

Vitamin D levels in Portuguese Navy military personnel: a cross-sectional study

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Summary

INTRODUCTION

OBJECTIVES

METHODS

RESULTS

DISCUSSION

CONCLUSIONS

Which is the main source of vitamin D?

- a) Food.
- b) Dietary supplements.
- c) Sun exposure.
- d) Medicines.



Which is the main source of vitamin D?

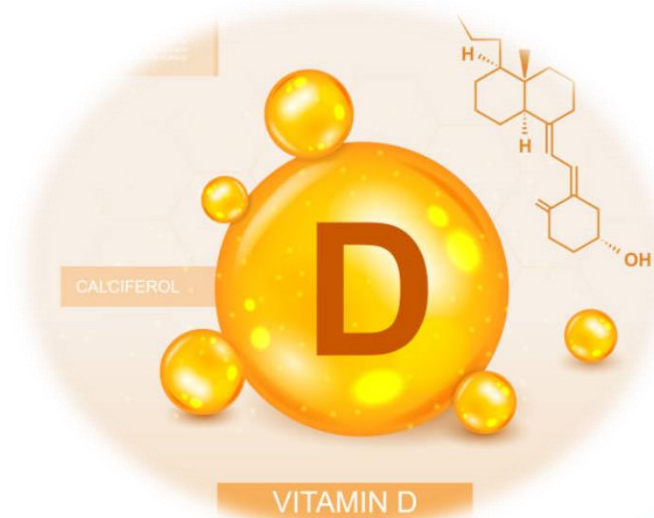
- a) Food.
- b) Dietary supplements.
- c) Sun exposure: 7-dehydrocholesterol is endogenously converted to vitamin D3 in the skin exposed to UV-B (290-315nm) (non-enzymatic synthesis).**
- d) Medicines.

Vitamin D levels in Portuguese Navy military personnel: a cross-sectional study

Introduction

Active-duty service members face incredibly demanding physical and psychological stressors.

Vitamin D's role in maintaining skeletal and extra-skeletal health, including mental health, is still a common research topic.¹⁻³



The (American) Endocrine Society recently published a new clinical practice guideline on vitamin D for disease prevention and no longer endorses the target 25-hydroxy-vitamin D (25(OH)D) level of 30 ng/mL nor does it endorse a specific 25(OH)D level to define vitamin D sufficiency, insufficiency and deficiency.⁴

Portuguese health authorities and laboratories still distinguish 25(OH)D levels as follows:

- deficiency (<20 ng/mL),
- insufficiency (≥ 20 ng/mL and <30 ng/mL), and
- sufficiency (≥ 30 ng/mL).⁵

Vitamin D deficiency prevalence in Portugal is high, and blood sample collection during winter is associated with a higher risk of deficiency.⁶

Working indoors is also a well-known risk factor for vitamin D deficiency.⁷

A previous study with Portuguese active-duty military personnel (n=1744) revealed a vitamin D deficiency prevalence of 34.6% (37.1% in 555 Navy military personnel).⁸

A recent systematic review on vitamin D status in active-duty Navy military personnel reported a high prevalence of vitamin D deficiency in the Navy and proposed further research on this topic, as their occupational settings can be particularly detrimental to endogenous vitamin D synthesis.⁹

Vitamin D levels in Portuguese Navy military personnel: a cross-sectional study

Objectives

Main objective

Evaluate vitamin D levels in a sample of Portuguese active-duty Navy military personnel during winter.

Secondary objective

Explore the relationship between Navy occupational settings and vitamin D levels.



Which food has more vitamin D levels per serving?

- a) Mushrooms, white, raw, sliced, exposed to UV light, ½ cup.
- b) Trout (rainbow), farmed, cooked, 3 ounces.
- c) Salmon (sockeye), cooked, 3 ounces.
- d) Cod liver oil, 1 tablespoon.



Which food has more vitamin D levels per serving?

- a) Mushrooms, white, raw, sliced, exposed to UV light, ½ cup: 9.2 mcg | 366 IU
- b) Trout (rainbow), farmed, cooked, 3 ounces: 16.2 mcg | 645 IU
- c) Salmon (sockeye), cooked, 3 ounces: 14.2 mcg | 570 IU
- d) **Cod liver oil, 1 tablespoon: 34.0 micrograms | 1,360 International Units**

A cross-sectional study was conducted including all Portuguese active-duty Navy military personnel (inclusion criteria) who collected a blood sample at the Naval Medicine Centre between 22 December 2023 and 20 March 2024.

Exclusion criteria included civilian or militarized personnel.

Four blood parameters (serum 25(OH)D, calcium, phosphorus and parathyroid hormone (PTH)) were added to their analysis request.

In addition, they were invited to complete a questionnaire.

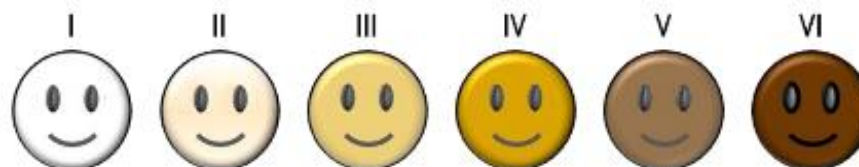
Personal data, such as sex, age, and military category (officer vs sergeant vs seaman), were retrieved from the analytical results form.

Questionnaire

(1) reason for carrying out analyses (occupational vs routine vs disease);

(2) occupational setting (ashore vs onboard; indoors vs outdoors; day shift vs night shift vs mixed shift);

(3) skin type (Fitzpatrick scale);



(4) sun exposure (≥ 30 min, 09:00-15:00, wearing only a t-shirt and shorts, without sunscreen), vitamin D-rich food intake (e.g., cod liver oil, fatty fish, oysters, liver, eggs, butter and/or milk fortified with vitamin D) and vitamin D supplements or medication intake (number of days in the last 30 days; 4-point Likert scale: 0 days, 1-10 days, 11-20 days and 21-30 days);

Blood sample analysis

Blood samples were processed at the Clinical Pathology Service (Lisbon) of the Portuguese Armed Forces Hospital.

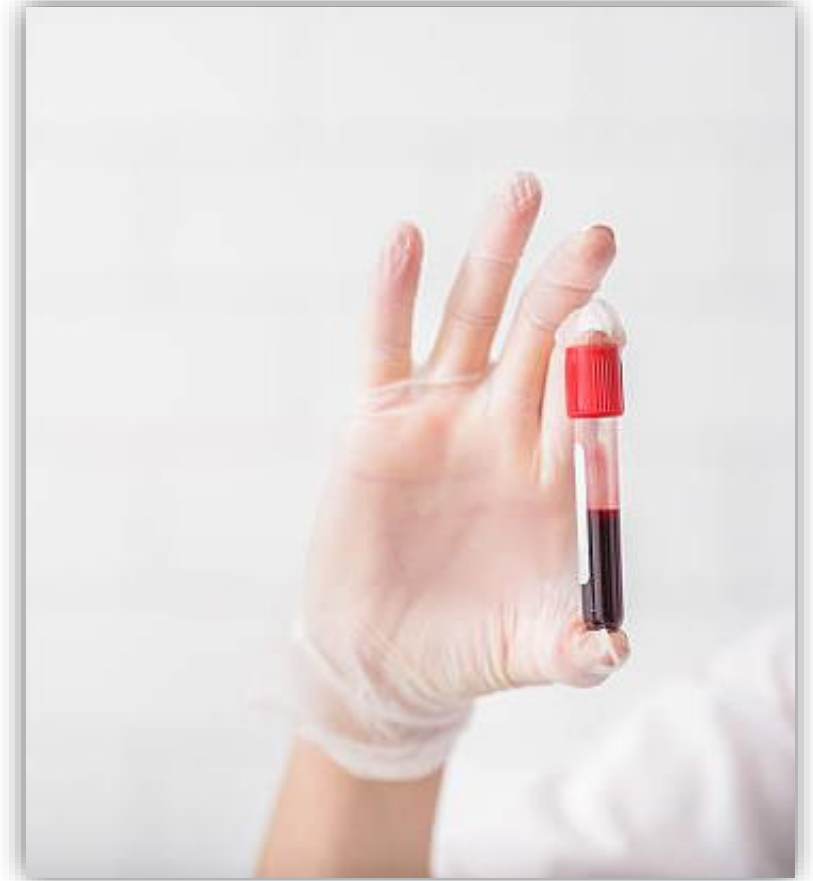
Chemiluminescence was used to perform 25(OH)D and PTH assays on the Alinity equipment (Abbott; certified by the Centers for Disease Control and Prevention – Vitamin D Standardization-Certification Program).

The o-cresolphthalein complexone and phosphomolybdate ultraviolet (UV) methods were applied to measure calcium and phosphorus levels, respectively, using the Cobas c501 equipment (Roche).

Blood sample analysis

Laboratory reference values:

- 25(OH)D:
 - <20 ng/mL (deficiency),
 - ≥20 ng/mL and <30 ng/mL (insufficiency), and
 - ≥30 ng/mL (sufficiency).
- Calcium: 8.1 to 10.4 mg/dL.
- Phosphorus: 2.7 to 4.5 mg/dL.
- PTH: 15 to 68.3 pg/mL.



Statistical analysis (IBM SPSS Statistics V.29)

Categorical variables: absolute and relative frequency.

Continuous variables: mean and standard deviation (normal distribution) or median and interquartile range (non-normal distribution).

Sample means comparison test (t-test) and median test: used to compare homologous means and medians, respectively.

Proportion comparison test (z-test): used to compare homologous prevalences of vitamin D deficiency, insufficiency and sufficiency (when median 25(OH)D values were statistically different).

Significance level: 5%.

Which is the Recommended Dietary Allowance for vitamin D in adults?

- a) 400 IU.
- b) 600 IU.
- c) 800 IU.
- d) 1000 IU.

400 IU

600 IU

800 IU

1000 IU

Which is the Recommended Dietary Allowance for vitamin D in adults?

- a) 400 IU: 0-12 months (adequate intake).
- b) 600 IU: 15 mcg.**
- c) 800 IU: >70 years.
- d) 1000 IU.

Vitamin D levels in Portuguese Navy military personnel: a cross-sectional study

Results

735 Portuguese active-duty Navy military personnel (38.27 ± 9.5 years old mean age; range 20–64 years old) were included (95.3% participation rate).

Distribution by personal characteristics and winter period:

		n (%)
Sex	Male	651 (88.6)
	Female	84 (11.4)
Age	<30 years old	176 (23.9)
	30-39 years old	188 (25.6)
	40-49 years old	275 (37.4)
	≥50 years old	96 (13.1)
Category	Officer	159 (21.6)
	Sergeant	231 (31.4)
	Seaman	345 (46.9)
Winter period	22 Dec to 20 Jan	127 (17.3)
	21 Jan to 20 Feb	386 (52.5)
	21 Feb to 20 Mar	222 (30.2)

Median (IQR) [Min.; Max.] serum levels:

- 25(OH)D: 21.8 (16.3–27.5) [5.3; 65.4] ng/mL,
- Calcium: 9.8 (9.6–10.0) [7.8; 10.9] mg/dL, and
- PTH: 51.3 (37.8–65.4) [11.9; 144.0] pg/mL.

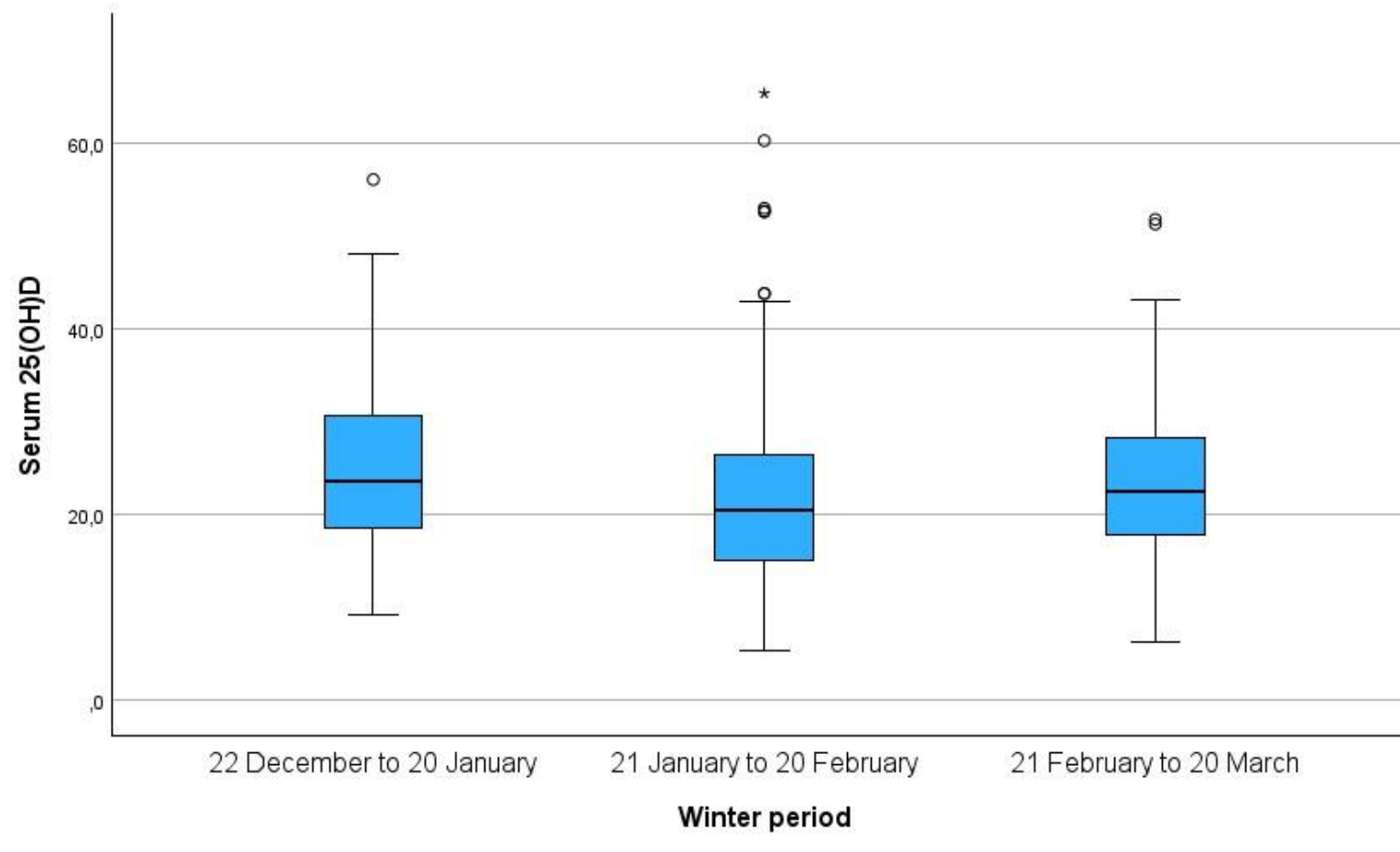
Mean±SD [Min.; Max.] serum phosphorus levels: 3.23±0.45 [1.60; 5.10] mg/dL.

The median calcium and mean phosphorus levels significantly differed between males and females ($p<0.001$; $p=0.000$).

Vitamin D levels in Portuguese Navy military personnel: a cross-sectional study

Results

The median 25(OH)D level was significantly lower in the middle of winter ($p=0.000$).



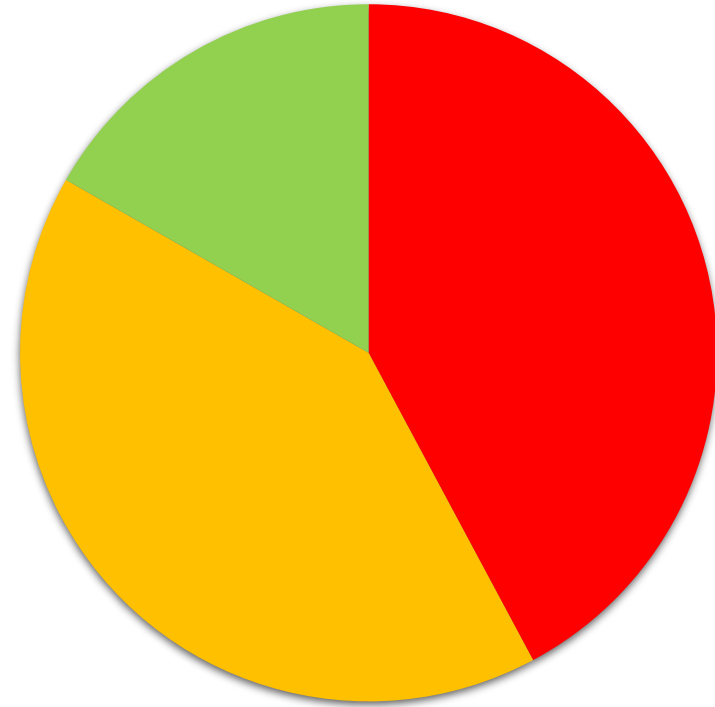
The median PTH level was significantly higher at the beginning of winter ($p=0.000$).

The youngest group's median PTH value was significantly lower than all older group's median PTH value ($p=0.000$).

PTH values were negatively correlated with 25(OH)D, calcium and phosphorus values, with statistical significance ($p<0.001$).

Vitamin D status:

- Deficiency: 310 (42.2%),
 - 21 (2.9%) subjects with 25(OH)D <10 ng/mL
- Insufficiency: 302 (41.1%),
- Sufficiency: 123 (16.7%).



Vitamin D deficiency prevalence was significantly higher in the second third of the winter than in the first third ($p=0.002$).

Vitamin D sufficiency prevalence was significantly higher in the first third of the winter.

Vitamin D status	Winter period			p ₁ -value*	p ₂ -value*
	22 nd Dec to 20 th Jan N (%)	21 st Jan to 20 th Feb N (%)	21 st Feb to 20 th Mar N (%)		
Deficiency	39 (30.7)	185 (47.9)	86 (38.7)	0.002	NSD
Insufficiency	52 (40.9)	151 (39.1)	99 (44.6)	NSD	NSD
Sufficiency	36 (28.3)	50 (13)	37 (16.7)	0.000	0.030

*Proportion comparison test (z- test). p_1 = “22nd Dec to 20th Jan” vs. “21st Jan to 20th Feb”. p_2 = “22nd Dec to 20th Jan” vs. “21st Feb to 20th Mar”. NSD = no statistical difference between groups.

Vitamin D levels in Portuguese Navy military personnel: a cross-sectional study

Results

609 participants agreed to complete the questionnaire (82.9% response rate).

Most of them (83.9%) underwent testing for occupational purposes and only 33 (5.4%) reported a disease under study.

Distribution by occupational setting:

		n (%)
Workplace	Ashore	338 (55.5)
	Onboard	271 (44.5)
Workstation	Indoors	411 (67.5)
	Outdoors	158 (25.9)
	Unknown	40 (6.6)
Work shift	Day shift	293 (48.1)
	Mixed shift	302 (49.6)
	Night shift	2 (0.3)
	Unknown	12 (2.0)

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Distribution by skin type and sun exposure, vitamin D-rich food intake and vitamin D supplements or medication intake:

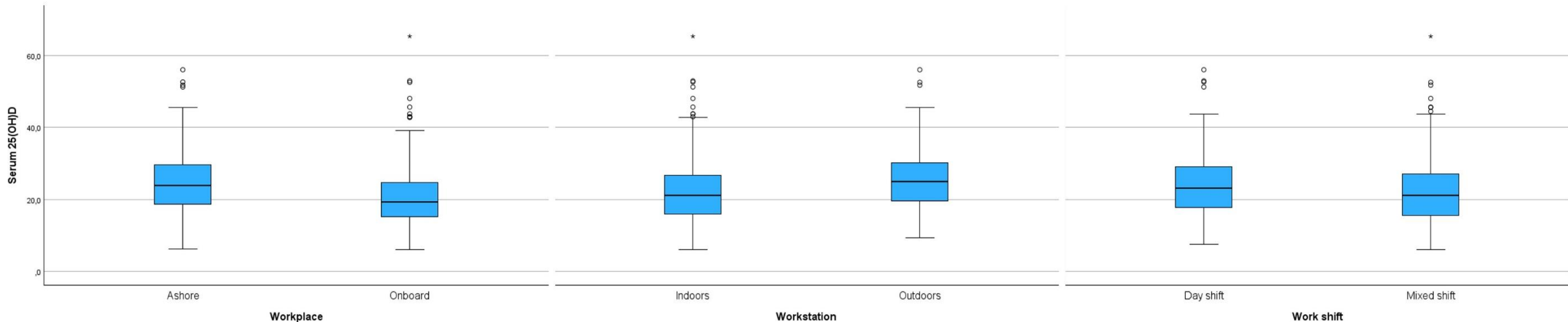
		n (%)
Skin type	I	18 (3.0)
	II	136 (22.3)
	III	288 (47.3)
	IV	140 (23.0)
	V	18 (3.0)
	VI	0 (0)
	Unknown	9 (1.5)

		n (%)
Sun exposure	0 days	232 (38.1)
	1-10 days	286 (47.0)
	11-20 days	55 (9.0)
	21-30 days	36 (5.9)
Vitamin D-rich food intake	0 days	31 (5.1)
	1-10 days	452 (74.2)
	11-20 days	75 (12.3)
	21-30 days	51 (8.4)
Vitamin D supplements or medication intake	0 days	464 (76.2)
	1-10 days	84 (13.8)
	11-20 days	17 (2.8)
	21-30 days	42 (6.9)
	Unknown	2 (0.3)

Vitamin D levels in Portuguese Navy military personnel: a cross-sectional study

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The median 25(OH)D level was significantly different between the workplace ($p<0.001$), workstation ($p<0.001$) and work shift ('day shift' vs 'mixed shift'; $p=0.030$) groups, with lower levels observed in the 'onboard', 'indoors' and 'mixed shift' groups.



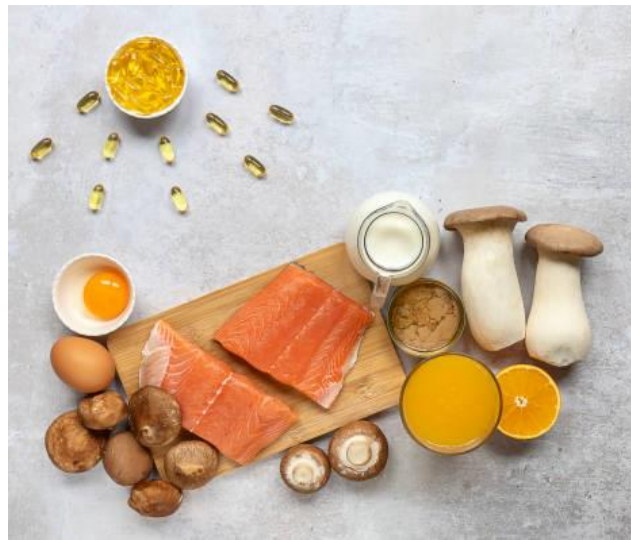
Vitamin D levels in Portuguese Navy military personnel: a cross-sectional study

Results

The median 25(OH)D level consistently increased with sun exposure and vitamin D-rich food intake.

The median PTH level was significantly different between workplace groups ($p=0.016$), with higher levels in the 'ashore' group.

The median PTH level consistently decreased with sun exposure and vitamin D-rich food intake.



Vitamin D deficiency prevalence was significantly higher ($p=0.000$) in the 'onboard' group.

Vitamin D sufficiency and insufficiency prevalences were significantly lower ($p=0.000$ and $p=0.003$, respectively).

Vitamin D deficiency prevalence was significantly higher ($p=0.000$) in the 'indoors' group.

Vitamin D sufficiency prevalence was significantly lower ($p=0.001$).

Vitamin D deficiency prevalence was significantly higher ($p=0.004$) in the 'mixed shift' group.

Vitamin D insufficiency prevalence was significantly lower ($p=0.040$).

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Results

When merging workstation or work shift with workplace, a lower median 25(OH)D level was revealed in the ‘onboard’ groups compared with ‘ashore’ groups:

	Onboard	Ashore
Indoors	19.0 ng/mL	22.8 ng/mL
Outdoors	24.2 ng/mL	25.8 ng/mL
Day shift	20.1 ng/mL	23.7 ng/mL
Mixed shift	18.9 ng/mL	24.3 ng/mL



In the 'indoors' group, vitamin D deficiency prevalence was significantly higher in the 'onboard' group ($p=0.000$).

Vitamin D insufficiency and sufficiency prevalences were significantly higher in the 'ashore' group ($p=0.009$ and $p=0.005$, respectively).

In the 'mixed shift' group, vitamin D deficiency prevalence was significantly higher in the 'onboard' group ($p=0.000$).

Vitamin D insufficiency and sufficiency prevalences were significantly higher in the 'ashore' group ($p=0.030$ and $p=0.000$, respectively).

In the 'day shift' group, vitamin D deficiency prevalence was significantly higher in the 'onboard' group ($p=0.001$).

Vitamin D sufficiency prevalence was significantly higher in the 'ashore' group ($p=0.038$).

Vitamin D sufficiency prevalence was significantly higher in the '21–30 days intake' group than in the '1–10 days intake' group ($p=0.036$).

Vitamin D sufficiency prevalence was significantly higher in the '21–30 days intake of vitamin D supplements or medication' group than in the 'no intake' group ($p=0.002$).

Which adults are at risk of vitamin D inadequacy?

- a) Those with limited sun exposure.
- b) Those with dark skin.
- c) Those with obesity.
- d) All of the above.



Which adults are at risk of vitamin D inadequacy?

- a) Those with limited sun exposure: unlikely to obtain adequate amounts of vitamin D from sunlight.
- b) Those with dark skin: greater amounts of the pigment melanin in the epidermal layer of the skin reduce the skin's ability to produce vitamin D from sunlight.
- c) Those with obesity: greater amounts of subcutaneous fat sequester more of the vitamin D, so people with obesity might need greater intakes of vitamin D to achieve levels similar to those of people with normal weight.
- d) All of the above.**

This study included a convenience sample of 735 subjects from the 6493 Portuguese active-duty Navy military personnel (11.3%; reference date 31 December 2023).

As anticipated, males (88.6%) and younger personnel (86.8% with <50 years old) were predominant.

Most of the participants were otherwise healthy and underwent testing for occupational purposes (e.g., pre-mission). Among those who reported a disease under study, none had a direct relation to vitamin D metabolism, except for one pregnant woman.

Vitamin D levels

The median serum 25(OH)D value was below 30 ng/mL considering all participants and across all characteristic subdivisions, and it was significantly lower in the middle of winter.

Differences in median calcium, mean phosphorus and median PTH levels according to sex and/or age were found, as expected, according to previously published studies.^{10 11}



Vitamin D levels

Vitamin D and PTH form a tightly regulated feedback cycle:

High number of cases with PTH value above the reference interval (154 in 730; 21.1%), which may indicate a biological effort to improve vitamin D serum levels.

Vitamin D and PTH play a crucial role in maintaining calcium and phosphate homeostasis:

Calcium and phosphorus serum levels were within reference intervals in 95.8% and 87.8% of the subjects, respectively.



Vitamin D status

The prevalence of vitamin D deficiency was 42.2% (2.9% of subjects had serum 25(OH)D value <10 ng/mL).

Portugal is among the countries with the highest prevalence of vitamin D deficiency.

The estimated prevalence of vitamin D deficiency in the adult Portuguese population (2011–2013) during winter was 76% (prevalence of levels of 25(OH)D ≤ 10 ng/mL was 24.3%).⁶



Vitamin D status

This reality underscores the need to implement occupational and population-wide interventions before and during winter, both at the individual and community levels.

The prevalence of vitamin D deficiency during winter was not statistically different between active-duty Navy military personnel and civilians (60.7% vs 56.7%, but it was between active-duty Navy and Army military personnel (60.7% vs 47.6%; $p=0.017$)).⁸

Some Navy occupational settings may pose a higher risk and should be specially addressed by Navy occupational health services.

Vitamin D status

For nonpregnant adults <50 years of age without established indications for vitamin D treatment, the (American) Endocrine Society's current guidelines suggest against empirical vitamin D supplementation, assuming that these adults follow the Recommended Daily Allowance (15 μg daily).⁴

However, the estimated mean vitamin D dietary intake (excluding dietary supplements) in the Portuguese adult population (18–64 years old) is 3.54 $\mu\text{g}/\text{day}$, and only 5.7% have a vitamin D intake above 10 $\mu\text{g}/\text{day}$ (estimated average requirement).¹²



Vitamin D status

These findings advocate the need to implement vitamin D food fortification policies in Portugal.

Special attention should be given to people living/working indoors, especially during months near winter solstice, to prevent vitamin D inadequacy.

Even though the optimal level of 25(OH)D to prevent disease likely depends on the clinical outcomes being evaluated, 10 to 20 µg of vitamin D per day may be adequate to avoid clinical vitamin D deficiency and maintain calcium homeostasis in healthy individuals.^{4 13}

Vitamin D status

Vitamin D supplementation should be specific to age group, body weight and skin type.¹³

Daily oral supplementation of cholecalciferol is the preferred method as it is effective, simple and safe—cholecalciferol can maintain physiological serum 25(OH)D levels between 30 and 50 ng/mL for a long time due to its pharmacokinetic profile characterized by prolonged storage and release on demand according to physiological needs.¹³



Vitamin D status

For generally healthy adults without established indications for 25(OH)D testing, the (American) Endocrine Society's new guidelines do not recommend routine 25(OH)D level screening to guide decision-making about vitamin D supplementation, as well as routine 25(OH)D level follow-up testing to guide vitamin D dosing.⁴

Rather than test in all situations that can be reasonable, it may be better to test only high-risk individuals, including people working long hours indoors.¹³

In the Portuguese Navy, there are various indoor occupational settings, and, especially for the submariners, 25(OH)D level screening should be considered case by case.



Vitamin D status

We may admit the excuse of 25(OH) D testing if empirical vitamin D supplementation became generalized, as it is a safe treatment—vitamin D toxicity prevalence is so rare that there is no explicit cut-off value above which it occurs and below which it does not—with no need for routine monitoring for long-term maintenance vitamin D doses of $\leq 50 \mu\text{g/day}$.¹³

Unfortunately, an expressive number of participants reported no vitamin D supplements or medication intake.



Vitamin D levels and Navy occupational settings

In this study, the median 25(OH)D level was significantly lower among indoor workers (as well as onboard workers, indeed because 86.9% of them were also indoor workers) and in mixed shift workers (65.8% of mixed shift workers were also indoor workers; as were 78.2% of day shift workers) as found in recent systematic reviews.^{7 14}

Vitamin D deficiency prevalence was also significantly higher in the ‘onboard’, ‘indoors’ and ‘mixed shift’ groups.

Vitamin D levels and Navy occupational settings

Onboard workers should be addressed specifically in the Portuguese Navy occupational health service recommendations regarding vitamin D intake, knowing that they presented a lower median 25(OH)D level, as well as a significantly higher vitamin D deficiency prevalence among indoor workers and mixed shift workers.

It is crucial to remember that these personnel often live 24/7 aboard warships or, more critically, in submerged submarines. Future studies should focus on submariners.



Vitamin D levels and Navy occupational settings

The median 25(OH)D level increased consistently with sun exposure and vitamin D-rich food intake. The same pattern was generally shown for vitamin D sufficiency prevalence.

That is why these three recommendations—sun exposure, dietary intake and supplementation—in sequence or preferably together, should be taken into account for the Portuguese active-duty Navy military personnel who are usually integrated into indoor occupational settings, despite the workplace and the kind of shift work.

Study strengths and limitations

First study addressing vitamin D levels in Portuguese active-duty Navy military personnel.

Convenience sample - not representative.

Single blood test conducted during winter - does not reflect long-term vitamin D status.

Respondents' attention - seven participants answered 'never' to all fatigue-related questions, despite two of them being inversely coded.

Recall bias - recall information about sun exposure, vitamin D-rich Food intake and vitamin D supplements or medication intake over the past 30 days.

How much daily sun exposure is required for sufficient vitamin D synthesis?

- a) 5 to 30 minutes, between 10 a.m. and 4 p.m., to the face, arms, hands, and legs, without sunscreen.
- b) 60 to 90 minutes, between 10 a.m. and 4 p.m., to the face, arms, hands, and legs, with sunscreen.
- c) 60 to 90 minutes, between 4 p.m. and 8 p.m., to the face, arms, hands, and legs, without sunscreen.
- d) 5 to 30 minutes, between 10 a.m. and 4 p.m., to the face, arms, hands, and legs, with sunscreen.



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- d) 5 to 30 minutes, between 10 a.m. and 4 p.m., to the face, arms, hands, and legs, with sunscreen.

During the winter, a sample of Portuguese active-duty Navy military personnel had a median serum 25(OH)D level below 30 ng/mL, with a vitamin D deficiency prevalence of 42.2%.

The median serum 25(OH)D level and the prevalence of vitamin D deficiency were significantly different across workplace, workstation and work shift groups, with the worse values observed in the 'onboard', 'indoors' and 'mixed shift' groups.

Portuguese Navy occupational health services must implement measures to promote optimal vitamin D levels for all military personnel.

Future research should focus on submariners.

Thank you for your attention!

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