The relationship between vitamin D deficiency and oxidative stress can be independent of age and gender

Editor's
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Abstract: The active vitamin D (1,25(OH)₂D) acts through a nuclear receptor to perform several functions in cellular metabolism. 1,25(OH)₂D participates directly in calcium homeostasis, regulates the immune system, nervous system, blood pressure, insulin secretion, among others. Vitamin D deficiency could also be associated with several diseases and increased cellular oxidative damage. The present study aimed to investigate whether lipid peroxidation and/or protein oxidation are affected by vitamin D deficiency and whether sunlight exposure/diet, gender, and age might influence this relationship. Vitamin D concentrations were obtained from the Heart Hospital database and a questionnaire was applied among the 212 participants. We used the inactive vitamin D (25(OH)₂D) in the analyses since 1,25(OH)₂D has a short half-life and a low blood concentration. Lipid peroxidation and protein oxidation analyses were performed using spectrophotometry. Multivariate analyses suggested the participation of vitamin D deficiency (<30ng/mL) and sunlight/diet in oxidative stress ($p < 0.05$; R^2 MDA: 0.562; R^2 CG: 0.429). Multiple linear regression test show that the age and gender of patients are not interfering in the analyses ($p > 0.05$). Therefore, we suggest that the relationship between vitamin D deficiency and oxidative stress can be independent of age and gender.

Keywords: Vitamin D, oxidative stress, lipid peroxidation, protein oxidation

Introduction

Vitamin D (VD) deficiency is a growing epidemiological problem, affecting a large number of individuals. It is estimated that more than 50% of the world's elderly do not have satisfactory VD levels and more than 1 billion people worldwide have VD insufficiency or deficiency [1, 2]. Approximately 50% of participants in a study conducted by a group of US researchers had low vitamin concentrations. VD deficiency was also observed in 30% to 50% of children and adults in Saudi Arabia, Australia, Turkey, India, and Lebanon [3]. Studies performed in other countries, including Brazil and involving patients of different ages, showed that VD concentrations were below than 30 ng/ml (75 nmol/L) in many patients [4].

The reference value varies according to the technique used for the measurement, but values above 75 nmol/L or 30 ng/mL are necessary to improve the beneficial effects of VD on health [5]. The VD requirements are 600 IU/day for individuals up to 70 years and 800 IU/day for individuals over 70 years but environmental, hormonal, genetic and

nutritional factors influence the serum concentration of vitamin D [6]. VD deficiency is related to several diseases, such as kidney diseases, diabetes, cardiomyopathies, and Alzheimer's disease. Besides that, VD low concentrations may be involved with increased cell damage induced by reactive oxygen species (ROS), since this vitamin regulates several cellular pathways that have a role in antioxidant synthesis [7, 8].

The cellular environment is usually reduced, this is important for normal cell function and survival. Any change to an oxidation state can lead to oxidative stress damage and cellular processes dysfunction since several proteins are sensitive to oxidation [9]. VD can be able to exert protective effects against ROS and nitric oxide and may prevent oxidative damages [10]. This vitamin regulates the gamma-glutamyl transpeptidase (g-GT) expression, which contributes to reduced glutathione (GSH) synthesis. GSH is essential to glutathione peroxidase (GPX) activity, which participates of the cellular enzymatic antioxidant defense system [10, 11]. VD can also increase glucose-6-phosphate dehydrogenase (G6PD), glutamate-cysteine

ligase (CGL) and glutathione reductase activity, increasing GSH formation [12, 13]. Indirectly this vitamin acts in antioxidants synthesis by regulating Klotho and Nrf2 (Nuclear factor erythroid 2-related factor 2) expression, which are important to ROS signaling pathway function. VD, acting with Klotho and Nrf2, regulates antioxidant systems expression, which could prevent oxidative stress [7].

Nrf2 is a stress-sensitive transcription factor and responds to ROS presence, activating several genes that encode antioxidant and detoxifying enzymes [14, 15]. When ROS levels increase in response to cellular stress, Nrf2 enters into the cell nucleus and binds to the antioxidant response element. This results in increased expression of several genes and antioxidants and detoxifying enzymes syntheses, such as Catalase, Glucose-6-Phosphate dehydrogenase, GSH, GPX, Superoxide Dismutase (SOD) 1 and 2 (antioxidants), Alcohol dehydrogenase, carbonyl dehydrogenase, cytochrome P450, prostaglandin reductase, and NADPH (detoxifiers). In the absence of cell stress and ROS, Nrf2 activity is repressed [16, 17]. In this way, Nrf2 remains bound to Keap 1 protein, does not enter the nucleus and is signalized for degradation [18].

Klotho is able to prevent aging and oxidative stress, this ability may depend on the inhibition of the insulin/IGF1 (insulin-like growth factor 1) pathway, resulting in FOXO activation. FOXO regulates antioxidant enzymes expression such as Catalase and mitochondrial Superoxide dismutase [19, 20, 21]. Klotho can also prevent aging and oxidative stress by increasing peroxiredoxins and thyroxine reductase 1 expression, which act together to reduce ROS [22].

VD deficiency can activate RAGE (receptor for advanced glycation end products). RAGE can increase oxidative stress by NADPH oxidase activation, which induces ROS formation [9]. VD may have antioxidant properties by modifying some of antioxidant enzymes and supplementation with VD may improve glycemic control and oxidative stress in type 2 diabetes mellitus [23]. Another study suggests that the beneficial effects of VD could occur directly through antioxidant mechanisms instead of gene expression. In this study, homocysteine has been shown impairing mitochondrial function and inducing changes in cardiac tissue redox status, which were reversed by VD [24]. Halicka and colleagues [25] have reported activated VD could be considered a kind of natural antioxidant that helps repair damaged DNA. Finally, VD can be related to chronic diseases prevention by regulating oxidative stress and inducing the expression of antioxidant defense system molecules. Once VD induces GSH, GPX, SOD expression, and suppresses NADPH oxidase expression, directly or indirectly, this vitamin could be similar to a membrane antioxidant, however, few in vivo studies have examined this hypothesis [26].

The interaction of $1,25(\text{OH})_2\text{D}$ with its intracellular receptors modulates vitamin D-dependent gene transcription and activation of vitamin D-responsive elements, which triggers multiple second messenger systems. Therefore, hypovitaminosis D might increase the incidence and severity of several common diseases, such as metabolic disorders that are linked to oxidative stress [27]. One study has demonstrated that magnesium-zinc-calcium-vitamin D co-supplementation for 6 weeks may reduce biomarkers of inflammation and oxidative stress [28]. Sinha et al. showed that treatment of vitamin D deficient humans with cholecalciferol improves the mitochondrial oxidative phosphorylation rate. Therefore, the interplay between vitamin D-VDR, ROS signalling, and the antioxidant system is complex [29].

Although some antioxidant properties of VD have been described, a significant focus has been to exploit the VD in oxidative damage. Preclinical studies have been strongly encouraging; however, to date literature trials have delivered conflicting findings. In order to generate additional evidence about VD protector effects, this work aims to analyze whether lipid peroxidation and protein oxidation are affected by vitamin D deficiency and whether sunlight exposure/diet, gender, and age can influence this relationship.

Materials and Methods

Materials

The following materials were used: trichloroacetic acid PA-ACS, hydrochloric acid PA-ACS (Dinâmica, química contemporânea Ltda.), sodium dodecyl sulfate (Reagen, Quimibrás Industrias Químicas S.A), phosphoric acid PA-ACS, 2,4-dinitrophenylhydrazine, 2-thiobarbituric acid, *butylated hydroxytoluene* (Sigma-Aldrich), ethyl acetate (Synth) and Guanidine hydrochloride (Uniscience).

The trial design and participants

All experimental procedures were approved by the Human Research Ethics Committee at the Federal University of Rio Grande do Norte (CEP/UFRN No. 67240817.3.0000.5537) and written informed consent was obtained from all patients. Regarding the inclusion and exclusion criteria, patients from Heart Hospital (HCOR/Natal/RN/Brazil) were invited to participate; the invited patients were those submitted to VD dosage. Men, women, and individuals from 12 years of age were allowed to participate in the research. Hospitalized patients were not included in the study, only patients undergoing routine exams. The 212

participants answered a questionnaire, which includes information such as age, sunlight exposure, and food preference. The experimental group included patients with low levels of VD (less than 30 ng/mL), that is, people with VD insufficiency or deficiency. The control group included patients with normal levels or sufficiency of VD (greater than 30 ng/mL). The power of our sample is 99% (Gpower).

Blood Samples

The blood samples were collected during patients' routine exams. Tubes (5ml) without anticoagulant were used for VD dosage. Tubes (4 ml) with Ethylenediamine tetraacetic acid (EDTA) were used for cellular oxidative measurement.

VD dosage

The dosage was performed through Chemiluminescent Microparticle Immunoassay - CMIA, using serum samples. The results were provided by 'HCOR'. In the research, 25 Hydroxy Vitamin D was used because it is the most abundant and the most stable metabolite. 25 Hydroxy Vitamin D circulating levels directly reflects the VD ingested and/or synthesized. Patients with concentrations less than 29.9 ng/mL are considered VD deficient/insufficient (experimental group). Concentrations between 30 and 100 ng/mL are considered sufficient (control group) [30]. We used the 25 Hydroxyvitamin D (25OHD) in analyses. 1,25(OH)²D was not used since it has a blood concentration 1000 times lower than 25 Hydroxyvitamin D (25OHD) and a short half-life. Therefore 1,25(OH)²D is not indicated in the VD sufficiency assessment [31].

Storage and samples transport

EDTA tubes containing the samples were transported in a refrigerated box (2°–6 °C) following the biosafety standards from HCOR to Bioscience Center of UFRN. The samples were stored in a refrigerator until the centrifugation time. Samples were not stored for more than two hours.

Measurement of cellular oxidative stress markers

Measurement of Malondialdehyde (MDA)

In order to obtain blood plasma, the blood samples were centrifuged at 1500 x g, at 4°C, for 10 minutes in a refrigerated centrifuge. For MDA measurement, in a 200 µL plasma was added one solution of 85% phosphoric acid (H₃PO₄) and also 0.4 mg of butylhydroxytoluene (BHT) dissolved in 80% ethanol and 2.94 mg of 2-thiobarbituric acid (TBA) 98% dissolved in 500µL of distilled water.

These plasma samples were incubated for 45 minutes at 90°C. After incubation, 90% sodium dodecyl sulfate dissolved in distilled water was added. Subsequently, the samples were homogenized and centrifuged at 3000 x g, at 4°C, for 10 minutes. After centrifugation, the absorbance was read at 532nm on the Ultrospec 2100 pro spectrophotometer. Finally, the amount of lipids that react with TBA was calculated using the formula: $A_{532nm} = e \cdot b \cdot c$. Molar extinction coefficient (e) was $1.56 \cdot 10^5 M^{-1}cm^{-1}$ and cuvette optical path (b) was 1 cm. The mol/L values were converted to µmol/L.

Measurement of proteins carbonyl group (CG)

Firstly, 4.89 M dinitrophenylhydrazine dissolved in 12M hydrochloric acid was added in 100µL of plasma. The tubes were incubated at 37°C for 90 minutes and 0.14 ml of 100% trichloroacetic acid diluted in 0.36 ml of distilled water was added. The samples were homogenized for 3 minutes and centrifuged for 3 minutes at 3830 x g, at 4°C. The supernatant was discarded; the pellet was suspended in 0.5 mL of 1:1 ethyl acetate and centrifuged again for 6 minutes at 3830 x g, at 4°C. The supernatant was discarded again and the precipitate was resuspended in 0.5 ml of a solution with 280 mg of 10.36 M guanidine hydrochloride. The samples were centrifuged at 3830 x g for 3 minutes, at 4°C and absorbance was read at 360 nm. The concentration of CG was determined using the formula: $A_{532nm} = e \cdot b \cdot c$. Molar extinction coefficient (e) was $21000 M^{-1}cm^{-1}$ and cuvette optical path (b) was 1 cm. The mol/L values were converted to µmol/L.

Analysis of sunlight exposure and diet data

For this analysis, the Likert scale was used. A questionnaire was carried out among the research participants, including information about sunlight exposure and diet. A scale from zero to four points was assigned, ranging from rare to daily sunlight exposure (for 20 minutes or more), respectively. In addition, a scale from zero to four points was also attributed in relation to consumption of foods rich in VD. For example, patients who consume any of these foods daily: Liver, Fish, Milk and /or Eggs, were assigned four points in the scale to them, patients who rarely consume these foods were assigned zero to them. The values of both scales (points) were summed (general scale) to obtain a value that represents the VD concentration (Table 1).

Statistical analyses

Data were analyzed using statistical software IBM SPSS 23.0 (SPSS Inc.) for Windows and Python programming language. For the statistical analyses, the Mann-Whitney and

Table 1. Descriptive analyzes of variables

	Population data (n = 212)			Men data (n = 55)			Women data (n = 157)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Age (years)	61.27	13	97	64.27	24	89	60.22	13	97
Vitamin D (ng/mL)	36.3	14	110.3	38.65	18.9	107	35.5	14	110.3
MDA (μmol/L)	0.82	0.044	3.256	0.74	0.044	3.256	0.84	0.05	3.23
CG (μmol/L)	30.34	10.52	64.38	29.24	10.52	64.38	30.73	10.57	64.35
Sunlight/diet scale	5.17	1	8	5.24	1	8	5.15	1	8

MDA, Malondialdehyde; CG, Carbonyl group.

multiple linear regression tests were applied; regarding correlation test, Spearman coefficient were used. Values of $p < 0.05$ were considered as the criterion for significance.

Descriptive analyses

All descriptive data were expressed as mean $\pm$ standard deviation, median, minimal and maximal values for the whole population and according to the gender (Table 1).

Univariate analyses

Univariate analyses (Mann-Whitney) and correlation test were performed in order to investigate the relationship between VD concentrations, lipid peroxidation, protein oxidation, age, gender and sunlight exposure/VD rich diet (Table 2).

We investigated the relationship between VD and MDA. MDA median was calculated. Patients with MDA levels greater than the median were inserted in the High 'group' (n = 106 patients) and patients with MDA levels less than or equal to the median were inserted in the 'Low' group (n = 106).

We also investigated the relationship between VD and CG; for this, the median of CG concentrations was also calculated. The patients were divided into two groups: those with CG concentrations greater than the median (High, n = 106) and those with CG concentrations less than or

equal to the median (Low, n = 106). Moreover, the patients were divided into two groups: those with sufficient VD levels, i.e., greater than or equal to 30 ng/mL (High) and those with VD deficiency/insufficiency, that is, concentrations less than 30 ng/mL (Low).

An analysis of the relationship between sunlight exposure/diet and oxidative damage was also performed. The median of the general scale values (sunlight and diet) were calculated based on Likert scale. The patients were divided into two groups: those with values above the median (132 patients in the 'High' group) and those with values below the median (80 patients in the 'Low' group). To evaluate the relationship between solar exposition/diet and oxidative damage, the median was used.

The relationship between age and oxidative stress was analyzed and the median of the patient's age was calculated. The patients were divided into two groups: those who were older than the median age (110 patients in the 'High' group) and those who were younger than the median age (102 patients in the 'Low' group). Additionally, patients with age greater than the median (High 'group') and patients with age less than the median ('Low' group) were analyzed separately. We evaluated the relationship between VD-MDA and VD-CG in 'High' and 'Low' groups.

To evaluate the relationship between gender and oxidative stress, the patients were divided into two groups: men

Table 2. Multiple Linear Regression. Model 1 (Malondialdehyde)

Summary ^a				
Degree of freedom	F value		p value	R square
2 (regression)	133.385		<0.05 ^b	0.562
209 (residual)				
Coefficients				
	Coefficient	Standardized coefficient (β)	t value	p value
(Constant)	2.4	–	23.254	<0.05
Vitamin D	–0.020	–0.373	–6.959	<0.05
Sunlight exposure/diet	–0.165	–0.484	–9.034	<0.05
Equation: MDA = 2.4 + (–0.020 × Vitamin D)+(–0.165 × Sunlight Exposure/diet) ^c				

a. Dependent variable: Malondialdehyde; b. Variables included: (Constant), Vitamin D, Sunlight exposure/diet. c. Predictors variables.

(55) and women (157). We evaluated the relationship between VD-MDA and VD-CG in both groups in order to verify if the gender was interfering in the analyses.

Multivariate analyses

In addition, we performed multivariate analyses (Tables 3 and 4). For the analyses, we selected two multivariable models (lipid peroxidation and protein oxidation) in order to investigate the multivariable relationship between VD deficiency and lipid peroxidation/protein oxidation, adjusted on age, gender and sunlight exposure/VD rich diet. Multiple linear regression test was used to verify and validate the impact of the variables on our models.

Results

Descriptive analyses

The descriptive analyses of the 212 participants are presented in Table 1. In the research, 143 people have VD sufficiency, while 69 have VD deficiency/insufficiency. In other words, 33% of participants do not have satisfactory levels of VD. Patients from 13 to 97 years participated in the research, the mean age in population was 61 years. Besides, 55 men and 157 women were randomly included in the research. The VD mean in men group was 38.65 ng/mL while in women group, it was 35.5 ng/mL. The mean age in men group was 64 years and the mean age in women group was 60 years. Finally, it was found that the MDA mean in men group was 0.74 $\mu\text{mol/L}$ while in women, it was 0.84 $\mu\text{mol/L}$; The CG mean in men group was 29.24 while in women, it was 30.73. There was no statistical difference regarding VD, MDA and CG concentrations between men and women groups ($p > 0.05$).

In order to classify the groups included in the analyses, the median values were used. The median value of sunlight/diet scale was five. The mean VD concentration in

the 'High' group of sunlight/diet was 42.13 ng/mL, while in the 'Low' group, it was 26.67 ng/mL. The mean MDA concentration in the 'High' group of sunlight/diet was 0.44 $\mu\text{mol/L}$, while in the 'Low' group, it was 1.44 $\mu\text{mol/L}$ and the mean CG concentration in the 'High' group was 24.25 $\mu\text{mol/L}$, whereas, in the 'Low' group, it was 40.37 $\mu\text{mol/L}$.

The median age was 64 years. The mean MDA concentration in the 'High' group of age was 0.77 $\mu\text{mol/L}$, while in the 'Low' group, it was 0.86 $\mu\text{mol/L}$ (Figure 2a). The CG concentration mean in the 'High' group (ages over 64 years) was 29.82 $\mu\text{mol/L}$, in the 'Low' group (ages under 64 years), it was 30.89 $\mu\text{mol/L}$ (Figure 2b). The VD concentration mean in the 'High' group of age was 37.62 ng/mL while in the 'Low' group, it was 34.87 ng/mL (Figure 2c).

The mean MDA concentration in the 'men' group was 0.74 $\mu\text{mol/L}$, while in the 'women' group, it was 0.84 $\mu\text{mol/L}$ (Figure 4a). The CG concentration mean in the 'men' group was 29.23 $\mu\text{mol/L}$, in the 'women' group, it was 30.72 $\mu\text{mol/L}$ (Figure 4b). The VD concentration mean in the 'men' group was 38.65 ng/mL, in the 'women' group, it was 35.47 ng/mL (Figure 4c).

The median MDA concentration was 0.627 $\mu\text{mol/L}$ (Figure 5b). The mean concentration of VD in the group of patients with a high MDA concentration was 28.62 ng/mL, the VD mean in the group of patients with low MDA concentration was 43.97 ng/mL. The median of CG was 29.37 $\mu\text{mol/L}$ (Figure 5d). The VD concentration mean in the patient's group with high CG concentration was 29.60 $\mu\text{mol/L}$, whereas, in the group with low CG concentration, the VD mean was 42.99 ng/mL.

Multivariate analysis

Sunlight exposure, diet, VD and oxidative damage

According to Figure 1a, patients with high VD concentrations have significantly higher sunlight exposure/VD-rich diet than patients with low levels of VD concentrations,

Table 3. Multiple Linear Regression. Model 2 (Carbonyl Group)

Summary ^a				
Degree of freedom	F value		p value	R square
2 (regression)	78.582		<0.05 ^b	0.429
209 (residual)				
Coefficients				
	Coefficient	Standardized coefficient (β)	t value	p value
(Constant)	55.590	–	26.210	<0.05
Vitamin D	–0.449	–0.462	–7.438	<0.05
Sunlight exposure/diet	–1.733	–0.283	–4.595	<0.05
Equation: CG = 55.590 + (–0.449 × Vitamin D) + (–1.733 × Sunlight Exposure/diet) ^c				

a. Dependent variable: Carbonyl Group; b. Variables included: (Constant), Vitamin D, Sunlight exposure/diet; c. Predictors variables.

showing that sunlight/diet might represent VD concentrations and can be used to oxidative damage analyses. Such analyses are important in suggesting that VD deficiency may be involved in oxidative damage. According to Figure 1b, patients with high lipid peroxidation have significantly lower sunlight exposure/VD-rich diet than patients with low levels of lipid peroxidation. Finally, Figure 1c suggests that patients with high protein oxidation have significantly lower sunlight exposure/VD-rich diet than patients with low protein oxidation.

Age, lipid peroxidation, protein oxidation and VD

No statistical difference was observed between the groups of age regarding to the concentrations of VD, MDA and CG. Our analyses (Figure 2) suggest that age has no influence on the relationship between VD deficiency and oxidative damage analyses (lipid peroxidation and protein oxidation). The Figures 4 and 7 provide more evidence for this finding. According to Figure 3, patients with high lipid peroxidation and high protein oxidation have a significantly lower concentration of VD than patients with low levels of

Figure 1. A) VD concentration distribution of patients with general scale values (sum of sunlight exposure scale and VD-rich diet scale) greater than or equal to 5 (High, $n = 132$) and less than five (Low, $n = 80$). B) Malondialdehyde (MDA) concentration distribution of patients with general scale values (sum of sunlight exposure scale and VD-rich diet scale) greater than or equal to 5 (High, $n = 132$) and less than five (Low, $n = 80$). C) GC concentration distribution of patients with general scale values (sum of sunlight exposure scale and VD-rich diet scale) greater than or equal to 5 (High, $n = 132$) and less than 5 (Low, $n = 80$). * $p < 0.05$ when compared to the "High" group with the respective "Low" group.

Table 4. Univariate analysis

	MDA (μmol/L)		CG (μmol/L)	
	Spearman correlation	p value (Mann-Whitney)	Spearman correlation	p value (Mann-Whitney)
Age (years)	−0.057	0.464	−0.075	0.697
Vitamin D (ng/mL)	−0.828	0.0003	−0.731	0.0006
Sunlight/diet Scale	−0.712	0.0008	−0.542	0.0005

MDA, Malondialdehyde; CG, Carbonyl group.

Figure 2. A) Malondialdehyde (MDA) concentration distribution of patients with ages greater than or equal to 64 years old (High, n = 110) and less than 64 years old (Low, n = 102). B) CG concentration distribution of patients with ages greater than or equal to 64 years old (High, n = 110) and less than 64 years old (Low, n = 102). C) VD concentration distribution of patients with ages greater than or equal to 64 years old (High, n = 110) and less than 64 years old (Low, n = 102). p = 0.46 (MDA), p = 0.69 (CG), p = 0.17 (VD) when compared the “High” group with the respective “Low” group.

Figure 3. A) Malondialdehyde (MDA) concentration distribution of patients with VD greater than or equal to 30 ng/mL (High, $n = 78$) and patients with VD less than 30 ng/mL (Low, $n = 32$). Analyses were performed using patients older than 64 years old. B) MDA concentration distribution of patients with VD greater than or equal to 30 ng/mL (High, $n = 65$) and patients with VD less than 30 ng/mL (Low, $n = 37$). C) CG concentration distribution of patients with VD greater than or equal to 30 ng/mL (High, $n = 78$) and patients with VD less than 30 ng/mL (Low, $n = 32$). Analyses were performed on patients older than 64 years old. D) CG concentration distribution of patients with VD greater than or equal to 30 ng/mL (High, $n = 65$) and patients with VD less than 30 ng/mL (Low, $n = 37$). Analyses were performed on patients younger than 64 years old. * $p < 0.05$ when compared to the "High" group with the respective "Low" group.

lipid peroxidation, even when we analyzed both groups of patients apart ($p < 0.05$). We can suggest that the patients' ages are not interfering in the results.

Gender, lipid peroxidation, protein oxidation and VD

No statistical difference was observed between the groups of gender regarding to the concentrations of VD, MDA

and CG. The analyses (Figure 4) suggest that gender has no influence on the relationship between VD deficiency and oxidative damage analyses (lipid peroxidation and protein oxidation). The Figures 6 and 8 provide more evidence for this finding. According to Figure 5, patients with high lipid peroxidation and high protein oxidation have a significantly lower concentration of VD than patients with low

Figure 4. A) Malondialdehyde (MDA) concentration distribution of men ($n = 55$) and women ($n = 157$). B) CG concentration distribution of men ($n = 55$) and women ($n = 157$). C) VD concentration distribution of men ($n = 55$) and women ($n = 157$). $p = 0.87$ (MDA), $p = 0.15$ (CG), $p = 0.71$ (VD) when compared the "High" group with the respective "Low" group.

levels of lipid peroxidation, even when we analyzed both groups of men and women patients apart ($p < 0.05$). Therefore, gender did not influence our results.

The multiple linear regression test evidenced the participation of VD deficiency and sunlight/diet in oxidative stress, since VD deficiency and sunlight/diet impact on

MDA and CG levels ($p < 0.05$). However, there is a greater influence of VD levels in MDA ($R^2: 0.390$) and CG ($R^2: 0.371$) concentrations when compared to the influence of diet/sunlight exposure on MDA ($R^2: 0.171$) and CG concentrations ($R^2: 0.059$). The multiple linear regression test shows that the age and gender of patients do not impact in

Figure 5. A) Malondialdehyde (MDA) concentration distribution of patients with VD greater than or equal to 30 ng/mL (High, $n = 107$) and patients with VD less than 30 ng/mL (Low, $n = 50$). Analyses were performed using women B) MDA concentration distribution of patients with VD greater than or equal to 30 ng/mL (High, $n = 36$) and patients with VD less than 30 ng/mL (Low, $n = 19$). Analyses were performed on men. C) CG concentration distribution of patients with VD greater than or equal to 30 ng/mL (High, $n = 107$) and patients with VD less than 30 ng/mL (Low, $n = 50$). Analyses were performed on women. D) CG concentration distribution of patients with VD greater than or equal to 30 ng/mL (High, $n = 36$) and patients with VD less than 30 ng/mL (Low, $n = 19$). Analyses were performed on men. * $p < 0.05$ when compared to the “High” group with the respective “Low” group.

VD, MDA and CG levels ($p > 0.05$) (Tables 2 and 3). Therefore, once again we suggest that the relationship between vitamin D deficiency and oxidative stress can be independent of age and gender.

Univariate Analyses

VD and lipid peroxidation

According to Figure 5a and 1b, patients with high lipid peroxidation have a significantly lower concentration of VD

Figure 6. A) Malondialdehyde (MDA) concentration distribution of patients with VD greater than or equal to 30 ng/mL (High, $n = 143$) and patients with VD less than 30 ng/mL (Low, $n = 69$). B) Distribution of VD concentrations (ng/mL) of patients with MDA greater than 0.627 $\mu\text{mol/L}$ (High, $n = 106$) and less than or equal to 0.627 $\mu\text{mol/L}$ (Low, $n = 106$). C) CG concentration distribution of patients with VD greater than or equal to 30 ng/mL (High, $n = 143$) and patients with VD less than 30 ng/mL (Low, $n = 69$). D) Distribution of VD concentrations (ng/mL) of patients with CG greater than 29.37 $\mu\text{mol/L}$ (High, $n = 106$) and less than or equal to 29.37 $\mu\text{mol/L}$ (Low, $n = 106$). * $p < 0.05$ when compared to the "High" group with the respective "Low" group.

than patients with low levels of lipid peroxidation ($p < 0.05$) (Table 4). In addition, we found an inversely and strong correlation between vitamin D and MDA (Spearman correlation: -0.828).

VD and protein oxidation

Figures 5c and 1d suggest that patients with high levels of CG have a significantly lower VD concentration than patients with low levels of CG (Table 4). In addition, we

found an inversely and strong correlation between vitamin D and CG (Spearman correlation: -0.731).

Correlation analyses

VD, lipid peroxidation and age

Figure 7a shows that age is not correlated with MDA concentration, however, VD levels are correlated with MDA

Figure 7. A) Distribution of age values and malondialdehyde (MDA) concentrations of patients with VD concentrations less than 30 ng/mL (Low, $n = 69$) and equal to or greater than 30 ng/mL (High, $n = 143$). Correlation MDA-VD: -0.828 ; correlation age-MDA: -0.057 (Spearman correlation coefficient). B) Distribution of age values and CG concentrations of patients with VD concentrations less than 30 ng/mL (Low, $n = 69$) and equal to or greater than 30 ng/mL (High, $n = 143$). Correlation CG-VD: -0.731 . Correlation age-CG: -0.075 (Spearman correlation coefficient).

levels. Patients with high VD concentrations have different ages and present low MDA concentrations (no more than $1 \mu\text{M/L}$). Patients with low VD concentrations also have different ages, although the majority of them present high MDA concentrations. Indeed, the correlation between MDA and VD is inversely proportional and strong, and the correlation between age and MDA is almost non-existent (Table 4).

VD, protein oxidation and age

Figure 7b suggests that the VD concentration, but not the age, is correlated with CG concentration. Patients with high VD concentrations have different ages and have lower CG concentrations than patients with low VD levels, who also have varied ages. The correlation between CG and VD is inversely proportional and strong, and the correlation between AGE and MDA is weak (Table 4).

VD, lipid peroxidation, protein oxidation and gender

Figure 8a suggests that gender is not correlated with MDA concentration, however, VD levels are correlated with MDA levels. Indeed, the correlation between MDA and VD is inversely proportional and strong, and the correlation between gender and MDA is almost non-existent. Figure 8b suggests that gender is not correlated with CG concentration, however, VD levels are correlated with CG levels. Indeed, the correlation between CG and VD is inversely

proportional and strong, and the correlation between gender and CG is almost non-existent.

Discussion

The present study aimed to investigate whether lipid peroxidation and protein oxidation are affected by vitamin D deficiency and whether sunlight exposure/diet, gender, and age might influence this relationship. To achieve this aim, some analyses were performed. Multivariate analyses showed that VD deficiency may be related to lipid peroxidation and protein oxidation. In fact, VD deficient patients had significantly higher concentrations of malondialdehyde, an indicator of lipid peroxidation, when compared to VD sufficient patients. VD deficient patients also had significantly higher concentrations of carbonyl group, an indicator of protein oxidation when compared to VD sufficient patients (Figure 5, Tables 2–4). We could suggest that VD can modulate the oxidative stress, being able to exert beneficial contributions to the organism, since oxidative stress is related to pathological conditions.

One study demonstrated that vitamin deficiency D increased the cytotoxicity mediated by ROS. It is likely that vitamin D deficiency induces VDR ablation, ROS generation and in consequence deleterious effects on the

Figure 8. A) Distribution of VD and malondialdehyde (MDA) concentrations of male and female patients. Correlation gender-MDA: -0.04 (Spearman correlation coefficient). B) Distribution of VD and MDA concentrations of male and female patients. Correlation gender-GC: -0.071 (Spearman correlation coefficient).

mitochondrial function [29, 32]. VD supplementation significantly decreased MDA levels in women with polycystic ovary syndrome [33]. In addition, VD has been described as responsible for protection against cell damage associated with the CG by regulating cell death pathways and regulating the ROS and NOS synthesis [34]. This information corroborates with our findings. Recent studies have shown a poor antioxidant status among individuals with VD deficiency. However, results have not revealed a clear link between VD concentrations and oxidative stress biomarkers [35].

Other studies have shown that VD supplementation reduces oxidative stress in diabetic patients [23, 36, 37], but the VD administration did not significantly reduce oxidative stress in women with the polycystic ovarian syndrome [38]. A possible role of VD as a newly recognized antioxidant in diabetes is discussed [39], in this way, VD3 is suggested as a key player in the regulation of the oxidative metabolism in diabetes [40]. It has been observed that VD protects against non-alcoholic fatty liver disease by attenuating oxidative stress and increasing antioxidant enzymes expression in a rat model [41]. VD insufficiency contributes to the inflammatory process and oxidative stress imbalance in patients with HCV. The profile of oxidative stress markers in these patients depends on VD levels, which probably change intracellular signaling pathways and increase inflammation and liver injury [42].

VD also reduced oxidative stress in hippocampus, adipose tissue and rat heart [43, 44, 45]. A study indicated that

VD deficiency leads to mild oxidative stress in the muscle which may act as a trigger for increased proteolysis in the VD deficient muscle [46]. In our study, we found that VD deficiency leads to accentuated oxidative stress in human plasma (Figures 1). Although the relationship between VD, endothelium, and cardiovascular disease is well established, little is known about the antioxidant effect of VD [47].

In the other hand, reduced glutathione (GSH), oxidized glutathione (GSSH) and glutamine plasma levels were lower in the VD sufficient group [48] and no association was observed between VD and oxidative DNA damage [49]. Thus, the association between VD status and oxidative stress markers remains contradictory [50]. Taken together, these data suggest that VD deficiency may intensify cellular oxidative stress, while satisfactory levels of VD, may contribute reducing the oxidative stress. In this way, VD may be able to exert several beneficial contributions, since oxidative stress is related to several pathological conditions.

In addition, analysis was performed to verify if a high solar incidence and a VD-rich diet, together, are associated with a low level of cellular oxidative stress (Figure 1, Tables 2–4). In this context, we found that patients who stayed longer in the sun and consumed VD-rich foods frequently had lower levels of MDA and CG compared to patients who did not stay in the sun long enough and did not consume foods rich in VD regularly. These data, although not confirming the relationship between VD deficiency, lipid peroxidation, and protein oxidation, can be interpreted as

an indication of such relationship, since sunlight directly affects VD_3 synthesis [50–54]. VD is also founded in foods, such as liver, fish, eggs, and milk.

According to Krzywanski J et al. (2016), sunlight exposure can be more efficient than oral supplementation with VD. The equator proximity is a strong stimulus for VD synthesis, that is, efficiency in VD production is due to ultraviolet radiation availability [52]. Additionally, one study showed that the relationship between VDR genotype and VD concentration can be influenced by sunlight exposure [53]. Our results also showed that patients with higher VD concentrations present higher sunlight exposure/VD-rich diet than patients with low levels of VD concentrations (Figure 1, Tables 2–4). However, it is not clear how VD concentrations vary according to changes in solar UV radiation, during different seasons of the year for example. Hypovitaminosis D usually reflects reduced sunlight exposure and this condition can be treated with adequate sunlight exposure [54].

Related to age, some studies showed that elderly patients have reduced VD synthesis due to some endogenous factors [7, 55]. Our analysis, however, did not reveal an inverse and statistically significant relationship between VD concentration and patient age (Figure 2, Tables 2–4), possibly due to the random sample of predominantly elderly patients. In addition, we correlated the VD concentration, oxidative stress and patient's age. We have shown that VD concentration is inversely correlated with MDA concentration. The VD concentration was also inversely correlated with CG concentration (Figure 7, Table 2).

Aging may be associated with several VD-regulated processes, including modifications in gene transcripts, destabilization in mitochondrial energy metabolism, a decline in antioxidant defenses and destabilization in signaling pathways. In addition, VD deficiency results in deregulation of calcium homeostasis, affecting the action potential, which can be finished earlier, an aging characteristic. Finally, increased intracellular calcium stimulates calcineurin, inducing memory loss [7].

We cannot say that VD deficiency is the cause of increased MDA and CG. However, we can demonstrate that the correlation between VD and MDA is stronger than the correlation between VD and CG. That is, VD deficiency is more correlated with lipid peroxidation than with protein oxidation, although both correlations are strong. These analyses can suggest that VD deficiency may be involved with dyslipidemias. Additionally, according the multivariate analyses, the patients' ages and gender are not interfering in our results, that is, the increased oxidative stress is not due to the high patients' ages and gender (Tables 2 and 3).

The effects resulting from VD deficiency may vary according to gender, age, population and VD deficiency degree [56]. The role of VD in oxidative stress prevention

is showed here by VD-25 Hydroxy concentrations and lipid peroxidation/protein oxidation; this role is reinforced by sunlight exposure and the VD-rich diet data. However, it is not clear how VD variates according to changes in solar Ultraviolet radiation. As very few foods, i.e. fish, egg yolks and milk, are rich in vitamin D, the main source of the vitamin is synthesized in the skin in the presence of ultraviolet B rays from sunlight. Therefore, in countries such as Canada, sufficient amounts of VD cannot be synthesized using sunlight during the fall and winter months, unlike Brazil for example. Consequently, there is a concern that many people around the world are not reaching adequate levels of VD, since VD is a prohormone that is essential to good health [57].

In summary, we clearly demonstrated that patients with VD insufficiency have greater lipid peroxidation and protein oxidation when compared with sufficient VD patients. The VD status, the sunlight exposure and a VD-rich diet were strongly and inversely correlated with malondialdehyde and carbonyl group concentrations. Regarding multivariate analyses, lipid peroxidation and/or protein oxidation are affected by vitamin D deficiency. In addition, age and gender did not influence this relationship. Taking together, information demonstrated here suggests that VD deficiency may be related to oxidative stress.

Regarding the strengths of our study, we highlight the power of the sample (99%) due to the participation of 212 patients, in other words, at a given significance level (0.05), the power of the test is increased by having a larger sample size. Moreover, this study was the first to demonstrate that these strong correlations were not being influenced by the age and gender of the patients. Regarding the weakness of the study, it would be important to evaluate the body mass index of participants in order to verify whether patients' body mass index could interfere in oxidative stress. Also, dosing antioxidant enzymes could evidence the relationship between vitamin D and oxidative stress. Finally, it would be interesting to evaluate whether oxidative stress affects vitamin D metabolism.

New studies will be required to establish more relationships between VD deficiency and oxidative stress. Currently, little information is available regarding the molecular antioxidant mechanisms of VD. Data integration involving transcriptional and genomic data in combination to VD measurement in long-term experiments certainly will shed a light on VD role in oxidative stress. New understandings of vitamin D-related advances in metabolomics, transcriptomics, epigenetics, in relation to its ability to control oxidative stress in conjunction with micronutrients, vitamins, and antioxidants, following normalization of serum $25(\text{OH})\text{D}$ and tissue $1,25(\text{OH})_2\text{D}$ concentrations, likely to promise cost-effective better clinical outcomes in humans [27].

References

- Mithal, A., Wahl, D.A., Bonjour, J.P., Burckhardt, P., Dawson-Hughes, B., & Eisman, J.A., et al. (2009) Global vitamin D status and determinants of hypovitaminosis D. *Osteoporos Int.* 20 (11): 1807–1820.
- Naeem, Z. (2010) Vitamin D deficiency – an ignored epidemic. *Int J Health Sci.* 4 (1): 5.
- Alfawaz, H., Tamim, H., Alharbi, S., Aljaser, S., & Tamimi, W. (2014) Vitamin D status among patients visiting a tertiary care center in Riyadh, Saudi Arabia: a retrospective review of 3475 cases. *BMC Public Health.* 14 (1): 159.
- Van Schoor, N.M., & Lips, P. (2011) Worldwide vitamin D status. *Best Pract Res Clin Endocrinol Metab.* 25 (4): 671–680.
- Maeda, S.S., Borba, V.Z., Camargo, M.B.R., Silva, D.M.W., Borges, J.L.C., & Bandeira, F., et al. (2014) Recomendações da Sociedade Brasileira de Endocrinologia e Metabologia (SBEM) para o diagnóstico e tratamento da hipovitaminose D. *Arq Bras Endocrinol Metab.* 58 (5): 411–33.
- Alves, M., Bastos, M., Leitão, F., Marques, G., Ribeiro, G., & Carrilho, F. (2013) Vitamina D – importância da avaliação laboratorial. *Rev Port Endocrinol, Diabetes Metab.* 8 (1): 32–39.
- Berridge, M.J. (2015) Vitamin D cell signalling in health and disease. *Biochem Biophys Res Commun.* 460 (1): 53–71.
- Holick, M.F. (2005) The vitamin D epidemic and its health consequences. *J Nutr.* 135 (11): 2739S–2748S.
- Bhattacharyya, A., Chattopadhyay, R., Mitra, S., & Crowe, S.E. (2014) Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. *Physiol Rev.* 94 (2): 329–354.
- Garcion, E., Nataf, S., Berod, A., Darcy, F., & Brachet, P. (1997) 1, 25-dihydroxyvitamin D3 inhibits the expression of inducible nitric oxide synthase in rat central nervous system during experimental allergic encephalomyelitis. *Mol Brain Res.* 45 (2): 255–267.
- Lin, A.M., Chen, K.B., & Chao, P.L. (2005) Antioxidative effect of vitamin D3 on zinc-induced oxidative stress in CNS. *Ann N Y Acad Sci.* 1053 (1): 319–329.
- Huun, M.U., Garberg, H.T., Escobar, J., Chafer, C., Vento, M., & Holme, I.M., et al. (2018) DHA reduces oxidative stress following hypoxia-ischemia in newborn piglets: a study of lipid peroxidation products in urine and plasma. *J Perinat Med.* 46 (2): 209–217.
- Gawel, S., Wardas, M., Niedworok, E., & Wardas, P. (2004) Malondialdehyde (MDA) as a lipid peroxidation marker. *Wiad. Lek.* 57 (9–10): 453–455.
- Bobilev, I., Novik, V., Levi, I., Shpilberg, O., Levy, J., & Sharoni, Y., et al. (2011) The Nrf2 transcription factor is a positive regulator of myeloid differentiation of acute myeloid leukemia cells. *Cancer Biol Ther.* 11 (3): 317–329.
- Hayes, J.D., & Dinkova-Kostova, A.T. (2014) The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. *Trends Biochem Sci.* 39 (4): 199–218.
- Lee, D.H., Gold, R., & Linker, R.A. (2012) Mechanisms of oxidative damage in multiple sclerosis and neurodegenerative diseases: therapeutic modulation via fumaric acid esters. *Int J Mol Sci.* 13 (9): 11783–11803.
- Tsai, C.W., Lin, C.Y., & Wang, Y.J. (2011) Carnosic acid induces the NAD (P) H: Quinone Oxidoreductase 1 expression in rat clone 9 cells through the p38/Nuclear Factor Erythroid-2 Related Factor 2 Pathway. *J Nutr.* 141 (12): 2119–2125.
- Mitsuishi, Y., Motohashi, H., & Yamamoto, M. (2012) The Keap1-Nrf2 system in cancers: stress response and anabolic metabolism. *Front Oncol.* 2200.
- Kurosu, H., Yamamoto, M., Clark, J.D., Pastor, J.V., Nandi, A., & Gurnani, P., et al. (2005) Suppression of aging in mice by the hormone Klotho. *Science.* 309 (5742): 1829–1833.
- Yamamoto, M., Clark, J.D., Pastor, J.V., Gurnani, P., Nandi, A., & Kurosu, H., et al. (2005) Regulation of oxidative stress by the anti-aging hormone Klotho. *J Biol Chem.* 280 (45): 38029–38034.
- Urakawa, I., Yamazaki, Y., Shimada, T., Iijima, K., Hasegawa, H., & Okawa, K., et al. (2006) Klotho converts canonical FGF receptor into a specific receptor for FGF23. *Nature.* 444 (7120): 770.
- Zeldich, E., Chen, C.D., Colvin, T.A., Bove-Fenderson, E.A., Liang, J., & Zhou, T.B.T., et al. (2014) The neuroprotective effect of Klotho is mediated via regulation of members of the redox system. *J Biol Chem.* 289 (35): 24700–24715.
- Saif-Elnasr, M., Ibrahim, I.M., & Alkady, M.M. (2017) Role of vitamin D on glycemic control and oxidative stress in type 2 diabetes mellitus. *J Res Med Sci.* 22, 22.
- Longoni, A., Kolling, J., Siebert, C., Santos, J.P., Silva, J.S., & Pettenuzzo, L.F., et al. (2017) 1, 25-dihydroxyvitamin D3 prevents deleterious effects of homocysteine on mitochondrial function and redox status in heart slices. *Nutr Res.* 38, 52–63.
- Halicka, H.D., Zhao, H., & Li, J. (2012) Attenuation of constitutive DNA damage signaling by 1, 25-dihydroxyvitamin D3. *Aging (Albany NY).* 4 (4): 270.
- Mokhtari, Z., Hekmatdoost, A., & Nourian, M. (2017) Antioxidant efficacy of vitamin D. *J Parathyroid Dis.* 5 (1): 11–16.
- Wimalawansa, S.J., & Vitamin, D. (2019) Deficiency: Effects on oxidative stress, epigenetics, gene regulation, and aging. *Biology (Basel).* 8 (2): 30.
- Jamilian, M., Mirhosseini, N., Eslahi, M., Bahmani, F., Shokrpour, M., & Chamani, M., et al. (2019) The effects of magnesium-zinc-calcium-vitamin D co-supplementation on biomarkers of inflammation, oxidative stress and pregnancy outcomes in gestational diabetes. *BMC Pregnancy and Childbirth.* 19 (1): 107.
- Dzik, K.P., & Kaczor, J.J. (2019) Mechanisms of vitamin D on skeletal muscle function: oxidative stress, energy metabolism and anabolic state. *Eur J Appl Physiol.* 119 (4): 825–839.
- Holick, M.F., Binkley, N.C., Bischoff-Ferrari, H.A., Gordon, C. M., Hanley, D.A., & Heaney, R.P., et al. (2011) Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 96 (7): 1911–1930.
- Brooks, M.H., Bell, N.H., Love, L., Stern, P.H., Orfei, E., & Queener, S.F., et al. (1978) Vitamin-D-dependent rickets type II: resistance of target organs to 1, 25-dihydroxyvitamin D. *N Engl J Med.* 298 (18): 996–999.
- Dzik, K., Skrobot, W., Flis, D.J., Karnia, M., Libionka, W., & Kloc, W., et al. (2018) Vitamin D supplementation attenuates oxidative stress in paraspinal skeletal muscles in patients with low back pain. *Eur J Appl Physiol.* 118 (1): 143–151.
- Nasri, K., Akrami, S., Rahimi, M., Taghizadeh, M., Behfar, M., & Mazandarani, M.R., et al. (2018) The effects of vitamin D and evening primrose oil co-supplementation on lipid profiles and biomarkers of oxidative stress in vitamin D-deficient women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. *Endocr Res.* 43 (1): 1–10.
- Shymanskyi, I.O., Lisakovska, O.O., Mazanova, A.O., Labudzynski, D.O., Khomenko, A.V., & Veliky, M.M. (2016) Prednisolone and vitamin D3 modulate oxidative metabolism and cell death pathways in blood and bone marrow mononuclear cells. *Ukr Biochem J.* 88 (5): 38–47.
- Wang, E.W., Siu, P.M., Pang, M.Y., Woo, J., Collins, A.R., & Benzie, I.F. (2017) Vitamin D deficiency, oxidative stress and antioxidant status: only weak association seen in the absence

- of advanced age, obesity or pre-existing disease. *Br J Nutr.* 118 (1): 11–16.
36. Anandabaskar, N., Selvarajan, S., Dkhar, S.A., Kamalanathan, S.K., Tamilarasu, K., & Bobby, Z. (2017) Effect of vitamin D supplementation on vascular functions and oxidative stress in type 2 diabetic patients with vitamin D deficiency. *Indian J Endocrinol Metab.* 21 (4): 555.
 37. Javanbakht, M.H., Hamed Mohammady, K.F., Razzaghi, M., Zarei, M., & Djalali, M. (2016) Are serum levels of F2-Isoprostane and oxidized-LDL related to vitamin D status in type 2 diabetic patients? A case-control study. *Rep Biochem Mol Biol.* 5 (1): 26.
 38. Maktabi, M., Jamilian, M., & Asemi, Z. (2018) Magnesium-zinc-calcium-vitamin D co-supplementation improves hormonal profiles, biomarkers of inflammation and oxidative stress in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. *Biol Trace Elem Res.* 182 (1): 21–28.
 39. Nikooyeh, B., & Neyestani, T.R. (2016) Oxidative stress, type 2 diabetes and vitamin D: past, present and future. *Diabetes Metab Res Rev.* 32 (3): 260–267.
 40. Labudzynski, D.O., Zaitseva, O.V., Latyshko, N.V., Gudkova, O.O., & Veliky, M.M. (2015) Vitamin D3 contribution to the regulation of oxidative metabolism in the liver of diabetic mice. *Ukr Biochem J.* 87 (3): 75–90.
 41. Zhu, C.G., Liu, Y.X., Wang, H., Wang, B.P., Qu, H.Q., & Wang, B. L., et al. (2017) Active form of vitamin D ameliorates non-alcoholic fatty liver disease by alleviating oxidative stress in a high-fat diet rat model. *Endocr J.* 64 (7): 663–673.
 42. Almeida, J.P.S., Liberatti, L.S., Barros, F.E.N., Kallaur, A.P., Lozovoy, M.A.B., & Scavuzzi, B.M., et al. (2016) Profile of oxidative stress markers is dependent on vitamin D levels in patients with chronic hepatitis C. *Nutrition.* 32 (3): 362–367.
 43. Farhangi, M.A., Mesgari-Abbasi, M., Hajiluian, G., Nameni, G., & Shahabi, P. (2017) Adipose tissue inflammation and oxidative stress: the ameliorative effects of vitamin D. *Inflammation.* 40 (5): 1688–1697.
 44. Farhangi, M.A., Nameni, G., Hajiluian, G., & Mesgari-Abbasi, M. (2017) Cardiac tissue oxidative stress and inflammation after vitamin D administrations in high fat-diet induced obese rats. *BMC Cardiovasc Disord.* 17 (1): 161.
 45. Hajiluian, G., Abbasalizad Farhangi, M., Nameni, G., Shahabi, P., & Megari-Abbasi, M. (2018) Oxidative stress-induced cognitive impairment in obesity can be reversed by vitamin D administration in rats. *Nutr Neurosci.* 21 (10): 744–752.
 46. Bhat, M., & Ismail, A. (2015) Vitamin D treatment protects against and reverses oxidative stress induced muscle proteolysis. *J Steroid Biochem and Mol Biol.* 152, 171–179.
 47. Uberti, F., Lattuada, D., Morsanuto, V., Nava, U., Bolis, G., & Vacca, G., et al. (2014) Vitamin D protects human endothelial cells from oxidative stress through the autophagic and survival pathways. *J Clin Endocrinol Metab.* 99 (4): 1367–1374.
 48. Alvarez, J.A., Grunwell, J.R., Gillespie, S.E., Tangpricha, V., & Hebbbar, K.B. (2018) Vitamin D deficiency is associated with an oxidized plasma cysteine redox potential in critically ill children. *J Steroid Biochem Mol Biol.* 175, 164–169.
 49. Krivošíková, Z., Gajdoš, M., & Šebeková, K. (2015) Vitamin D levels decline with rising number of cardiometabolic risk factors in healthy adults: association with adipokines, inflammation, oxidative stress and advanced glycation markers. *PLoS one.* 10 (6): e0131753.
 50. Souza, C.S., & Vieites, F.M. (2014) Vitamina D3 e seus metabólitos para frangos de corte. *Arch Zootec.* 63 (241): 11–24.
 51. Webb, A.R., Aseem, S., Kift, R.C., Rhodes, L.E., & Farrar, M.D. (2016) Target the message: a qualitative study exploring knowledge and cultural attitudes to sunlight and vitamin D in greater Manchester. UK. *Br J Dermatol.* 175 (6): 1401–1403.
 52. Krzywanski, J., Mikulski, T., Krysztofiak, H., Mlynczak, M., Gaczynska, E., & Ziemia, A. (2016) Seasonal vitamin D status in polish elite athletes in relation to sun exposure and oral supplementation. *PLoS One.* 11 (10): e0164395.
 53. Livingstone, K.M., Celis-Morales, C., Hoeller, U., Lambrinou, C. P., Moschonis, G., & Macready, A.L., et al. (2017) Weekday sunlight exposure, but not vitamin D intake, influences the association between vitamin D receptor genotype and circulating concentration 25-hydroxyvitamin D in a pan-European population: the food4me study. *Mol Nutr Food Res.* 61 (2): 1600476.
 54. Uwitonze, A.M., & Razzaque, M.S. (2018) Role of magnesium in vitamin D activation and function. *J Am Osteopath Assoc.* 118 (3): 181–189.
 55. Ribeiro, A.F.D.C., Serakides, R., Nunes, V.A., Silva, C.M.D., & Ocarino, N.D.M. (2003) Osteoporosis and the endocrine disturbances of thyroid and gonads. *ABE&M.* 47 (3): 228–236.
 56. Hooshmand, B., Lökk, J., Solomon, A., Mangialasche, F., Miralbell, J., & Wahlund, L.O., et al. (2014) Vitamin D in relation to cognitive impairment, cerebrospinal fluid biomarkers, and brain volumes. *J Gerontol A Biol Sci Med Sci.* 69 (9): 1132–1138.
 57. McGee, M. (2019) Vitamin D: insufficiency, uncertainty and achievability. *Int J Vitam Nutr Res.* 13, 1–4.

History

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper. The authors declare they have received written informed consents signed by each research participant.

Publication ethics

All experimental procedures were approved by Human Research Ethics Committee at Federal University of Rio Grande do Norte (CEP/UFRN No. 67240817.3.0000.5537) and a written informed consent was obtained from all patients.

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