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ABSTRACT

Context: Military personnel engage in vigorous exercise, often resulting in higher bone mineral density; however, lower leg bone injuries are common in this population. Predictors of change in tibial bone quality and strength need to be characterized in this high-risk population. **Objective:** This study aimed to examine the effects of an eight-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. Additionally, we sought to investigate what outcome variables (biomarkers, body composition, physical performance) would be predictive of estimated tibial bone strength in college-aged Reserve Officers' Training Corps (ROTC) members. **Design:** Prospective Cohort Study. **Setting:** XXX University. **Patients of Other Participants:** ROTC (n=14 male; n=4 female) were matched for sex, age, and body mass to physically active Controls (n=14 male; n=4 female). ROTC engaged in an eight-week training intervention, while physically active Controls made no changes to their exercise routines. **Main Outcome Measures:** Pre general health questionnaires and pre, mid, and post intervention bone scans (DXA, pQCT), serum blood draws (parathyroid hormone and sclerostin), and physical performance measures (muscle strength and aerobic capacity) were tested. **Results:** ROTC participants exhibited significantly increased hip bone density and content (all $p \leq 0.03$) after the eight-week intervention. Sclerostin, not PTH, was a significant positive correlate and predictor in all ROTC models for estimated bone strength at the fracture prone 38% tibial site.

Both groups decreased total body and regional fat mass and ROTC increased aerobic capacity (all $p \leq 0.05$). **Conclusions:** All bone, body composition, and performance measures either improved or were maintained in response to ROTC training and sclerostin should be further investigated as a potential early indicator of changes in estimated tibial bone strength in military cohorts.

Keywords: Military, Biomarkers, Estimated Bone Strength, Physical Performance

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Key Points:

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

Bone turnover is a continual dynamic process where skeletal tissue responds to stimuli to meet the body's demands 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, signaling bone resorption, followed by bone formation. Bone injury can occur when vigorous and potentially damaging bouts of physical activity are repeated, without adequate time for bone formation to occur. The disturbance of this reparative process may reduce the integrity of the bone and increase bone injury risk [1]. One of the most common bone injuries is a stress fracture, characterized as 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 suffer from a high incidence of bone injuries [4] suggesting 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 mineralized material's ability to resist bending forces. It is often estimated based on measures of bone mineral density and content (e.g. areal or volumetric density) and bone morphology and geometric properties (e.g. cortical diameter or bone) [5,6]. Beck et al. [5] used both DXA and pQCT and found recruits who presented with fractures had poorer physical fitness and body composition, smaller mid-thigh muscle cross-sectional area (mCSA), and estimated thigh and tibiae bone strength compared with injury free recruits. In college-aged adults at the US Naval Academy, low total body areal bone mineral density (aBMD) was inversely correlated with fracture risk during eight weeks of training [4]. Most studies in young-adult military cohorts assess differences between those who have already fractured and those who have not; less research has

been conducted on predictors of estimated tibial bone strength using a prospective design [6]. While 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, thus reducing their utility.

Serum markers of bone resorption and formation have been used to understand skeletal responses to military training for over 30 years, but to date many of these results are contradictory. Prospective studies following military personnel yielded inconsistent findings as bone turnover markers of resorption and formation were either strongly associated [7] 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. PTH responses to military training are inconsistent with studies reporting chronically elevated serum PTH levels post-basic training in male [10] and female [11] military recruits, while it did not change in elite male combat trainees [9], and it decreased in male and female recruits over a 16-week basic training period [12]. Due to the variable response of PTH to military training, additional research is needed to fully elucidate its role in bone resorption in this cohort. Sclerostin is another biomarker produced by osteocytes, the bone cells responsible for sensing mechanical stimuli imposed upon the skeleton. Increased mechanical loading downregulates sclerostin expression, whereas unloading conditions have been shown to upregulate sclerostin expression [13]. Serum sclerostin levels are often increased 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 injury free soldiers [6]; however, in young-adult female military recruits it significantly decreased but was not

predictive of 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 this study was to examine the effects of an eight-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 matched Controls. The second aim was to determine what variables are most predictive of post-intervention estimated bone strength in the fracture-prone 38% tibia site. We hypothesized that the ROTC population would exhibit greater changes in bone and biomarkers and have superior body composition and performance measures than active Controls. 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

The (REDACTED) Institutional Review Board approved this study (IRB#XXX) and was in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants. The ROTC inclusion criteria included males and females 18-30 years old and were active members of either Army or Navy ROTC program that serves to prepare college students for future service in the US military as officers. Inclusion criteria for Controls were males and females who were physically active ≥3x/week, and matched for sex, age (±2 years), and body mass (±2.5 kg) to an enrolled ROTC participant. Exclusion criteria for ROTC and Controls included having reported a history of musculoskeletal diseases and/or injury, current or past smoking, metal implants, pregnancy, or menstrual irregularities. This study required six visits

(Figure 1). In visit one, participants completed all paperwork and became familiarized with testing procedures. Visit two included a fasted blood draw, DXA and pQCT scans, and 1 repetition maximum (1RM) leg press and bench press. Visit three included an aerobic capacity treadmill test. Mid-intervention testing included a DXA scan and 1RM leg press and bench press. Post-intervention testing repeated the exact methods from visits 2 and 3. For each visit, Controls were tested ± 11 days to their respective matched ROTC.

Exercise Intervention

All ROTC participants completed the same biweekly, eight-week structured training program designed, administered, and controlled for specificity and progression by ROTC training staff, with over 95% attendance rates. This program was a shift from more traditional basic training protocols that included distance runs, rucking/marching, push-ups, pull-ups, and sit-ups that have been associated with high injury risk [4,7,26]. Exercises in the new training program were categorized as high-intensity interval training, resistance training, or aerobic training and all 16 training sessions aimed to incorporate all three types of exercises. Figure 2 highlights an example circuit that was completed twice and followed by a three-mile run. Control participants were included if they reported exercising ≥3x/week and completed training logs to characterize their frequency and type of training. It is important to note that the research team did not interfere with either groups' exercises and the newly designed intervention implemented by this ROTC program is not necessarily a reflection of ROTC training programs at other Universities.

DXA and pQCT Measures

Participants underwent DXA and pQCT scans for the assessment of body composition and bone measures as previously described [15,16]. DXA (Lunar Prodigy, enCORE 16, GE

Healthcare, Madison, WI) was used to measure whole-body total fat mass (FM) (g), % fat mass (BF%), bone-free lean body mass (BFLBM) (g), and bone mineral content (BMC) (g). aBMD was measured using total body, lumbar spine (L1-L4), and proximal femora (total hip, femoral neck, trochanter) scans. The in vivo coefficients of variation (CV%) for aBMD and body composition variables ranges from 0.4 - 2.7%.

A pQCT scanner (XCT 3000, v.6.00, Stratec Medizintechnik GmbH, Pforzheim, Germany) was used to measure non-dominant tibia characteristics as previously described [15,16]. Tibial scans were obtained at 4%, 38%, and 66% of tibia length proximal to the distal end of the tibia. The 4% slice is composed primarily of trabecular bone, while the 38% and 66% sites are primarily cortical bone. The 38% site also provides key bone geometry data that is helpful for stress fracture research as this area of the tibia commonly sustains the greatest loads [5]. While the 66% site is where muscle cross-sectional area is greatest. At the distal tibia (4%) measures included: total vBMD (mg/cm^3), total bone area (mm^2), trabecular vBMD (mg/cm^3), trabecular area (mm^2), and estimated bone strength index (mg/mm^4) (BSI). 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 (Ipolar) (mm^4), and estimated bone strength, described as the torsional polar strength for strength-strain index (pSSI) (mm^3). Muscle cross-sectional area (mm^2) (mCSA) 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 [17]. In our laboratory the CV% for all pQCT measurements ranges from 0.3 - 1.2%. The same qualified trained technician performed all quality assurance tests, scans, and analyses for DXA and pQCT measurements.

Serum Biomarkers

Participants were instructed to not exercise 24 hours prior and be at least eight hours fasted for blood draws. Approximately 10 mL were collected via venipuncture between 8:00 - 9:00 am. Clotted samples were centrifuged, aliquoted, and stored at -80°C. All samples were assayed in duplicate using DRG International Inc., Springfield, NJ. (Cat# EIA3645) for PTH and TECO medical Quidel Corp., Santa Clara, CA and Sissach, Switzerland (Cat# TE1023-HS) for sclerostin. For all assays the intra and inter-assay CV%'s ranged from 0.2 - 9.4% and 4.7% - 8.5%, respectively.

Physical Performance Testing

Participants completed maximal muscle strength and aerobic capacity testing. One repetition maximum (1RM) protocols were used to test decline leg press (Body Solid, Forest Park, IL) and flat bench press (Cybex, Medway, MA) strength, intraclass correlation coefficients were between 0.997 - 0.999. Maximal aerobic capacity was measured using a modified Balke treadmill protocol with open-circuit spirometry (ParvoMedics; Sandy, UT) and continuous heart rate monitoring (Polar T31, Bethpage, NY). At the end of each stage, participants reported their Rating of Perceived Exertion (RPE) and VO_2 peak was calculated as the average of the two highest consecutive 30-second VO_2 measurement. Average respiratory exchange ratio was 1.14, max heart rate was 196 bpm, and RPE was 19. After visual inspection of VO_2 kinetics, 56 of the 64 exercise tests demonstrated a clear plateau in oxygen consumption and six of the remaining eight tests reached all other criteria for VO_2 peak [18].

Questionnaires

Participants completed multiple questionnaires. The in-house general health questionnaire detailed participant's medical history and medication usage, smoking and physical activity habits. Physical activity and training logs included frequency, intensity, duration, and time of exercise. ROTC also answered questions regarding auxiliary physical activity outside of the ROTC mandated training sessions and injury history such as medial tibial stress syndrome or stress fractures. The validated 7-day calcium intake questionnaire [19] estimated daily calcium intake (mg/day). The validated Bone and Physical Activity Questionnaire (BPAQ) [20] quantifies past, current, and total osteogenic loads associated with the reported forms of exercise. Lastly, female participants completed 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 past 12 months.

Statistical Analysis

All statistical procedures were performed using IBM SPSS (v25, Armonk, New York) and significance was set at $p \leq 0.05$. Most dependent variables were normally distributed based on the Shapiro-Wilks test and are reported as unadjusted means ± standard deviations (SD) in tables and means ± standard errors (SE) in figures. Calcium intake was non-normally distributed, so a Mann-Whitney U test was used to analyze baseline group differences. Two-way repeated measures ANOVA were used to determine group×time interactions and main effects for group and/or time, significant interaction effects were decomposed using paired t-tests. Pearson's Correlation Coefficients were calculated for post-intervention biomarkers and post-intervention 38% pSSI. Stepwise linear regression was used to identify predictors of post 38% SSI (estimated tibial bone strength), such as post PTH and sclerostin, BFLBM and BF%, and VO_2 peak, 1RM

bench/leg press as these values have previously been implicated with tibial bone injuries in military [4,21]. Two variable models were considered due to sample size.

An a priori power analysis using G*Power [22] from two previously published studies using group×time interaction designs was performed to determine sample size. Gaffney-Stromberg et al. [23] investigated biomarker changes in military personnel over separate times of the year. Effect sizes for summer PTH and sclerostin ranged from 0.26–0.61, suggesting a sample size between 8–32. Evans et al. [12] investigated aerobic performance and body composition in military recruits over 4 months. Effect sizes for VO_2 and body fat percentage ranged from 0.34–0.52 suggesting a sample size range of 10–20 for 80% power. Effect sizes were calculated as: [(mean^1–mean^2)/pooled standard deviation. Due to the small number of females enrolled, a sex comparison could not be performed with adequate power.

RESULTS

Participant Characteristics

Initially, 42 participants (ROTC n=20, Controls n=22) were screened for the study. 36 participants (ROTC n=18; Controls n=18) were included in the analysis. Two ROTC were excluded prior to participation, one for prolonged illnesses and another withdrew after visit 1. Four Controls were excluded: one for inability to maintain body mass within the matching criteria for their ROTC counterpart, one for voluntary termination, and two for sustained injuries unrelated to this study. All matching criteria were maintained for the 36 participants with no significant changes in height or body mass over time for either group. Females comprised 22% of the sample and 50% of females reported using oral contraceptives while an additional 13% reported using an implant contraceptive method.

No significant differences were found between ROTC and Controls at baseline for all anthropometrics, questionnaire data, or calcium intake ($p \geq 0.06$) (Table 1), except VO_2 peak was greater in ROTC ($p=0.02$; Table 2). Body mass index (BMI) ranged from 18.9 – 26.8 kg/m² with only 11 participants (ROTC n=6; Controls n=5) in the overweight category. Calcium intake was above the recommended 1000 mg/day and was not different between groups [24]. Both ROTC and Controls met the American College of Sports Medicine physical activity guidelines[18].

Bone and Biomarkers

Significant group×time interactions (Figure 3) show dominant total hip and femoral neck aBMD significantly increased in ROTC (Panel A, $p \leq 0.02$), while the greater trochanter did not change (Panel C, $p=0.39$). No significant findings emerged for other hip sites or for other aBMD or BMC measures (all $p \geq 0.14$, see Table 3 and Supplemental Table 1). Significant group×time interactions were found for the lumbar spine aBMD and BMC, however, these differences were no longer significant after post hoc analysis (post hoc $p \geq 0.08$). All participants had normal aBMD values (Z-Scores > −2.0) [25]. No significant differences were found for pQCT variables at the 4% or 38% tibia sites (all $p \geq 0.16$). Significant time effects were found for 66% total BMC and muscle CSA, which significantly increased from pre to post (both $p \leq 0.02$) (Table 3 and Supplemental Table 2).

No significant effects were found for either biomarker (all $p \geq 0.15$, Table 3) but post sclerostin was significantly positively correlated with post 38% pSSI for ROTC (Panel A, $p<0.01$) but not Controls ($p>0.56$) and post PTH was not significantly correlated with post 38% pSSI for either group (Panel B, $p \geq 0.80$) (Figure 4). Exclusion of biomarker outliers (two box plots) did not change statistical outcomes thus all reported outcomes include all data.

Predictors of Estimated Bone Strength

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

Body Composition and Performance

Total BF% and arm and leg fat mass significantly decreased while total body BFLBM increased from pre to mid in both groups (all $p \leq 0.05$; see Table 2). Both groups significantly improved their relative VO_2 peak values over time ($p=0.01$), but ROTC had greater pre and post values ($p<0.01$). Additionally, leg press and bench press 1RM increased from pre to mid for both groups (all $p \leq 0.04$).

DISCUSSION

As bone injuries cost the U.S. military over $100 million dollars per year [26] and stress fractures specifically are considered the leading cause of injury related discharge reported in both the United States Marine Corps and Naval basic training programs [27], new predictors of lower leg estimated bone strength are necessary to improve our understanding of skeletal changes in this at-risk population with the ultimate goal of injury risk reduction. This study documented positive effects on bone after eight weeks of ROTC training. Additionally, sclerostin combined with measures of body composition and physical performance predicted 46–66% of estimated bone strength variance at the fracture-prone 38% site, while PTH was less consistently predictive in ROTC. Our data suggest 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 found positive skeletal changes to ROTC over the study period. Dominant femoral neck and total hip aBMD increased in ROTC over the study period. While the magnitude of these changes (0.8%) was less than the DXA precision (0.9%) if the trend continued would most likely exceed measurement error in a few weeks. One possible reason for the skeletal changes observed at the hip and not in the tibia was the introduction of multidirectional loading vectors. Previous exercise training was comprised of mainly running, marching, and rucking which would all load the hips and tibia in a similar vertical direction. However, the new training protocol included a wide variety of multidirectional exercises that may have introduced novel loading vectors at the hip such as the buddy drag, burpees, and kettlebell swings from Figure 2. Although, pQCT measures did not significantly change, the values were similar to uninjured military recruits as described by Davey et al. [28]. Davey and colleagues also reported bone strength at the 38% site displayed strong correlations to injury risk in 1,000+ military recruits, further exacerbating the need for a better understanding of this site. We found multiple significant prediction models for post 38% pSSI. In ROTC, sclerostin significantly 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 ROTC, supporting Milgrom et al. [29] who reported those with greater calf muscle CSA would be capable of reducing bone loads and potentially reduce the risk of tibial fractures during military training. Bennell et al. [1] also reported in athletes that 66% mCSA is 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

297 unclear. For instance, Hughes et al. [14] reported a 5.7% decline in sclerostin after eight weeks in
298 female Army basic combat training recruits, but after adjusting for age, race/ethnicity, and BMI
299 these changes were no longer significant. Our study confirms these findings. It is important that
300 future studies continue to elucidate how, and to what extent, sclerostin responds to long-term
301 exercise and how this biomarker may be used to gain a better understanding of skeletal
302 metabolism in both tactical and competitive athletes.

303 High serum PTH levels have been reported to be associated with stress fracture in
304 military recruits [10,11]; however, contrary findings have been observed in athletic and other
305 military populations [12,14]. We did not detect significant PTH changes over two time points in
306 either group confirming the findings of Hughes et al. [14] who investigated PTH responses over
307 eight weeks in Army basic training. In contrast, Evans et al. [12] reported PTH levels significantly
308 decreased after eight weeks of basic training, but returned to baseline levels by the post (16
309 weeks) testing period in male Israeli Defense Forces recruits. Seasonal effects on Vitamin D and
310 dietary calcium intake are two factors that could influence PTH responses to training. In the
311 present study, controls provided blood draws within 11 days of their matched ROTC participant,
312 reducing potential confounding effects from sunlight-related Vitamin D activation and its
313 subsequent effect on serum ionized calcium levels and PTH production. Additionally, dietary
314 calcium intake was above the recommended 1000 mg/day [24] and similar between groups and
315 time points suggesting no impetus for altered PTH responses. However, seasonal variation may
316 still have an impact on the extent of positive bone adaptations to training. Gaffney-Stromberg et
317 al. [23] found Marine recruits improved estimated distal tibial bone strength to a greater extent
318 when training in July, August, and September, than when training in February, March, and April.
319 Data collection for our study also occurred during these winter months which may explain the

lack of significant responses in estimated tibial bone strength. PTH does not appear to provide any additional information to changes in estimated tibial bone strength during this short time period in this ROTC cohort.

Performance and Body Composition Responses to Training

Instead of using common field tests such as number of push-ups or a timed two-mile run, we utilized laboratory based maximum tests such 1RM and VO_2 peak. All participants' bench press:body-weight and leg press:body weight ratios were over 1.1 and 1:2.5 which is considered good and excellent, respectively [18]. Muscular strength increased from pre- to mid-intervention for both groups; however, these measures either plateaued or returned to baseline values by the post test. We were not able to quantify the training protocols at mid- and post-intervention time points, thus, we are not able to determine how the training type, time, intensity, volume, and frequency may have contributed to these findings. ROTC male and female participants exhibited relative VO_2 peak values in good and excellent categories at the beginning and end of the study[18]. Evans et al. [12] reported a 5% increase in estimated aerobic capacity over the 16 week basic training period; the current study demonstrated nearly half of that magnitude of improvement in half the duration suggesting similar results.

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

This study has limitations and strengths, which presented challenges and unique opportunities with this cohort. This specific cohort was selected as these individuals are training to become future officers in the US military and provide the opportunity to utilize more precise measures such as DXA and pQCT. Additionally, maximum performance testing was conducted allowing for more comprehensive assessments of physical capacity; however, these methods can be difficult to perform for large-scale studies within basic training populations and minimizes the ability to make direct comparisons of performance. It is important to note that the acute responses of sclerostin and PTH may not be indicative of the long-term effects of exercise on skeletal mass. Lastly, the findings of this study describe how collegiate ROTC programs may prepare these individuals for future military service but these findings should not be generalized to other types of basic training where the frequency, intensity, and duration of training are likely to surpass this eight-week intervention.

In conclusion, our findings suggest that this newly adopted ROTC training program is not detrimental to bone, body composition, or performance. Additionally, sclerostin should be further investigated in tactical and competitive athlete cohorts as a potential biomarker for providing information about skeletal metabolism and estimated tibial bone strength.

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FIGURE LEGENDS

Figure 1. Study schedule of testing and description of testing measures completed throughout the study. DXA: dual energy x-ray absorptiometry; TB: total body scan, L1-L4: lumbar spine scans of vertebral bodies 1-4; TH: Dual total hip scans; pQCT: peripheral quantitative computed tomography: 1RM: 1 repetition maximum.

Figure 2. Sample ROTC schematic for a high-intensity circuit training session. This circuit was completed two times and followed by a three mile run.

Figure 3. Dominant and non-dominant hip areal bone mineral density (aBMD) changes over time. * Significantly different than pre. Significant changes were found for dominant total hip (panel a) and femoral neck (panel b) but no significant changes were found for the dominant greater trochanter (panel c) or any of the three non-dominant hip measures.

Figure 4. Pearson's correlations between post 38% pSSI and post sclerostin (Panel a) and post PTH (Panel b) by group. ROTC correlations are closed circles and Control correlations are open circles. Sclerostin was significantly positively correlated with post 38% pSSI for ROTC ($p<0.01$) but not Controls ($p=0.56$). PTH was not significantly correlated with post 38% pSSI for either group ($p\geq0.80$).

Table 1. Baseline Participant Characteristics - means (SD).

	ROTC (n=18)	CON (n=18)
Age (years)	20.4 (2.4)	21.2 (1.8)
Height (m)	1.76 (9.1)	1.78 (6.7)
Body mass (kg)	73.4 (10.9)	74.5 (10.7)
Calcium intake (mg/day)	1165 (571)	1072 (725)
BPAQ- past	46.9 (27.8)	53.2 (36.1)
BPAQ- current	6.2 (4.2)	6.8 (5.5)
BPAQ- total	26.6 (14.6)	29.9 (19.0)
Days/week PA	4.9 (1.4)	4.9 (1.0)
Days/week RT	4.1 (1.6)	3.5 (1.8)
Days/week AT	3.5 (1.7)	2.4 (1.8)

* Significance set at $p \leq 0.05$; BPAQ: Bone Physical Activity Questionnaire; PA: Physical Activity; RT: Resistance Training; AT: Aerobic Training;

Table 2. Pre-Post DXA, pQCT, and Biomarker Measures - mean (SD).

	ROTC (n=18)		CON (n=18)	
Measures	**Pre**	**Post**	**Pre**	**Post**
DXA				
Total Body aBMD (g/cm^2)	1.33 (0.11)	1.31 (0.13)	1.35 (0.12)	1.34 (0.12)
Total Body BMC (g)	3023 (521)	3041 (519)	3020 (500)	3019 (493)
L1-L4 aBMD (g/cm^2)	1.31 (0.11)	1.31 (0.12)	1.33 (0.16)	1.32 (0.15)
4% pQCT				
Total vBMD (mg/cm^3)	344.3 (27.7)	344.8 (28.2)	357.0 (37.3)	355.7 (36.9)
Total BMC (mg/mm)	395.0 (64.9)	394.9 (64.8)	398.2 (74.9)	398.2 (74.6)
Trabecular vBMD (mg/cm^3)	308.6 (27.1)	308.6 (26.9)	312.6 (32.3)	311.7 (32.7)
Total BSI (mg^2/mm^4)	136.6 (28.3)	136.8 (28.6)	143.2 (36.4)	142.6 (36.1)
Trabecular BSI (mg^2/mm^4)	100.9 (25.1)	100.6 (24.8)	99.4 (29.7)	99.5 (29.6)
38% pQCT				
Total vBMD (mg/cm^3)	934.7 (55.1)	936.0 (54.7)	945.8 (65.4)	945.9 (65.4)
Total BMC (mg/mm)	396.7 (53.1)	398.0 (54.0)	411.8 (67.3)	411.9 (67.1)
Cortical vBMD (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% pQCT				
Total vBMD (mg/cm^3)	714.8 (87.6)	698.4 (50.8)	725.8 (68.1)	726.9 (67.9)
Total BMC (mg/mm)	435.3 (61.0)	436.3 (61.2) *	450.3 (74.0)	450.9 (74.1) *
Cortical vBMD (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 CSA (mm^2)	7534 (1039) *	7734 (1050)	7972 (750) *	8043 (1789)
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)

Significance set at $p \leq 0.05$; * Time effect as both groups increase from pre to post. aBMD: areal Bone Mineral Density; BMC: Bone Mineral Content; L1-L4: lumbar Spine 1-4; BFLBM: bone free lean body mass; vBMD: volumetric BMD; BSI: Bone Strength Index; CSA: cross-sectional area.

Table 3. Body Composition and Physical Performance Over Time - mean (SD).

	ROTC (n=18)			CON (n=18)		
Measures	**Pre**	**Mid**	**Post**	**Pre**	**Mid**	**Post**
Body Composition						
Total Body Fat Percent	20.8 (5.5)	20.3 (5.7) *****	20.4 (5.8)	21.8 (6.6)	21.2 (6.2) *****	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) *****	22.8 (6.5)	23.6 (8.1)	22.6 (8.1) *****	23.3 (7.7)
Arm Fat Mass (kg)	18.4 (7.0)	17.9 (7.1) *****	17.9 (7.2) *****	19.5 (7.8)	18.9 (7.3) *****	18.9 (7.4) *****
Total Body BFLBM (kg)	55.3 (9.7)	55.9 (10.1) *****	55.8 (10.4)	56.5 (11.5)	57.0 (11.7) *****	56.9 (11.8)
Leg BFLBM (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 BFLBM (kg)	7.4 (2.0)	7.5 (1.9) *****	7.5 (2.1) ‡	7.4 (2.4)	7.4 (2.4) *****	7.4 (2.4) ‡
Physical Performance						
1RM Bench Press (kg)	80.0 (30.6)	83.7 (32.2) *****	82.8 (30.0) *****	77.9 (36.0)	81.7 (38.3) *****	80.6 (35.0) *****
1 RM Leg Press (kg)	251.9 (80.4)	277.6 (81.8) *****	283.7 (80.7) *****	257.1 (106.8)	283.6 (109.2) *****	284.9 (112.2) *****
VO_2 Max (ml/kg/min)	52.5 (7.8) †	---	53.8 (8.1) * †	47.1 (4.8)	---	48.5 (5.6) *****
Respiratory Exchange Ratio	1.15 (0.06)	---	1.14 (0.07) *****	1.16 (0.07)	---	1.12 (0.04) *****

Significance set at $p \leq 0.05$; ***** significantly different than Pre for both groups; ‡ significantly greater than Mid; † significantly greater than CON; BFLBM: Bone Free Lean Body Mass.

Figure 1

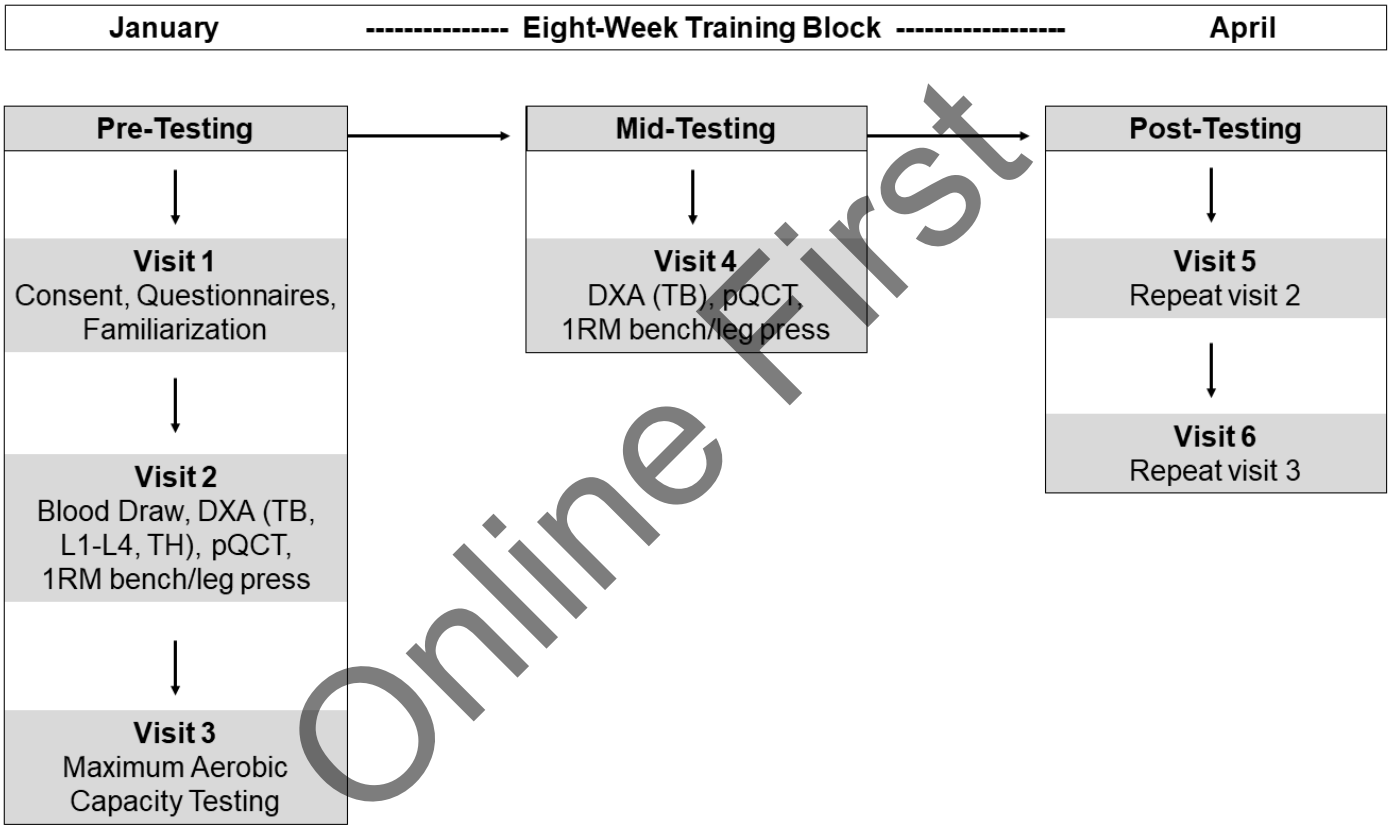


Figure 2

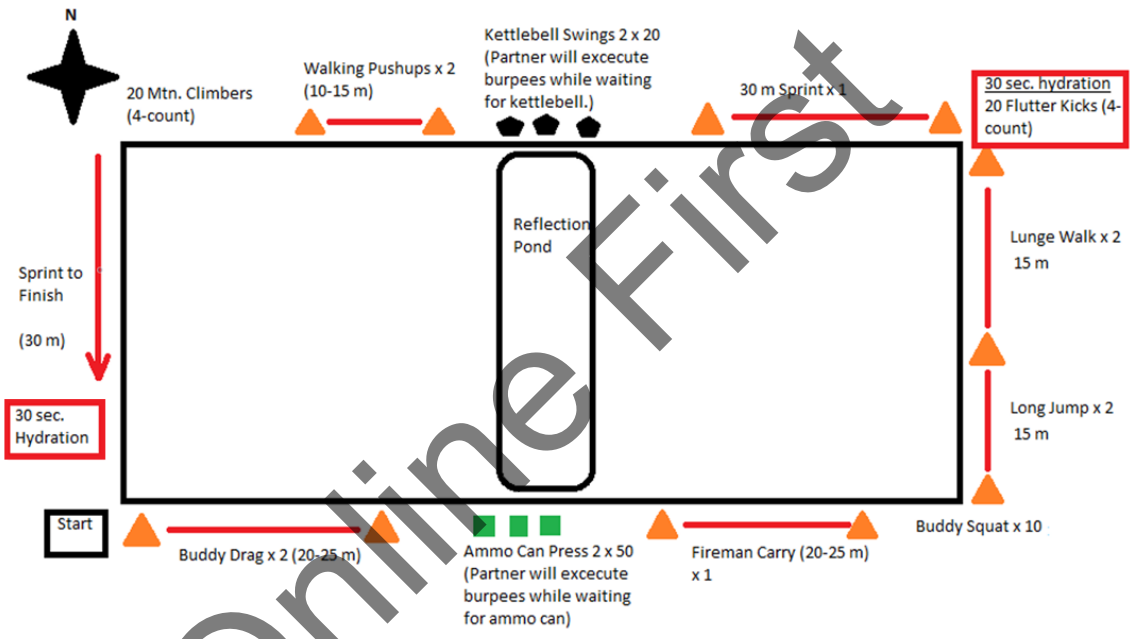


Figure 3

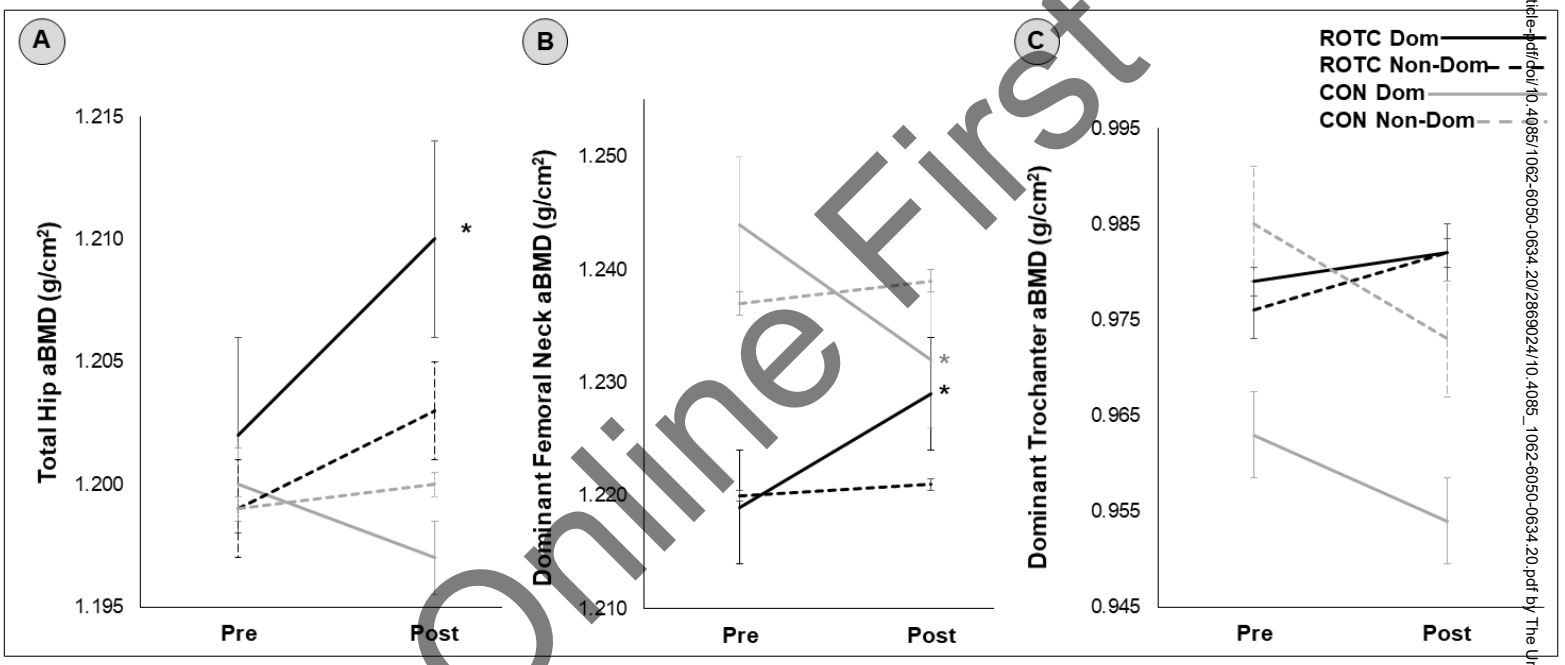
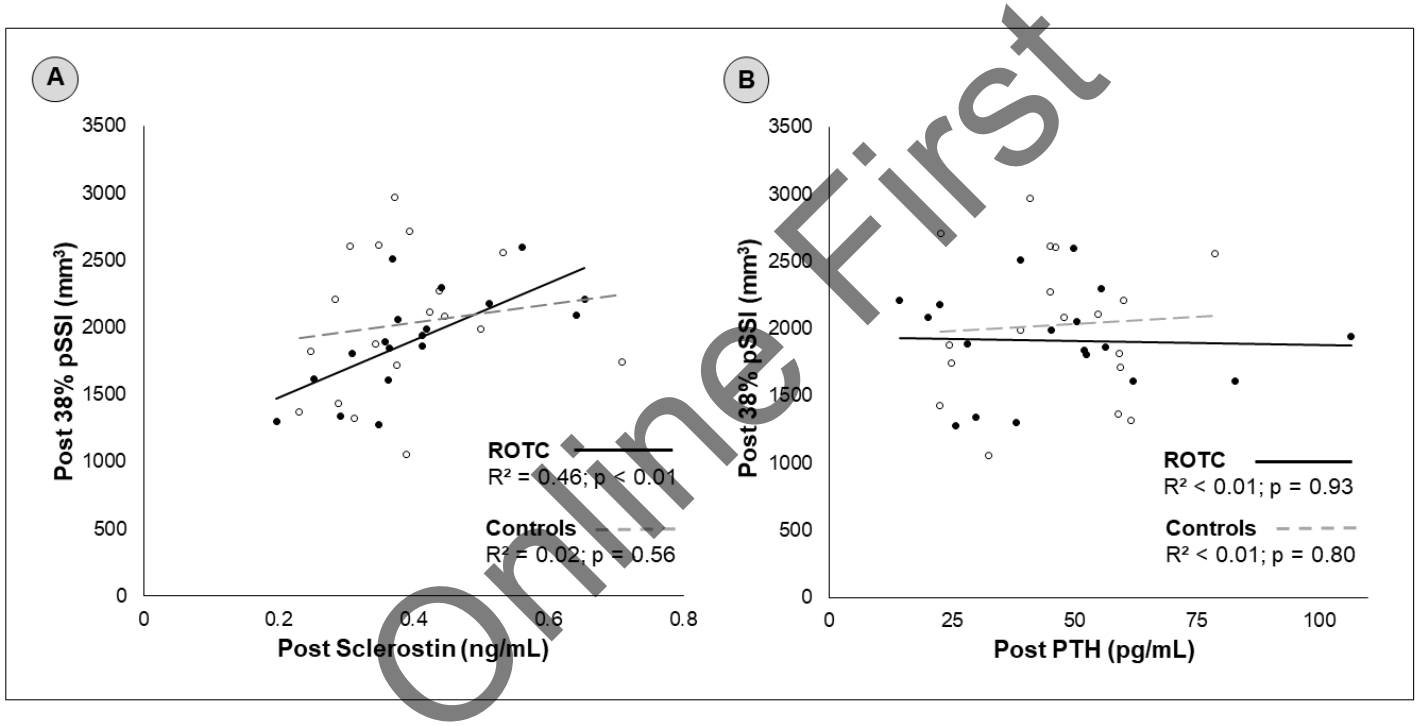


Figure 4



Supplemental Table 1. Additional DXA Measures Over Time - means (SD).

	ROTC (n=18)		Controls (n=18)	
Measures	**Pre**	**Post**	**Pre**	**Post**
Dominant Hip				
Femoral Neck aBMD (g/cm^2)	1.22 (.14)	1.23 (.14) *†	1.24 (.15)	1.23 (.15) *
Greater Troc. aBMD (g/cm^2)	0.98 (.12)	0.98 (.12)	0.96 (.15)	0.95 (.15)
Total Hip aBMD (g/cm^2)	1.20 (.13)	1.21 (.13) *†	1.20 (.16)	1.99 (.16)
Non-Dominant Hip				
Femoral Neck aBMD (g/cm^2)	1.22 (.14)	1.22 (.14)	1.24 (.17)	1.24 (.17)
Greater Troc. aBMD (g/cm^2)	0.98 (.12)	0.98 (.12)	0.99 (.18)	0.97 (.16)
Total Hip aBMD (g/cm^2)	1.19 (.13)	1.20 (.13)	1.19 (.18)	1.20 (.17)

Measures	**Pre**	**Mid**	**Post**	**Pre**	**Mid**	**Post**
Arms Bone and Fat						
BMC (g)	434 (106)	435 (102)	435 (107)	418 (99)	419 (98)	417 (97)
Percent Fat	18.4 (7.0)	17.9 (7.1) *	17.9 (7.2) *	19.5 (7.8)	18.9 (7.3)	18.9 (7.4)
Fat Mass (kg)	1.7 (.5)	1.6 (.5) *	1.6 (.5) *	1.7 (.4)	1.7 (.4)	1.6 (.4)
Legs Bone and Fat						
BMC (g)	1139 (219)	1139 (215)	1140 (221)	1090 (224)	1111 (219)	1134 (226)
Percent Fat	23.5 (6.5)	23.0 (6.8) *	22.8 (6.5)	23.6 (8.1)	22.6 (8.1)	23.3 (7.7)
Fat Mass (kg)	6.0 (1.7)	5.9 (1.6) *	5.9 (1.7)	6.1 (1.6)	5.8 (1.5)	5.9 (1.4)

Significant time effects $p<0.05$: * significantly different than Pre; † significantly different than Mid; Dom: Dominant; N-Dom: Non-Dominant; FN: Femoral Neck; GT: Greater Trochanter; TH: Total Hip; aBMD: areal Bone Mineral Density; BMC: Bone Mineral Content.

Supplemental Table 2. Additional pQCT Measures Over Time - means (SD).

	ROTC (n=18)		Controls (n=18)	
Measures	**Pre**	**Post**	**Pre**	**Post**
4% tibia				
Total Area (mm²)	1149.4 (181.2)	1147.2 (179.6)	1118.8 (200.2)	1123.7 (203.7)
Trab. BMC (mg/mm)	324.4 (61.6)	323.5 (60.6)	314.9 (68.8)	315.9 (68.6)
Trab. Area (mm²)	1051.5 (175.8)	1048.3 (173.5)	1008.4 (195.5)	1015.0 (198.7)
Periosteal Circ. (mm)	119.8 (9.6)	119.7 (9.5)	118.1 (10.5)	118.4 (10.7)
38% tibia				
Total Area (mm²)	425.8 (62.2)	426.6 (62.9)	439.5 (88.6)	439.7 (88.8)
Cort. BMC (mg/mm)	381.3 (49.9)	382.4 (50.7)	394.7 (62.9)	394.7 (62.3)
Cort. Area (mm²)	324.7 (45.2)	325.6 (45.5)	338.1 (60.1)	338.2 (59.4)
Periosteal Circ. (mm)	73.0 (5.4)	73.0 (5.5)	73.9 (7.6)	74.0 (7.7)
Endosteal Circ. (mm)	35.3 (5.0)	35.3 (4.9)	35.0 (7.0)	35.0 (7.1)
iPolar (mm⁴)	31230 (8436)	31356 (8405)	33067 (11996)	33105 (12082)
66% tibia				
Total Area (mm²)	619.5 (118.2)	627.7 (97.6)	625.9 (117.9)	625.8 (117.7)
Cort. BMC (mg/mm)	396.5 (53.9)	397.1 (56.4)	407.4 (64.6)	408.2 (65.1)
Cort. Area (mm²)	349.3 (50.4)	349.9 (52.4)	359.6 (62.4)	360.0 (62.6)
Periosteal Circ. (mm)	87.8 (8.9)	88.6 (7.0)	88.3 (8.6)	88.3 (8.5)
Endosteal Circ. (mm)	57.5 (9.9)	58.7 (6.5)	57.3 (8.3)	57.2 (8.2)
iPolar (mm⁴)	57755 (17938)	58624 (16222)	58221 (19431)	58224 (19351)

* Significant $p<0.05$; Trab: Trabecular; Circ: Circumference; Cort: Cortical; iPolar: polar moment of inertia.