TriService Nursing Research Program Final Report Cover Page

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Signatures

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The views expressed are those of the author and do not reflect the official policy of the Department of the Army, the Department of Defense or the U.S. Government.
The investigators have adhered to the policies for protection of human subjects as prescribed in 45 CFR 46.

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Abstract

Purpose: Warfighters are at risk for musculoskeletal injuries and metabolic disorders which negatively impact physical performance and military readiness; vitamin D adequacy may mitigate these problems. The purpose of this study was to assess baseline serum 25(OH)D, treat levels below 30 ng/mL with supplementation, and correlate 25(OH) D with self-reported symptoms and body composition to develop a phenotype for low vitamin D status.

Design: Prospective longitudinal clinical trial with 3 groups

Methods: Subjects were assigned to the Treatment Group (TG) if 25(OH)D levels were below 30 ng/mL, and randomized to D3 1000 IU or 5000 IU supplement daily for 3 months. In Comparison Group 25(OH)D levels were at or above 30 ng/mL and no supplementation given. Diet, sun exposure, physical activity, symptoms, biomarkers, and body composition were assessed; primary outcome was 25(OH)D at 3 mos (T2) and 15 mos (T3). Targeted gene expression analysis was performed to evaluate treatment response.

Sample: 130 AD SMs comprised the predominantly male (62%) cohort, average age 32 yrs, 59% married, and 68% in enlisted ranks. Groups were similarly diverse in race, gender, and ethnicity. Deficiency status was highest in Caucasians (56%), followed by Asians/Hispanics (26%), then Blacks (18%).

Analysis: Descriptive statistics and RM-ANOVA procedures were conducted using R v3.4. Statistical significance set at $p < .05$.

Findings: Serum 25(OH)D significantly improved for both arms of the TG; greatest increase for D3 5000 IU after 3 months. Symptom scores significantly improved with higher 25(OH)D at T2 and declined at T3. No significant differences observed in gene expression in response to supplementation.

Implications for Military Nursing: Lacking gene-based or military-specific guidance for optimal vitamin D levels, clinicians must address deficiency by prescribing supplements and recommending fortified foods and judicious sun exposure, to minimize adverse health conditions and improve readiness and performance status.

TSNRP Research Priorities that Study or Project Addresses

Primary Priority

Force Health Protection:	☒ Fit and ready force ☐ Deploy with and care for the warrior ☐ Care for all entrusted to our care
Nursing Competencies and Practice:	☐ Patient outcomes ☐ Quality and safety ☐ Translate research into practice/evidence-based practice ☐ Clinical excellence ☐ Knowledge management ☐ Education and training
Leadership, Ethics, and Mentoring:	☐ Health policy ☐ Recruitment and retention ☐ Preparing tomorrow's leaders ☐ Care of the caregiver
Other:	☐

Secondary Priority

Force Health Protection:	☐ Fit and ready force ☐ Deploy with and care for the warrior ☐ Care for all entrusted to our care
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Other:	☐

Progress towards Achievement of Specific Aims of the Study

Findings related to each specific aim, research questions, and hypotheses.

There is ongoing controversy regarding optimal serum levels of vitamin D, in fact, published, evidence-based recommendations can vary by age group and lifestyle. This research was developed with a goal of informing military health policy directed at both garrison and deployment health related to assessment and monitoring of vitamin D status to ensure the highest levels of readiness and resilience. The research team set out to address the phenotypic and genotypic expression of vitamin D deficiency in a cohort of SMs who represent the vast number of military men and women who are prone to musculoskeletal injury and related symptoms, such as muscle weakness and fatigue. Also of interest was cardiovascular risk which was assessed by blood pressure readings, body composition, and physical activity levels. Our team, experienced with high priority issues of Warfighter health, conducted the following measurements (Table 1) to address study aims and research questions which are described in detail below.

Table 1. Summary of Measures and Data Collection Timepoints

	Time 1 Baseline	Time 2 3 months	Time 3 15 months
Demographic Questionnaire Part I and II	X	-	-
Demographic Questionnaire Part II only		X	X
Nutrition Assessment/ Body Composition/ BP	X	X	X
General Health Symptom Surveys (PROMIS)	X	X	X
DEXA	X		X*
Biomarkers (bone, immune, endocrine status)	X	X	X**
Gene Expression Analyses	X	X	-
Vitamin D Supplementation for 25(OH)D < 30 ng/mL	3-month intervention following baseline measures		-

PROMIS Patient-Reported Outcomes Measurement System; DEXA Dual Energy Xray Absorptiometry; 25(OH)D 25-Hydroxyvitamin D; ng nanograms; mL milliliters
*50% of study participants only
**serum 25(OH)D only

Specific Aim 1: Explore the phenotypic expression of vitamin D status in a cohort of Service Members (SMs) to determine if there is a risk profile of symptoms, biomarkers, or behaviors associated with deficiency states.

RQ1: Are there symptoms, biomarkers, or biobehavioral phenomena characterizing low vitamin D status that can be captured with existing instruments and methodologies?

This prospective, longitudinal trial initially consented 152 active duty service members with 130 returning for group assignment. Immediately following enrollment, all subjects had baseline 25(OH)D levels drawn. Subjects were assigned to the Treatment Group (TG) if they had 25(OH)D levels less than 30 ng/mL. A pharmacist then conducted a within–group randomization to assign subjects to an oral vitamin D3 low dose (1000 IU) or high dose (5000 IU) supplement daily for 3 months (mos). The Comparison Group (CG) had levels at or above 30 ng/mL and did not receive any supplementation. The Pharmacist also labeled and dispensed the supplement purchased online from Nature Made®, a reputable

manufacturer carrying the United States Pharmacopeia Verified Mark. This indicates that the supplements contain the amount of active ingredient stated on the label, they don't contain contaminants, and they will dissolve in time to be absorbed. Research staff and subjects were blinded to supplement dose until completion of the 3-month study supplement intervention. This time interval matches the standard-of-care vitamin D repletion period of 90 days, although doses vary due to provider preference. The 12-month period of follow-up post-supplementation was representative of a deployment period when a SM may or may not interface regularly with Brigade or Battalion medical resources, especially for mild symptoms. The plan was to document the trajectory of serum 25(OH)D over this period of time, as well as any symptoms. Dietary intake, sun exposure, physical activity, symptoms, blood and bone biomarkers, blood pressure, and body composition measures were assessed at Baseline (T1), 3 mos (T2) and 15 mos (T3); for blood biomarkers, only 25(OH)D was measured at T3. DEXA bone mineral density was assessed in all subjects at Baseline and in a randomized subset of the study population at T3.

Demographics (Table 2) revealed a cohort (N=130) of mostly males (62%), an average age of 32 years, of whom 59% were married, and 68% were in enlisted ranks. Groups were similarly diverse in race, gender, and ethnicity. A 38% participation by females was notable, as was the relatively high rate of participation by Asians (n=25, 19%). Deficiency status was highest in Caucasians (56%), followed by Asians/Hispanics (26%), then Blacks (18%). Results for Baseline measures for the cohort according to vitamin D status upon enrollment (sufficient or deficient) are listed in Table 3.

Table 2. Demographic variables by group assignment

	Control Group (n=43)		**Treatment Group (n=87)**	
Variable	**n**	**%***	**n**	**%**
Sex				
Male	25	58	56	64
Black	4	9.3	10	11.5
White	17	39.5	30	34.5
Other**	4	9.3	16	18.4
Female	18	41.8	31	36
Black	2	4.6	4	4.6
White	16	37.2	13	14.9
Other**	0	0	14	16.1
Officer Rank	18	41.9	19	21.8
Age (y), Mean(sd)	32.8 (10.5)		31.6 (8.2)	
Married	18	41.8	42	48.3
Medical History				
High blood pressure	4	9	4	4.6
Broken bones	16	37	20	23
Family history bone disease	7	16.3	9	10.3
Overuse injury/Stress fracture	7	16.3	16	18.4
Taking vitamins	7	16.3	9	10.3
Tobacco use = Never	22	51	43	49.4
Alcohol use = 0-1 times/mo	13	30	39	44.8
Exercise				
Moderate (150-300 mins/wk)	33	77	64	73.5
Vigorous (75-150 mins/wk)	29	67	59	67.8

Muscle strengthening (2d/wk)	34	79	61	70
Sun Exposure				
Days in sun/week	4.1(1.6)		3.8 (2.0)	
Minutes per week in sun	86 (87.5)		80.8 (84.6)	
Sunscreen use = rarely/never	15	35%	36	41.4%
Skin type				
Fair	5	11.6	7	8
Moderate	29	67.4	53	61
Dark	9	21	25	29

* Equals % of assigned group

** Includes American Indian, Asian, Native Hawaiian, Hispanic

Table 3. Baseline biomarkers, body composition, and dietary intake by vitamin D status

	Sufficient n=43	**Deficient n=86**	**Reference values**
Variable	**Mean (SD)**	**Mean (SD)**	
Calcium	9.2 (0.35)	9.3 (0.32)	8.5 – 10.2 mg/dL
25(OH)D	37.8 (5.6)	22.6 (4.9)*****	Sufficiency ≥ 30 ng/mL *Endocrine Society, 2011*
IGF-1	187.2 (52)	189.2 (53)	Age 26-30 (M) 98-282 ng/mL; (F) 78-270 ng/mL, Age 31-35 (M) 88-246 ng/mL; (F) 73-243 ng/mL
IL-6	1.4 (1.9)	1.8 (6.2)	0-15.5 pg/mL
Osteocalcin	M 29.2 (30.8) F 21.1 (10.1)	M 19.9 (8.2) F 17.9 (8.7)	(M) 3.2-39.6 ng/mL (F) Premenopausal 4.9-30.9 ng/mL
PTH	39.5 (11.9)	41.8 (12.4)	15-65 pg/mL with normal calcium
SHBG	M 33.5 (15) F 117.7 (65.6)	M 35.9 (16.5) F 78.6 (44)*****	(M) 11-80 nmol/L (F) 15-155 nmol/L
Body Fat %	M 21.3 (6.2) F 28.5 (6.7)	M 21.7 (6.3) F 31.7 (7.8)	Age 21-27 (M) 22%; (F) 32% Age 28-39 (M) 24%; (F) 34%
BP Systolic Diastolic	116.9 (13.3) 66.2 (8.9)	116.3 (11.8) 67.3 (8.8)*	Systolic < 120 mmHg Diastolic < 80 mmHg *Arnett et al. 2019*
BMI	M 27.4 (3.8) F 24.8 (2.6)	M 27.6 (3.8) F 25.6 (3.4)	Per AR 600-9: (M) 27.2 Max; (F) 25.6 Max
Weight (lbs)	M 190.0 (33.3) F 149.2 (19.0)	M 189.0 (32.7) F 149.4 (20.8)	Per AR 600-9: Varies by height and age
Femoral neck BMD	1.1 (0.1)	1.1 (0.2)	Bone density is within 1 SD (±1) of the young adult mean (WHO, 2018)
Spine L1-L4 BMD	1.3 (0.1)	1.3 (0.2)	
Calcium intake, mg/d	894 (618)	1039 (583)	RDA 1000 mg daily
Vitamin D intake, IU/d	253.8 (193)	284 (185)	RDA 600 IU daily

25(OH)D serum 25-hydroxyvitamin D; IGF-1 Insulin–like Growth Factor-1; IL Interleukin-6; PTH Parathyroid Hormone; SHBG Sex Hormone Binding Globulin; BP Blood Pressure; BMI body mass index; BMD bone mineral density; mg milligrams; ng nanograms; IU international units; pg picograms; mL milliliters; nmol nanomole; d day; M male; F female; AR Army Regulation; RDA Recommended Dietary Allowance

* $p < .05$ between normal & deficient grp

The repeated measures statistical modeling technique was used to analyze all outcomes. Demographic and biomarker variables were used as independent variables in the repeated measures procedure. This technique showed the changes in three time points accounting for other independent variables. The SAS mixed procedure was used to run the repeated measure analysis. Statistical analyses included Analysis of Variance (ANOVA) procedures followed by a *post hoc* test examining which group(s) were different in light of a significant ANOVA test. Tukey's Studentized Range (Honestly Significant Difference/HSD) was used for the pairwise comparisons yielding the following results. This test is one of the most conservative comparison tests but adequately controls the Type I experiment-wise error rate.

Biomarker results: **Serum 25(OH)D** significantly improved for both Treatment Groups (TGs) at T2 (Table 4, Tables 5a-c); response was not significant by gender or race (data not shown). The TGs showed a statistically significant trend back towards Baseline by T3 (Table 4). Interestingly, the CG with 25(OH)D levels greater than 30 ng/mL at Baseline, decreased at each subsequent measurement point over the 15-month study, although less than either TG from T2 to T3.

In addition to the 25(OH)D biomarker, significant group differences (using ANOVA techniques) were identified between the CG and TG (low) at T2 for **SHBG** in males ($p = .02$), and for the CG and TG (high) in females ($p = .04$) (Tables 6a-d). The group x time interaction was not significant. Of note, synthetic estrogens in various forms of contraceptives reportedly increase SHBG values and 17 of 49 (35%) females reported use of oral contraceptives or implanted birth control measures at Baseline (Rickenlund et al. 2004).

The only other blood biomarker that changed significantly was **IL-6** in the TG where levels decreased from 1.88 (6.2) pg/mL at Baseline to 1.18 (1.3) pg/mL, $p = .04$ at T2. The normal range for IL-6 is 0 – 15.5 pg/mL. When elevated, this cytokine can be a signal of inflammation, infection, autoimmune disorders or cardiovascular disease. It is also important for bone maintenance, brain function, and body temperature regulation, all of which can reflect a healthy endocrine system.

Table 4. Serum 25(OH) D response by group assigned

Group	Time 1 (Baseline) n, M (sd)	Time 2 (3 mos) n, M (sd)	Time 3 (15 mos) n, M (sd)	p
CG	43, 37.8 (5.6)	42, 34.5 (9.8)	37, 32.2 (8.1)	<.0001
TG 1 (low)	45, 22.2 (5.0)	40, 30.8 (10.0)	32, 23.7 (8.4)	<.0001
TG 5 (high)	43, 22.9 (4.7)	39, 40.1 (7.3)	32, 25.3 (6.9)	<.0001

CG: Comparison group; TG: Treatment group, low = D3 1000 IU, high = D3 5000 IU

Table 5a. ANOVA for Vitamin D biomarker group x time

R-Square	Coeff Var		Root MSE	25(OH)D Mean	
0.428698	24.95956		7.5087	30.1	
Source	DF	Type III SS	Mean Square	F Value	p
timepoint	2	4764.99	2382.49	42.26	<.0001
group assigned	2	5140.87	2570.44	45.59	<.0001
timepoint*grp_assign	4	4579.41	1144.85	20.31	<.0001

Table 5b. Tukey's Studentized Range (HSD) for 25(OH)D by time

Alpha	0.05
Error Degrees of Freedom	344
Error Mean Square	56.382

Critical Value of Studentized Range	3.33

Time Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
Baseline – Time 2	-7.4995	-9.728	-5.271	***
Time 2 – Time 3	7.761	5.38	10.142	***

*** Comparisons significant at the p < .05 level

Table 5c. Tukey's Studentized Range (HSD) Test for 25(OH)D by group

Group Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
CG – TG (low)	9.429	7.143	11.717	***
CG – TG (high)	5.478	3.176	7.781	***
TG (low) – TG (high)	3.951	1.625	6.277	***

CG: Comparison group; TG: Treatment group, low = D3 1000 IU, high = D3 5000 IU
*** Comparisons significant at the p < .05 level

Table 6a. Sex Hormone Binding Globulin (SHBG) for Females, group x time

R-Square		Coeff Var	Root MSE	SHBG Mean	
0.077284		60.95529	56.77011	93.13	
Source	DF	Type III SS	Mean Square	F Value	p
timepoint	1	45.5740	45.57396	0.01	0.91
group assigned	2	22086.2444	11043.12219	3.43	**0.04**
timepoint*group_assi	2	2057.5733	1028.78668	0.32	0.73

Table 6b. Tukey's Studentized Range (HSD) Test for SHBG, Female by group

Alpha	0.05
Error Degrees of Freedom	91
Error Mean Square	3222.846
Critical Value of Studentized Range	3.37

Group Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
CG – TG (Low)	28.08	-5.28	61.44	
CG –TG (High)	34.44	1.08	67.80	***
TG (Low) – TG (High)	6.35	-28.00	40.71	

CG: Comparison group; TG: Treatment group, low = D3 1000 IU, high = D3 5000 IU
*** Comparisons significant at the p < .05 level

Table 6c. Sex Hormone Binding Globulin (SHBG), Male, group x time

R-Square		Coeff Var	Root MSE	SHBG Mean	
0.58922		44.75111	15.53008	34.70	
Source	DF	Type III SS	Mean Square	F Value	p

timepoint	1	178.7452	178.7452	0.74	0.39
group assigned	2	1996.3167	998.1584	4.14	**0.02**
timepoint*grp_assign	2	31.1140	15.5570	0.06	0.94

Table 6d. Tukey's Studentized Range (HSD) Test for SHBG, Male

Alpha	0.05
Error Degrees of Freedom	149
Error Mean Square	241.1833
Critical Value of Studentized Range	3.35

Group assigned comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
CG - TG (High)	7.136	-0.181	14.453	
CG – TG (Low)	8.235	1.019	15.450	***
TG (High) –TG (Low)	1.099	-6.080	8.278	

CG: Comparison group; TG: Treatment group, low = D3 1000 IU, high = D3 5000 IU
*** Comparisons significant at the $p < .05$ level

Blood pressure (BP), systolic and diastolic measurement, was chosen as a biomarker for cardiovascular health. Upon enrollment, four subjects in each group reported a history of high blood pressure; this was verified by the reports of antihypertensive medications in our enrollment data set. At Baseline, the group with deficient vitamin D status had a significantly higher mean diastolic BP compared with the sufficient vitamin D group (Table 3.), although not clinically significant as the reading was well below the 2019 American Heart Association/ American College of Cardiology (Arnett et al. 2019) criteria for hypertension (>120/80 mmHg). This trend continued throughout all measurement timepoints, but again, readings never reached the level of pre-hypertension or hypertension (data not shown). In 2016, 5% of AC Soldiers had hypertension (USAPHC, 2017) so we believe this remains an important parameter of health assessments.

Another measure of cardiovascular health was the time spent doing **physical activity (PA)** each week, categorized as moderate, vigorous, and strengthening exercise, compared with the Department of Health and Human Services (DHHS) Physical Activity Guidelines for Americans (Piercy et al. 2018). [Incidentally, the Office of the Army Surgeon General (OTSG) endorses these same guidelines.] For approximately 70% of CG and TG (low/high) participants, minutes reported for moderate (150-300 mins) and/or vigorous (75-150 mins) exercise each week, as well as strengthening exercises (2 or more days/week), at both study timepoints (Baseline, T3), met the DHHS/OTSG Physical Activity Guidelines. In 2015, broadly distributed service-level surveys found active duty service members met or exceeded Healthy People 2020 (HP2020) targets for physical activity; 78.5% of Army respondents reported moderate PA for at least 150 minutes/week or vigorous PA for at least 75 minutes exceeding the target of 47.9% (Meadows et al., 2018). In addition, Army-specific data from the 2016 Health of the Force Report reflects an acceptable score of 81/100 for meeting OTSG targets for activity goals and standards, even if this equates to only 54% of the Force (USAPHC, 2016). Some reasons given for not meeting PT goals were related to work responsibilities and 'profiles' limiting physical activity due to injuries. Military populations typically have less difficulty meeting these physical activity recommendations due to unit-based physical training, and our healthy cohort was no exception. However, ~30% of our subjects were not meeting established recommendations which may have been related to the fact that the Unit Commander did not mandate regular PT formations, or similar barriers as those mentioned in the DA surveys. Army Physical Fitness Test (APFT) run times could not be verified and therefore are not reported.

Body composition measures included weight, lean and fat mass/percent body fat, body mass index (BMI), and bone mineral density (BMD). In addition to the important information inherent in these measurements for health assessment, increases in the anthropometrics can be indicative of metabolic syndrome or other endocrine abnormalities. These indices are relevant to vitamin D metabolism as higher body fat percentage and higher BMI have been shown to be inversely correlated with vitamin D levels. Vitamin D is a fat soluble vitamin that remains sequestered in adipose tissue with unregulated release into the circulation. The exact mechanism of its storage and release has not been clearly elucidated from scientific investigations. Yet, at least one published report recommends weight-based dosing in order to achieve optimal 25(OH)D levels (Ekwaru et al. 2014). In a military sample, one is not as likely to deal with overweight or obese men and women yet this research team has devoted years of time to studying overweight service members. Sadly, the numbers continue to rise with 19.5% of the military force on JBLM meeting BMI obesity criteria (USPHC, 2017). Mean body weight, percent body fat, and BMI results for male and female subjects were within the military fitness standards in Army Regulation 600-9. There was no correlation between body weight/body fat and 25(OH)D levels for this sample. Of note, body fat measurements were also extracted from DEXA reports which include body tissue and bone compartment readings. The body fat results from DEXA were higher for both males and females and require further investigation for cross-validation with bioelectrical impedance analysis. We examined BMD as part of our overall health assessment, as well as for correlation with vitamin D levels. This young, healthy population had normal readings for bone density according to the World Health Organization standard using young adult mean BMD in both the sufficient and the deficient vitamin D group (Table 3.). This may be a reflection of the fact that the deficient group had a mean 25(OH)D of 22.6 (4.9) ng/mL which does not meet the level of true deficiency of 10 ng/mL according to the Endocrine Society (2004) and thus, BMD was likely sustained through diet, physical activity, and genetic influences. This does not diminish the role of circulating 25(OH)D in bone health and in prevention of disease. Given the high rate of musculoskeletal injuries with significant impact on readiness, health care utilization and cost, and quality of life, it remains critically important to educate young service members about modifiable ways to promote bone health, particularly before reaching peak bone mass at ~25-30 years old (Heaney et al. 2000). Exciting work done by Gaffney-Stomberg and her team has found that the vitamin D receptor and vitamin D binding protein single nucleotide polymorphisms were associated with 25(OH)D status and bone turnover, and those with the highest genetic risk score required the greatest vitamin D intake to improve 25(OH)D during initial military training (Gaffney-Stomberg et al. 2017). More research in needed to acquire a better understanding of the physiologic mechanisms underlying vitamin D metabolism which is crucial to determining supplementation specifics of how much, for how long, and for whom. Based on a recent excellent systematic review, the best evidence is available for positive effects of calcium intake and physical activity, especially during the late childhood and peripubertal years—a critical period for bone accretion. Good evidence is also available for a role of vitamin D and dairy consumption. However, more work is needed on physical activity dose response and the potential interaction between physical activity and diet quality. (Weaver et al. 2016)

Dietary intake of calcium and vitamin D was assessed using a valid and reliable tool, the Vitamin D and Calcium Intake and Frequency Questionnaire (Taylor et al. 2009). We administered this tool at the 3 study timepoints Baseline, T2, and T3; mean intake values at each timepoint are shown in Table 7a. We found statistically significant vitamin D intake for specific study intervals (Table 7b-c). Calcium intake, in general, was just below the RDA yet intake was statistically significantly higher in the TG (high) when compared with the CG (Table 8a-b). Of note is that at Baseline, vitamin D intake was 45.6% of the RDA of 600 IU/daily, and calcium was 99% of the RDA of 1000 mg/daily. The low vitamin D intake was most pronounced in the CG; ironically, all of the CG had serum 25(OH)D levels above 30 ng/mL upon enrollment. Dietary intake of vitamin D exceeded the RDA at 15 mos (T3) for all groups. While not studied closely yet, our data set indicates that the increase can be attributed to both dietary intake and supplement consumption. Outreach efforts with subjects assigned to the TGs included emails once a week for 11 weeks with content specific to the benefits of vitamin D, along with recommendations for food and

supplemental sources of the nutrient. By T3, 41% of all returning participants reported vitamin D or multivitamin supplement use following the intervention period, either on their own or by MD prescription. Intake levels may have reflected an increase in dietary consumption of vitamin D-containing foods alone. Most subjects reported high quantity and frequency of cheese consumption which helps explain the sufficient calcium levels in spite of the fact that many no longer drink milk by preference or due to gastrointestinal intolerance.

Table 7a. Average Vitamin D Intake (IU)/Day over Time

Timepoint	N	Mean	Std Dev
1	43	273.90	188.6
2	41	269.00	230.50
3*	35	787.80	915.5

* Includes supplements at T3; RDA = 600 IU/day

Table 7b. ANOVA for Dietary Vitamin D Intake, group x time

R-Square		Coeff Var	Root MSE	Dietary Vitamin D Mean	
0.089341		186.2919	780.2556	418.8350	
Source	DF	Type III SS	Mean Square	F Value	p
timepoint	2	19384690.08	9692345.04	15.92	**< .0001**
group assigned	2	916940.39	458470.19	0.75	0.47
timepoint*grp_assign	4	1102900.79	275725.20	0.45	0.78

Table 7c. Tukey's Studentized Range (HSD) Test for Dietary Vitamin D Intake, across time

Alpha	0.05
Error Degrees of Freedom	245
Error Mean Square	46262.2
Critical Value of Studentized Range	3.33

Timepoint comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
Time 3 - Baseline	504.12	261.43	746.81	***
Time 3 – Time 2	509.15	263.39	754.91	***

CG: Comparison group; TG: Treatment group, low = D3 1000 IU, high = D3 5000 IU
***Comparisons significant at p < .05.

Table 8a. ANOVA for Dietary Calcium Intake, group x time

R-Square		Coeff Var	Root MSE	Dietary Calcium Mean	
0.030862		59.83443	570.3462	953.2074	
Source	DF	Type III SS	Mean Square	F Value	p
timepoint	2	285461.269	142730.635	0.44	0.64

group assigned	2	3119257.969	1559628.984	4.79	**0.009**
timepoint*grp_assign	4	158611.934	39652.983	0.12	0.97

Table 8b. Tukey's Studentized Range (HSD) Test for Dietary Calcium Intake by group

Alpha	0.05
Error Degrees of Freedom	345
Error Mean Square	325294.8
Critical Value of Studentized Range	3.32888

Group Comparison	Difference between Means	Simultaneous 95% Confidence Limits		
TG (high) - CG	226.11	51.96	400.25	***
TG (high) – TG (low)	82.68	-93.99	259.34	
TG (low) - CG	143.43	-30.33	317.19	

CG: Comparison group; TG: Treatment group, high = D3 5000 IU, low = D3 1000 IU
***Comparisons significant at $p \leq .05$

Sun exposure in minutes per day, and days per week, was collected to address the potential influence of natural production of vitamin D on serum 25(OH)D levels. The CG and TG subjects reported similar amounts of sun exposure with ~4 days per week for a total of 80-86 minutes per day, and ~40% in both groups selected 'rarely or never' applied sunscreen (Table 2). While use of sunscreen is advocated for preventing sunburn, skin damage, or melanoma, an SPF of 30 is 97% effective in blocking UVB rays which are critical to stimulating cutaneous vitamin D production. With over 60% of the study population vitamin D insufficient or deficient, and similar results in several previous studies by this Team, it appears that environmental or lifestyle factors other than just sunscreen use impact vitamin D status. Sunlight exposure remains the most significant source of vitamin D and only 15 minutes a day, midday, with sufficient skin exposure can maintain vitamin D stores. However, time in the sun is very challenging for Soldiers who are fully covered in the Army Combat Uniform throughout the day whether their job is indoors or outdoors. It is also important for Unit Leaders to avoid heat casualties due to training activities in midday temperatures so Soldiers are often indoors or out of the sun at this time. The study population was part of the Joint Base Lewis-McChord community situated at latitude 47.1 degrees in the Pacific Northwest, which along with season and time of day, greatly influences the sun's zenith angle and thus ability to stimulate sufficient quantities of vitamin D in the skin (Holick, 2017). Subjects reported residing in the local community for an average of 14 months indicating all had experienced the full 4 seasons prior to study participation. Recruiting took place year-round with approximately 25% of study subjects enrolled in each of the 4 seasons; each subject participated for 15 months involving exposure to all seasons again. Using the Fitzpatrick Skin Type Category tool, subjects were asked about skin tone. The majority of subjects selected "Moderate" suggesting a low-to-moderate tendency for sunburn and gradual tanning with repeated sun exposure. This was unexpected since the greatest number of participants were Caucasians, followed by Asians and Hispanics. Positive correlations were found between amount of time in the sun each week and gender ($p=.0005$), working or exercising outdoors ($p < .0001$), days of moderate exercise ($p < .0001$), and minutes of moderate exercise ($p = .0003$). Negative correlations were identified for sun exposure and rank ($p = .004$). We found no relationship between baseline 25(OH)D level and sun exposure or season.

Patient Reported Outcomes Measurement Information System (PROMIS) surveys using online instruments captured symptoms (cognitive function, sleep-related impairment, fatigue, pain interference, physical function, global health, mental health, and physical health) relevant to this study population and

research aims. Our goal was to assess symptoms over time and to correlate responses with vitamin D status. The Assessment Center at Northwestern University compiled the responses from the instruments administered online at 3 timepoints and forwarded them to the research team for further analysis. The online manual states that "PROMIS…and many of the NIH Toolbox® measures use a T-score metric in which 50 is the mean of a relevant reference population and 10 is the standard deviation (SD) of that population. A higher PROMIS T-score represents more of the concept being measured." (US Dept HHS, 2019). Table 9 summarizes mean (standard deviation) scores for study domains by group assignment across time. The study population was comprised of healthy young men and women and scores generally reflect this with all domains reaching a score of 50 $\pm$ 10, except the composite score for global health.

Fatigue scores were lowest for the TG taking D3 5000 IU/day (Mean = 47.0 $\pm$ 8.1) compared with the TG taking 1000 IU/day (Mean 50.9 $\pm$ 7.5); scores significantly improved when serum 25(OH)D levels improved and worsened when 25(OH)D dropped to low baseline levels. In addition, results showed that race and gender were potentially important predictors of fatigue (Table 11c.). **Physical function** demonstrated a similar pattern with *minimally important differences* along the trajectory of 25(OH)D levels over time, but none were statistically significant. Statistically significant improvements were seen across *groups* for **cognition** ($p < .0001$), **sleep** ($p = .0008$), **fatigue** ($p = .002$), **global health** ($p < .0001$) as well as the subcomponents global **mental health** ($p < .0001$), and **physical health** ($p < .0025$), and **pain** ($p = .003$), and over *time* for **sleep** ($p = 0.02$). (See Tables 10-13, select symptoms only.)

Table 9. PROMIS Domains, Mean (sd) for assigned group across time

Domain	Baseline n=129			3-month Follow Up n=124			15-month Follow Up n=103		
	CG	TG-Low	TG - High	CG	TG-Low	TG-High	CG	TG-Low	TG-High
Cognitive function**	49.7 (7.8)	46.6 (6.6)	50.9 (9.4)	50.6 (7.7)	48.6 (6.7)	53.3 (8.8)	49.6 (8.8)	46.1 (7.7)	52.1 (8.9)
Sleep-related impairment**	51.5 (7)	53.0 (7)	48.3 (9.5)	49.3 (8.2)	49.5 (9.5)	45.2 (8.4)	48.9 (9.4)	52.6 (9.4)	48.6 (8.8)
Fatigue**	49.5 (8.2)	52.0 (7)	49.0 (8)	48.9 (7.8)	48.6 (7.6)	44.3 (8.5)	47.6 (9.2)	52.0 (8.5)	47.9 (8)
Pain interference**	46.9 (6.4)	48.4 (7.5)	47.4 (7.9)	45.6 (6.4)	49.1 (8.5)	46.3 (7.7)	46.8 (7.5)	51.5 (8.5)	47.7 (8.6)
Physical function	55.3 (5.6)	52.6 (6.6)	54.1 (6.5)	55.0 (5.7)	54.3 (6.4)	54.9 (5.4)	54.4 (6.7)	50.8 (7.8)	52.8 (6.3)
Global Health***	41.2 (5.0)	38.0 (5.9)	40.9 (6.2)	41.2 (4.8)	39.7 (6.1)	42.2 (5.7)	40.3 (5.8)	36.3 (8.0)	40.9 (6.1)
Global Health Mental**	55.6 (7.6)	50.4 (7.7)	55.5 (8.9)	55.0 (7.7)	52.8 (8.2)	57.7 (7.7)	54.1 (8.5)	49.5 (10.2)	56.1 (8.7)
Global Health Physical**	53.7 (6.4)	50.8 (6.7)	53.1 (7.7)	53.5 (6.2)	52.5 (7.2)	54.5 (7.2)	52.7 (7.7)	48.1 (8.1)	52.8 (8.2)

CG: Comparison group; TG: Treatment group, high = D3 5000 IU, low = D3 1000 IU

* Higher T score represents more of the concept being measured; e.g. higher score for cognitive domain means higher cognitive function; higher score for pain interference means higher level of pain

** Statistically significant change by group or time (see Tables 10-13)

***Global Health domain – used overall score, and separate mental and physical components

Table 10a. ANOVA for Sleep-related Impairment – PROMIS, group x time

R-Square		Coeff Var	Root MSE	Sleep - Mean	
0.06711		17.266	8.574	49.66	
Source	DF	Type III SS	Mean Square	F Value	p
timepoint	2	570.086	285.04	3.88	**0.02**
group assigned	2	1067.147	533.57	7.44	**0.0007**
timepoint*group_assi	4	182.562	45.64	0.62	0.65

Table 10b. Tukey's Studentized Range (HSD) Test for Sleep-related Impairment by group and time

Alpha	0.05
Error Degrees of Freedom	347
Error Mean Square	73.528
Critical Value of Studentized Range	3.33

Group Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
TG (Low) – TG (High)	4.299	1.654	6.944	***

CG: Comparison group; TG: Treatment group, high = D3 5000 IU, low = D3 1000 IU
*** Comparisons significant at the $p < .05$ level

Time Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
Baseline – Time 2	2.937	0.399	5.475	***

*** Comparisons significant at the $p < .05$ level

Table 11a. ANOVA for Fatigue – PROMIS, group x time

R-Square		Coeff Var	Root MSE	Fatigue - Mean	
0.069301		16.552	8.0872	48.86	
Source	DF	Type III SS	Mean Square	F Value	p
timepoint	2	542.167	271.083	4.14	**0.02**
group assigned	2	836.564	418.28	6.40	**0.0019**
timepoint*group_assi	4	341.818	85.45	1.31	0.26

Table 11b. Tukey's Studentized Range (HSD) Test for Fatigue by group and time

Alpha	0.05
Error Degrees of Freedom	347
Error Mean Square	65.403
Critical Value of Studentized Range	3.3288

Group Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	

TG (Low) – TG (High)	3.738	1.244	6.233	***

Time Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
Baseline – Time 2	2.865	0.471	5.259	***

CG: Comparison group; TG: Treatment group, high = D3 5000 IU, low = D3 1000 IU
*** Comparisons significant at the p < .05 level

Table 11c. Repeated measures - Predictors of Fatigue

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	p
Group assigned	2	97	7.71	**0.0008**
Timepoint	2	198	3.82	**0.0237**
Sex	1	97	22.01	**<.0001**
Race	2	97	5.09	**0.0079**
Grp_assign*Time	4	198	1.47	0.2133

Table 12a. ANOVA for Pain Interference – PROMIS, group x time

R-Square		Coeff Var	Root MSE	Pain - Mean	
0.0424		16.007	7.631	47.67	
Source	DF	Type III SS	Mean Square	F Value	p
timepoint	2	156.31	78.15	1.34	0.26
group assigned	2	683.84	341.92	5.87	**0.003**
timepoint*group_assi	4	125.27	31.32	0.54	0.71

Table 12b. Tukey's Studentized Range (HSD) Test for Pain Interference by group

Alpha	0.05
Error Degrees of Freedom	347
Error Mean Square	65.3198
Critical Value of Studentized Range	3.3288

Group Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
CG – TG (Low)	-3.075	-5.39	-0.760	***
TG (Low) – TG (High)	2.384	0.03	4.74	***

CG: Comparison group; TG: Treatment group, high = D3 5000 IU, low = D3 1000 IU
*** Comparisons significant at the p < .05 level

Table 13a. ANOVA for Global Health – PROMIS, group x time

R-Square		Coeff Var	Root MSE	Global Health - Mean	
0.07487		14.8745	5.9708	40.141	
Source	DF	Type III SS	Mean Square	F Value	p
timepoint	2	190.72	95.36	2.67	0.07
group assigned	2	761.96	380.98	10.7	**<.0001**
timepoint*group_assi	4	84.67	21.17	0.59	0.67

Table 13b. Tukey's Studentized Range (HSD) Test for Global Health

Alpha	0.05
Error Degrees of Freedom	345
Error Mean Square	35.650
Critical Value of Studentized Range	3.33

Group Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
CG – TG (Low)	2.831	1.012	4.650	***
TG (Low) – TG (High)	-3.244	-5.094	-1.395	***

CG: Comparison group; TG: Treatment group, high = D3 5000 IU, low = D3 1000 IU
*** Comparisons significant at the $p < .05$ level

Specific Aim 2: Examine the effect of vitamin D levels on broad gene expression from carefully chosen candidate genes known to influence vitamin D metabolism, bone density, and immune function.

RQ 1. Can we identify genes predictive of the biologic processes expressed in vitamin D metabolism, bone density, and immune function collectively?

H_0: There will be no difference in gene expression between the comparison group receiving no supplementation and the treatment group receiving either D3 low dose (1000 IU) or high dose (5000 IU) from Baseline to T2 (3 months).

H_A: Gene expression analysis will demonstrate a more robust biologic response over time to D3 high dose (5000 IU) supplementation compared to low dose (1000) supplementation and no supplementation.

The Research Team had no choice but to accept the null hypothesis.

Even though the laboratory expertise, the sample collection and preparation, and the sequencing techniques were exceptionally well-planned and executed, results from the **gene expression** analyses were extremely disappointing. We meticulously prepared the targeted gene panel (McCarthy N15-009 grant proposal) to focus efforts on vitamin D metabolism and related biological functions, such as apoptosis and immune response, mineralization and bone development, response to stress and DNA repair, signal transduction and signaling for innate and adaptive immune response. Research Scientist, Dr.

Stanley Langevin, with expertise in genomics, was contracted to conduct the digital mRNA-sequencing with 80 paired samples, before and after vitamin D supplementation. The work was completed at the Research Acceleration and Innovation Network (RAIN) Laboratory, in partnership with the University of Washington –Tacoma. Samples were collected at Baseline and at 3 months (T2) following the 90-day treatment period. Initial sample preparation was done per protocol at Madigan Army Medical Center in the Department of Clinical Investigation Laboratory by Research Physiologist Laurie Gillette. Pellets were then transferred on dry ice by POV to the RAIN Lab at UW Tacoma. The 12 housekeeping genes described in the Hossein-nezhad et al. (2013) paper were found to be stable in our vitamin D samples and therefore, these genes were used to normalize the study data (Personal communication S.L. May 16, 2018). To make absolutely certain that the samples were of sufficient quality control, the samples were analyzed for differences between male and female responders to vitamin D supplementation. Twenty three (23) differentially expressed (DE) genes were identified; these were sex-linked genes providing confidence in both sample and data quality. However, again, there was no signal for a statistically significant difference between treatment responders and non-responders or low dose treatment compared to high dose treatment. The current study was largely informed by the Hossein-nezhad et al. (2013) paper in hopes that a greater number of subjects and a modified supplementation regimen would yield even more relevant and valuable results.

The steps in the process of total RNA extraction and mRNA sequencing sample preparation included:

a. Total RNA from each peripheral blood mononuclear cell (PBMC) pellet was isolated using RNAzol in combination with ZYMO Direct-zol MagBead kit; high quality RNA was obtained ranging from 50 ng – 200 ng with the average input of total RNA per reaction = 50 ng,

b. mRNA transcripts were selected by polyT priming and digital expression profiles were generated using the Lexogen QuantSeq mRNA-Seq library kit,

c. All samples from the same subject were paired together from RNA extraction to sequencing,

d. 10X multiplexed per HiSeq 2500 lane,

e. Assessed sequence read quality and trimmed low quality/adaptor sequences,

f. Aligned high quality sequence reads to human genome [all sequences aligned to 3' end of polyadenylated RNA transcripts – STAR aligner], and

g. Compared read counts between samples/treatment groups to identify genes that were differentially expressed.

The analysis averaged 10-25 million raw 50 base pair reads per sample; most samples had <10% reads removed due to low quality. Reads mapped to hg38 reference human genome. Differential expression analysis identified 538 genes that significantly changed. One downregulated gene, *EPB41L4A*, known to be involved in beta-catenin signaling and expressed in over 25 tissues including gallbladder, thyroid, kidney, and liver was identified. One paper describes its potential role in colorectal cancer. It is unclear of the significance of the gene to this experiment although we plan to follow up on the possible colorectal cancer link to see if additional research has examined a preventative role for vitamin D. ***However, comparing high dose to controls, low dose to controls, and high and low dose, revealed no significant differences between study groups.***

Table 14. Top abundant differentially expressed genes comparing before and after Vitamin D treatment

lymphotoxin beta	LTB	6958.842815	0.294641563	0.0044338
ribosomal protein lateral stalk subunit P2	RPLP2	5986.080909	0.284179004	0.0009997
ZFP36 ring finger protein	ZFP36	5560.494221	-0.360327384	0.0036167
Kruppel like factor 6	KLF6	4536.869623	-0.204230229	0.001058
integrin subunit beta 2	ITGB2	3151.896751	0.415110069	0.0007868
RNA, U4atac small nuclear	RNU4ATAC	3014.486567	-0.481892339	0.0004331
eukaryotic translation elongation factor 2	EEF2	2262.409555	0.302251941	0.0006326
BCL2 associated X, apoptosis regulator	BAX	2112.467658	-0.356279681	0.0007264
ribosomal protein L37	RPL37	2025.4302	0.292014071	0.0006055
bromodomain containing 2	BRD2	1937.396025	0.354094903	2.41E-05
DEAD-box helicase 5	DDX5	1713.765948	-0.218328018	0.0025511
actin gamma 1	ACTG1	1633.190686	0.326629139	0.0009438
ribosomal protein L11	RPL11	1614.236809	0.317498111	0.000986
ubiquitin conjugating enzyme E2 D3	UBE2D3	1585.530093	0.189767533	0.0006515
solute carrier family 25 member 6	SLC25A6	1539.766994	0.309115078	0.0013949
ZFP36 ring finger protein like 2	ZFP36L2	1225.731138	0.311146314	0.0007381
thymosin beta 10	TMSB10	1145.402815	0.253160543	0.003578
talin 1	TLN1	1143.917607	-0.191046725	0.0013428
glutamate ionotropic receptor NMDA	GRINA	1012.933776	-0.363508936	0.0008494
DNA J heat shock protein family (Hsp40)	DNAJC7	1001.561303	0.314279093	0.0007312

Dr. Zach Colburn, a Bioinformaticist on staff at Madigan Army Medical Center, volunteered to repeat the analysis using the data output file from RAIN – UW Tacoma Lab to confirm the results. He formally tested effects of vitamin D supplementation on genome-wide gene expression considering significantly DE probes at an FDR < 5% for any fold-change in an unadjusted model and did not observe any significant differences in gene expression between groups at Baseline or at T2, 3 months after treatment. Unfortunately, none of the 538 DE genes identified in either analysis matched any of the selected panel of genes nor did they react to different doses of vitamin D supplementation.

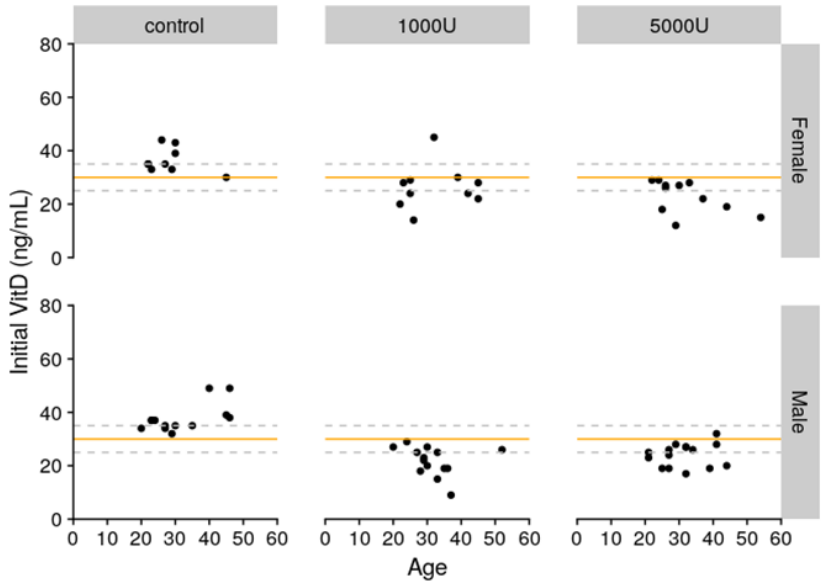

Fig. 1. Gene expression samples at baseline by age, gender, and vitamin D status

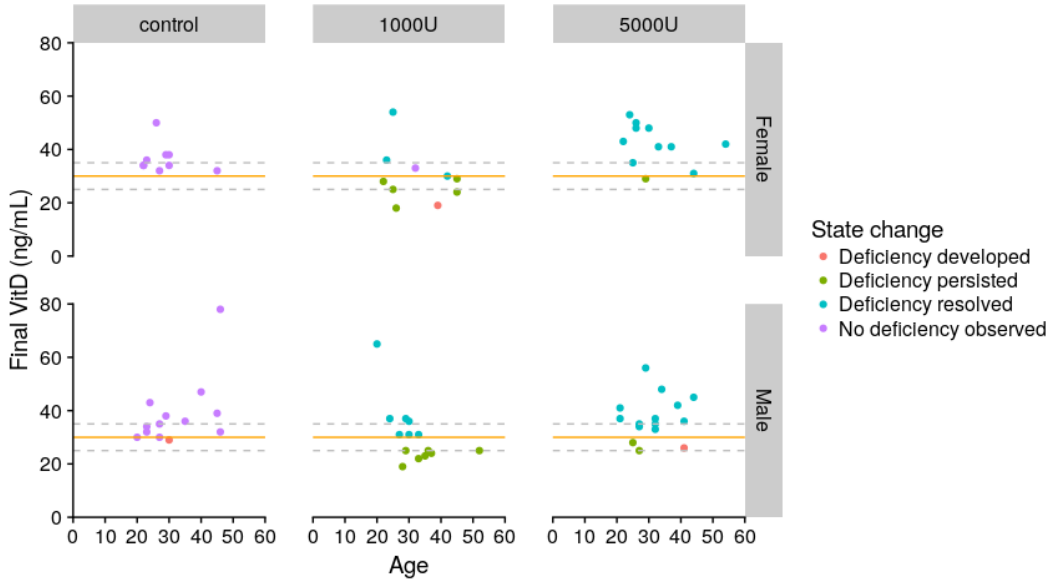

Fig. 2. Gene expression samples by age, gender, and vitamin D status at 3 months (T2)

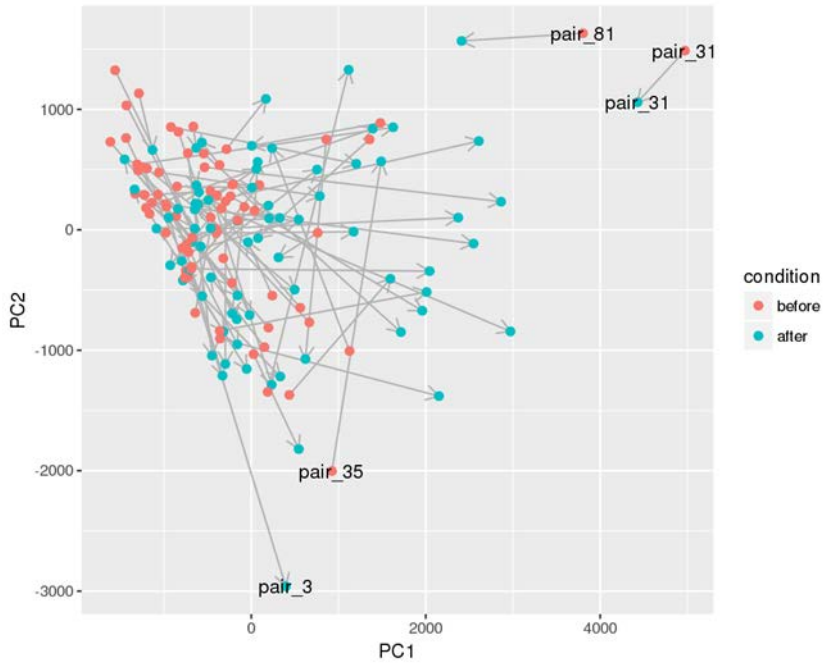

Figure 3. Transcriptome profiles comparing before and after Vitamin D treatment

Specific Aim 3: Evaluate changes in gene expression levels between and within groups supplemented with low vs high vitamin D, and compare to healthy controls.

RQ3: In deficient individuals, how will supplementation with vitamin D alter gene expression, and how will low versus high dose supplementation affect this expression?

H0: There will be no difference in upregulation or downregulation of target genes for healthy controls and subjects receiving high dose vitamin D supplementation.

HA: High dose vitamin D supplementation will impact gene regulation, for targeted genes, to a greater extent than low dose vitamin D supplementation.

Again, the Research Team had no choice but to accept the null hypothesis.

In-depth analytic techniques were used by 2 experts and essentially no meaningful results were obtained for this exploratory look at gene expression. The analysis conducted by Dr. Zach Colburn did lead to observations that may be worth further investigation. In his expert opinion, we may not be able to say that the genes were up/down-regulated, but it would be accurate to say that they were *dysregulated* between treatments. There are a number of transcription regulation-related genes with interactions annotated in the Reactome Project. The majority of interactions involved the two genes, CREBBP or RBL2. The CREBBP gene is now known to play critical roles in embryonic development, growth control, and homeostasis by coupling chromatin remodeling to transcription factor recognition. RBL2 gene is highly expressed in numerous tissues including lymph nodes, testis, and others; the family of retinoblastoma protein family are key factors in cell-cycle regulation and stand at the crossroads of multiple pathways dictating cell fate decisions. The relevance to this study on vitamin D metabolism is not yet clear. Table 15 details 11 DE genes that were identified in 2 separate analyses, although not responsive to vitamin D supplementation.

Low vs. High Vitamin D Supplementation

The analysis team reported limited confidence in the list of DE genes that differentiated individuals with low and high vitamin D levels. Only one gene had an adjusted p value less than 0.05. Another 10 genes had an adjusted p value of just under 0.6. However, some of these were differentially expressed using the Wilcoxon rank sum test. Although they were not statistically significant, the literature supports the idea that at least some of them may be differentially expressed. Of these 11 genes total, 3 stood out and were graphed: APTX, BLVRB, and JDP2. The three genes exhibited limited discriminatory ability via principal components analysis and linear discriminant analysis. No interactions were found for these genes in the Reactome Project.

Persistence vs. Resolution of Vitamin D Deficiency

Three genes exhibited an adjusted p value of < 0.05, and 10 exhibited an adjusted p value of < 0.1. Of these 10 genes, 3 were also DE by the Wilcoxon rank sum test. After excluding one outlier gene, only ARHGEF9 and SCN3A remained. SCN3A is a sodium voltage-gated channel subunit. Interestingly, ARHGEF9, a Cdc42 guanine nucleotide exchange factor, interacts with GABRA2, which is a GABA (neurotransmitter) receptor subunit. SCN3A mutations have been associated with autism, as have ARHGEF9 mutations. GABRA2 downregulation/mutations are also associated with autism.

Table 15. Differentially expressed genes following vitamin D supplementation

Gene	Role in Vitamin D Metabolism	Reference
NOS3	Vitamin D is a direct transcriptional regulator of the NOS3 gene	Andrukhova, O., Slavic, S., Zeitz, U., et. al. (2014). Vitamin D is a regulator of endothelial nitric oxide synthase and arterial stiffness in mice. *Mol Endocrinol,* 28(1),53-64.
SNX12	SNX12 is the target of miRNA that is differentially expressed between individuals with low and high vitamin D.	Enquobahrie, D.A., Williams, M.A., Qiu, C., et. al. (2011). Global maternal early pregnancy peripheral blood mRNA and miRNA expression profiles according to plasma 25-hydroxyvitamin D concentrations. *J Matern Fetal Neonatal Med,* 24(8),1002-12.
PDPK1	PDPK1 is down regulated following vitamin D supplementation	Chiang, K.C., Yeh, C.N., Lin, K.J., et. al. (2014). Chemopreventive and chemotherapeutic effect of dietary supplementation of vitamin D on cholangiocarcinoma in a Chemical-Induced animal model. *Oncotarget, 5*(11):3849-61.
CREBBP	Known to play critical roles in embryonic development, growth control, and homeostasis by coupling chromatin remodeling to transcription factor recognition.	Castillo, A., Jimenez-Lara, A.M., Tolon, R.M., Aranda, A. (1999). Synergistic activation of the prolactin promoter by vitamin D receptor and GHF-1: role of the coactivators, CREB-binding protein and steroid hormone receptor coactivator-1 (SRC-1). *Mol Endocrinol, 13*(7),1141-54.
RBL2	RBL2 is up regulated in MCF-7 and MDA-MB-231 breast cancer cell lines	Fleet JC, DeSmet M, Johnson R, Li Y. (2012) Vitamin D and cancer: a review of molecular mechanisms. *Biochem J.,* 441(1),61-76.

	following vitamin D supplementation.	
USP48	USP48 regulates MDM2 protein levels via a mechanism that is independent of its de-ubiquitination functions. Moreover, MDM2 binds and inhibits vitamin D receptor.	Cetkovská K, Šustová H, Uldrijan S. (2017) Ubiquitin-specific peptidase 48 regulates Mdm2 protein levels independent of its deubiquitinase activity. *Sci Rep,*7, 43180. Heyne K, Heil TC, Bette B, Reichrath J, Roemer K. (2015). MDM2 binds and inhibits vitamin D receptor. *Cell Cycle,* 14(13),2003-10.
IWS1	The S. cerevisiae IWS1 homolog gene has been shown to be a target of the VDR transcription factor.	Appears to have a role in HIV but no clear connection to vitamin D.
NR1H4	NR1H4 and VDR are closely related. In rats, vitamin D treatment induces an up-regulation of NR1H4 protein (farnesoid receptor X).	Staudinger JL, Woody S, Sun M, Cui W. (2013). Nuclear-receptor-mediated regulation of drug- and bile-acid-transporter proteins in gut and liver. *Drug Metab Rev,*45(1),48-59. Chow EC, Maeng HJ, Liu S, et.al. (2009). 1alpha,25-Dihydroxyvitamin D(3) triggered vitamin D receptor and farnesoid X receptor-like effects in rat intestine and liver in vivo. *Biopharm Drug Dispos,* 30(8),457-75.
SIGLEC5	Siglec-5 associates with PSGL-1. Vitamin D treatment alters glycosylation of PSGL-1. PSGL1 mRNA is lower in patients with sufficient vitamin D.	Pepin M, Mezouar S, Pegon J, et. al. (2016). Soluble Siglec-5 associates to PSGL-1 and displays anti-inflammatory activity. *Sci Rep,*28(6),37953. Riek AE, Oh J, Sprague JE, et. al. (2012). Vitamin D suppression of endoplasmic reticulum stress promotes an antiatherogenic monocyte/macrophage phenotype in type 2 diabetic patients. *J Biol Chem,* 287(46), 38482-94.
CXorf40A	CXorf40A expression is affected by vitamin D	Feigert, CE. The effect of vitamin D2, vitamin D3 or vitamin D2 in mushroom powder supplements on broad gene expression in human white blood cells. Boston University Theses & Dissertations [5434]. https://open.bu.edu/handle/2144/15093

Specific Aim 4 (Exploratory): Examine the relationship between vitamin D deficiency and the clinically relevant outcomes of stress fracture and high blood pressure using genomic analysis before and after supplementation to a therapeutic plasma level of vitamin D.
RQ4: What, if any, relationship exists between low vitamin D and clinically relevant outcomes for the target population, stress fracture and high blood pressure?

H0: There will be no relationship between regulation of targeted genes and rates of stress fractures or high blood pressure before and after low dose or high dose vitamin D supplementation.

HA1: There will be a relationship between expression of targeted genes and rates of stress fractures before and after low dose or high dose vitamin D supplementation.

HA2: There will be a relationship between expression of targeted genes and rates of high blood pressure before and after low dose or high dose vitamin D supplementation.

While gene expression results could not be leveraged to better understand biologic processes in play with low vitamin D, we did document a higher rate of recent stress fractures in the deficient group at Baseline. The rate was positively correlated with amount of time spent on strength training which could be an important observation to share with the Holistic Health and Fitness Teams on JBLM. These Teams are comprised of a registered dietitian, physical therapist, occupational therapist, and a physician assistant who are embedded with FORSCOM units to enhance readiness and resilience by reinforcing healthy nutrition, physical activity, and sleep behaviors. The gene expression analyses did not yield any meaningful results which we could further analyze for a relationship between 25(OH)D and the number of subjects with high blood pressure. Both of these variables were of relatively low frequency in the data set which was unexpected but reflects a more favorable profile of health for this cohort. For further discussion of stress fractures and blood pressure see the earlier discussions related to Specific Aim 1.

Relationship of current findings to previous findings.

Over the years of studying health promotion, and bone health in particular, with military populations, our team has not seen a high rate of participation from females, or Asian Soldiers. In this study we enrolled 49 or 37.7% females, and 25 (19%) subjects who selected a primary race of Asian, 11 of whom were females. While still low in numbers, we were able to conduct analyses where gender and race were identified as potentially important predictors in model fitting.

Serum 25(OH)D level was the primary outcome and as expected, this level responded to a greater degree to D3 5000 IU supplementation for 3 months. The lower dose of D3 1000 IU was more in line with the National Academy of Medicine recommendation for 600 IU daily and the Endocrine Society recommendation for 1500 – 2000 IU/day yet results were less impressive, in spite of a significant increase in serum 25(OH)D levels. Serum 25(OH)D has long been established as a biomarker of exposure to vitamin D (from sun, food, and dietary supplements), however, the extent to which such levels serve as a biomarker of effect (i.e., health outcomes) has not been clearly established (IOM, 2010). Furthermore, while serum 25(OH)D levels increase in response to increased vitamin D intake, the relationship is non-linear for reasons that are not entirely clear (IOM, 2010). The increase varies, for example, by baseline serum levels and duration of supplementation. Increasing serum 25(OH)D to >20 ng/mL requires more vitamin D when attempting to increase levels from a baseline < 20 ng/mL. There is a steeper rise in serum 25(OH)D when the dose of vitamin D is < 1,000 IU/day; a lower, more flattened response is seen at higher daily doses. However, no dose was able to sustain levels deemed “sufficient” over the course of this study. This finding is not unique for this Research Team that has been studying vitamin D metabolism and treatment response for years. Is it simply that young active adults in the military constantly utilize stores of vitamin D to promote bone health and tissue/organ development and when lifestyle includes frequent physical demands, inadequate nutrition, stressful work, and poor sleep, the demand is greater than the available stores? The concept of attaining *full genetic potential for bone strength* means optimizing all modifiable environmental factors, such as nutrients and mechanical loading, to favor skeletal health (Heaney, 2000). There is much still to learn about the mechanisms for achieving optimal vitamin D levels and sustaining them; further research involving new genomic approaches may help address the enigma surrounding the role of vitamin D in health, and readiness.

Sex hormone binding globulin (SHBG) is relevant to clinical studies examining health conditions that are associated with the endocrine system, including overweight, obesity, Type 2 diabetes mellitus, metabolic syndrome, or thyroid disorders. Disordered nutrient metabolism, including that of vitamin D,

can be further evaluated using SHBG and expert consultation with an Endocrinologist. While its significance is not yet clear in this study, a paper by Valimaki et al. (20004) reported the first documented result of serum SHBG as an independent positive predictor of bone turnover rate, which also positively correlated with serum 25(OH)D levels. The Valimaki et al. study enrolled only males so more work is needed to evaluate the role of sex steroids in the SHBG response in females, when many are prescribed oral contraceptives like the 16 out of 49 females in this study. It is also unclear what the response means when taking a vitamin D supplement. In their study, serum SHBG levels were positively associated with serum 25(OH)D levels, even after adjusting for weight, which is known to suppress both SHBG and 25(OH)D concentrations (Valimaki et al., 2004). As in the Valimaki study, SHBG levels in our study were highest in the CG at Baseline which had the highest levels of 25(OH)D for both males and females; no level exceeded the normal range for SHBG. Weight/body fat for this cohort was well within the acceptable military standards so these factors were not examined separately. Our Research Team has followed SHBG levels for years in previous military populations and may not have sizable numbers of male and female participants that could be used to expand our analysis for a contribution to the literature on a topic lacking implications for clinical care.

Interleukin-6 was selected as a biomarker of immune health. Recent studies have reported an association between vitamin D deficiency and self-reported symptoms, including musculoskeletal pain and sleep disorders, manifested by high levels of IL-6, a pro-inflammatory cytokine (Azzizieh et al. 2017). Patient-Reported Outcomes Scores (PROMIS) for pain had no significant correlation with IL-6, but sleep scores did improve significantly at Time 2 when IL-6 decreased (Table 9). We captured missed work days to assess for a correlation with vitamin D status but subjects in all groups reported an average of only 1.4 (1.8) days of illness in the year before study enrollment, and 1.2 (1.7) days for the year of enrollment, with no significant correlation to vitamin D status. It is important to further explore correlations between vitamin D status, IL-6, missed work, and PROMIS symptoms (e.g. pain, sleep, physical function) as this constellation of signals may have a direct impact on Warfighter performance.

We methodically prepared our list of genes from published literature specifically related to vitamin D supplementation and developed a research plan loosely based on a vitamin D supplementation study by Hossein-nezhad et al. (2013) who had enrolled only 8 subjects and performed a final analysis on 5 subjects. Published results stated “Our data suggest that any improvement in vitamin D status will significantly affect expression of genes that have a wide variety of biologic functions of more than 160 pathways linked to cancer, autoimmune disorders, and cardiovascular disease that have been associated with vitamin D deficiency.” While their conclusions appear to exaggerate the impact of results from a final sample of only 5 subjects, their work remains essentially unchallenged. Unfortunately our results cannot verify or refute their results. A recent publication in the Journal of the American Medical Association/Cardiology (Barbarawi et al. 2019) reports that results from a very large, robust meta-analysis involving 83,000 individuals demonstrated vitamin D supplementation was not associated with reduced major adverse cardiovascular events, individual CVD end points (myocardial infarction, stroke, CVD mortality), or all-cause mortality. The authors concluded that vitamin D supplementation does not confer cardiovascular protection. Another recent publication reported that among people at high risk for type 2 diabetes, vitamin D3 supplementation of 4000 IU/day did not result in a significantly lower risk of diabetes than placebo (Pittas et al. 2019). This means further research is needed to elucidate the role of vitamin D in chronic disease prevention, using novel affordable and rapid next-generation sequencing technologies.

Effect of problems or obstacles on the results.

We designed the research as we have done many times with our past successful research; we envisioned the most rigorous methods possible but acknowledged a need for a pragmatic approach given the constraints of the sample population and time. For this reason, we opted for a prospective longitudinal clinical trial but chose not to randomize all subjects. We knew from previous work that ~60% of our population would be vitamin D insufficient or deficient and they would be strong candidates for

supplementation. However, this meant 40% would be at sufficient levels and lacking guidelines for safe upper levels of vitamin D and dosing limits, we felt these subjects should be a Comparison group, rather than a Control group which requires randomization. We felt that randomization to low dose, high dose, or placebo might do a disservice to those in the deficient group who should receive supplementation and not a placebo. Now that we have conducted 3 investigations involving vitamin D and no harm has occurred for any subject, I would feel more comfortable randomizing all subjects to all potential dosage groups to achieve a more rigorous study design.

The most difficult aspect of this study was maintaining ongoing communication with a Team that included several experts outside the institution who had busy schedules and limited time for team meetings. The Core Team (McCarthy, Elshaw, Szekely) met almost weekly and relayed important developments to other team members as necessary. The Geneticist Consultant was from a local hospital and he was invited to be a Consultant for his expertise and his "connections" with genomics laboratories capable of performing the gene expression work necessary for this proposal. His principal contact was a friend and fellow Geneticist who operated an enormous and reputable genomics lab in Beijing, China and who offered to run the samples at no charge. Needless to say, we were unable to engage with that company and we were left to find alternative labs with limited funds designated for the work. Many months of coordination followed by rejection of our requests for RNA sequencing repeatedly, ultimately resulted in the RAIN-University of Washington (UW) Lab accepting the project. Costs were negotiated that could be supported with the existing grant budget. We did retain the Geneticist in order to leverage his expertise in interpreting the gene expression analysis results. The analysis never led to any useful results so we did not really benefit from including the Geneticist Consultant. For future studies, my preference would be to establish deliverables with hourly contracts rather than offering salary support.

The feedback we received from the RAIN-UW Lab was valuable. Dr. Langevin, the Research Scientist managing the project, felt that digital mRNA-Seq was a cost effective way to perform global gene expression studies. He also felt the quality of the peripheral blood mononuclear cell samples was optimal for mRNA-Seq studies despite the fact that differentially expressed gene analyses revealed that treatment with vitamin D did not alter the global mRNA-transcript profiles of subjects tested. Future vitamin D gene expression studies should implement additional temporal sampling and sample collection at relevant time points for mRNA-Seq profiling which is early and often. We established a good working relationship with Dr. Langevin and he will assist with the sequencing work for the McCarthy Precision Nutrition (N18-B15) Study.

Retention is a challenge for research involving a military population particularly when the protocol is 15 months long. Our recruiting efforts stressed that participants must remain available for follow up for the 15 months but there is always a possibility that a change of duty station or discharge from the military is unexpected. The intervention period began with 23 fewer subjects due to failure to report for initial blood draw (21) or ineligibility per screening criteria (2). Initially we requested to enroll 132 SMs in our IRB protocol assuming we would experience ~20% attrition and meet our goal sample size. However, when we realized attrition may be as high as 30%, we requested to enroll an additional 30 subjects for a total of 152. This was approved and we did enroll 152 with a final N = 103 or 32% attrition which did not impact our ability to conduct the planned analyses for biomarkers, body composition, and reported symptoms.

Limitations.

This study was a single center trial in a geographic location known for low levels of sunlight for ~6 months of the year. The Research Team had experience with monitoring serum levels of 25(OH)D in previous studies and therefore anticipated the high rate (60%) of low vitamin D, but also recognized that this was subject to many uncontrollable factors. Soldiers are a highly mobile population and previous assignments and deployments, race, gender, age, and state of health are factors that should be accounted for in data analyses. We did our best to control for some of these variables. Other limitations included the

fact that we did not interrupt enrollment for seasonal considerations as done in other vitamin D studies, but we collected data on season of enrollment, which also reflects season of blood collection.

We were not able to insure that all participants in the TG took all of their D3 supplements as instructed but we sent text reminders throughout the intervention period and we asked the subjects to bring back their bottle of pills at the 3-month follow up appointment. Even though we instructed them not to, some participants may have made changes to their diet over the course of the study, or may have taken supplements containing vitamin D, which they did not report to us. Another issue with studying the highly dynamic nature of vitamin D metabolism is that results can change rapidly; for example, ten participants (24%) in the CG became deficient in 25(OH)D between the initial and second blood draw, which may have led to unknown effects on gene expression. A large number of variables impact sun exposure, including season, sunscreen use, and travel to sunny and/or southern destinations. It is possible that sun exposure was a factor in changes in serum vitamin D in some of our participants and we did not fully capture this exposure. (See discussion of sun exposure previously.)

The sample size was low compared to many of our previous studies with the AD military population. Our focus was on getting a sufficient number of subjects enrolled quickly so we could act on the genomics aims while waiting for the final follow-up at 12 months post-supplementation. As mentioned previously, the conceptual model for this study was loosely based on a highly cited research investigation involving vitamin D gene expression following supplementation in only 8 subjects with only 5 available for the final analysis (Hossein-nezhad et al. 2013). Therefore, we anticipated we would far exceed the number of subjects needed to conduct a similar gene expression analysis if we had n=30-35 in each group. While we did accomplish this, the sample size was more important for analyzing other variables as the gene expression analysis had negligible yield.

Conclusion.

The results thus far are congruent with current literature describing a continued high prevalence of vitamin D insufficiency and deficiency globally. A great deal of attention has been devoted to research examining non-skeletal benefits of vitamin D; recent publications have shed new light on its lack of benefit in preventing cardiovascular morbidity and mortality, as well as protecting at risk individuals from developing type 2 diabetes. However, there is much we have not resolved regarding vitamin D, such as optimal serum levels, weight-based dosing, genetic influences on bone or tissues stemming from the ubiquitous vitamin D receptors, and links between 25(OH)D levels and somatic symptoms as we documented in this study.

This mixed gender and race cohort was highly representative of the local military population, and the Army composition as well. The latest figures for JBLM show that over 78% of the population is under 35 years old, 13.8% are female, chronic disease diagnoses are lower than the Army average except for obesity at 19.5% (Army avg 17.5%), and physical activity rates reflect that over 83% meet the OTSG/DHHS recommendations (Health of the Force, 2017). No differences between study groups were identified for body composition, bone mineral density, calcium intake, physical activity, sun exposure, or sunscreen use. Reported incidence of overuse injuries/stress fractures was 2.3 times higher in the vitamin D deficient group compared to the sufficient group at Baseline (16/86 vs 7/43; p = NS) but there was no correlation between the two variables nor was there any change in the incidence of stress fractures between groups by the end of the study. There was a positive correlation between the incidence of stress fractures and history of broken bones (p = .0085) and amount of strength training (p =.048). This could imply that overuse injuries were occurring due to muscle fatigue from increased amounts of strength training.

Vitamin D insufficiency and deficiency is widespread in soldiers, regardless of season. Negative symptoms related to cognition, fatigue, sleep, pain, and global health, which encompassed mental and physical health, were reported more frequently in soldiers with low vitamin D levels. Our results indicate that symptoms improved and serum 25(OH)D responded better to D3 5000 IU daily. The low sample size may not provide sufficient justification for a vitamin D dose or frequency, but results can and should

inform Defense Health Agency policies related to pre- and post-deployment health assessments. Experts recommend advising adults to establish daily habits of consuming vitamin D-containing foods and beverages or taking a daily supplement of at least D3 1000 - 2000 IU, and incorporating sensible sun exposure. This advice will help ensure active duty military are proactive in their go-to-war preparations of medical readiness and mental resilience for performance optimization.

Significance of Study or Project Results to Military Nursing.

Our efforts to create a phenotype for the young military service member (SM) with low vitamin D were met with mixed success; based on this study, we can characterize this individual as a 30 year old enlisted male who is generally healthy and of normal weight, body fat, body mass index, and bone density, who leads an active lifestyle involving high levels of moderate and/or vigorous physical activity and strengthening exercises weekly, but is subject to suboptimal sun exposure and a vitamin D-deficient diet. When presenting to an advanced practice nurse or perhaps a clinical nurse specialist embedded with a FORSCOM unit, complaints may be vague and include fatigue, sleep-related impairment, pain interfering with usual activities, changes in cognitive function, or general mental or physical alterations such as mood disturbance or muscle weakness. These symptoms clearly do not distinguish a low vitamin D level from many other possible conditions but may warrant closer evaluation. The advanced practice nurse interacting with the active or reserve component SM prior to training exercises or deployment has a responsibility to ensure each SM is physically and mentally mission-ready. By intervening early in the pre-deployment health assessment period, supplementation of vitamin D or other potential nutritional deficiencies, such as iron or folate, can restore inadequate levels and in doing so, optimize warfighter readiness and performance. It is critical to allow sufficient time for monitoring and re-assessing as most published reports agree with our findings that levels decline unpredictably over the course of 3-12 months following supplementation. At a minimum, if repletion has been prescribed prior to a deployment then reassessment of vitamin D levels should be done upon redeployment. While supplements do not stimulate the healthy immune response resulting from natural sunlight exposure and have a variable dose-response across body types, they are the primary treatment available. There remains a lack of knowledge about the non-skeletal health benefits of vitamin D status, the optimal blood level for such benefits, and the most therapeutic dose to achieve these benefits so the emphasis for military SMs should be on maintaining skeletal integrity, preventing musculoskeletal injuries, and supporting strenuous physical activities.

It is apparent from our data pre- and post-supplementation that Warfighters are subject to the same risk factors, if not more, for low serum 25(OH)D levels as most adults living in the United States; some risks are modifiable and others are not. Advanced practice nurses, clinic nurses, and licensed practical nurses can all educate SMs on the benefits of a healthy diet, adequate nutrient stores, and an active lifestyle to build and maintain strong bones. Every encounter with a SM is an opportunity to stress the Performance Triad or Fit for Performance initiatives designed to guide behaviors that lead to operational readiness and personal fitness.

The findings from this study are not remarkable but they continue to address the gap in our clinical knowledge of vitamin D metabolism and our appreciation for the impact it can have on health and wellness. We attempted to leverage exciting new genomics technologies to help explain the impact of vitamin D on gene expression for genes having a role in mineralization and bone development, apoptosis and immune function, response to stress and DNA repair, and others, however, either the timing of sample collection or the candidate gene selection was flawed. While not necessarily avoidable, we should continue to develop a cadre of nurses with expertise in genomics who will be uniquely positioned to advance the military healthcare agenda by incorporating new sequencing techniques and accessing omics platforms that offer unlimited possibilities for explaining complex metabolic phenomena. Until precision health and all its accompanying technologies are user-friendly and at the point-of-care, many clinicians will shy away from adopting such unfamiliar diagnostic tools.

For now, clinicians must use their best judgment when correcting low vitamin D levels with supplements, and recommend fortified foods and judicious sun exposure, along with regular moderate to vigorous physical activity to maximize bone strength, minimize adverse health conditions, and optimize readiness and resilience.

Changes in Clinical Practice, Leadership, Management, Education, Policy, and/or Military Doctrine that Resulted from Study or Project

None to date.

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Summary of Dissemination

Type of Dissemination	***Citation***	***Date and Source of Approval for Public Release***
Publications	McCarthy, M.S., Elshaw, E.B., Szekely, B.M., Pflugeisen, B. (2017). Health promotion research in active duty army soldiers: The road to a fit and ready force. *Nurs Outlook, 65*(5S), S6-S16. http://dx.doi.org/10.1016/j.outlook.2017.06.009. McCarthy, M.S., Elshaw, E.B., Szekely, B.M., Raju, D. A prospective cohort study of vitamin D supplementation in AD Soldiers: Preliminary findings (Invited). *Mil Med*, 2019;184 (March/April Suppl):498-505. doi:10.1093/milmed/usy393.	*PAO approval dated:* *9/10/18* *5/10/18*
Published Abstracts	McCarthy, M.S., Elshaw, E., Szekely, B.M. Warfighter Vitamin D Supplementation, 25(OH)D Response, and Bone Health Status. Military Health System Research Symposium, Kissimmee, FL. August 2017. (Conference proceedings) McCarthy MS, Elshaw E, Szekely BM, and Raju D. Longitudinal Assessment of Warfighter Vitamin D Supplementation. Military Health System Research Symposium, Kissimmee, FL. August 23, 2018. (Conference proceedings)	*PAO approval dtd:* *8/28/17* *8/14/18*

Podium Presentations		*PAO approval dtd:*
	McCarthy, M.S. Using genomics to evaluate vitamin D deficiency and supplementation. MultiCare Institute of Research and Innovation, MultiCare Health System, Tacoma, WA. November 18, 2016.	*10/25/16*
	McCarthy M.S. Vitamin D Supplementation and Warfighter Nutritional Resilience. Triservice Nursing Research Program Research and EBP Dissemination Course. Ellicott City, MD. April 25, 2017.	*3/24/17*
	McCarthy, M.S. Warfighter Vitamin D Supplementation 25(OH)D response and bone health status. Military Health System Research Symposium. Kissimmee, FL. August 28, 2017.	*8/28/17*
	McCarthy, M.S. An RCT Examining Vitamin D Supplementation, 25(OH)D Response, and Bone Health Status. Western Institute of Nursing Conference. Spokane, WA. April 13, 2018.	*4/19/18*
	McCarthy, M.S. An RCT Examining Vitamin D Supplementation, 25(OH)D Response, and Bone Health Status. TriService Nursing Research Program Research and EBP Dissemination Course, San Antonio, TX. May 3, 2018.	*4/25/18*
	McCarthy, M.S. An RCT Examining Vitamin D Supplementation, 25(OH)D Response, and Bone Health Status. Madigan Research Day, Tacoma, WA. May 4, 2018.	*NA*
	McCarthy, M.S. Longitudinal Assessment of Warfighter Vitamin D Supplementation. Military Health System Research Symposium, Kissimmee, FL. August 23, 2018.	*8/14/18*
	McCarthy, M.S. A Nutrigenomics Approach to Vitamin D Treatment & Response. Council for the Advancement of Nursing Science, Washington, D.C. September 14, 2018	*8/14/18*

Poster Presentations		*PAO approval dtd 2/5/18*
	Szekely, B., McCarthy, M.S. A Randomized Controlled Trial to Examine Vitamin D Supplementation, 25(OH)D Response, and Bone Health Status. Seattle Nursing Research and EBP Conference. Lynnwood, WA. January 30, 2018.	
	McCarthy, M.S. An RCT Examining Vitamin D Supplementation, 25(OH)D Response, and Bone Health Status. TriService Nursing Research Program Research and EBP Dissemination Course, San Antonio, TX. May 2, 2018.	*4/18/18*

Media Reports	JBLM Northwest Guardian. June 9th online, June 10th print edition, 2016. Boosting your vitamin D safely in the summer sun. (Newspaper article.) JBLM Northwest Guardian. September 15, 2016 online. Precision medicine a key focus. (Newspaper article.)	*PAO sponsored media therefore no separate PAO approval required*

Reportable Outcomes

Reportable Outcome	Detailed Description
Applied for Patent	None
Issued a Patent	None
Developed a cell line	None
Developed a tissue or serum repository	None
Developed a data registry	None

Recruitment and Retention Table

1) Summary Table: Human Subjects Research with Control Group

Recruitment and Retention Aspect	Number of Subjects This Reporting Period		Total Number of Subjects Since Study or Project Began	
Number of Subjects Projected in Grant Application	103		152	
Subjects Available				
Subjects Contacted or Reached by Approved Recruitment Method	0		5692	
Subjects Screened	0		258	
Subjects Ineligible	0		83	
Subjects Refused	0		23	
Human Subjects Consented	0		152	
Subjects withdrew prior to lab draw, not ID'd as control or intervention group	0		19	
Subjects consented but later found to be ineligible	0		2	
Subjects Intervention Group / Control Group	0	0	88	43
Intervention Group / Control Group Subjects Who Withdrew	3	1	23	5
Intervention Group / Control Group Subjects Who Completed Study	9	6	65	38
Intervention Group / Control Group Subjects With Complete Data	7	6	61	35
Intervention Group / Control Group Subjects With Incomplete Data*	2	0	4	3

*Missing at least one data point.

Demographic Characteristics of the Sample

Characteristic	
Age (yrs)	32 ± 9.3
Women, n (%)	57 (37.5)
Race	
White, n (%)	94 (61.8)
Black, n (%)	26 (17.1)
Hispanic or Latino, n (%)	14 (9.2)
Native Hawaiian or other Pacific Islander, n (%)	2 (1.3)
Asian, n (%)	28 (18.4)
Other, n (%)	2 (1.3)
Military Service or Civilian	
Air Force, n (%)	4 (3)
Army, n (%)	148 (97)
Marine, n (%)	0 (0)
Navy, n (%)	0 (0)
Civilian, n (%)	0 (0)
Service Component	
Active Duty, n (%)	152 (100)
Reserve, n (%)	0 (0)
National Guard, n (%)	0 (0)
Retired Military, n (%)	0 (0)
Prior Military but not Retired, n (%)	0 (0)
Military Dependent, n (%)	0 (0)
Civilian, n (%)	0 (0)

Program Budget Summary Report

Company: The Geneva Foundation
User: Robinson, Kathleen

Period Start Date: 1/1/2015
Period End Date: 4/30/2019

Current Fringe Rate: 35.50%
Current G&A Rate: 10.00%

Contract: 10391 - Genomics of Vitamin D Supplementation and War
Award Amount: 535,346.00
Total Estimated: 535,346.00
Total Funded: 535,346.00

Contract PoP: 4/1/2015 - 3/31/2019
Customer: TRISERVICE NURSING RESEARCH PROGRAM
Customer Contract ID: HU0001-15-1-TS05
Contract Manager: Robinson, Kathleen

Category	Budget	Period	Cumulative	Commitments	Cumul. + Commit.	Remaining Balance
Direct Expenditures						
Personnel						
Personnel Salary & Wages	330,737.00	251,124.88	251,124.88	0.00	251,124.88	79,612.12
Fringe Benefits (Burden)	0.00	79,130.32	79,130.32	0.00	79,130.32	-79,130.32
Total Personnel	**330,737.00**	**330,255.20**	**330,255.20**	**0.00**	**330,255.20**	**481.80**
Non-Personnel						
Equipment	0.00	0.00	0.00	0.00	0.00	0.00
Travel	1,948.49	1,948.49	1,948.49	0.00	1,948.49	0.00
Supplies	779.96	779.96	779.96	0.00	779.96	0.00
Other	70,015.84	70,015.84	70,015.84	0.00	70,015.84	0.00
Consultant	13,375.00	13,375.00	13,375.00	0.00	13,375.00	0.00
Subcontractor	31,958.00	31,957.71	31,957.71	0.00	31,957.71	0.29
Total Non-Personnel	**118,077.29**	**118,077.00**	**118,077.00**	**0.00**	**118,077.00**	**0.29**
Total Direct Expenditures	**448,814.29**	**448,332.20**	**448,332.20**	**0.00**	**448,332.20**	**482.09**
Indirect Expenditures						
G&A Burden	86,620.39	86,195.17	86,195.17	0.00	86,195.17	425.22
Other Indirect Costs	-88.68	0.00	0.00	0.00	0.00	-88.68
Total Indirect Expenditures	**86,531.71**	**86,195.17**	**86,195.17**	**0.00**	**86,195.17**	**336.54**
Total Dir. + Indir. Expenditures	**535,346.00**	**534,527.37**	**534,527.37**	**0.00**	**534,527.37**	**818.63**
Fee Amount	0.00	0.00	0.00	0.00	0.00	0.00
Total Expenditures + Fee	**535,346.00**	**534,527.37**	**534,527.37**	**0.00**	**534,527.37**	**818.63**