Hypovitaminosis D Is Associated With Visceral Adiposity, High Levels of Low-Density Lipoprotein and Triglycerides in Alternating Shift Workers

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Abstract

Background: Studies suggest that there is a strong association between low vitamin D levels and cardiovascular disease (CVD) and its risk factors (RFs). Hypovitaminosis D (25(OH)D < 30 ng/mL or 75 nmol/L) is a recent public health problem that has reached different populations. The objective of the study was to investigate whether hypovitaminosis D is an additional mechanism to explain the disturbances in the lipid profile as well as the excess of abdominal fat presented by alternating shift workers of a mining company in the region of Inconfidentes, Minas Gerais, Brazil.

Methods: A cross-sectional study was conducted in a sample of 391 adult males, aged 20 - 57 years old and working alternating shifts, who had at least one risk criterion for CVD. Demographic, behavioral, clinical, and anthropometric and body composition variables were obtained. A blood sample was drawn for determining 25(OH)D, parathyroid hormone intact molecule, lipid profile, blood glucose, insulin, C-reactive protein, and adipokines.

Results: The average age of the 391 study participants was 36.1 ± 7.3 years. The percentage of hypovitaminosis D and dyslipidemia was 73% and 74.2%, respectively. Excess visceral fat was significant in the hypovitaminosis D group, with an odds ratio (OR) of 2.4 (95% confidence interval (CI): 1.1 - 5.2). Dyslipidemia showed 25(OH)D levels significantly lower (OR: 2.7, 95% CI: 1.6 - 4.3) than in individuals with normal levels of cholesterol and fractions, and triglycerides. After adjusted the analysis by age and seasonality, the vitamin levels had a significant inverse association and dose-dependent with low-density lipoprotein (OR: 5.9), triglycerides (OR: 2.3) and visceral fat area (OR: 2.4).

Conclusion: Hypovitaminosis D and dyslipidemia were found in the majority of our mining company shift workers. Furthermore, excess visceral adiposity, hypertriglyceridemia and high low-density lipoprotein cholesterol levels are strong predictors of hypovitaminosis D.

Keywords: Alternating shift workers; Occupational health; Low-density lipoprotein; Triglycerides; Atherosclerosis; Visceral fat; Hypovitaminosis D; Metabolic syndrome

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Introduction

Vitamin D comprises a group of secosteroid molecules, classically acting on bone metabolism and calcium homeostasis. Calcidiol (25(OH)D) is the biochemical marker that best reflects vitamin D status, and the active metabolite, calcitriol $(1,25(OH)_2D_3)$, acts as a hormone steroid [1]. This exerts its biological effect via the nuclear receptor (VDR), which is widely distributed in tissues, and provides a range of physiological effects in the body [2]. Humans get vitamin D through the skin by exposure to solar ultraviolet B rays (UVB) and dietary intake, which meets only 10-20% of the body's needs [3].

Observational studies suggest there is a strong vitamin D deficiency associated with cardiovascular disease (CVD) and its risk factors (RFs), such as dyslipidemia and adiposity. Several authors [4-8] observed that the low levels of vitamin D increases the risk of CVD and its FRs, but the mechanisms that explain this association are still being explored. Clinical and experimental studies are needed to determine an appropriate vitamin D status that could contribute to the prevention of CVD.

According to Ding et al (2012) [9], the connection of vitamin D with adipose tissue is due to signaling through the VDR, which inhibits adipogenic transcription factors and lipid accumulation during adipocyte differentiation. The association between increased adiposity and reduced serum calcidiol has been reported for more than three decades, suggesting, as the primary hypothesis, the facility to be stored in adipose tissue due to lipid solubility [10]. Thus, the low bioavailability of calcidiol compromises the function of adipose tissue.

Hypovitaminosis D (25(OH)D < 30 ng/mL or 75 nmol/L) is a recent public health problem, which has been reaching different populations in different age groups around the world [11]. Advanced age, female gender, higher latitude, air pollution, winter season, darker skin pigmentation, less sunlight exposure, dietary habits, and the absence of vitamin D fortification in foods are the main factors that are significantly associated with low levels of 25(OH)D [12].

A study by Ward et al (2011) was the first to draw attention to the risk of vitamin D deficiency according to some occupational standards, such as night shifts and long hour shifts [13]. Hypovitaminosis D primarily accompanies individuals who have changes in one or more components of the lipid profile [14-16], and are overweight or obese [17]. However, among the studies of shift workers that evaluated the association with cardiovascular RFs, few paid attention to the relationship of vitamin D with changes in the levels of dyslipidemia components and high adiposity affecting this occupational group.

Although studies show the association of shift work with an increased risk for CVD [18, 19], the pathways that could explain the development of these RFs are not yet fully known and little is known about their manifestations in alternating shift workers in the mining area. Therefore, this study aimed to assess whether hypovitaminosis D is an additional mechanism to explain lipid profile disorders and excess abdominal adiposity, represented by the volume of visceral fat presented by alternating shift workers of a mining company operating in the Inconfidentes region, located in the state Minas Gerais, in southeastern Brazil.

Materials and Methods

A cross-sectional study was carried out with 391 adult men, off-road truck drivers operating at four mines in a mining company located in the region of Inconfidentes, Mariana and Ouro Preto municipalities, in the State of Minas Gerais, Brazil. Men with kidney disease or chronic liver disease and using statin and/or vitamin D supplements were excluded. The drivers work under conditions of alternating shifts, with clockwise rotation (morning/afternoon/evening), including 6 and 12 h of rest.

The socio-demographic characteristics were obtained by means of a semi-structured self-administered questionnaire. Skin color was self-reported. To determine alcohol consumption, we used the "Alcohol Use Disorders Identification Test - AUDIT" (WHO, 1992) [20] with individuals classified as low-risk consumption and risk drinking. Smoking was evaluated by "Fagerstrom Nicotine Dependence Test - FTND" [21] with individuals classified as smokers and non-smokers. The level of physical activity was measured by version 8 of the "International Physical Activity Questionnaire - IPAQ" [22].

Three blood pressure measurements were performed with automatic digital blood pressure monitor HEM - 705CP® device (Omron). The worker sat with the cuff pressure firmly set and adjusted and kept at heart level. The interval between measurements was 1 - 2 min. At the end of the measurements, the lower value blood pressure measurement was considered.

Systolic blood pressure (SBP) ≥ 140 mm Hg and/or diastolic blood pressure (DBP) ≥ 90 mm Hg and/or use of antihypertensive medication were classified as arterial hypertension [23]. Alternatively, we adopted a lower cut-off point to classify blood pressure as "changed", for those with SBP ≥ 130 mm Hg or DBP ≥ 85 mm Hg or use of antihypertensive.

Body weight, body fat percentage (BF%), and the visceral fat area (VFA) were obtained by bioimpedance (BIA), the segmented body composition monitor InBody model 720® (Biospace Co. Ltd. Factory) and height was measured in centimeters by Estadiometer digital HM-210 model D® (Charder). The body mass index (BMI) was calculated using the formula weight (kg)/height^2 (m) and classified into pre-obese (BMI: 25 - 29.9 kg/m^2) and obesity (BMI ≥ 30 kg/m^2) according to the World Health Organization (2000) [24]. Waist circumference (WC), hip circumference (HC) and circumference neck (NC) were obtained with a simple, inelastic tape measure [25]. The waist/hip ratio (WHR) was calculated by the $WC_{(cm)}/HC_{(cm)}$ ratio and the waist/height ratio (WHER) was calculated by the $WC_{(cm)}/height_{(m)}$ ratio. Blood collection was performed after fasting for 12 h and held in the collection tube S-Monovette® (Sarstedt) of 7.5 mL without anticoagulant to obtain serum for biochemical analysis and S-Monovette® tube (Sarstedt) 2.7 mL containing fluoride to obtain plasma for blood glucose level.

The concentration of vitamin D (25(OH)D) was determined in Liaison® apparatus (DiaSorin), a chemiluminescence immune analyzer. The levels of parathyroid hormone intact molecule (iPTH) were determined by electrochemiluminescence in Cobas apparatus E 6000/601® (Roche). Serum levels of vitamin D were classified according to Maeda et al (2014) [26] as sufficient (25(OH)D ≥ 30 ng/mL or 75 nmol/L) and low (25(OH)D < 30 ng/mL or 75 nmol/L). iPTH levels were ranked according to the respective kit manufacturer as normal (≤ 65pg/mL) and high (> 65pg/mL).

Dosages of triglycerides (TG), total cholesterol (TC), and high-density lipoprotein (HDL-c) fraction were determined by enzyme-colorimetric method (Human Brazil, Itabira, Brazil) in the Auto-Analyzer Chemwell R6® (Technology Awareness). Low-density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c) fractions were obtained by mathematical calculation using the Friedewald formula for triglyceride concentration less than or equal to 400 mg/dL [27]. The components of the lipid profile were classified according to the V Brazilian Guidelines for dyslipidemia and prevention of atherosclerosis [28]. Dyslipidemia was considered when one or more criteria were met according to NCPE/ATP III (2002) [29]: total cholesterol ≥ 200 mg/dL, LDL-c ≥ 130 mg/dL, HDL-c < 40 mg/dL, TG ≥ 150 mg/dL and VLDL-c ≥ 30 mg/dL.

Fasting blood glucose was determined by enzymatic colorimetric analysis (Bioclin, Belo Horizonte, Brazil) in CM20 apparatus (Wiener). Blood glucose was classified according to the IDF (2005) [30] as normal (< 100 mg/dL) and impaired (≥ 100 mg/dL).

Fasting insulin levels (pg/mL), adiponectin (pg/mL), and C-reactive protein (mg/L) (CRP) were determined by Milliplex® Human Panel adipokine magnetic bead method. Insulin and adiponectin levels were classified according to the values

Table 1. Distribution of Categories of Demographic and Behavioral Variables According to the Levels of 25(OH)D

Variables	25(OH)D < 30 ng/mL (75 nmol/L)	25(OH)D ≥ 30 ng/mL (75 nmol/L)	Total	n	Odds ratio (95% CI)	P value*
Age (years), mean ± SD	36.7 ± 7.5	34.7 ± 6.4	36.1 ± 7.3	391	-	0.016
Skin color			%			0.835
White	100 (35.1)	36 (34)	34.8	136	1.0	
Non-white	185 (64.9)	70 (66)	65.2	255	0.95 (0.59 - 1.52)	
Total				391		
Education			%			0.175
≤ 8 years	32 (11.2)	7 (6.6)	10.0	39	1.0	
> 8 years	253 (88.8)	99 (93.4)	90.0	352	0.56 (0.24 - 1.31)	
Total				391		
Time spent in shift work			%			0.427
≤ 5 years	135 (47.4)	55 (51.9)	48.6	190	1.0	
> 5 years	150 (52.6)	51 (48.1)	51.4	201	1.19 (0.76 - 1.87)	
Total				391		
Seasonality			%			< 0.0001
Spring	221 (77.5)	61 (57.5)	72.1	282	1.0	
Summer	64 (22.5)	45 (42.5)	27.9	108	0.72 (0.68 - 0.77)	
Total				391		
Smoking			%			0.183
No	220 (82.1)	87 (87.9)	83.7	307	1.0	
Yes	48 (17.9)	12 (12.1)	16.3	60	1.58 (0.80 - 3.12)	
Total				367		
Alcohol consumption			%			0.135
Low risk	246 (86.3)	85 (80.2)	84.7	331	1.0	
Risk	39 (13.7)	21 (19.8)	15.3	60	0.64 (0.35 - 1.15)	
Total				391		
Physical activity			%			0.103
≥ 3 days/week	42 (14.7)	9 (8.5)	87.0	340	1.0	
< 3 days/week	243 (85.3)	97 (91.5)	13.0	51	1.86 (0.87 - 3.97)	
Total				391		
Hypertension[a]			%			0.057
No	90 (33.6)	23 (23.2)	69.2	113	1.0	
Yes	178 (66.4)	76 (76.8)	30.8	254	0.6 (0.35 - 1.01)	
Total				367		
Dyslipidemia[b]			%			< 0.0001
No	58 (20.4)	43 (40.6)	25.8	101	1.0	
Yes	227 (79.6)	63 (59.4)	74.2	290	2.7 (1.6 - 4.3)	
Total				391		

[a]Systolic blood pressure ≥ 140 mm Hg and diastolic blood pressure ≥ 90 mm Hg or use of antihypertensive (VI Diretrizes Brasileira de Hipertensao, 2010). [b]One or more ATPIII criteria: total cholesterol ≥ 200 mg/dL, LDL-c ≥ 130 mg/dL, HDL-c < 40 mg/dL, TG ≥ 150 mg/dL, VLDL-c ≥ 30 mg/dL.

below and above the 75th percentile and CRP levels were classified as low risk/moderate (< 3 mg/L) and high risk (≥ 3 mg/L) according to the American Society of Cardiology and the Centers for Disease Control and Prevention (CDC) [31].

Student's *t*-test and analysis of variance (ANOVA) were used to estimate statistical significance between mean values of age and levels of lipid profile components, PTH, blood glucose, insulin, and adiponectin according to the categories of 25(OH)D. Univariate analysis was used to cross-classified the categories of 25(OH)D (< 30 ng/mL or < 75 nmol/L and ≥ 30 ng/mL or ≥ 75 nmol/L) with demographic and behavioral variables and biochemical, anthropometric, clinical and body composition characteristics to generate odds ratios (ORs) with 95% confidence intervals (CIs) to guide interpretation. Multiple logistic regression models (enter method) were performed using CVD RF variables significantly associated with the categories of 25(OH)D in univariate analysis. To adjust the models, only the variables with $P \le 0.25$, biological plausibility, and epidemiological relevance were considered. The significance level for all tests was set at 5% ($P < 0.05$). Statistical analyses were performed using SPSS software version 22.0.

This study was approved by the Ethics Committee on Research With Human of the Ouro Preto Federal University. Participation in the study was entirely voluntary: workers consent and signed informed consent of each participant were obtained prior to the study.

Results

The average age of the 391 study participants was 36.1 ± 7.3 years (ranging from 20 to 57), and most of them with more than 8 years of education (90%). Among workers, 51% had more than 5 years of work shift, and the majority were non-smokers and non-alcoholic (83.7% and 84.7%). The study population was largely sedentary, where only 22.3% reported doing physical activities outdoors for 10 min or more for a minimum of three times a week, but only 22.7% had excess visceral fat. The prevalence of hypertension and dyslipidemia was 30.8% and 74.2%, respectively, and the prevalence of hypovitaminosis D (serum 25(OH)D below 30 ng/mL or 75 nmol/L) in this population was high (73%). The prevalence of overweight (BMI: 25 - 29.9 kg/m^2) and obesity (BMI ≥ 30 kg/m^2) was 48.4% and 17.2%, respectively.

Table 1 shows the distribution of the categories of demographic and behavioral variables according to vitamin D levels observed among these workers. Workers whose blood had been collected in the summer (29.7 ± 8.1 ng/mL or 74.2 nmol/L) had significantly higher vitamin D levels ($P < 0.0001$) than that collected in the spring (24.5 ± 7.2 ng/mL or 61.2 nmol/L).

Table 2 shows the biochemical, anthropometric, clinical and body composition characteristics according to levels of 25(OH)D regarded as insufficient and sufficient in this population. All anthropometric variables were significant enough for the insufficient vitamin D group (< 30 ng/mL or 75 nmol/L), highlighting the waist/height ratio that had the highest value of OR 2.38 (95% CI: 1.46 - 3.88). For body composition variables, visceral fat was significant with an OR of 2.9 (95% CI:

1.46 - 5.75). Dyslipidemia had significantly lower vitamin D levels (OR: 2.7, 95% CI: 1.6 - 4.3) than individuals with normal cholesterol levels and fractions, and triglycerides. Between the cholesterol fractions, only the HDL was not significant when comparing the sufficient and insufficient groups. Hyperglycemic workers had significantly lower levels of vitamin D than euglycemic workers ($P < 0.05$).

The concentrations of biochemical variables were distributed in quartiles of 25(OH)D and there was a significant difference for all variables except for HDL-c and adiponectin (Table 3). Among the biochemical variables, as the levels of 25(OH)D increased, the levels of triglycerides, total cholesterol, LDL-c and VLDL-c fractions, fasting glucose, fasting insulin, and PTH decreased. The same pattern was seen in the VFA, BMI, and systolic and diastolic blood pressure. Conversely, the HDL-c fraction followed a pattern increasing from quartiles 1 through 4.

A binary logistic regression model was created using the anthropometric, body composition, and biochemical variables adjusted for age and seasonality. The variables that remained significant were visceral fat, LDL-c, and triglycerides (Table 4). In relation to workers with sufficient levels of vitamin D, those with insufficient levels most often presented with excess visceral fat in the range 11 - 20 cm^3 (OR: 2.4; 95% CI: 1.1 - 5.2) with excess LDL-c above 160 mg/dL (OR: 5.9; 95% CI: 1.3 - 25.7), and excess triglycerides above 150 mg/dL (OR: 2.3; 95% CI: 1.3 - 4.0).

Table 5 shows the modification of risk analysis adjusted for age and seasonality for the insufficient and sufficient groups with regard to the TG levels and VFA. Considering TG levels < 150 mg/dL and VFA ≤ 10 cm^3 as the reference group, the risk of vitamin D insufficiency was 2.2 times higher (95% CI: 1.23 - 3.83) in the presence of only TG ≥ 150 mg/dL and 2.0 times higher (95% CI: 0.80 - 4.89) in the presence of only VFA > 10 cm^3. The risk was 8.23 (95% CI: 2.38 - 28.51) in the presence of both factors. Risk modification analysis was adjusted for age and seasonality for the insufficient and sufficient groups for TG and LDL-c levels. Considering TG levels < 150 mg/dL and LDL-c < 160 mg/dL as the reference group, the risk of vitamin D insufficiency was 2.1 times higher (95% CI: 1.2 - 3.5) only in the presence of TG ≥ 150 mg/dL and 3.51 times higher (95% CI: 0.7 -16.1) only in the presence of LDL-c ≥ 160 mg/dL. It was 11.1 (95% CI: 1.4 - 84.2) in the presence of the two RFs.

Discussion

Our results show that 23% of the participants had deficiency and 50% for vitamin D insufficiency. Given that hypovitaminosis D includes both groups, this percentage reaches 73%. The average of the 25(OH)D levels was low in this population, equal to 26.1 ng/mL (65.2 nmol/L). A significant dose-response pattern between hypovitaminosis D was observed (25(OH)D < 30 ng/mL or 75 nmol/L) along with disturbances in lipid profile (triglycerides and LDL-c) and the visceral adiposity in this population.

The shift work schedule is subject to studies that show its

Table 2. Biochemical Characteristics, Anthropometric, and Clinical Body Composition According to Levels of 25(OH)D

Variables	25(OH)D < 30 ng/mL (75 nmol/L)	25(OH)D ≥ 30 ng/mL (75 nmol/L)	Mean (SD)	n	Odds ratio (95% CI)	P value*
BMI (kg/m^2)			26.6 (3.7)			0.015
< 25	83 (30.9)	44 (44.4)		127	1.0	
≥ 25	186 (69.1)	55 (55.6)		241	1.8 (1.11 - 2.87)	
Total				368		
WC (cm)			91.2 (11.2)			0.025
< 90	101 (37.5)	50 (50.5)		151	1.0	
≥ 90	168 (62.5)	49 (49.5)		217	1.69 (1.06 - 2.7)	
Total				368		
NC (cm)			39.3 (3.3)			0.008
< 39.5	129 (48)	63 (63.6)		192	1.0	
≥ 39.5	140 (52)	36 (36.4)		176	1.9 (1.2 - 3.0)	
Total				368		
WHR			0.9 (0.8)			0.017
< 0.90	128 (47.6)	61 (61.6)		189	1.0	
≥ 0.90	141 (52.4)	38 (38.4)		179	1.76 (1.1 - 2.8)	
Total				368		
WHER			0.52(0.06)			0.000
< 0.50	63 (23.6)	42 (42.4)		105	1.0	
≥ 0.50	204 (76.4)	57 (57.6)		261	2.38 (1.5 - 3.9)	
Total				366		
Visceral fat area (cm^3)			8.3 (5.8)			0.002
1 - 10	196 (73.1)	87 (88.8)		283	1.0	
11 - 20	72 (26.9)	11 (11.2)		83	2.9 (1.5 - 5.7)	
Total				366		
SBP (mm Hg)			133.0 (12.8)			0.06
< 140	179 (66.5)	76 (76.8)		255	1.0	
≥ 140	90 (33.5)	23 (23.2)		113	1.66 (1.0 - 2.8)	
Total				368		
DBP (mm Hg)			82.9 (8.9)			0.168
< 90	207 (77.2)	83 (83.8)		290	1.0	
≥ 90	61 (22.8)	16 (16.2)		77	1.52 (0.83 - 2.8)	
Total				367		
PTH (pg/mL)			31.4 (11.1)			0.217
< 65	275 (98.6)	105 (100)		380	1.0	
≥ 65	4 (1.4)	0 (0)		4	0.72 (0.7 - 0.8)	
Total				384		
TC (mg/dL)			195.3 (43.1)			0.05
< 240	239 (83.9)	97 (91.5)		336	1.0	
≥ 240	46 (16.1)	9 (8.5)		55	2.07 (1.0 - 4.4)	
Total				391		
HDL-c (mg/dL)			55.4 (17.1)			0.656
≥ 40	231 (81.1)	88 (83.0)		319	1.0	
< 40	54 (18.9)	18 (17.0)		72	1.14 (0.6 - 2.0)	
Total				391		

Table 2. Biochemical Characteristics, Anthropometric, and Clinical Body Composition According to Levels of 25(OH)D - *(continued)*

Variables	25(OH)D < 30 ng/ mL (75 nmol/L)	25(OH)D ≥ 30 ng/ mL (75 nmol/L)	Mean (SD)	n	Odds ratio (95% CI)	P value*
LDL-c (mg/dL)			109.8 (36.5)			0.003
< 160	241 (86.7)	101 (97.1)		342	1.0	
≥ 160	37 (13.3)	3 (2.9)		40	5.2 (1.5 - 17.1)	
Total				382		
TG (mg/dL)			155.8 (84.8)			0.000
< 150	140 (49.1)	75 (70.8)		215	1.0	
≥ 150	145 (50.9)	31 (29.2)		176	2.5 (1.5 - 4.0)	
Total				391		
VLDL-c (mg/dL)			30.0 (14.0)			0.000
< 30	140 (50)	75 (72.1)		215	1.0	
≥ 30	140 (50)	29 (27.9)		169	2.6 (1.6 - 4.2)	
Total				384		
Fasting glucose (mg/dL)			88.3 (16.6)			0.025
< 100	245 (88.8)	101 (96.2)		346	1.0	
≥ 100	31 (11.2)	4 (3.8)		35	3.2 (1.1 - 9.3)	
Total				381		
Adiponectin (pg/mL)**			6.5 (0.5)			0.895
< 6.77	142 (75.9)	51 (75)		193	1.0	
≥ 6.77	45 (24.1)	17 (25)		62	0.95 (0.5 - 1.8)	
Total				255		
Fasting insulin (pg/mL)**			2.45 (0.26)			0.110
< 2.6	56 (82.4)	135 (72.6)		191	1.0	
≥ 2.6	12 (17.6)	51 (27.4)		63	1.7 (0.8 - 3.5)	
Total				254		
CRP (mg/L)			8.7 (10)			0.820
< 3	20 (29.4)	52 (28.0)		72	1.0	
≥ 3	48 (70.6)	134 (72)		182	1.1 (0.6 - 2.0)	
Total				254		

BMI: body mass index; WC: waist circumference; NC: neck circumference; WHR: waist-hip ratio; WHER: waist-height ratio; TC: total cholesterol; HDL-c: low-density lipoprotein cholesterol; LDL-c: high-density lipoprotein cholesterol; TG: triglycerides; VLDL-c: very low-density lipoprotein cholesterol; PTH: parathyroid hormone; CRP: C-reactive protein; SBP: systolic blood pressure; DBP: diastolic blood pressure. **Amount expressed in the logarithmic form. *P value for Pearson's Chi-square < 0.05 was considered significant.

link to increased risk of developing metabolic disorders mainly related to components of Metabolic Syndrome (MS) [19, 32] and the etiology of atherosclerosis [33]. In an earlier study in this same shift worker population, Ambrosim (2014) [34] found a high prevalence of RFs for MS, such as dyslipidemia, obesity, and overweight.

Shift work is identified as a contributor to a pro-atherogenic profile of cardiovascular risk by means of an unfavorable lipid profile [35], which is characterized by hypertriglyceridemia, decreased HDL, and the formation of smaller particles from the LDL-c, the known small LDL and dense (LDLpd). These factors together characterize the artherogenic lipid phenotype of MS [36].

Even though considered a subclinical event, hypovitaminosis D is recognized as one of the most important factors that influence the health of the skeleton and some chronic diseases such as CVD [4]. Hypovitamnosis D is associated with the intensity of exposure to sunlight, which is significantly reduced due to the occupational environment, especially in individuals working in shifts or in an enclosed room [13]. Some authors [37] found levels of 25(OH)D in shift workers similar to those observed in this study, indicating the occupational environment as an important factor influencing sunlight exposure.

Among the biochemical parameters significantly associated with vitamin D insufficiency, LDL-c merits close attention. Hypovitaminosis D was 5.9 times more frequent in individuals with levels above 160 mg/dL compared to those with lower levels. Excessive amounts of LDL-c in circulation participate in the formation of the atheroma after being modified by oxidation. Increased concentrations of LDL-c increase cardiovas-

Table 3. Distribution of Serum Levels of Lipid Profile Components, PTH, Blood Glucose, Insulin, and Adiponectin According to the Quartile of 25(OH)D in the Group of Alternating Shift Workers in the Inconfidentes Region, Minas Gerais

Variables	Quartile 1 (0 - 19.9 ng/mL) (0 - 49.9 nmol/L)	Quartile 2 (20 - 24.6 ng/mL) (50 - 61.1 nmol/L)	Quartile 3 (24.7 - 29.7 ng/mL) (61.2 - 74.2 nmol/L)	Quartile 4 (29.8 - 60 ng/mL) (74.3 - 150 nmol/L)	P value*
TG (mg/dL)	191.1 ± 90.3^a	169.4 ± 89.9^a	134.8 ± 63.8^b	132.5 ± 79.8^b	< 0.001
TC (mg/dL)	$199.4 \pm 47.9^{a, b}$	202.4 ± 41.3^b	$197.0 \pm 43.6^{a, b}$	183.7 ± 38.1^a	0.010
HDL-c (mg/dL)	52.2 ± 17.2^a	54.6 ± 15.5^a	56.6 ± 18.0^a	57.8 ± 17.6^a	0.109
LDL-c (mg/dL)	$111.0 \pm 37.2^{a, b}$	115.7 ± 37.0^a	113.7 ± 39.5^a	100.0 ± 30.7^b	0.011
VLDL-c (mg/dL)	36.8 ± 15.5^a	32.1 ± 12.4^a	26.3 ± 11.2^b	25.3 ± 13.7^b	< 0.001
BMI (kg/m^2)	27.5 ± 2.8^a	28.0 ± 4.2^a	25.5 ± 3.5^b	25.5 ± 3.3^b	< 0.001
VFA (cm^3)	9.2 ± 3.0^a	9.1 ± 3.6^a	7.1 ± 2.8^b	7.8 ± 9.7^b	< 0.001
PTH (pg/mL)	35.4 ± 13.5^a	31.3 ± 10.6^b	29.6 ± 9.1^b	29.9 ± 10.3^b	0.001
FG (mg/dL)	93.2 ± 26.6^a	$89.4 \pm 12.7^{a, b}$	86.6 ± 14.0^b	85.0 ± 8.0^b	0.004
FI (pg/mL)*	2.5 ± 0.2^a	2.5 ± 0.3^a	2.4 ± 0.3^a	2.4 ± 0.3^a	0.017
ADP (pg/mL)*	6.6 ± 0.3^a	6.5 ± 0.3^a	6.5 ± 0.6^a	6.6 ± 0.4^a	0.288
SBP (mm Hg)	135.9 ± 12.6^a	$134.0 \pm 12.4^{a, b}$	$132.0 \pm 12.9^{a, b}$	130.3 ± 12.8^b	0.020
DBP (mm Hg)	84.9 ± 9.2^a	84.2 ± 9.4^a	$81.7 \pm 7.6^{a, b}$	80.8 ± 8.8^b	0.003

*Amount expressed in the logarithmic form one-way ANOVA TG. TC: total cholesterol; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; TG: triglycerides; VLDL-c: very low-density lipoprotein cholesterol; BMI: body mass index; VFA: visceral fat area; PTH: parathyroid hormone; FG: fasting glucose; FI: fasting insulin; ADP: adiponectin; SBP: systolic blood pressure; DBP: diastolic blood pressure. *P value for the ANOVA < 0.05 was considered significant. [a,b,c]Equal letters indicate similar means Tukey post-test.

cular risk, but it is known that its size can be an even more important predictor. Hypovitaminosis D has been considered an independent RF for atherosclerosis and is associated with endothelial dysfunction and increased lipid peroxidation occurring in plasma lipoproteins and other mechanisms [38, 39]. A study that evaluated the effect of vitamin D supplementation on endothelial function showed favorable effects [40]. Also, the concentration of substances reactive to thiobarbituric acid (TBARS), which are used as indicators of lipid peroxidation, significantly decreased after supplementation in individuals with inadequate levels of 25(OH)D. As the authors did not observe an association between flow-mediated dilation and 25(OH)D levels, it was concluded that the beneficial effects on endothelial function occurred due to a reduction in oxidative stress by 25(OH)D. Therefore, hypovitaminosis D associated with high levels of LDL-c may predispose the emergence of

dysfunction in vascular endothelium and increase CVD rates in alternating shift workers.

Another biochemical parameter independently associated with vitamin D insufficiency was TG. Hypovitaminosis D was 2.3 times more frequent in patients with TG levels greater than 150 mg/dL than in those with lower levels. There are some mechanisms suggested to explain the reduction of TG levels mediated by 25(OH)D. The first mechanism is related to the function of classical vitamin D to increase the absorption of calcium via the intestines with consequent increase of its plasma levels. The elevation of serum calcium decreases hepatic TG synthesis and secretion through its suppressive effect on a TG microsomal transfer protein known as MTP (microsomal triglyceride transfer protein) [41]. The second mechanism relates to vitamin D suppressive effect on PTH concentration in serum. The post-heparin lipase activity is reduced by high

Table 4. Risk Factors Adjusted for Age and Seasonality for Hypovitaminosis D (25(OH)D < 30 ng/mL or 75 nmol/L) in Alternating Shift Workers, Minas Gerais, Brazil

Variables	Crude odds ratio (95% CI)	P value	Adjusted odds ratio* (95% CI)	P value
Visceral fat area (cm^3)				
11 - 20	2.9 (1.5 - 5.7)	0.002	2.4 (1.1 - 5.2)	0.037
LDL-cholesterol level (mg/dL)				
≥ 160	5.2 (1.5 - 17.1)	0.003	5.9 (1.3 - 25.7)	0.018
Triglycerides level (mg/dL)				
≥ 150	2.5 (1.5 - 4.0)	0.000	2.3 (1.3 - 4.0)	0.003

CI: confidence interval. *Adjusting for age and seasonality.

Table 5. Bivariate Association of Risk for Hypovitaminosis D Between Serum Levels of Triglycerides (TG) > 150 md/dL, Visceral Fat Area (VFA) 11 - 20 cm^3 and LDL-c > 160 mg/dL

		Insufficient	Sufficient	Crude odds ratio (95% CI)	P value	Adjusted odds ratio* (95%CI)	P value
TG	VFA						
No	No	141 (66.8)	70 (33.2)	1.0	-	1.0	
No	Yes	27 (77.1)	8 (2.9)	1.7 (0.7 - 3.9)	0.22	2.0 (0.80 - 4.89)	0.14
Yes	No	105 (80.8)	25 (19.2)	2.1 (1.2 - 3.5)	0.005	2.2 (1.23 - 3.83)	0.008
Yes	Yes	50 (94.3)	3 (5.7)	8.2 (2.5 - 27.5)	< 0.001	8.2 (2.38 - 28.51)	0.001
LDL-c	TG						
No	No	164 (67.5)	79 (32.5)	1.0	-	1.0	
No	Yes	130 (81.3)	30 (18.7)	2.1 (1.3 - 3.4)	0.002	2.1 (1.2 - 3.5)	0.007
Yes	No	16 (88.9)	2 (16.1)	3.9 (1.9 - 17)	0.058	3.5 (0.7 - 16.1)	0.107
Yes	Yes	24 (96.0)	1 (4.0)	11.6 (1.5 - 87.0)	0.003	11.1 (1.4 - 84.2)	0.02

CI: confidence interval; TG: triglycerides; VFA: visceral fat area; LDL-c: high density lipoprotein. *Adjusting for age and seasonality.

concentrations of PTH and low serum PTH levels can reduce TG levels via enhanced peripheral removal [41]. In addition, two other mechanisms have also been implicated. Vitamin D increases the expression of VLDL-c receptors in some kinds of cells [42] promoting increased uptake of TG. Finally, insulin resistance may explain the association between 25(OH)D and TG: when vitamin D deficiency is present, it increases the risk of insulin resistance [43], which in turn is associated with an increase in VLDL-c and TG.

In the present study, hypovitaminosis D was 11 times more common in individuals with high concomitant levels of LDL-c and TG compared to no change in these factors and much more frequently than in the presence of these factors alone (OR$_{TG}$ = 2.1; OR$_{LDL-c}$ = 3.5). Vitamin D appears to have an effect on the regulation of lipid profile and is considered by some authors as one of the mechanisms that explain its relationship with the DCV [44]. Some observational studies claim that low levels of vitamin D are associated with an unfavorable lipid profile [14], but some clinical studies do not confirm these findings [14]. Gonzalez et al (2014) [16] found an inverse relationship between deficient levels of 25(OH)D (< 20 ng/mL or 50 nmol/L)) and hypertriglyceridemia. However, no association with dyslipidemia was observed in this study. Yet other authors [45] found similar results to our study, finding a significant inverse association between 25(OH)D and LDL-c and TG.

Additionally, the concomitant presence of excess visceral fat and hypertriglyceridemia indicated increased risk of hypovitaminosis D (OR: 8.2) compared with the absence of those factors and much higher when in the presence of these factors separately (OR$_{TG}$ = 2.2; OR$_{VFA}$ = 2.0). Visceral adiposity is another important factor involved in the etiology of atherogenic lipid phenotype present in the MS, which is considered the most pathogenic compartment and most often associated with the secretion of adipokines, among other factors [36]. Furthermore, in adults, the accumulation of visceral fat is strongly correlated with several other cardiovascular RFs such as hypertension, hypertriglyceridemia, impaired fasting glucose, MS, and insulin resistance [46-48]. Currently among the studies that aimed to assess the relationship of low levels

of 25(OH)D with obesity, few have included direct measurements of adiposity. However, it is still difficult to establish the pattern of adiposity that affects more potential levels of vitamin D. More research is needed to explore these potential relationships.

Some limitations should be considered in this study, such as the adopted cross-sectional design, which cannot establish a causal relationship between the analyzed factors and hypovitaminosis D. The absence of information on the use of sunscreen, along with the intake of vitamin D in the diet, could have influenced the low levels of vitamin D in this population. Finally, a third limiting factor is that using a single measurement of serum levels of 25(OH)D can only reflect recent exposure, not taking into account intra-individual seasonal variations, which may be present.

Conclusion

Our results suggest that alternate shift mine workers are exposed to excess body weight and dyslipidemia, and that these factors constitute strong predictors of hypovitaminosis D. Therefore, it is necessary to assess whether an intervention in the vitamin D status, combined with behavioral changes such as diet and physical activity, could influence the reduction of atherogenic lipid phenotype of the metabolic syndrome, thus contributing to an improvement in the health of this population.

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Conflicts of Interest

The authors declare no conflict of interests.

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