2 and BF% ranged from 0.34 to 0.52, suggesting a sample size ranging from 10 to 20 was needed for 80% power. Effect sizes were calculated as (mean[1] − mean[2])/pooled SD. Given the small number of females enrolled, a sex comparison could not be performed with adequate power. All statistical procedures were carried out using SPSS (version 25; IBM Corp), and significance was set at $P \leq .05$.

RESULTS

Participant Characteristics

Initially, 42 participants (20 ROTC members, 22 control individuals) were screened for the study. A total of 36 participants (18 ROTC members, 18 control individuals) were included in the analysis. Two ROTC members were excluded before participation: 1 for prolonged illnesses and 1 who withdrew after visit 1. Four control individuals were excluded: 1 for an inability to maintain body mass within the matching criteria for the ROTC counterpart, 1 for voluntary termination, and 2 for injuries unrelated to this study. All matching criteria were maintained for the 36 participants, with no changes in height or body mass over time for either group. Women composed 22% (n = 8/36) of the sample; 50% (n = 4/8) of the women reported using oral contraceptives, and 13% (n = 1/8) reported using an implant contraceptive method.

No differences were found between the ROTC and control groups at baseline for anthropometrics, questionnaire data, or calcium intake ($P > .05$; Table 1); however, VO_2 peak was greater in the ROTC group ($P = .02$; Table 2). Body mass index (BMI) ranged from 18.9 to 26.8, with only 11 participants (6 ROTC members, 5 control individuals) in the overweight category. Calcium intake was above the recommended 1000 mg/d.[26] Both ROTC and control participants met the American College of Sports Medicine's physical activity guidelines.[19]

Table 2. Body Composition and Physical Performance Over Time, Mean ± SD

Measure	Reserve Officers' Training Corps Group (n = 18)			Control Group (n = 18)		
	Preintervention	Midintervention	Postintervention	Preintervention	Midintervention	Postintervention
Body composition						
Total body fat, %	20.8 ± 5.5	20.3 ± 5.7[a]	20.4 ± 5.8	21.8 ± 6.6	21.2 ± 6.2[a]	21.3 ± 6.2
Total body fat mass, kg	15.2 ± 4.2	14.8 ± 4.1	14.9 ± 4.3	16.1 ± 4.2	15.6 ± 4.1	15.7 ± 3.9
Leg fat mass, kg	23.5 ± 6.5	23.0 ± 6.8[a]	22.8 ± 6.5	23.6 ± 8.1	22.6 ± 8.1[a]	23.3 ± 7.7
Arm fat mass, kg	18.4 ± 7.0	17.9 ± 7.1[a]	17.9 ± 7.2[a]	19.5 ± 7.8	18.9 ± 7.3[a]	18.9 ± 7.4[a]
Total body bone-free lean body mass, kg	55.3 ± 9.7	55.9 ± 10.1[a]	55.8 ± 10.4	56.5 ± 11.5	57.0 ± 11.7[a]	56.9 ± 11.8
Leg bone-free lean body mass, kg	18.7 ± 3.5	18.9 ± 3.6	19.0 ± 3.6	18.7 ± 4.6	20.0 ± 5.8	19.4 ± 4.5
Arm bone-free lean body mass, kg	7.4 ± 2.0	7.5 ± 1.9[a]	7.5 ± 2.1[b]	7.4 ± 2.4	7.4 ± 2.4[a]	7.4 ± 2.4[b]
Physical performance						
1-Repetition maximum bench press, kg	80.0 ± 30.6	83.7 ± 32.2[a]	82.8 ± 30.0[a]	77.9 ± 36.0	81.7 ± 38.3[a]	80.6 ± 35.0[a]
1-Repetition maximum leg press, kg	251.9 ± 80.4	277.6 ± 81.8[a]	283.7 ± 80.7[a]	257.1 ± 106.8	283.6 ± 109.2[a]	284.9 ± 112.2[a]
Peak oxygen consumption, mL/kg/min	52.5 ± 7.8[c]	Measurement not taken	53.8 ± 8.1[a,c]	47.1 ± 4.8	Measurement not taken	48.5 ± 5.6[a]
Respiratory exchange ratio	1.15 ± 0.06	Measurement not taken	1.14 ± 0.07[a]	1.16 ± 0.07	Measurement not taken	1.12 ± 0.04[a]

[a] Different from preintervention ($P \leq .05$).
[b] Greater than midintervention ($P \leq .05$).
[c] Greater than control group ($P \leq .05$).

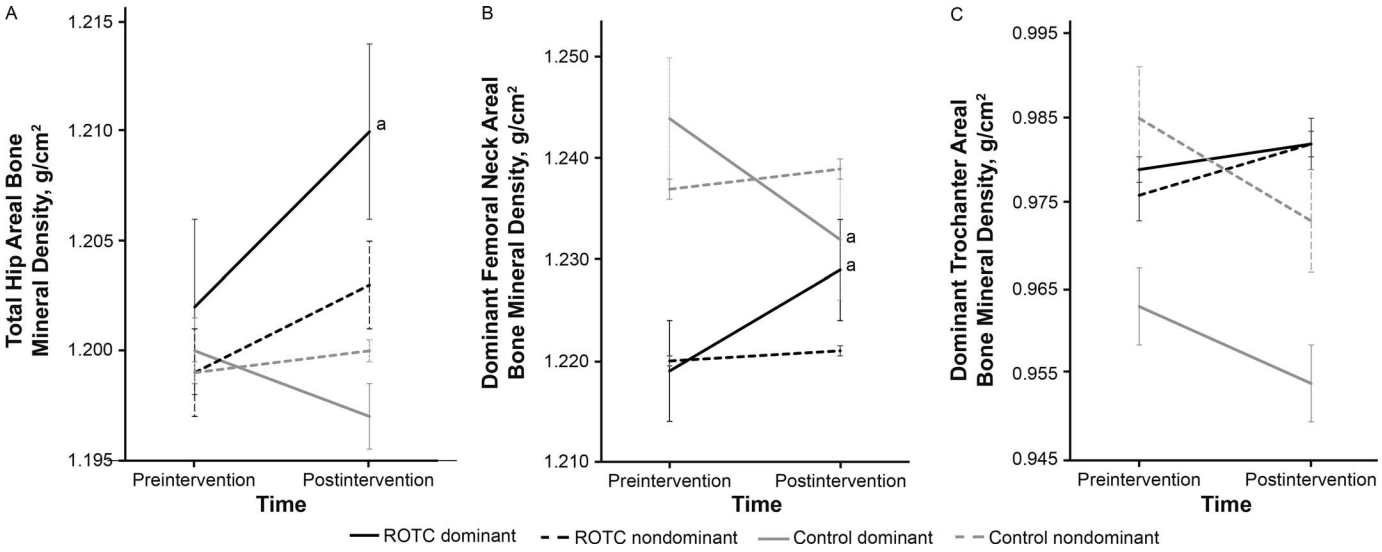

Figure 3. Dominant- and nondominant-hip areal bone mineral density over time. In the Reserve Officers' Training Corps (ROTC) group, changes were found for the dominant, A, total hip and, B, femoral neck but not for, C, the dominant greater trochanter or any of the 3 nondominant hip measures. In the control group, measures for, B, the dominant femoral neck were reduced. [a] Different from preintervention.

Bone and Biomarkers

Group-by-time interactions showed total hip and femoral neck aBMD of the dominant hip increased in the ROTC group (Figure 3A and B, respectively; $P \leq .02$), whereas greater trochanter aBMD did not change ($P \geq .39$; Figure 3C). No differences were observed for other hip sites or for other aBMD or BMC measures (all P values $\geq .15$; Table 3). We noted group-by-time interactions for the lumbar spine aBMD and BMC; however, these differences were not different after post hoc analysis ($P \geq .08$). All participants had normal aBMD values (Z-scores > -2.0).[27] No differences were found for pQCT variables at the 4% or 38% tibia sites (all P values $\geq .17$). Time effects were present for 66% total BMC and mCSA, which increased from pre- to postintervention (both P values $\leq .03$; Table 3; see Supplementa1 Table, available online at http://dx.doi.org/10.4085/1062-6050-0634.20.s1).

No effects were demonstrated for either biomarker (all P values $\geq .15$; Table 3), but postintervention sclerostin was positively correlated with postintervention 38% pSSI for the ROTC ($R^2 = 0.469$, $P = .01$) but not the control group ($R^2 = 0.029$, $P = .57$ Figure 4A), and postintervention PTH was not correlated with postintervention 38% pSSI for either group ($R^2 \leq 0.114$, $P \geq .81$; Figure 4B). Exclusion of biomarker outliers (2 box plots) did not change the statistical outcomes, and thus, all reported outcomes include all data.

Predictors of Estimated Bone Strength

All models constituting postintervention sclerostin and postintervention BF%, BFLBM, 1RM leg press, 1RM bench press, VO_2 peak, or 66% tibial mCSA were predictors of postintervention 38% pSSI for the ROTC and control groups (Table 4). Regression models with postintervention PTH were predictors of postintervention 38% pSSI for the ROTC and control groups but accounted for a lower proportion of the variance (R^2) than the sclerostin models (Table 4).

Body Composition and Performance

Total BF% and arm and leg FM decreased, whereas total body BFLBM increased from pre- to midintervention in both groups (all P values $\leq .05$; Table 2). Both groups improved their relative VO_2 peak values over time ($P = .02$), but the ROTC group had greater pre- and postintervention values ($P \leq .01$). Additionally, the 1RM leg press and bench press increased from pre- to midintervention for both groups (all P values $\leq .04$).

DISCUSSION

Given that bone injuries cost the US military >$100 million per year[28] and stress fractures specifically are considered the leading cause of injury-related discharge in both the US Marine Corps and Navy basic training programs,[15] new predictors of lower leg estimated bone strength are necessary to improve our understanding of skeletal changes in this at-risk population, with the goal of reducing the injury risk. We documented positive effects on bone after 8 weeks of ROTC training. Additionally, sclerostin combined with measures of body composition and physical performance predicted 46% to 66% of estimated bone strength variance at the fracture-prone 38% tibia site, whereas PTH was less consistently predictive in the ROTC group. Our data suggested that researchers who are interested in studying cohorts at risk for tibial bone injury may want to consider sclerostin as a predictive model component.

Bone and Biomarker Responses to Training

We observed positive skeletal changes in the ROTC group over the study period. Femoral neck and total hip aBMD of the dominant hip increased in the ROTC group. The magnitude of these changes (0.8%) was less than the DXA precision (0.9%), but would most likely exceed measurement error in a few weeks if the trend continued. One possible reason for the skeletal changes observed at the

Table 3. Preintervention-Postintervention Dual-Energy X-ray Absorptiometry, Peripheral Quantitative Computed Tomography, and Biomarker Measures, Mean ± SD

	Reserve Officers' Training Corps Group (n = 18)		Control Group (n = 18)	
Measure	Preintervention	Postintervention	Preintervention	Postintervention
Dual-energy x-ray absorptiometry				
Total body areal bone mineral density, g/cm^2	1.33 ± 0.11	1.31 ± 0.13	1.35 ± 0.12	1.34 ± 0.12
Total body bone mineral content, g	3023 ± 521	3041 ± 519	3020 ± 500	3019 ± 493
L1–L4 Areal bone mineral density, g/cm^2	1.31 ± 0.11	1.31 ± 0.12	1.33 ± 0.16	1.32 ± 0.15
4% Peripheral quantitative computed tomography				
Total volumetric bone mineral density, mg/cm^3	344.3 ± 27.7	344.8 ± 28.2	357.0 ± 37.3	355.7 ± 36.9
Total bone mineral content, mg/mm	395.0 ± 64.9	394.9 ± 64.8	398.2 ± 74.9	398.2 ± 74.6
Trabecular volumetric bone mineral density, mg/cm^3	308.6 ± 27.1	308.6 ± 26.9	312.6 ± 32.3	311.7 ± 32.7
Total bone strength index, mg^2/mm^4	136.6 ± 28.3	136.8 ± 28.6	143.2 ± 36.4	142.6 ± 36.1
Trabecular bone strength index, mg^2/mm^4	100.9 ± 25.1	100.6 ± 24.8	99.4 ± 29.7	99.5 ± 29.6
38% Peripheral quantitative computed tomography				
Total volumetric bone mineral density, mg/cm^3	934.7 ± 55.1	936.0 ± 54.7	945.8 ± 65.4	945.9 ± 65.4
Total bone mineral content, mg/mm	396.7 ± 53.1	398.0 ± 54.0	411.8 ± 67.3	411.9 ± 67.1
Cortical volumetric bone mineral density, mg/cm^3	1175.9 ± 25.8	1176.2 ± 24.9	1171.3 ± 28.2	1171.1 ± 28.7
Cortical thickness, mm	6.0 ± 0.6	6.0 ± 0.6	6.2 ± 0.7	6.2 ± 0.7
Stress-strain index, mm^3	1910.8 ± 385.4	1911.7 ± 382.5	2013.1 ± 532.5	2024.8 ± 536.8
66% Peripheral quantitative computed tomography				
Total volumetric bone mineral density, mg/cm^3	714.8 ± 87.6	698.4 ± 50.8	725.8 ± 68.1	726.9 ± 67.9
Total bone mineral content, mg/mm	435.3 ± 61.0	436.3 ± 61.2[a]	450.3 ± 74.0	450.9 ± 74.1[a]
Cortical volumetric bone mineral density, mg/cm^3	1136.9 ± 25.4	1136.8 ± 21.9	1136.1 ± 25.1	1136.8 ± 26.4
Cortical thickness, mm	4.8 ± 0.5	4.7 ± 0.5	4.9 ± 0.6	4.9 ± 0.6
Stress-strain index, mm^3	2904.9 ± 701.7	2939.8 ± 621.8	2994.2 ± 745.3	2991.7 ± 750.3
Muscle cross-sectional area, mm^2	7534 ± 1039	7734 ± 1050[a]	7972 ± 750	8043 ± 1789[a]
Biomarkers				
Sclerostin, ng/mL	0.42 ± 0.14	0.41 ± 0.12	0.39 ± 0.10	47.8 ± 19.5
Parathyroid hormone, U/L	40.8 ± 22.5	46.61 ± 22.4	0.39 ± 0.12	45.8 ± 16.1

[a] Time effect from pre- to postintervention ($P \leq .05$).

hip and not in the tibia was the introduction of multidirectional loading vectors. The previous exercise training protocol comprised mainly running, marching, and rucking, which all load the hips and tibia in a similar vertical direction. However, the new training protocol included a wide variety of multidirectional exercises, such as the buddy drag, burpees, and kettlebell swings (Figure 2), that may have introduced novel loading vectors at the

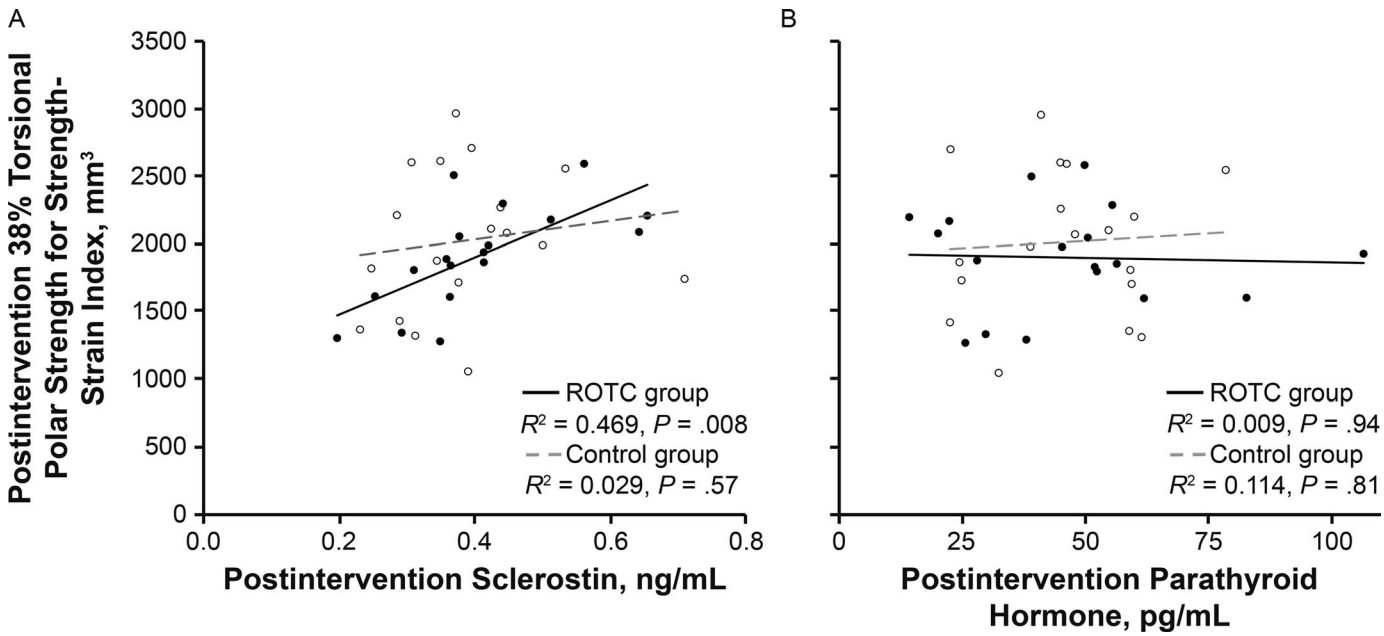

Figure 4. Pearson product moment correlations between postintervention 38% polar strength for strength-strain index (pSSI) and, A, postintervention sclerostin and, B, postintervention parathyroid hormone by group. Reserve Officers' Training Corps (ROTC) group correlations are represented by closed circles, and control group correlations by open circles. Sclerostin was positively correlated with postintervention 38% pSSI for the ROTC group but not the control group. Parathyroid hormone was not correlated with postintervention 38% pSSI for either group.

Table 4. Predictive Models of Postintervention 38% Polar Strength for Strength-Strain Index for the Whole Sample and ROTC and Control Groups Separately

Predictive Models of Postintervention 38% Polar Strength for Strength-Strain Index	Whole Sample		ROTC Group		Control Group	
	R^2	P	R^2	P	R^2	P
Total body fat percentage + sclerostin	0.337	.008[a]	0.464	.01[a]	0.589	<.001[a]
Total body fat percentage + parathyroid hormone	0.312	.009[a]	0.098	.50	0.585	<.001[a]
Bone-free lean body mass + sclerostin	0.689	<.001[a]	0.669	<.001[a]	0.729	<.001[a]
Bone-free lean body mass + parathyroid hormone	0.671	<.001[a]	0.680	<.001[a]	0.731	<.001[a]
Leg press + sclerostin	0.576	<.001[a]	0.568	<.001[a]	0.718	<.001[a]
Leg press + parathyroid hormone	0.562	<.001[a]	0.442	.02[a]	0.723	<.001[a]
Bench press + sclerostin	0.452	.005[a]	0.488	.008[a]	0.616	<.001[a]
Bench press + parathyroid hormone	0.450	.005[a]	0.338	.048[a]	0.575	<.001[a]
Peak oxygen consumption + sclerostin	0.223	.02[a]	0.494	.007[a]	0.394	.04[a]
Peak oxygen consumption + parathyroid hormone	0.029	.07	0.156	.28	0.350	.04[a]
66% Tibia muscle cross-sectional area + sclerostin	0.459	.004[a]	0.476	.008[a]	0.396	.04[a]
66% Tibia muscle cross-sectional area + parathyroid hormone	0.454	.05[a]	0.127	.37	0.372	.04[a]

Abbreviation: ROTC, Reserve Officers' Training Corps.

[a] Different ($P \leq .05$).

hip. Although the pQCT measures did not change, the values were similar to those of uninjured military recruits as described by Davey et al.[29] Davey et al[29] also noted that bone strength at the 38% tibia site displayed strong correlations with injury risk in >1000 military recruits, further highlighting the need for a better understanding of this site. We found multiple prediction models for postintervention 38% pSSI. In the ROTC group, sclerostin contributed to predictive models when combined with measures of body composition and physical performance. More specifically, sclerostin and 66% mCSA predicted 47% of 38% pSSI variability in the ROTC group, supporting the work of Milgrom et al,[30] who indicated that those with greater calf mCSA would be capable of reducing bone loads and potentially reducing the risk of tibial fractures during military training. Bennell and Brukner[1] also reported that 66% mCSA in athletes was negatively correlated with 38% and 66% pSSI and fracture rates, further supporting that 66% mCSA in addition to sclerostin should be considered when assessing changes in estimated tibial bone strength. Although sclerostin may be an important predictor of bone status in college-aged military personnel, to date, the magnitude of the sclerostin response to military training remains unclear. For instance, Hughes et al[14] ddescribed a 5.7% decline in sclerostin after 8 weeks in female US Army basic combat training recruits, but these changes were no longer different after adjusting for age, race or ethnicity, and BMI. With our data, we confirm these findings. Future researchers should assess how, and to what extent, sclerostin responds to long-term exercise and how this biomarker may be used to gain a better understanding of skeletal metabolism in both tactical and competitive athletes.

High serum PTH levels have been associated with stress fractures in military recruits[10,11]; however, contrary results were seen in athletic and other military populations.[12,14] We did not detect PTH changes over 2 time points in either group, confirming the findings of Hughes et al,[14] who investigated PTH responses over 8 weeks in US Army basic trainees. In contrast, Evans et al[12] demonstrated decreased PTH levels after 8 weeks of basic training that returned to baseline by the posttesting period (16 weeks) in male Israeli Defense Forces recruits. Seasonal effects on vitamin D and dietary calcium intake are 2 factors that could influence PTH responses to training. In our study, control individuals provided blood draws within 11 days of their matched ROTC participants, reducing the potential confounding effects from sunlight-related vitamin D activation and its subsequent effect on serum ionized calcium levels and PTH production. Additionally, dietary calcium intake was greater than the recommended 1000 mg/d[26] and similar between groups and time points, suggesting no impetus for altered PTH responses. Nonetheless, seasonal variation may still affect the extent of positive bone adaptations to training. Gaffney-Stomberg et al[25] found that US Marine recruits improved their estimated distal tibial bone strength to a greater extent when training in July, August, and September than when training in February, March, and April. Our data collection also occurred during these winter months, which may explain the lack of responses in estimated tibial bone strength. We noted that PTH did not appear to provide any additional information to changes in estimated tibial bone strength during this short period in the ROTC cohort.

Performance and Body Composition Responses to Training

Instead of using common field tests such as the number of pushups or a timed 2-mi (3.2-km) run, we used laboratory-based maximum tests such 1RM and VO_2 peak. All participants' bench press-to-body weight and leg press-to-body weight ratios were >1:1 and 1:2.5, which are considered good and excellent, respectively.[19] Muscular strength increased from pre- to midintervention for both groups; however, these measures either plateaued or returned to baseline values by postintervention. We were not able to quantify the training protocols at the mid- and postintervention time points, so we could not determine how the training type, time, intensity, volume, and frequency may have contributed to these results. The male and female ROTC participants exhibited relative VO_2 peak values in good and excellent categories at the beginning and end of the study.[19] Evans et al[12] reported a 5% increase in estimated aerobic capacity over the 16-week basic training period; we identified nearly half of that magnitude of improvement in half the duration, suggesting a similar outcome.

We found positive body composition changes in both the ROTC and control groups. Total BF% decreased 2.4% and 2.8%, whereas total body BFLBM increased 1.1% and 0.9% from pre- to midintervention points in the ROTC and control groups, respectively. The BF% changes exceeded the DXA measurement error, but the BFLBM changes did not. Armstrong et al[4] followed 31 incoming freshmen at the US Naval Academy with similar baseline characteristics as our cohort; however, their average total BF% was nearly 5% lower. These differences are most likely attributable to frequency and intensity differences between the exercise interventions.

Our study had limitations and strengths, which presented challenges and unique opportunities in this cohort. We selected this specific cohort because these individuals were training to become future officers in the US military and provided the opportunity to use more precise measures such as DXA and pQCT. In addition, we conducted maximum performance testing, allowing for more comprehensive assessments of physical capacity; yet these methods can be difficult to perform in large-scale studies of basic training populations and minimize the ability to directly compare performance. It is important to note that the acute responses of sclerostin and PTH may not indicate the long-term effects of exercise on skeletal mass. Lastly, our findings described how collegiate ROTC programs may prepare these individuals for future military service, but they should not be generalized to other types of basic training in which the frequency, intensity, and duration of training are likely to surpass those of this 8-week intervention.

CONCLUSIONS

We suggested that this newly adopted ROTC training program was not detrimental to bone, body composition, or performance. In addition, sclerostin should be further investigated in tactical and competitive athlete cohorts as a potential biomarker for information about skeletal metabolism and estimated tibial bone strength.

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