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ORIGINAL RESEARCH

Micronutrient Status During Military Training and Associations With Musculoskeletal Health, Injury, and Readiness Outcomes

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Objective: Micronutrient status, specifically vitamin D and iron, represent modifiable factors for optimizing military readiness. The primary purpose of this investigation was to determine associations between micronutrient deficiency (i.e., iron status and 25-hydroxy-vitamin D [25(OH)D]) and operationally relevant outcomes (i.e., skeletal health, musculoskeletal injury) at baseline and post-10 weeks of arduous military training. ***Methods:*** A total of 227 (177 men, 50 women) Marine Officer Candidates School (OCS) candidates who completed OCS training with complete data sets were included in this analysis. Vitamin D and iron status indicators were collected at two timepoints, pre (baseline) and post OCS. Musculoskeletal outcomes at the mid- and proximal tibial diaphysis were assessed via peripheral quantitative computed tomography. ***Results:*** Micronutrient status declined following OCS training in men and women and was associated with musculoskeletal outcomes including greater bone strength (strength strain index) at the mid-diaphysis site in those with optimal status ($M = 38.26$ mm^3, $SE = 15.59$) versus those without ($M = -8.03$ mm^3, $SE = 17.27$). In women ($p = .037$), endosteal circumference was greater in the deficient group ($M = 53.26$ mm, $SE = 1.19$) compared with the optimal group ($M = 49.47$ mm, $SE = 1.31$) at the proximal diaphysis. In men, greater baseline hepcidin concentrations were associated with an increased likelihood of suffering musculoskeletal injury during training. ***Conclusions:*** Vitamin D and iron status declined over the course of training, suggesting impaired micronutrient status. Differences in musculoskeletal outcomes by micronutrient group suggests optimal vitamin D and ferritin concentrations may exert beneficial effects on bone fatigability and fracture reduction during military training.

Keywords: peripheral quantitative computed tomography (pQCT), total 25-OH Vitamin D, iron status, Marine OCS training

United States Marine Corps Officer Candidates School (OCS) is an intensive and demanding 10-week military training course wherein male and female candidates execute the same rigorous training events in pursuit of the same highly coveted goal: to be commissioned as United States Marine Corps officers. Barriers to successful completion of OCS include musculoskeletal injury (MSKI), which has been identified as one of the predominant threats to military readiness. The burden of MSKI is particularly high during initial military training wherein recruits undergo high volume, prolonged, intensive and often unaccustomed mechanical loading (O'Leary, Wardle, Rawcliffe, et al., 2020). Known risk factors that affect MSKI include military-specific and repetitive weight-bearing activities such as load carriage (Hughes et al., 2019), military drill (Rice et al., 2018), and female sex (O'Leary, Wardle, Rawcliffe, et al., 2020). Bone stress injuries, a specific type of MSKI, represent a critical and pervasive military health issue, afflicting upward of 7% of men and 20% of women (Lovalekar et al., 2023) during initial military training, rendering bone a critical target tissue for MSKI reduction. Additionally, musculoskeletal outcomes may relate to physical performance which is a priority for military populations (Bird et al., 2022). Therefore, there is a need to optimize MSK health to limit injury, improve performance, and reduce attrition, particularly in women and during early career training.

One avenue of inquiry for understanding and mitigating MSKI risk relates to micronutrient status, which represents a modifiable factor for optimizing military readiness. Iron and vitamin D, in particular, are reciprocally related (Azizi-Soleiman et al., 2016) and have been separately investigated for effects on MSK health (Hennigar et al., 2016). Vitamin D and iron deficiencies are highly prevalent globally and are commonly seen in military personnel, with greater deficits observed in women (Andersen et al., 2010). Prior longitudinal observational studies have consistently demonstrated diminished serum 25-hydroxy-vitamin D (25[OH]D), the major circulating form of vitamin D, resulting from military training (Andersen et al., 2010). However, the definitive mechanism contributing to this diminished status remains unclear.

Prior work has also shown that diminished iron status resulting from military training is independent of energy deficits (O'Leary, Wardle, & Greeves, 2020) and further, that increased dietary intake during training is insufficient in circumventing this decline in men and women alike (Yanovich et al., 2015). While supplemental iron during military training has been shown to be efficacious in women (McClung et al., 2009), little data exists in men. Likewise, vitamin D and calcium supplementation imparts increases in bone parameters, including bone compressive strength in both men and women (Gaffney-Stomberg et al., 2014) and lowers the incidence of stress fracture in women (Lappe et al., 2008) without exacerbating iron status decline (Hennigar et al., 2016). Furthermore, even marginal iron deficiency has been shown to impair physical performance in women (Brownlie et al., 2002); however, results remain limited in young adult men and previous work often only included limited assessments of iron status. However, despite prior observations of vitamin D and iron deficiencies occurring simultaneously, little data exists examining both deficiencies concurrently in men and women undergoing the same intense training.

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Therefore, the primary purpose of this study was to characterize hematological status in male and female Marine OCS candidates and determine associations between micronutrient deficiency (i.e., iron status and 25-hydroxy-vitamin D) and operationally relevant outcomes (i.e., skeletal health, MSKI) at baseline and post-10 weeks of arduous military training. We hypothesized that candidates would suffer a combined decline in hematological status over the course of 10 weeks with more pronounced changes occurring in female candidates than in male. Furthermore, those candidates with compromised hematological status would also demonstrate attenuated bone quality and greater incidence of MSKI.

Materials and Methods

Experimental Approach to the Problem

The proposed study was an ancillary component to Institutional Review Board-approved protocol involving developing a Warfighter mobility signature and predictive algorithm for MSKI risk during Marine Corps Officer Candidate School, Grant Number: N00014-21-1-2725 (Bird et al., 2022; Koltun et al., 2022; Koltun, Bird, Forse, et al., 2023; Koltun, Bird, Lovalekar, et al., 2023; Lovalekar et al., 2023). This study was approved by the University of Pittsburgh Institutional Review Board and Office of Naval Research Human Research Protection Office, endorsed by the OCS Human Research Program, and performed in accordance with the Declaration of Helsinki.

Participants

Candidates were recruited for the OCS study via large, in-person information sessions and group briefings by study personnel. No OCS leadership was present during briefings to remove the potential threat of coercion. Study participants were healthy male and female United States Marine Corps officer candidates aged ≥18 years. All individuals entering the 10-week training course were eligible to participate. A study team member briefed the volunteers who provided written informed consent prior to participation. Candidates were not excluded on the basis of age, prior injuries, or health conditions due to being deemed “fit for duty” by Marine Corps standards. Baseline characteristics by sex can be found in Table 1.

Experimental Procedures

The proposed study was a prospective, longitudinal study in OCS candidates, which consisted of two assessment time points, baseline, and posttesting. A total of 12 ml of blood was collected during medical screening that occurred prior to the initiation of the 10-week program and again following completion of it. Officer Candidates were not fasted for blood draws. Serum blood tubes sat at room temperature for 30 min to clot and were then centrifuged at 1,500*g* for 10 min. Plasma tubes were centrifuged at 1,500*g* for 10 min at room temperature immediately after collection. Supernatant was aliquoted and stored on dry ice, then transferred overnight on dry ice to the University of Pittsburgh, where it was stored at −80 °C until further analysis. In addition to blood collection, candidates performed peripheral quantitative computed tomography (pQCT) scans.

Outcome Measures

Anthropometric Variables

Prior to the start of physical training, height and weight were recorded by a stadiometer and digital scale (Healthometer Professional 500KL) and used to calculate body mass index.

Table 1 Baseline Characteristics by Sex

Baseline characteristics	Women ($n = 50$)	Men ($n = 177$)
Age (years)	24 ± 3	23 ± 3
Race/ethnicity (*n*, %)		
White	41 (82%)	103 (58%)
Asian	2 (4%)	6 (3%)
Black or African American	2 (4%)	6 (3%)
Hispanic or Latino	3 (6%)	14 (8%)
Other	1 (2%)	5 (3%)
Not specified	3 (6%)	43 (24%)
Height (cm)	165.00 ± 5.87[a]	177.05 ± 6.74[a]
Body mass (kg)	65.49 ± 6.27[a]	80.76 ± 8.67[a]
Body mass index (kg/m^2)	23.9 ± 2.0[a]	25.9 ± 2.2[a]
Season testing occurred (*n*, %)[b]		
Fall	5 (10%)	27 (15%)
Winter	14 (28%)	82 (46%)
Summer	31 (62%)	68 (38%)
Lower-extremity overuse injury	15 (30%)	31 (17.5%)

[a]Significantly different by sex.
[b]Season determined by arrival date.

Musculoskeletal Variables

Parameters of volumetric bone density, bone geometry, and estimated bone strength were assessed via pQCT (XCT2000, Stratec) with a voxel size of 0.4 mm at both timepoints. Three tibial sites were imaged: the metaphysis (4%), mid-diaphysis (38%), and proximal diaphysis (66%). For analysis, sites were down selected to only the two proximal sites. The two proximal sites were chosen as we have demonstrated previously that predominantly cortical sites are associated with bone stress injury (BSI) during OCS (Koltun et al., 2022). Tibial scans were performed of the nondominant leg, except in instances of metal hardware implantation or history of previous fracture. A trained technician measured tibial length (tibial plateau to the midline of the medial malleolus, mm) at the baseline visit. Image analysis was performed in accordance with expert recommendations (Bone Diagnostic LLC; Rantalainen et al., 2011). Cortical volumetric bone mineral density and endosteal and periosteal circumference were acquired at the proximal diaphyseal regions (38, 66%). Polar strength strain index (SSI = moment of resistance = polar moment of inertia of the cortical bone area [mm × 4]/[max distance to the center] density weighted section modulus) is a measure of bone strength and quantifies bone’s resistance to bending and torsional loads.

Hematological Variables

Total serum iron (reference range: 28–170 µg/dl) was measured via 2,4,6-Tri-(2-pyridyl)-5-triazine (TPTZ) using Beckman Coulter AU680 and AY5800 analyzers. Serum ferritin (reference range: 10–282 ng/ml) was measured via the immunoenzymatic method using Beckman Coulter UniCel Dxl 800. Total iron-binding capacity (TIBC; reference range: 250–420 µg/dl) was measured via immunotubidimetric method using Beckman Coulter AU680 and AU5800 analyzers. TIBC is the sum total of serum iron and unsaturated iron-binding capacity and was calculated as = (Transferrin) × (1.4). Hepcidin was measured via biotechne ELISA (Krepuska et al., 2024). Transferrin saturation (reference range:

25%–50%) was calculated as (serum iron/TIBC) × 100 and reported as a percentage. 25-hydroxy vitamin D (reference range: 25–100 ng/ml) was measured by mass spectrometry on a Water's XEVO Triple Quadrupole tandem liquid chromatography–mass spectrometry. Sample preparation was completed using a solid-phase extraction in a 96-well plate using Water's Oasis plates. Iron deficient was defined as ferritin cutoff of ≤30 ng/ml. Iron replete was defined as ferritin of >30 ng/ml. While thresholds vary, iron overload is typically defined as ferritin of >200 ng/ml. Vitamin D deficient was defined as 25(OH)D <20 ng/ml. Vitamin D insufficient was defined as 25(OH)D 20–30 ng/ml and sufficient was defined as 25(OH)D >30 ng/ml.

Musculoskeletal Injury

Injury data was deidentified from internal OCS records and collected on candidates who presented to the Physical Therapy Department for MSKI treatment. All BSIs were diagnosed by trained medical staff based on radiographs or magnetic resonance imaging. Only lower extremity overuse MSKI occurring during OCS and not preexisting were included in the present analysis.

Statistical Methods

Statistical analysis was performed using SPSS Statistics (version 28.01.1). Paired samples *t* tests were performed to evaluate significant within-subject differences. For variables that did not meet assumptions, data transformation was attempted and, if unsuccessful, Wilcoxon signed-rank tests were used to determine significance. Significance was set a priori at .05 (two sided). Changes in vitamin D and iron classification from baseline to posttesting were assessed using McNemar's test. Iron and vitamin D concentrations were assessed with 2 × 2 (Sex [male vs. female] × Time [pre vs. post OCS]) mixed-measures analyses of variance. Mean percentage change from pre to post was reported. To assess the effect of baseline micronutrient status on bone and muscle outcomes, 2 × 2 (Sex [male vs. female] × Group [Group 1: optimal (i.e., no deficiency) vs. Group 2: vitamin D insufficient + deficient + iron deficient]) analyses of variance were performed. The association between micronutrient concentrations and performance was measured using Pearson correlation coefficients. Binomial logistic regression analyses were performed to assess the effect of baseline micronutrients on development of lower extremity overuse MSKI during OCS training. Separate analyses were conducted for men and women.

Results

A total of 227 (177 men, 50 women) Marine OCS candidates who completed OCS training were included in this analysis. Men were taller ($p < .001$), heavier ($p < .001$) faster ($p < .001$), and had a greater body mass index ($p < .001$). Men and women did not significantly differ by age, race, or lower-extremity MSKI (all $p > .053$; Table 1).

Micronutrient Status at Baseline and Posttesting

Micronutrient classification data are presented in Table 2. At baseline, 75.0% of men were sufficient in 25(OH)D, as were 82.0% of women. At posttesting, the percentage of candidates with vitamin D insufficiency significantly increased in men (22.7–38.6%, $p < .001$), but not women ($p = .267$). There was no change in the percentage of men and women with vitamin D deficiency over time ($p > .50$). Conversely, at baseline, 90.3% of men were replete in iron status and just 54.0% of women. The percentage of candidates who were iron replete declined in men ($p = .011$) and women ($p < .001$), which resulted in a greater percentage of men ($p = .036$) and women ($p < .001$) with iron deficiency posttesting.

Changes in concentrations of biomarkers are presented in Table 3. A main effect of time for 25(OH)D (41.65 ± 1.13 to 36.21 ± 0.81 ng/ml, $p < .001$, $\eta^2 = .174$), total iron (107.13 ± 3.08 to 75.17 ± 2.40 μg/dl, $p < .001$, $\eta^2 = .327$), ferritin (62.99 ± 4.39 to 42.31 ± 3.37 ng/ml, $p < .001$, $\eta^2 = .207$), iron saturation (31.33 ± 1.08 to 21.72 ± 0.97%, $p < .001$, $\eta^2 = .284$), and hepcidin (59,765.81 ± 3,201.91 to 40,871.28 ± 3,127.26 ng/ml, $p < .001$, $\eta^2 = .126$) indicates that concentrations of biomarkers associated with candidates' micronutrient status decreased during OCS. There was a significant Sex × Time interaction, such that TIBC concentration decreased over time in men (342.72 ± 47.88 to 330.12 ± 50.06 μg/dl, $p < .001$, $\eta^2 = .096$), but not women ($p = .150$). For 25(OH)D, there was a main effect of sex, such that women demonstrated greater concentrations than men ($p = .019$, $\eta^2 = .019$). There was no main effect of time on 25(OH)D. Men demonstrated greater total iron ($p < .001$, η^2=.073), ferritin ($p < .001$, η^2=.130), hepcidin

Table 2 Nutritional Status Groupings by Sex

		Baseline	Posttesting	McNemar test (*p*)
Vitamin D groups				
Sufficient (25(OH)D > 30 ng/ml)	M	132/176 = 75.0%	104/176 = 59.1%	**<.001**
	W	41/50 = 82.0%	34/50 = 68.0%	.065
Insufficient (25(OH)D 20–30 ng/ml)	M	40/176 = 22.7%	68/176 = 38.6%	**<.001**
	W	8/50 = 16.0%	13/50 = 26%	.267
Deficient (25(OH)D < 20 ng/ml)	M	4/174 = 2.3%	4/174 = 2.3%	1.00
	W	1/50 = 2.0%	3/50 = 6.0%	.500
Iron groups				
Replete (ferritin > 30 ng/ml)	M	159/176 = 90.3%	144/176 = 81.8%	**.011**
	W	27/50 = 54.0%	10/50 = 20.0%	**<.001**
Iron deficient (ferritin cutoff of ≤30 ng/ml)	M	17/176 = 9.7%	29/176 = 16.5%	**.036**
	W	23/50 = 46.0%	39/50 = 78.0%	**<.001**

Note. $n = 1$ (1%) woman missing from post 10-week vitamin D; $n = 4$ (2%) men missing from post 10-week iron; and $n = 1$ (2%) woman missing from post 10-week iron. $p < .05$ results are in bold.

Table 3 Change in Nutritional Status and Performance Metrics Following 10 Weeks of OCS Among Men and Women

					Effect		
		Baseline	Posttesting	Change (%)	Sex	Time	Sex × Time
Hematological measures							
Total 25-OH Vitamin D (ng/ml)	M	40.86 ± 13.82	34.85 ± 9.22	−8.9	**.019**	**<.001**	ns
	W	44.80 ± 15.88	38.98 ± 12.16	−10.64			
Total iron (µg/dl)	M	115.14 ± 37.26	86.60 ± 31.00	−20.49	**<.001**	**<.001**	ns
	W	102.43 ± 32.68	63.33 ± 26.89	−35.39			
Ferritin (ng/ml)	M	86.75 ± 60.29	63.56 ± 46.67	−18.85	**<.001**	**<.001**	ns
	W	41.33 ± 26.33	21.80 ± 13.93	−37.12			
TIBC (µg/dl)	M	342.72 ± 47.88	330.12 ± 50.06	−3.81	**<.001**	ns	**<.001**
	W	366.95 ± 82.48	382.08 ± 76.24	5.71			
Transferrin saturation (%)	M	34.18 ± 11.95	26.28 ± 10.56	−18.04	**<.001**	**<.001**	ns
	W	28.43 ± 10.41	17.19 ± 10.53	−37.78			
Hepcidin (ng/ml)	M	77.69 ± 46.27	60.59 ± 43.27	−14.22	**<.001**	**<.001**	ns
	W	49.59 ± 36.03	29.93 ± 42.78	−26.72			

Note. TIBC = total iron binding capacity; ns = nonsignificant ($p \geq .05$); OCS = Marine Officer Candidates School.
$p < .05$ results are in bold.

($p < .001$, $\eta^2 = .081$) concentrations, and percent iron saturation ($p < .001$, $\eta^2 = .102$) than women.

Micronutrient Status and pQCT Musculoskeletal Parameters

Differences between micronutrient status groups for pQCT-derived musculoskeletal outcomes at baseline and posttesting are presented in Table 4. At baseline, a significant Sex × Group interaction was present for endosteal circumference at the 66% proximal site (interaction effect: $p = .023$), such that in women ($p = .037$, $\eta_p^2 = .089$) but not men ($p = .298$, $\eta_p^2 = .011$), endosteal circumference was greater in the deficient group ($M = 53.26$ mm, $SE = 1.19$) compared with the optimal group ($M = 49.47$ mm, $SE = 1.31$). No other significant interaction effects or differences between micronutrient groups were observed at baseline.

When examining changes in musculoskeletal parameters based on micronutrient status, only SSI at the 38% site was significant. Specifically, at the tibial mid-diaphysis (38% site), there was a main effect of group in absolute change (posttesting minus baseline) in SSI ($p = .049$, $\eta_p^2 = .030$), such that the mean of the optimal group was significantly greater ($M = 38.26$ mm^3, $SE = 15.59$) than was the deficient group ($M = -8.03$ mm^3, $SE = 17.27$). No other significant interaction effects or differences between micronutrient groups were observed.

Micronutrients and Operationally Relevant Outcomes

MSKI data are presented in Table 1. A binomial logistic regression model was performed to ascertain the effects of each baseline micronutrient on the likelihood that participants suffer a lower extremity overuse MSKI during OCS training. In men but not women, baseline hepcidin, $\chi^2(1) = 3.915$, $p < .048$ explained 3.6% of the variance in injury and correctly classified 82.5% of cases. Increased baseline hepcidin was associated with an increased likelihood of suffering a MSKI in men. No other micronutrient parameters were predictive of injury (all $p > .07$).

Discussion

This study sought to characterize the hematological status of male and female Marine OCS candidates and determine associations between micronutrient deficiency and operationally relevant outcomes, including bone health and MSKI. Our major findings include: (1) vitamin D status declined over the course of 10 weeks, however, due to men and women beginning training with robust vitamin D concentrations, posttesting levels remained, on average, sufficient; (2) men and women demonstrated evidence of degraded iron status following OCS with women suffering greater magnitude declines than did men; (3) those with optimal micronutrient status exhibited more positive bone morphology and improved adaptation in response to training compared with those who were micronutrient deficient; and (4) in men but not women, baseline hepcidin was the only micronutrient parameter predictive of lower extremity overuse injury sustained during OCS training.

> Vitamin D status declined over the course of 10 weeks. However, due to men and women beginning training with robust Vitamin D concentrations, posttesting levels remained, on average, sufficient.

We found significant declines in vitamin D following military training in men and women. The decline observed in women is consistent with prior literature such as Andersen et al. (2010) who observed a 13.17% decline in 25(OH)D in women following 8 weeks of basic training (Andersen et al., 2010). In contrast with prior work, we found that women presented with significantly greater 25(OH)D concentrations than men did at baseline but suffered a greater magnitude decline over the course of 10 weeks. Interestingly, men and women in the present study demonstrated higher than average 25(OH)D concentrations compared with prior studies at baseline (82% of women and 75% of men were sufficient) and while status declined over time, on average, concentrations remained within sufficient range in both sexes. For example, a study of female Army recruits found that 57% entered BCT

Table 4 Baseline and Changes in Select Bone Parameters Based on Micronutrient Status

Effect						
Sex	**Baseline group**	***N***	**Mean ± *SD***	**Sex**	**Group**	**Sex × Group**
38% cortical density (mg/cm^3)						
Men	Optimal	78	1,131.67 ± 30.79	**<.001**	.279	.773
	Deficiency	32	1,127.59 ± 30.84			
Women	Optimal	22	1,164.91 ± 22.25			
	Deficiency	26	1,157.87 ± 22.66			
Δ 38% cortical density (mg/cm^3)						
Men	Optimal	78	2.37 ± 17.41	.210	.149	.437
	Deficiency	32	−5.62 ± 20.90			
Women	Optimal	22	−4.93 ± 15.97			
	Deficiency	26	−7.33 ± 20.15			
38% periosteal circumference (mm)						
Men	Optimal	78	77.91 ± 4.63	**<.001**	.963	.150
	Deficiency	32	76.72 ± 4.93			
Women	Optimal	22	68.04 ± 3.54			
	Deficiency	26	69.16 ± 3.99			
Δ 38% periosteal circumference (mm)						
Men	Optimal	78	0.13 ± 0.76	.475	.333	.060
	Deficiency	32	0.38 ± 0.88			
Women	Optimal	22	0.83 ± 3.07			
	Deficiency	26	0.07 ± 0.78			
38% endosteal circumference (mm)						
Men	Optimal	78	34.06 ± 4.58	**.001**	.305	.177
	Deficiency	32	33.81 ± 4.24			
Women	Optimal	22	30.44 ± 2.81			
	Deficiency	26	32.28 ± 4.49			
Δ 38% endosteal circumference (mm)						
Men	Optimal	78	0.030 ± 0.53	.569	.902	.198
	Deficiency	32	0.16 ± 0.67			
Women	Optimal	22	0.24 ± 0.87			
	Deficiency	26	0.08 ± 0.39			
38% SSI (mm^3)						
Men	Optimal	76	2,218.41 ± 357.92	**<.001**	.993	.116
	Deficiency	31	1,543.12 ± 199.51			
Women	Optimal	22	2,117.04 ± 384.08			
	Deficiency	26	1,628.84 ± 281.27			
Δ 38% SSI (mm^3)						
Men	Optimal	76	25.09 ± 110.00	.906	**.049**	.313
	Deficiency	31	2.38 ± 92.62			
Women	Optimal	22	51.43 ± 210.36			
	Deficiency	26	−18.44 ± 48.96			
66% cortical density (mg/cm^3)						
Men	Optimal	71	1,085.42 ± 34.56	**<.001**	.075	.699
	Deficiency	30	1,072.10 ± 36.74			
Women	Optimal	22	1,124.36 ± 33.71			
	Deficiency	27	1,115.77 ± 28.45			
Δ 66% cortical density (mg/cm^3)						
Men	Optimal	71	0.54 ± 19.85	**.021**	.971	.519
	Deficiency	30	2.93 ± 17.48			
Women	Optimal	22	−6.04 ± 20.14			
	Deficiency	27	−8.69 ± 24.14			

(continued)

Table 4 *(continued)*

Effect Sex	Baseline group	*N*	Mean ± *SD*	Sex	Group	Sex × Group
66% periosteal circumference (mm)						
Men	Optimal	71	92.61 ± 5.91	**<.001**	.540	.099
	Deficiency	30	91.51 ± 6.48			
Women	Optimal	22	80.42 ± 4.78			
	Deficiency	27	82.81 ± 5.70			
Δ 66% periosteal circumference (mm)						
Men	Optimal	71	0.22 ± 0.90	.380	.962	.474
	Deficiency	30	0.11 ± 0.72			
Women	Optimal	22	−0.04 ± 0.91			
	Deficiency	27	0.08 ± 0.78			
66% endosteal circumference (mm)						
Men	Optimal	77	58.11 ± 6.55	**<.001**	.312	**.023**
	Deficiency	33	56.64 ± 6.20			
Women	Optimal	22	49.47 ± 4.77			
	Deficiency	27	53.26 ± 7.08			
Δ 66% endosteal circumference (mm)						
Men	Optimal	77	0.16 ± 0.98	.634	.703	.895
	Deficiency	33	0.11 ± 0.66			
Women	Optimal	22	0.28 ± 1.48			
	Deficiency	27	0.18 ± 0.97			
66% SSI (mm^3)						
Men	Optimal	71	3,325.93 ± 576.60	**<.001**	.852	.191
	Deficiency	30	3,218.86 ± 645.04			
Women	Optimal	22	2,306.12 ± 391.41			
	Deficiency	27	2,422.94 ± 412.48			
Δ 66% SSI (mm^3)						
Men	Optimal	71	35.84 ± 111.78	**.003**	.527	.807
	Deficiency	30	27.15 ± 109.54			
Women	Optimal	22	−25.95 ± 77.32			
	Deficiency	27	−45.54 ± 155.83			

Note. 38, 66% SSI were LN transformed due to failure to meet assumptions; Δ = delta (posttesting minus baseline); SSI = polar strength strain index (SSI = moment of resistance = polar moment of inertia of the cortical bone area [mm × 4]/[max distance to the center]) density weighted section modulus); optimal group = sufficient vitamin D concentrations and replete ferritin concentrations; deficient group = insufficient or deficient vitamin D concentrations and/or deficient ferritin concentrations.
$p < .05$ results are in bold.

as vitamin D deficient or insufficient (Fogleman et al., 2022). Similarly, a study of female Marines found that 27% entered training as deficient and 36% as insufficient (Maloney & Goolkasian, 2020). In the absence of measuring supplementation, determining the degree of vitamin D supplementation currently used by new recruits prior to starting OCS training is needed to determine what factors contribute to higher micronutrient status compared with previous research.

> Men and women demonstrated evidence of degraded iron status following OCS with profoundly greater magnitude declines occurring in women.

In the present study, men and women demonstrated a decline in total iron, serum ferritin, iron saturation, and hepcidin, indicative of degraded iron status and more specifically, of iron deficiency. Though men demonstrated degraded status, due to starting with more than double the ferritin concentrations compared with women, substantially less men concluded training meeting the threshold of iron deficiency (≤30 ng/ml) compared with women. During the 10 weeks, TIBC significantly decreased in men, whereas in women it increased. This TIBC observation in women is likely due to the increases in transferrin levels with declining iron status (Cichoń et al., 2022). Moreover, transferrin saturation concentrations fell below the clinical threshold of 20% in women, indicative of iron deficiency. These findings are consistent with prior studies in military training including a marked decline in iron, transferrin saturation, and ferritin in male Israeli Defense Forces combatants undergoing 4 months of basic training (Moran et al., 2012). Similar declines in ferritin of −15% (Gofrit et al., 2023) and −32% (Martin et al., 2019) were observed in female combatants during military training of the same duration (4 months).

In the present study, the percentage of women who entered training iron deficient was 4.6× greater than the percentage of men and nearly 5× greater following training. Intense exercise has been shown to disturb iron homeostasis, even in situations of excessive iron intake (Yanovich et al., 2011). Greater decrements in iron status attributed to women in the present study could be the result of a multitude of factors including menstrual blood loss, insufficient dietary intake, and greater relative physical demands during training compared to their male counterparts (O'Leary et al., 2023). Additional research is warranted to understand why the rate of micronutrient decline is greater in female recruits. While observational studies have indicated a positive relationship between iron and vitamin D status, these results suggest that despite optimal vitamin D, women remain at high risk of iron status degradation entering and following arduous training.

> Those with optimal micronutrient status exhibited preferential bone morphology and improved adaptation in response to training compared with those who were micronutrient deficient.

In the present study, bone strength (SSI) over the course of training significantly increased in the optimal group (M = 38.26 mm^3, SE = 15.59) compared with a decline in the deficient group (M = −8.03 mm^3, SE = 17.27). Moreover, micronutrient deficient women exhibited greater endosteal circumference at the proximal tibia, despite similar periosteal circumference and density. In men, cortical mass is placed further from the neutral axis of the long bone, increasing cross-sectional area, and bestowing greater resistance to bending. Conversely, women typically construct bones that are narrower than are men's due to greater periosteal apposition in the male skeleton during puberty. To compensate for this difference, women tend to maintain structural integrity via endosteal apposition and increased mineral density at proximal sites (Hart et al., 2017). However, in the present study, results suggest that women suffering from micronutrient deficiency exhibited greater endosteal circumference, without greater periosteal circumference or density to compensate for resultant thinner cortices, leaving them at mechanical disadvantage.

It's theorized that female Soldiers experience greater physiological strain compared with men when exposed to the same absolute strains (Yanovich et al., 2015), due in part to differences in load attenuation (Burghardt et al., 2009). Bruce and Edwards (2023) emphasized this dimorphism by conducting finite element analysis to predict bone strains during running, and the authors observed 10% greater peak strain and 80% greater strained volume in the narrower tibia belonging to the average female compared to the average male (Bruce & Edwards, 2023). Prior work has consistently shown that military recruits and athletes alike with more narrow tibias were at a greater risk of stress fracture and BSI (Popp et al., 2021). Moreover, proximal densitometric measures are of special interest to military trainees, as most tibial stress fractures incurred during training occur at the diaphysis (shaft), where long bones experience high bending and torsion stress during activity and we have previously demonstrated an association with BSI risk during OCS (Koltun et al., 2022).

> Increased serum hepcidin concentrations in men, not women, exclusively predicted lower extremity overuse injury sustained during OCS training.

Increased circulating hepcidin results in increased iron sequestering thereby limiting biologically available iron for physical performance (McClung, 2019). Increased hepcidin has been observed in female marathoners (Roecker et al., 2005) and in military personnel following arduous operational activity (McClung, 2019), while others found that hepcidin concentrations decreased in women undergoing exhaustive running training (Auersperger et al., 2013). In the present study, hepcidin declined in both men (−14.22%) and women (−26.72%) following training and, in men only, was the only micronutrient parameter to predict incidence of lower extremity overuse injury sustained during OCS training, such that men who became injured exhibited greater hepcidin concentrations at baseline. Prior work has consistently demonstrated that regulatory factors that exert a significant influence on hepcidin activity include baseline iron status and hepcidin levels preexercise (Fensham et al., 2023). The decline in hepcidin seen here may indicate a protective adaptation due to the candidates declining iron status whereby hepcidin is suppressed so that dietary iron absorption and replenishment of iron stores can increase and recover from a deficient state (Peeling et al., 2014). Interestingly, this occurred despite the majority of men beginning and ending training with replete status. Further study is warranted to discern the change in hepcidin in response to military training, specifically as it relates to declines in iron status and MSKI risk.

Limitations

The present study has some limitations. This study did not measure soluble transferrin receptor or hemoglobin, negating our ability to diagnose iron deficiency anemia based on clinical thresholds. However, according to the Centers for Disease Control, the sensitivity of serum ferritin is 89% for diagnosis of iron depletion compared with hemoglobin, which is only 26% (Pfeiffer et al., 2013). We did not measure parathyroid hormone, limiting our ability to fully elucidate the plausible disturbance to calcium homeostasis resulting from intense exercise. We did not control for menstrual cycle or status in female candidates, which may have helped to explain our results. Due to the limited access to candidates typical to military field environments, candidates were not exclusively fasted prior to their blood draw, and physical activity prior to testing was not controlled for, therefore diurnal variation and changes in response to exercise and nutrition may have influenced our results. As previously noted, supplementation data was not directly measured. Finally, pQCT is able to measure volumetric bone mineral density but lacks the necessary resolution to examine bone microarchitecture.

Practical Applications and Conclusions

Parameters of vitamin D and iron status changed over the course of Marine OCS training, suggesting a decline in micronutrient status. However, robust vitamin D concentrations in women and high iron stores in men prior to the onset of training appeared to attenuate declines. Optimal micronutrient status was associated with improved bone strength and a more mechanistically advantageous tibial shaft, suggesting optimal vitamin D and ferritin concentrations may exert beneficial consequences on bone fatigability and fracture reduction during military training. Moreover, hepcidin was predictive of lower-extremity injury risk suggesting the need for additional research to more carefully investigate this potential association. While additional research is needed to further elucidate this relationship, dietary and supplemental intake of vitamin D and iron should be prioritized both preceding and during military training to counteract detriments to bone observed under conditions of insufficiency.

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