Vitamin D Correlation with Testosterone Concentration in Male US Soldiers and Veterans

L M. Wentz,[1] C S. Berry-Cabán,[2] Q Wu,[3] J D. Eldred[4]

Abstract

Background: Vitamin D has been positively correlated with testosterone in older men, but these hormonal relationships have not been examined in military personnel.

Purpose: The purpose of this study was to identify significant correlations between vitamin D and testosterone concentrations in male soldiers and veterans.

Material and Methods: This study examined unique cases of serum vitamin D assessments ordered at Womack Army Medical Center, Fort Bragg, NC, from January 2012 – September 2013. Inclusion criteria were male soldiers or veterans who had a testosterone assessment within 21 days of vitamin D assessment, yielding 796 subjects. General linear models were used to test the effect of vitamin D on total testosterone.

Results: Mean serum vitamin D concentrations were 29.2 ± 11.1 ng/ml, with 55.7% of subjects in the deficient or insufficient range of <30 ng/ml according to the US Army Medical Department guidelines. Mean total testosterone concentrations were 426.9 ± 178.6 ng/dl. Subjects in the lowest vitamin D quintile had significantly lower testosterone concentrations, younger age, and higher BMI than subjects in the highest quintile. When BMI, age, and time of testosterone measurement were included in the model to predict testosterone concentrations, the significance of vitamin D was eliminated.

Conclusion: These data show a high prevalence of vitamin D deficiency in male soldiers and veterans assessed in the southeast region of the United States. Since vitamin D deficiency may be related to hypothalamo-pituitary dysfunction in service members, future research should prospectively assess vitamin D status in comprehensive treatment plans for endocrine disorders

Keywords: 25-hydroxyvitamin D; androgen; endocrine hormones; human performance; military

Introduction

Previous research shows that vitamin D is positively correlated with testosterone concentration in older men.[1,2] Like testosterone, vitamin D functions as a hormone and is synthesised endogenously from cholesterol. Vitamin D is made in the skin from exposure to sunlight.[3] However, vitamin D is also consumed in the diet, with sources including oil-rich fish such as salmon, mackerel and herring; egg yolks and fortified foods such as milk. Vitamin D undergoes a series of hydroxylating reactions that alter its structure to form the biological active compound that binds vitamin D receptors to regulate gene expression for pathways essential to physical and cognitive performance.

Initially, vitamin D enzymes were thought to be exclusive to the liver and kidney but have recently been identified in other tissues as well. Notably, vitamin D metabolising enzymes and receptors have been identified in the testes, indicating that vitamin D may play a role in regulating testosterone production.[4] Emerging evidence strengthens support for the relationship between vitamin D and testosterone production by showing that 25-hydroxyvitamin D (25(OH)D) production occurs in the Leydig cells of testes, the site of testosterone production.[5]

Limited research has been published on vitamin D deficiency in active duty personnel.[6] A retrospective analysis of archived serum samples from 990 service members found that 35% of subjects had serum 25(OH)D concentrations in the deficient range of less than 20 ng/ml.[7] A study among female recruits entering basic training found that 57% of subjects had serum 25(OH)D concentrations less than 30 ng/ml at baseline and that 75% of subjects were in this range after completing 8 weeks of outdoor training.[8] These results suggest that outdoor training in tactical gear prevents adequate skin exposure, since basic combat training occurred during autumn in South Carolina.

A few studies in male military personnel have found high rates of vitamin D deficiency as well.[9,10] Furthermore, operational stress of military training has been shown to suppress testosterone concentrations in healthy men.[11] Considering the high prevalence of vitamin D deficiency and a potential role in the testes, it is hypothesised that poor vitamin D status limits testosterone synthesis in male military personnel. Therefore, the purpose of this study was to identify significant correlations between vitamin D and testosterone concentrations in male soldiers and veterans. Low testosterone concentrations have the potential to reduce muscle mass, initiate fatigue, limit performance, and have been shown to increase the risk for PTSD.[12,13] Vitamin D is commonly assessed at the time of testosterone measurement in military medicine. As a result, male service members tend to have testosterone assessments more frequently and at younger age than civilians.

Materials and Methods

This retrospective study examined 796 unique cases of serum vitamin D assessments ordered at Womack Army Medical Center, Fort Bragg, NC, between January 2012 and September 2013. Inclusion criteria were male soldiers or veterans who had a total testosterone assessment within 21 days of vitamin D assessment. Age at the time of the test was identified for all subjects, while body mass index (BMI), a ratio of weight to height-squared, was available for only 560 subjects. Race and ethnicity identifiers were too limited to be included in the analysis. This study was approved by Womack Army Medical Center Institutional Review Board.

Measurement of vitamin D and testosterone assessments were conducted through Womack Army Medical Center. Serum 25(OH)D concentrations were determined by liquid chromatography-tandem mass spectrometry (Quest Diagnostics, Chantilly, VA) with a detection limit of 4 ng/ml and a 8.3% coefficient of variation. Serum total testosterone concentrations were also determined by liquid chromatography-tandem mass spectrometry (Quest Diagnostics, Chantilly, VA) with a detection limit of 1.0 ng/dl and a 10.0% coefficient of variation. Since serum 25(OH)D has a half-life of three weeks,[3] subjects were included only if testosterone assessment was conducted within 21 days of vitamin D assessment. Therefore, no adjustment for season was warranted.

Serum vitamin D ranges for the purposes of data analysis in this study were based on the laboratory ranges used by the U.S. Army Medical Department (AMEDD) standards of care.[14] The AMEDD laboratory ranges follow guidelines from the Endocrine Society

Clinical Practice Guideline,[3] that defines deficient as 25(OH)D less than 20 ng/ml, insufficient as 20-29 ng/ml, and sufficient as 30-100 ng/ml.

According to the Endocrine Society Clinical Practice Guideline,[12] low testosterone is defined as less than 300 ng/dl. However, the U.S. AMEDD standards of care[14] use an age-stratified reference range for males, with low testosterone cut-offs of 270 ng/dl for men aged 20-49 years and 212 ng/dl for men aged greater than 49 years.

Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC). Summary statistics for categorical variables include frequencies, means, and standard deviations. Marginal relationships between age, vitamin D, and total testosterone were assessed using Pearson's correlations. Student's t-test and ANOVA were used to compare means. Quintiles for vitamin D were calculated using PROC ANOVA. Linear regression analysis using PROC regression was used to test the effect of vitamin D on total testosterone. In these models, age of soldiers was a covariate, and active duty vs. veteran was a fixed factor. Since testosterone varies with circadian rhythm, a categorical variable was created for assessments taken between 0700h and 0900h and those measured at other times of day. BMI was included as a covariate in step-wise regression. Interactions were tested and removed from the models if they were not statistically significant at a level of $P = 0.05$.

Results

Mean serum 25(OH)D concentrations for all 796 male soldiers and veterans were 29.2 ± 11.1 ng/ml (range 5-99 ng/ml). Seventeen percent of subjects tested were deficient in vitamin D, while 38.7% had insufficient status, and 44.3% had sufficient status. Overall mean total testosterone concentrations were 426.9 ± 178.6 ng/dl (range 12-972 ng/dl). According to the Endocrine Society Clinical Practice Guideline,[12] 24.1% of this sample had low testosterone. However, only 17.2% of the sample had low testosterone using the age-adjusted range in the U.S. AMEDD standards of care.[14]

Table 1 shows descriptive data by vitamin D status, from which a trend was observed toward lower testosterone in vitamin D deficient subjects but it was not significant ($P = 0.087$). When active duty personnel and veterans were compared, as expected, veterans were significantly older with higher BMI values and lower testosterone concentrations (Table 2). Distribution of testosterone concentrations according to 25(OH)D quintiles is shown in Table 3. Subjects in the lowest vitamin D quintile had

Table 1. Service Member age, BMI, and testosterone concentrations according to 25-hydroxyvitamin D status

	Deficient (<20 ng/ml) (n=135)	Insufficient (20-29 ng/ml) (n =308)	Sufficient (30-100 ng/ml) (n=353)
25(OH)D (ng/ml)	15.2 ± 3.3	25.0 ± 2.8	38.3 ± 9.6
Age (yrs)	39.8 ± 10.0	40.9 ± 9.7	41.1 ± 10.1
BMI (kg/m^2)	30.4 ± 4.2	29.8 ± 4.4	29.5 ± 3.7
Testosterone (ng/dl)	396.1 ± 162.5	435.6 ± 177.1	431.7 ± 184.1

Data are means ± standard deviation. BMI, Body Mass Index – data available for 560/796 subjects. No significant differences were observed at P < 0.05.

Table 2. Service Member age, BMI, and testosterone according military status

	Active Duty (n=684)	Veteran (n =112)	P-value
25(OH)D (ng/ml)	29.0 ± 10.3	30.7 ± 14.8	0.237
Age (yrs)	38.5 ± 7.9	54.6 ± 9.8	<0.001
BMI (kg/m^2)	29.6 ± 4.0	31.1 ± 4.6	0.003
Testosterone (ng/dl)	436.8 ± 178.2	366.1 ± 169.4	<0.001

Data are means ± standard deviation. BMI, Body Mass Index – data available for 560/796 subjects.

Table 3. Service Member age, BMI, and testosterone concentrations according to 25-hydroxyvitamin D quintiles

	Quintile 1 ≤21 ng/ml	Quintile 2 22-26 ng/ml	Quintile 3 27-31 ng/ml	Quintile 4 32-36 ng/ml	Quintile 5 >36 ng/ml
Testosterone (ng/dl)	398.3 ± 165.1	426.3 ± 189.6	440.3 ± 178.5*	424.4 ± 168.1	447.5 ± 189.6*
Age (yrs)	40.3 ± 9.6	40.7 ± 9.9	40.8 ± 10.0	39.6 ± 9.3	42.5 ± 10.7*+
BMI (kg/m^2)	30.6 ± 4.4	29.5 ± 4.3*	29.9 ± 4.2	29.5 ± 3.9	29.2 ± 3.6*

*Data are means ± standard deviation. BMI, Body Mass Index – data available for 560/796 subjects. Comparison between 25(OH)D quintiles were performed using PROC ANOVA. *P < 0.05 compared to quintile 1. +P < 0.05 compared to quintile 4.*

Table 4. General linear models to predict testosterone concentrations in male Soldiers and Veterans

Independent variable	Testosterone in all men (n=796)		Testosterone in men with BMI (n=560)	
	β	p	β	p
Vitamin D	1.258	0.026	0.754	0.257
Age	-3.041	<0.001	-2.615	0.001
AM Testosterone	26.120	0.037	27.870	0.066
BMI			-7.325	<0.001
Model R^2	0.039		0.065	

Figure 1. Testosterone plotted vs 25(OH)D concentrations in subjects with deficient and insufficient vitamin D. Darker areas indicate high concentration or data points. r=0.131 p=0.006
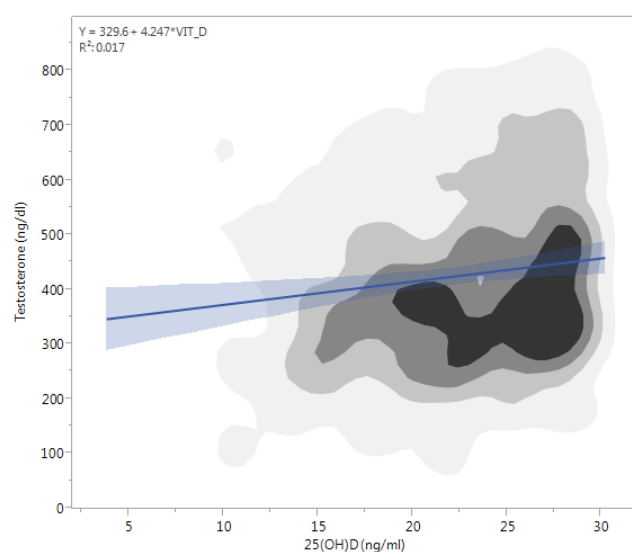
significantly lower testosterone concentrations, younger age, and higher BMI than subjects in the highest quintile. Testosterone concentration across quintiles did not show a U-shaped relationship.

Serum 25(OH)D concentrations were positively (but weakly) correlated with total testosterone concentrations in all subjects (r = 0.065; P = 0.066). However, when subjects with deficient and insufficient vitamin D status were isolated (n = 443), the Pearson's correlation was strengthened to a significant positive association (Figure 1; r = 0.131; P = 0.006).

The general linear model to predict testosterone concentrations in all subjects was significant when controlling for age and time of testosterone assessment, although the β coefficient for vitamin D was small (Table 4). When BMI was added as a covariate, the significance of vitamin D and time of testosterone measurement were eliminated.

Discussion

This study shows a high prevalence of poor vitamin D status in male military personnel and that men in the lowest vitamin D quintile had significantly lower testosterone concentrations compared with men in the highest quintile. Vitamin D concentrations showed a weak positive correlation with total testosterone although this correlation was stronger in men with deficient/insufficient vitamin D status. These results indicate that maintaining sufficient vitamin D may play a role in maintaining testosterone status. However, controlling for age, BMI, and time of testosterone measurement reduced the association of vitamin D with testosterone. To our knowledge, this

is the first report linking vitamin D to testosterone in military personnel.

Previous research has identified positive associations between 25(OH)D and testosterone in male subjects, for which potential mechanisms have been hypothesised.[1,2,15] Vitamin D receptors and metabolising enzymes are expressed in the testes, most notably in testosterone-producing Leydig cells,[4] In vitro, the bone-modulating protein osteocalcin stimulates production of both 25(OH)D and testosterone in Leydig cells, indicating that bone metabolism may regulate hormonal synthesis in testes.[5] Furthermore, hypogonadism develops in vitamin D receptor knock-out mice, characterised by reduced sperm count and motility along with abnormal testicular development.[16] Vitamin D deficient rats show similar testicular abnormalities and reduced survival as well as reduced fertility and mating.[17] In human males, vitamin D deficiency is associated with hypogonadism, characterised by a combination of low testosterone and low luteinizing hormone concentrations.[15] These results suggest that vitamin D deficiency limits hypothalamo-pituitary axis function, thus altering reproductive hormone synthesis.

It is well established that military veterans with traumatic brain injury have a high prevalence of hypothalamo-pituitary axis dysfunction, including low testosterone along with additional symptoms of hypogonadism.[18,19] Therefore, poor vitamin D and testosterone status in service members may reflect more widespread endocrine dysfunction. Symptoms of vitamin D deficiency are consistent with endocrine dysfunction, such as fatigue, depression, cognitive deficiencies, and loss of neuromuscular function that may impair human performance.[18,20,21] However, no research has been published on vitamin D status in hypothalamo-pituitary dysfunction or traumatic brain injury. Since vitamin D status alters expression of nearly 300 genes related to cellular differentiation, DNA replication, and transcription, among other functions,[22] it is plausible that vitamin D deficiency inhibits androgen expression.

In our study, greater than half of male soldiers and veterans had deficient or insufficient 25(OH)D. These results are consistent with previous research showing a high prevalence of poor vitamin D status in military personnel.[7-10] In fact, mean 25(OH)D concentrations in our study were higher than concentrations measured in other male soldiers. In a study of 204 male Finnish recruits, the median 25(OH)D concentrations were 18 ng/ml.[10] while a study of male Lithuanian soldiers had a mean 25(OH)D concentration of 12.5 ± 4.5 ng/ml, with 95% of the 262 men deficient in vitamin D.[9] National Health

and Nutrition Examination Survey (NHANES) data shows that 29% of male US civilians have 25(OH) D concentrations less than 20 ng/ml and 76% are below 30 ng/ml.[23]

A potential explanation for our results is the 35.1°N latitude of Fort Bragg, in which ultraviolet rays support a longer period for endogenous vitamin D synthesis.[24] All measurements were taken at Womack Army Medical Center, although subject permanent residence and outdoor activities were unknown. However, despite the latitude, only 44.3% of subjects had sufficient 25(OH)D status in this retrospective analysis, presenting the possibility that true deficiency rates are higher if all military personnel were screened for vitamin D status. Even in a southern climate, tactical gear may interfere with endogenous vitamin D synthesis, as evidenced by our subjects having similar vitamin D status and rates of deficiency to female soldiers training at a similar latitude.[8]

Normal ranges for total testosterone vary by reference laboratory. In this study, mean concentrations were normal according to both the Endocrine Society Guidelines[12] and AMEDD Standards of Care.[14] Our mean serum testosterone was similar to the mean value of 430 ng/dl measured in 124 male soldiers (aged 28.8 ± 5 years) entering Survival Training[11] but was lower than the mean value of 684 ± 75 ng/dl measured in 23 male soldiers (aged 23.0 ± 2.8 years) entering the Ranger School.[25] With a mean age of 40.8 ± 9.9 years (38.5 ± 7.9 years for active duty only), our subjects were considerably older and their testosterone concentrations reflect the age-related decline in this hormone. NHANES data from US civilians also mirror this age-related decline, showing that men aged 20 years have mean testosterone concentrations of 393 ng/dl and these levels decline to 376 ng/dl by 50 years of age.[26]

Previous research has shown a stronger linear relationship between 25(OH)D and testosterone at low vitamin D concentrations compared to sufficient vitamin D status.[1] However, our regression models showed that 25(OH)D was not a significant predictor for testosterone after correction for age, BMI, and time of testosterone measurement. These results are similar to a European study of men aged 40-79 years, in which 25(OH)D concentrations were not significantly correlated with total testosterone following adjustment for age and additional confounders.[15] The lack of testosterone association observed in subjects with sufficient vitamin D status suggests that the relationship between vitamin D and testosterone is not linear at higher levels. Other researchers have found a U-shaped association, showing men with the lowest and highest quintiles

of 25(OH)D had lower testosterone concentrations compared to men in middle quintiles.[27] On the contrary, we found men in the lowest 25(OH)D had significantly lower testosterone than men in the highest 25(OH)D quintile.

Unlike our data, a previous study found that 25(OH)D was significantly associated with total testosterone in a large sample of men after controlling for age and BM.[12] Our results support lower testosterone concentrations in older men with higher BMI, but we did not find lower 25(OH)D concentrations in older men. In fact, men in the highest 25(OH)D quintile were significantly older than subjects in the lowest quintile, and there was no significant difference between 25(OH)D concentrations in veteran and active duty service members.

We also found that BMI was highest in the lowest quintile for 25(OH)D. These findings are similar to other previous research.[1] The mean BMI in our study was 29.8 ± 4.1 kg/m2 (range 17.4 – 46.2), suggesting that most subjects were overweight, although we studied an active sample of men. Additionally, BMI data were available for only 70% of our subjects. Therefore, BMI may not accurately represent body composition in this population.

Limitations of this study include the retrospective nature of the medical record review in which we could establish correlations but not causal relationships. Furthermore, the sample was limited to subjects whose physicians ordered vitamin D and testosterone and is therefore not necessarily representative of all male service members. Lastly, data were not available for confounding variables such as race, physical activity, dietary habits, sun exposure and sex hormone binding globulin. The study was strengthened by only including assays completed within 3 weeks of one another to control for a season of vitamin D analysis, and medical records were reviewed for one geographical location to limit the effect of latitude. Future studies should consider prospective trials in service members in which vitamin D and testosterone are measured across multiple time points along with a full metabolic profile.

Conclusions

In conclusion, these data show a high prevalence of vitamin D deficiency in male soldiers and veterans assessed in the southeast region of the United States. At low 25(OH)D concentrations, a linear relationship with testosterone concentrations emerged that indicates vitamin D deficiency may limit testosterone synthesis and potentially limit human performance. However, the general linear model for all ranges

of vitamin D showed BMI and age had a stronger relationship with testosterone than vitamin D. Military personnel undergo unique physical and operational training, deployment schedules, and have greater risk of injury than civilians, thereby increasing stress to their neuroendocrine system. Since both vitamin D deficiency and hypothalamo-pituitary dysfunction plague service members, future research should prospectively assess vitamin D status in comprehensive treatment plans for endocrine disorders to optimise human performance, improve resiliency, and reduce morbidity of warriors and veterans.

Corresponding author: Laurel M. Wentz
wentzl@ecu.edu
Authors L M. Wentz,[1] C S. Berry-Cabán,[2] Q Wu,[3]
J D. Eldred[4]
Author Affiliations:
[1] Department of Nutrition Science, East Carolina University
[2] Department of Clinical Investigation, Womack Army Medical Center
[3] Department of Biostatistics, East Carolina University
[4] School of Medicine, University of New Mexico

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