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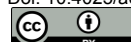Universidade Estadual de Maringá
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CIÊNCIAS DA SAÚDE / HEALTH SCIENCES

Study of the relationship between vitamin D deficiency, sunlight incidence and skeletal/extra skeletal diseases

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ABSTRACT. It is estimated that more than 1 billion people worldwide have vitamin D insufficiency or deficiency. Vitamin D participates in bone mineralization, and is therefore important in osteoporosis, osteomalacia and rickets prevention. However, vitamin D deficiency could also be associated with several other pathologies. The present study aimed to investigate the relationships between vitamin D deficiency and vitamin D deficiency-related disorders in patients. In addition, this study aims to verify if countries with low solar incidence have higher extraskeletal disease death rates when compared to countries with high solar incidence. The vitamin D concentrations were obtained from the Heart Hospital database (Natal/Brazil). The relationship between solar incidence and death rate for vitamin D deficiency-related disorders was verified. Death rate data were extracted from the 'World Life Expectancy' repository and data about solar incidence were obtained from NASA's Surface Meteorology and Solar Energy project. These data were statistically processed with IBM SPSS v23.0 software and R programming language. Our results showed that patients with vitamin D insufficiency/deficiency showed significantly more bone diseases, thyroid diseases, hypercholesterolemy, hypertriglyceridemia, cancers, diabetes, hepatobiliary diseases, and urinary system diseases. Moreover, countries with high solar incidence have low cancer and multiple sclerosis death rates. This work suggests the participation of vitamin D and sunlight incidence in several diseases.

Keywords: hypercholesterolemy; hypertriglyceridemia; cancer; diabetes; hepatobiliary diseases; urinary system diseases.

Introduction

Vitamin D (VD) has a unique metabolism, mainly being obtained through synthesis in the skin under the influence of sunlight, whereas intake by nutrition traditionally plays a relatively minor role (Pliz et al., 2018). VD is an essential nutrient with hormone-like activity which regulates calcium and bone metabolism. It is known that non-skeletal cells express vitamin D receptor (VDR), and as such interact with the active form of vitamin D, i.e. 1,25-dihydroxyvitamin D. Furthermore, most of these cells express extra-renal forms of 1α-hydroxylase enzyme, and the active form of vitamin D is known to regulate quite a number of genes, including those implicated in proliferation, differentiation, and apoptosis. It has recently been described that the pathophysiology of autoimmune thyroid disease, adrenal disease, and hyperparathyroidism has a VD component (Muscogiuri & Bhattoa, 2018).

VDR are present in numerous tissues such as the gut, renal tubules, bone, pancreas, skin, brain, prostate, breast, colon, immune system cells, hematopoietic cells, and muscle cells (Holick, 2007; Mandarino, Monteiro Júnior, Salgado, Lages, & Salgado Filho, 2015). Vitamin D plays a role in regulating cell proliferation and differentiation and in modulating the innate and adaptive immune response. In addition, vitamin D correlates with changes in cytokines and prevents inflammation induced by changes in myometrial cells mediated by the nuclear factor kappa B pathway (Kjalarsdottir et al., 2019). In addition, vitamin D regulates calcium influx through the R-Type voltage-gated calcium channel during glucose-stimulated insulin secretion, thereby modulating the capacity of beta cells to secrete insulin (Momentti, Estadella, & Pisani, 2018).

Vitamin D is also involved in promoting peripheral tolerance and limiting chronic inflammatory diseases. Renal transplant recipients are likely to have low vitamin D levels, which may influence their

immune status (Myszka et al., 2018). Recent studies have examined the role of vitamin D in immunomodulation, including the development and regulation of iNKT cells. Indeed, iNKT cells and CD4/CD8 intraepithelial lymphocytes are developmentally and functionally dependent on sufficient levels of vitamin D. Although the effects of vitamin D on iNKT cell development have been elucidated, it is not clear as to whether vitamin D deficiency (or VDR knockout) results in a proinflammatory or anti-inflammatory state (Smyk et al., 2018). As a result, vitamin D can be related to many diseases.

Vitamin D deficiency can lead to musculoskeletal diseases such as rickets and osteomalacia, but vitamin D supplementation may also prevent extra skeletal diseases such as respiratory tract infections, asthma exacerbations, pregnancy complications, and premature deaths (Pliz et al., 2018). Moreover, the genetic variations of VDR have revealed their association with the risk of metabolic syndrome (Karuwanarint et al., 2018). Relatively high rates of subclinical vitamin D deficiency have been reported in infants, children, and adolescents in several studies, especially from low-income countries (Salerno et al., 2018). Studies have shown that a high prevalence of vitamin D deficiency was observed associated with metabolic disorders in both obese adults and adolescents. Therefore, it is necessary to develop strategies for the prevention and control of obesity and vitamin D deficiency (Teixeira et al., 2018).

Numerous drugs with vitamin D activity are available for clinical use, and it may not be easy for a non-specialist to select the most suitable drugs for individual patients. The clinical activity of some vitamin D analogs is such that they can be employed in diseases like cancer and autoimmunity. Available drugs with a vitamin D-like activity do not have the same pharmacological actions and present side effects. They have specific characteristics which may be useful to know in order to select the best choice to prescribe to individual patients (Mazzaferro, Goldsmith, Larsson, Massy, & Cozzolino, 2014). In view of the above, this study aims to verify a relationship between vitamin D levels and some disorders in order to generate additional information about vitamin D protector effects.

Material and methods

Patient analyses

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 received from all patients. Patients from the Heart Hospital (HCOR/Natal/RN/Brazil) were invited to participate and the invited patients were those who were submitted to vitamin D dosage. Hospitalized patients were not included in the study.

Next, the participants answered a questionnaire. The experimental group included patients with low levels of vitamin D, meaning people with vitamin D insufficiency (less than 30 ng mL^{-1}) or deficiency (less than 20 ng mL^{-1}). The control group included patients with normal or sufficient levels of vitamin D (greater than 30 ng mL^{-1}). [14] A total of 212 patients participated in the study, in which 157 were women and 55 were men. Patient ages ranged from 13 to 97 years.

Blood samples for measuring 25-hydroxyvitamin D

The blood samples were collected during patients' routine exams in August/September 2017. Tubes (5ml) without anticoagulant were used for vitamin D dosage. The dosage was performed by 'HCOR' pharmacists using Chemiluminescent Microparticle Immunoassay (CMIA) and serum samples. CMIA measures the vitamin D status according to international standards. The results were provided by the 'HCOR' hospital without financial interests. We used 25-hydroxyvitamin D in this study because it is the most abundant and the most stable metabolite. Its circulating levels directly reflect the vitamin D ingested and/or synthesized. We did not use 1,25-dihydroxyvitamin D because it has a 1000 times lower blood concentration than 25-hydroxyvitamin D and a short half-life. Therefore 1,25-dihydroxyvitamin D is not indicated in the vitamin D sufficiency assessment. Patients with concentrations less than 29.9 ng mL^{-1} are considered vitamin D deficient/insufficient (experimental group). Concentrations between 30 and 100 ng mL^{-1} were considered sufficient (control group). This cut-off was used for both pediatric and adult populations, as described by Holick et al., 2011.

Country analyses

Data selection: Disease death rate

The death rate data relating to Coronary Heart disease, Diabetes Mellitus, Parkinson's disease, cancers, multiple sclerosis, and hypertension (number of deaths per 100,000 people) were obtained from the 'World Life Expectancy' repository (www.worldlifeexpectancy.com) in 2017. World life expectancy contains data related to the death rate for several diseases in many countries around the world. The repository has data from 172 countries. All of them were used in the analysis.

Data selection: Life expectancy

Life expectancy data from the 172 countries were obtained from the 'World Life Expectancy' (WLE) repository (www.worldlifeexpectancy.com) in 2017. Data available in WLE were provided by the World Health Organization, the World Bank, and the United Nations. A life expectancy average for men and women was calculated. Life expectancy was considered at 25 years.

Data selection: Solar incidence

Solar incidence data were obtained from 'NASA's Atmospheric Science Data Center' (https://eosweb.larc.nasa.gov/). The geographical coordinates of the five most populous cities of each country were collected and then the solar incidence was retrieved for each city. The annual solar incidence of a country was obtained by the average annual solar incidence of the five most populous cities in the country. Solar incidence was used to represent the VD levels in many countries.

Statistical analyses

Data were analyzed using IBM SPSS 23.0 (SPSS Inc.) statistical software for Windows. We used the Kolmogorov-Smirnov test (large samples) to test the distribution normality. The Mann-Whitney test and the Spearman correlation coefficient were used for the statistical analyses because the analyzed variables (non-parametric) did not show normal distribution. Values of $p<0.05$ were considered as the criterion for significance.

Results

Vitamin D deficiency and associated conditions

A total of 143 people had vitamin D sufficiency in this study, while 69 had vitamin D deficiency/insufficiency. In other words, 33% of participants did not have satisfactory levels of vitamin D. Patients from 13 to 97 years participated in the study; however, only one patient (0.47%) was not an adult (under 18 years old). The mean age in the population was 61 years. In addition, 55 men and 157 women were randomly included in the study. The vitamin D mean in the male group was 38.65 ng mL^{-1}, while it was 35.5 ng mL^{-1} in the female group. The mean age in the male group was 64 years, and the mean age in the female group was 60 years. There was no statistical difference regarding vitamin D concentrations between the male and female groups ($p>0.05$).

According to the data from NASA, there are no significant seasonal variations in Natal/Brazil and the solar incidence is almost constant throughout the year (NASA Earth Data, 2019). Thus, we evaluated the relationship between some metabolic disorders/diseases, and vitamin D concentrations in 'HCOR' patients (August, 2017). The presence of hypercholesterolemy, hypertriglyceridemia, hypertension, cardiovascular diseases, type 2 diabetes mellitus or insulin resistance, thyroid diseases, cancer, skeletal diseases, neurological diseases, hepatobiliary diseases, and urinary system diseases was verified.

The presence of hypercholesterolemy and hypertriglyceridemia were confirmed by consulting the patients' exams in the HCOR database and the presence of hypertension and specific diseases were confirmed by medical diagnosis. The number of patients with each disorder was measured and we demonstrated the number and percentage of patients with specific disorders in the vitamin D sufficient and deficient groups (Figures 1, 2). According to Figures 1 and 2, patients with vitamin D deficiency had significantly more bone diseases, thyroid diseases, hypercholesterolemy, hypertriglyceridemia, cancers, diabetes, hepatobiliary diseases, and urinary system diseases when compared to patients with vitamin D sufficiency (Table 1, Mann-Whitney and Spearman correlation, tests $p < 0.05$).
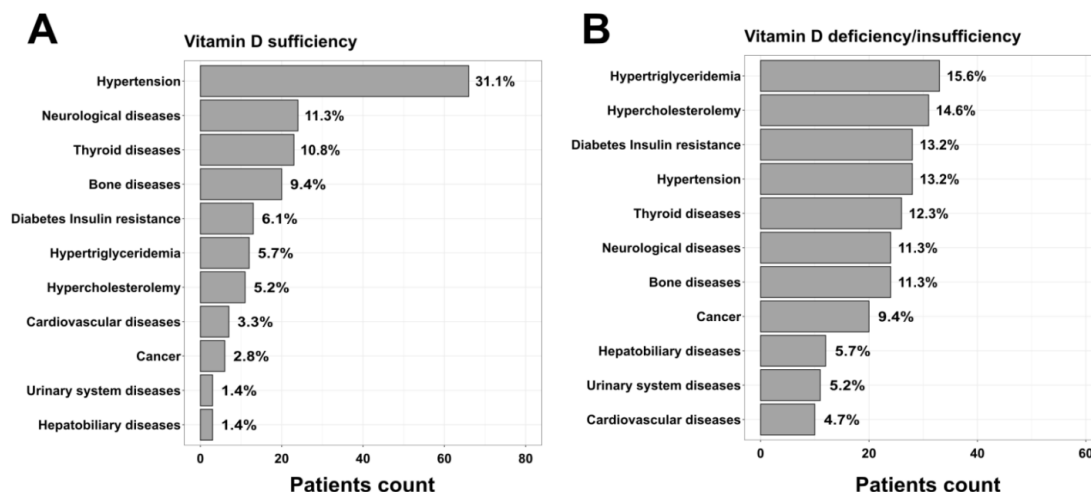
Figure 1. Distribution of patients with specifics disorders. A) Number and percentage of patients with vitamin D sufficiency (n=143) B) Number and percentage of patients with vitamin D insufficiency/deficiency (n=69). Asterisk marks on bars represent the statistical difference between sufficient and insufficiency/deficiency groups regarding each disorder. *p < 0.05 when comparing the 'sufficiency' and 'insufficiency/deficiency' groups (Wilcoxon-Mann-Whitney test). Charts were generated using R language.
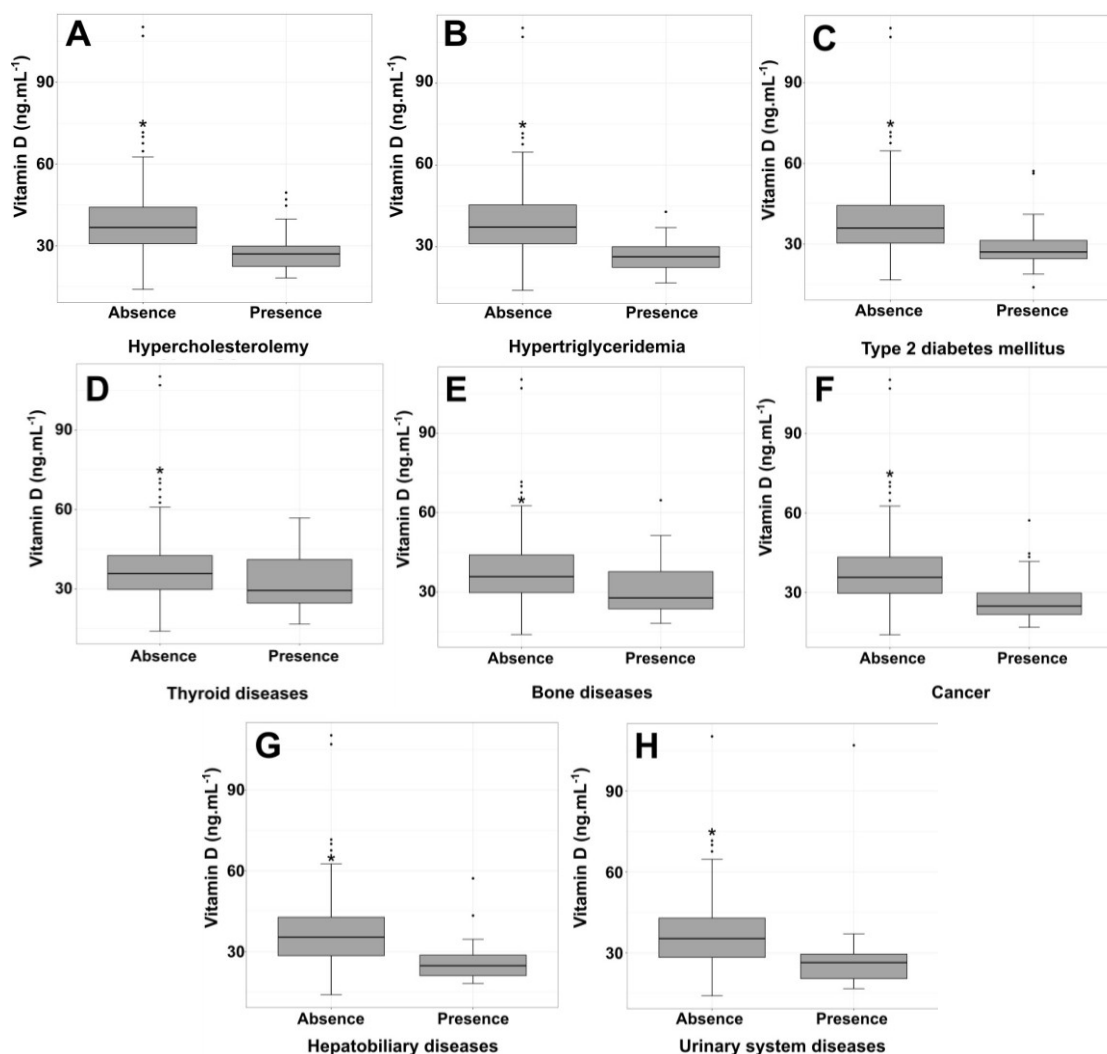

Figure 2. Distribution of vitamin D concentrations (ng mL^{-1}) in patients with (presence) or without (absence) specific diseases. Analyses were performed using 212 patients and 8 diseases groups a) Hypercholesterolemy b) Hypertriglyceridemia c) Type 2 Diabetes Mellitus d) Thyroid diseases e) Bone diseases f) Cancer g) Hepatobiliary diseases h) Urinary system diseases. Boxes represent the IQR, the line inside it represents the median, and the bottom and top lines of the box are the first and the third quartiles, respectively. Whiskers limits are the lowest and the highest observation within 1.5 of IQR from the lower and upper quartiles. The black dots represent the outliers. *p < 0.05 (Wilcoxon-Mann-Whitney test) when compared with respective 'Low' group. Statistics and charts were generated using SPSS v23.0 and R programming language, respectively. IQR, interquartile range.

Table 1. Disorder, number of patients with each disorder per groups (VD sufficiency (High) or VD deficiency (Low)), p-values regarding the significance between the 'high' and 'low' groups and disorders, and rho number regarding the correlations between the disorders and VD levels.

Disorder	Number of patients per vitamin levels	p value (Mann-Whitney)	rho (Spearman correlation)	p value (Spearman correlation)
Hypercholesterolemy	High-11 Low-31	<0.05*	-0.437	<0.05*
Hypertriglyceridemia	High-12 Low-33	<0.05*	-0.451	<0.05*
Hypertension	High-.66 Low-28	0.44	0.052	0.44
Cardiovascular disease	High-7 Low-10	0.066	-0.165	0.075
Diabetes Mellitus 2	High-13 Low-28	<0.05*	-0.373	<0.05*
Thyroid diseases	High-23 Low-26	<0.05*	-0.240	<0.05*
Cancer	High-6 Low-20	<0.05*	-0.354	<0.05*
Bone disease	High-20 Low-24	<0.05*	-0.240	<0.05*
Neurological disease	High-24 Low-24	0.073	-0.201	0.063
Hepatobiliary diseases	High-3 Low-12	<0.05*	-0.279	<0.05*
Urinary system diseases	High-3 Low-11	<0.05*	-0.261	<0.05*

*$p < 0.05$. Statistics were generated using SPSS v23.0.

Solar incidence (Sunlight) and Vitamin D-related disorders

The average vitamin D concentration is not known in many countries, making an epidemiological analysis on a large-scale difficult. There were no large-scale data available about serum vitamin D concentrations in patients with coronary heart disease, diabetes mellitus, Parkinson's disease, cancers, hypertension, or multiple sclerosis (MS). Therefore, we did a supplementary analysis to investigate the relationship between the death rates of these diseases and the average annual solar incidence of 172 countries for which data were available. Solar incidence ranges from 2.12 kWh m^{-2} day^{-1} in Iceland to 6.37 kWh m^{-2} day^{-1} in Niger. The median (5 kWh m^{-2} day^{-1}) was used to classify the countries into two groups: those with average solar incidence above the median (high incidence group, containing 106 countries) and those with the average solar incidence below the median (low incidence group, containing 106 countries).

According to Figure 3, countries with high solar incidence have significantly lower cancers and MS death rates than countries with low solar incidence ($p < 0.05$). Regarding cancers, we observed 92.48 deaths per 100,000 people on average in high sunlight countries and 124.85 deaths per 100,000 people on average in low sunlight countries. Regarding MS, 0.21 deaths per 100,000 people on average can be observed in high sunlight countries, and 0.46 deaths per 100,000 people on average in low sunlight countries. Both analyses including hospital patients and analyzes including countries showed no relationship between vitamin D deficiency and cardiovascular disease, hypertension, and Parkinson's disease.

In addition, we evaluated the life expectancy of the 172 analyzed countries to evaluate if the cancer death rate could be biased by life expectancy. We analyzed the subgroups of countries with life expectancy below and equal to or above the median (75 years). Figure 4a shows that low solar incidence countries have a significantly greater cancer death rate when compared with high solar incidence countries, even when we only analyzed the high life expectancy countries (greater than 75 years). Figure 3b shows that low solar incidence countries have a significantly greater cancer death rate when compared with high solar incidence countries when we only analyzed the low life expectancy countries (lower or equal than 75 years).
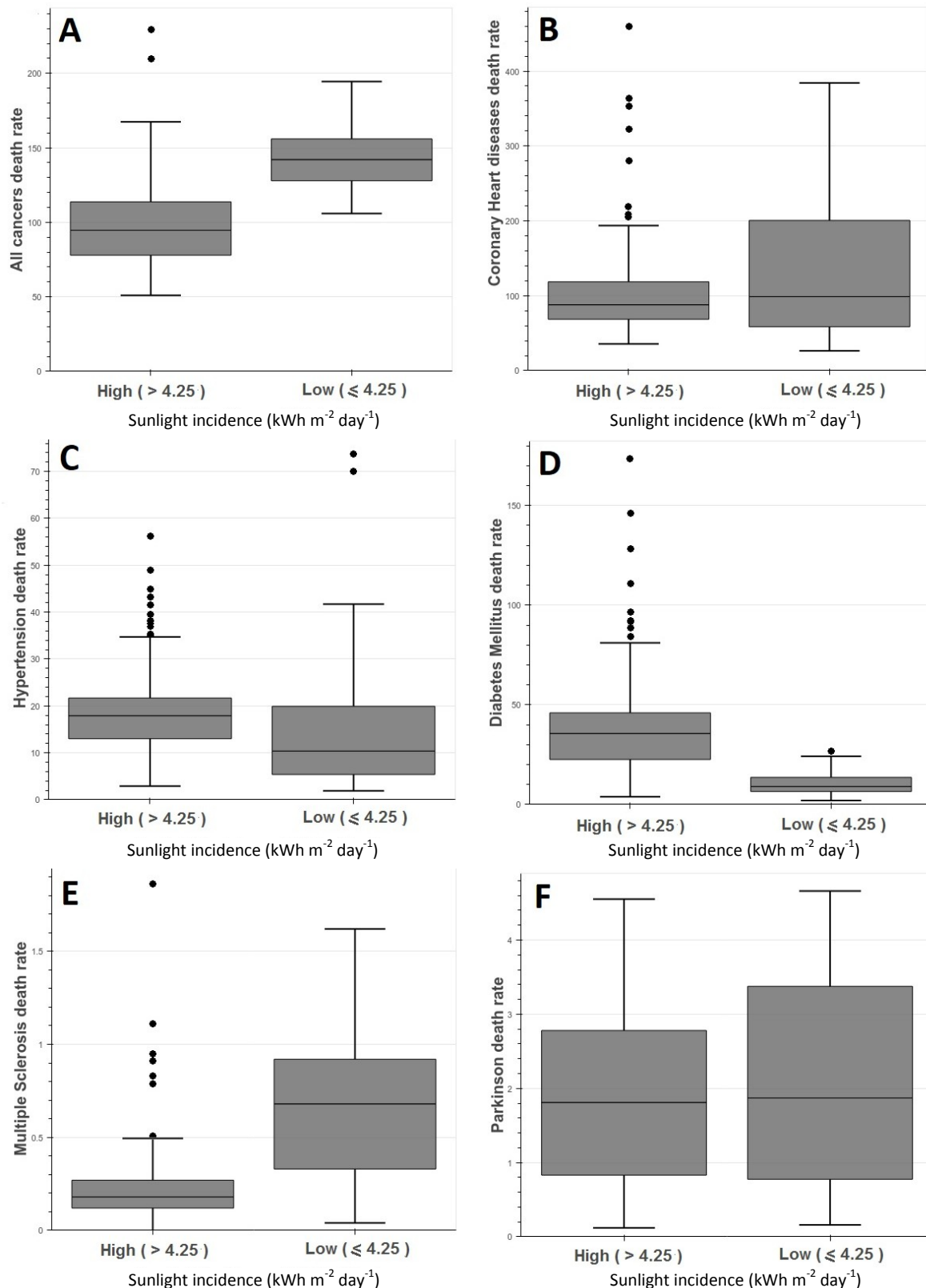
Figure 3. Diseases death rates in countries with high (n=86) and low (n=86) sunlight incidence. Distribution of diseases death rates per 100.000 habitants in countries with average annual solar incidence greater than 5 kWh m^{-2} day^{-1} (high) and equal or lower than 5 kWh m^{-2} day^{-1} (low). Analyses were performed using 172 countries present at World Life Expectancy repository. a) All cancers death rate b) Coronary Heart disease death rate c) Hypertension death rate d) Diabetes Mellitus death rate e) Multiple Sclerosis death rate f) Parkinson death rate. Boxes represent the IQR, the line inside it represents the median, and the bottom and top lines of the box are the first and the third quartiles, respectively. Whiskers limits are the lowest and the highest observation within 1.5 of IQR from the lower and upper quartiles. The black dots represent the outliers. *p < 0.05 when compared to 'Low' group (Wilcoxon-Mann-Whitney test) when comparing to respective low group. Statistics and charts were generated using SPSS and R programming language, respectively. IQR, interquartile range.
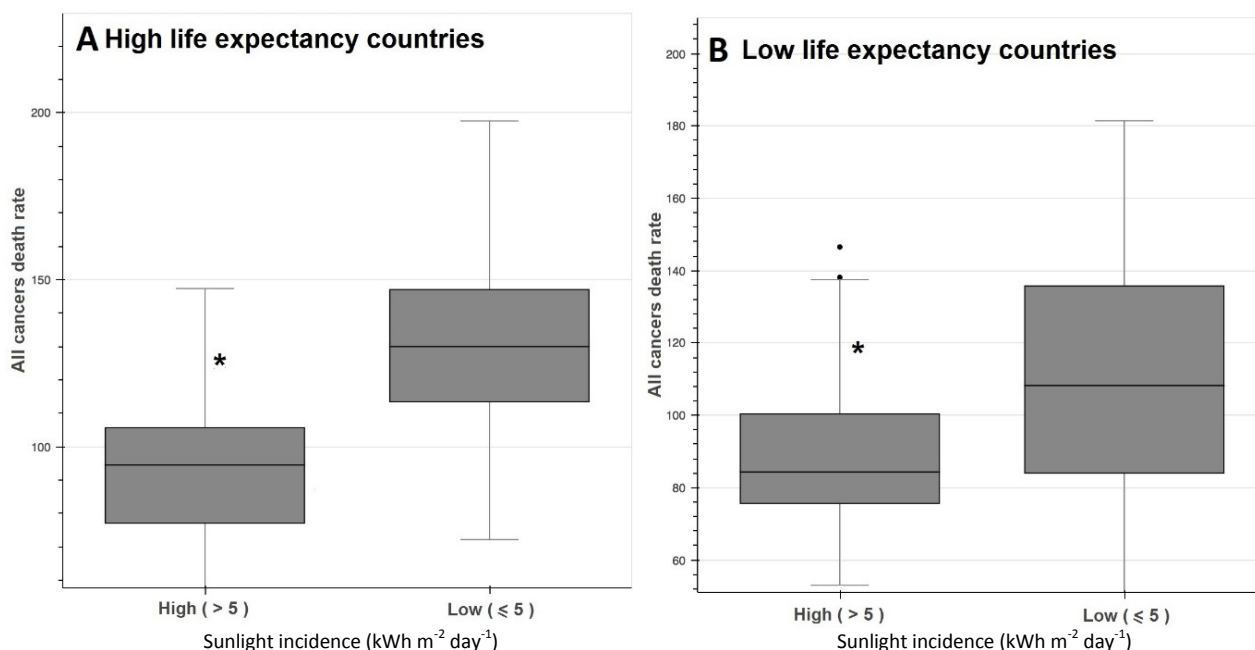
Figure 4. Diseases death rates in countries with high (n=86) and low (n=86) sunlight incidence. Distribution of diseases death rates per 100.000 habitants in countries with average annual solar incidence greater than 5 kWh m^{-2} day^{-1} (high) and equal or lower than 5 kWh m^{-2} day^{-1} (low). Analyses were performed using 172 countries present at World Life Expectancy repository. a) All cancers death rate in high expectancy life countries b) All cancers death rate in low expectancy life countries. Boxes represent the IQR, the line inside it represents the median, and the bottom and top lines of the box are the first and the third quartiles, respectively. Whiskers limits are the lowest and the highest observation within 1.5 of IQR from the lower and upper quartiles. The black dots represent the outliers. *p < 0.05 when compared to 'Low' group (Wilcoxon-Mann-Whitney test) when comparing to respective low group. Statistics and charts were generated using SPSS and R programming language, respectively. IQR, interquartile range.

Discussion

Regarding the association between vitamin D and diseases, clinical and epidemiological studies are still controversial and randomized control trials in humans do not yet exist to conclusively support a beneficial effect of vitamin D in health (Feldman, Krishnan, Swami, Giovannucci, & Feldman, 2014). A study suggested that vitamin D deficiency potentially increases the risk for diseases caused by higher adiposity and oxidative stress (Zhang et al., 2014). Our analyses have shown that vitamin D insufficiency/deficiency is significantly prevalent in patients with hypercholesterolemy, hypertriglyceridemia, cancer, diabetes, hepatobiliary diseases and urinary system diseases (Figure 1, 2), which may be related to increased oxidative stress in some patients. However, further studies are necessary in order to understand the molecular mechanisms for which vitamin D deficiency may influence the onset of these diseases.

As in our study (Figure 1, 2), other studies support an association between vitamin D and cholesterol levels (Cutillas-Marco, Prosper, Grant, & Morales-Suárez-Varela, 2013). Vitamin D supplementation in pregnant women with gestational diabetes mellitus had beneficial effects on glycemia, total-cholesterol, and LDL-cholesterol concentrations, but did not affect inflammation and oxidative stress (Asemi, Hashemi, Karamali, Samimi, & Esmaillzadeh, 2013). Vitamin D also protected against atherosclerosis in hypercholesterolemic swine by controlling cholesterol efflux, macrophage polarization, and increasing the CYP27A1 activation (Yin et al., 2015). VDR activation increases CYP7A1 expression to decrease cholesterol levels (Chow et al., 2014). Additionally, vitamin D supplementation might improve serum lipid concentrations in statin-treated patients with hypercholesterolemy. Moreover, Vitamin D might be an adjuvant therapy for patients with hypercholesterolemy (Qin, Zhao, Chen, Yin, & Wang, 2015).

In addition, low vitamin D levels appear to be associated with hypertriglyceridemia, insulin resistance, and metabolic syndrome in obese patients (Jiang, Peng, Chen, Wu, & Zhang, 2019). There is a possible beneficial contribution of plasma vitamin D in the hypertriglyceridemia pathogenesis through a decrease in inflammation (Guasch et al., 2012). Low serum vitamin D concentrations are associated with high triglyceride levels (Rodríguez-Rodríguez, Ortega, González-Rodríguez, & López-Sobaler, 2011); this is also shown in our work (Figures 1, 2). A study found that decreased vitamin D levels are associated with

increased insulin resistance, triglyceride levels, and blood pressure in older Korean adults, and confirmed the risk of 'hypertriglyceridemia' in vitamin D deficient subjects (Park et al., 2012).

In addition to the relationships between vitamin D and metabolic diseases, epidemiologic data also suggest that the incidence and severity of many types of cancer inversely correlate with indices of vitamin D status. Vitamin D regulates numerous cellular pathways which could have a role in determining cancer risk and prognosis. Although epidemiological and early clinical trials are inconsistent, results from preclinical and some clinical studies strongly suggest that vitamin D deficiency increases the risk of developing cancer (Feldman et al., 2014). The VDR is expressed in epithelial cells at risk for carcinogenesis, including cells in skin, breast, prostate, and colon, providing a direct molecular link by which vitamin D status impacts carcinogenesis (Welsh, 2012).

Furthermore, vitamin D associated polymorphisms were associated with colorectal, breast, and prostate cancer risk (Ordóñez-Mena & Brenner, 2014). New studies are needed to answer important questions which remain about the timing of VD exposure in relation to cancer etiology during the life span (e.g. adolescence or adulthood), the VD dose-response/optimal levels required for the most benefits, and in which stages of carcinogenesis (incidence or progression) VD is most relevant (Giovannucci, 2008).

There is a high prevalence of vitamin D deficiency in children with non-alcoholic fatty liver disease; however, no association was found between vitamin D deficiency and the severity of the disease on biopsies. This differs from adult studies in which vitamin D deficiency correlates with histological severity, suggesting diff erences in the risk factors for consequences of pediatric non-alcoholic fatty liver disease (Hourigan et al., 2015). Another study showed that vitamin D levels are inversely associated with non-alcoholic steatohepatitis in children with non-alcoholic fatty liver disease (Nobili et al., 2014). These data corroborate with those observed in our study, in which we observed that vitamin D status was inversely related to the incidence of liver diseases (Figures 1, 2). Further studies are needed to determine whether patients at risk of developing impaired liver function should be screened for vitamin D deficiency for preventive purposes (Skaaby et al., 2014). Although the exact vitamin D mechanisms have not been fully elucidated in chronic liver diseases, VD can be beneficial in treating these disorders; therefore, new studies are needed to validate VD clinical application (Chen, Shi, & Tang, 2014).

Another group in which a high prevalence of VD deficiency is observed is the adult and pediatric patients' with chronic kidney disease (CKD), as one of the characteristics of CKD is that it can be caused by VD metabolism deregulation (Dusso, González, & Martin, 2011). A recent meta-analysis found an association between treatment with active VD and decreased mortality in patients with CKD (Bover et al., 2015). The presence of CKD and diabetes mellitus could be associated with resistance to correct the vitamin D deficiency with therapy. The underlying mechanism needs to be evaluated in prospective studies. Furthermore, the disturbed signaling of VDR has been demonstrated in patients with cardiac disorders and chronic kidney disease ((Alshayeb, Wall, Showkat, Quarles, & Mangold, 2013). Finally, some findings suggest that glomerular filtration rate and parathyroid hormone are significantly associated with VD metabolism in men without CKD (Karhapää et al., 2012). No significant statistical differences were observed in relation to neurological diseases, cardiovascular diseases or hypertension (Figure 1, 2). However, other mechanisms, pathways, and mediators in addition to vitamin D are involved with the pathophysiology of neurological diseases, cardiovascular diseases, and hypertension. Overall, vitamin D deficient patients have significantly more illnesses or disorders when compared to sufficient vitamin D patients ($p < 0.05$).

Moreover, we found relationships between bone diseases, thyroid diseases, and VD levels. However, it is important to highlight that findings from recent meta-analyses regarding vitamin D supplementation without co-administration of calcium have not shown fracture prevention, possibly because of inappropriate doses or because the intervention was not targeted to deficient populations. Almost half of older adults (older than 50 years) continue to use VD supplements. The widespread use of vitamin D for osteoporosis prevention without specific risk factors for VD deficiency seems to be inappropriate (Reid, Bolland, & Grey, 2014). Supplementation with low doses of vitamin D in children additionally did not improve bone mineral density (Milart, Jobs, Tłustochowicz, Pogonowska, & Kalicki, 2018).

Furthermore, we evaluated whether countries with low solar incidence have higher mortality rates by cancer, diabetes mellitus, coronary heart disease, multiple sclerosis, hypertension, and Parkinson's disease when compared to countries with high solar incidence (Figure 3). Our results showed that countries with

low annual solar incidence have significantly higher cancer death rates and multiple sclerosis death rates. This may be due to the participation of vitamin D as a regulator of cellular oxidative stress (Mokhtari, Hekmatdoost, & Nourian, 2017) and the immune system (Yang, Leung, Adamopoulos, & Gershwin, 2013). The sunlight can reflect the VD concentrations because our analysis is consistent with a possible variation in serum vitamin D concentration between countries. Besides, an extra analysis (Figure 4) showed that low solar incidence countries have significantly greater cancer deaths rate when compared with high solar incidence countries, even when we analyzed both high and low life expectancy countries apart (See Chart in the Supplementary Material). In this way, the life expectancy is not significantly biasing our cancer analyses.

Although the precise causes of multiple sclerosis remain unknown, some evidence points towards hypovitaminosis D. Vitamin D is an immunomodulation factor and has a potential to be effective in preventing and treating autoimmune diseases, including MS (Collongues, Patte-Mensah, De Seze, Mensah-Nyagan, & Derfuss, 2018). Studies point to a participation of vitamin D in inducing T regulator cell and inhibiting Th1 and Th17 cells, acting in the pathogenesis of autoimmune processes (Altieri et al., 2017). In fact, epidemiological studies linking vitamin D to MS range from half-century-old findings of latitude gradients and emigrational risk patterns to modern, nested, case-control bio bank studies. These observations show an association although causation has yet to be proven (Salzer, Biström, & Sundström, 2014).

Alterations in the oxidant/antioxidant balance and lower vitamin D levels may contribute to the pathophysiology of MS (Polachini et al., 2016). There is epidemiological evidence that the risk of developing MS is increased in association with low levels of sun exposure, possibly because this is associated with low vitamin D status (Lucas, Byrne, Correale, Ilschner, & Hart, 2015). Another study suggested a protective effect from sun exposure and/or vitamin D in MS, as our study suggests. These data include geographic variations in MS occurrence, temporal trends, genetics, biobank, and questionnaire data (Salzer et al., 2014). To sum up, vitamin D deficiency is considered a risk factor for MS; however, there is no direct evidence for the effects of vitamin D on MS progression (Alharbi, 2015).

Related to cancer, the sunlight was inversely correlated with mortality rates for breast, colon, esophageal, gastric, and rectal cancers. In addition, low cancer survival rates may be due to lower serum 25-hydroxyvitamin D for black Americans. This may be attributed to low vitamin D production from solar ultraviolet-B irradiance due to darker skin (Grant, 2006). In another work, our group demonstrated that countries with high solar incidence have significantly lower AD death rates when compared to countries with low solar incidence, suggesting that vitamin D deficiency is involved with Alzheimer's disease progression (Câmara, Souza, & Dalmolin, 2018). Optimal serum VD levels are reported to be associated with many health benefits; however, few studies have determined predictive factors using national-level data. In another study, the strongest predictor of vitamin D inadequacy was non-Hispanic Black ethnicity. Other potential predictors included smoking, vitamin D supplements, collecting samples in winter, female gender, and lack of health care, among others (Lee, Lee, Maneno, Johnson, & Wutoh, 2019).

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. The region in which the study was conducted can be considered another strength since there are no significant seasonal variations in Natal/Brazil and the solar incidence is almost constant throughout the year (NASA Earth Data, 2019). New analyses are required to evaluate the role of VD deficiency in the disorders mentioned here and others. However, the numerous causal factors of some diseases, such as viruses or drugs, make it even more difficult to evaluate the influence of vitamin D on some diseases. Additionally, the exact role of VD deficiency in the establishment and progression of several diseases is not yet well elucidated and further research is essential to uncover the molecular mechanisms for which vitamin D may be involved in the onset and/or development of these diseases.

Conclusion

According to this research and literature, vitamin D insufficiency/deficiency can be associated with bone diseases, thyroid diseases, hypercholesterolemy, hypertriglyceridemia, cancers, diabetes, hepatobiliary diseases, and urinary system diseases. The role of vitamin D in cancers and Multiple Sclerosis can be reinforced by sunlight data. In addition, we suggest that the role of vitamin D in cancers is not influenced by

life expectancy. However, further research is necessary to elucidate some vitamin D actions. These studies may bring some therapeutic contributions to several diseases.
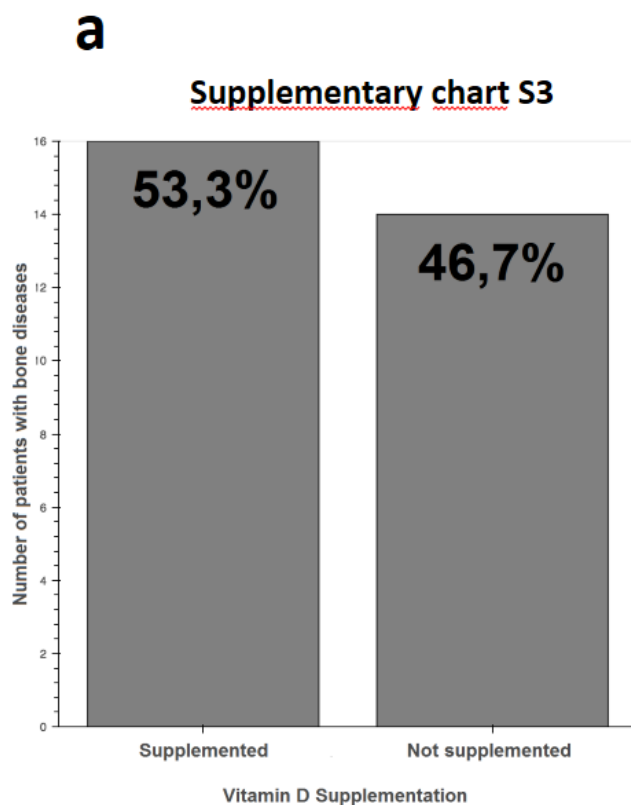
Acknowledgements

References

Alharbi, F. M. (2015). Update in vitamin D and multiple sclerosis. *Neurosciences*, *20*(4), 329-335. DOI: 10.17712/nsj.2015.4.20150357

Alshayeb, H. M., Wall, B. M., Showkat, A., Quarles, L. D., & Mangold, T. (2013). Chronic kidney disease and diabetes mellitus predict resistance to vitamin D replacement therapy. *The American Journal of the Medical Sciences*, *345*(4), 314-320. DOI: 10.1097/MAJ.0b013e31826af2d3

Altieri, B., Muscogiuri, G., Barrea, L., Mathieu, C., Vallone, C. V., Mascitelli, L, ... Casa, S. D. (2017). Does vitamin D play a role in autoimmune endocrine disorders? a proof of concept. *Reviews in Endocrine and Metabolic Disorders, 18*(3), 335-346. DOI: 10.1007/s11154-016-9405-9

Asemi, Z., Hashemi, T., Karamali, M., Samimi, M., & Esmaillzadeh, A. (2013). Effects of vitamin D supplementation on glucose metabolism, lipid concentrations, inflammation, and oxidative stress in gestational diabetes: a double-blind randomized controlled clinical trial. *The American Journal of Clinical Nutrition*, *98*(6), 1425-1432. DOI: 10.3945/ajcn.113.072785

Bover, J., Egido, J., Ferández-Giráldez, E., Praga, M., Solozábal-Campos, C., Torregrosa J. V, & Martínez-Castelao, A. (2015). Vitamin D, vitamin D receptor and the importance of its activation in patients with chronic kidney. *Nefrologia*, *35*(1), 28-41. DOI: 10.3265/Nefrologia.pre2014.Sep.11796

Câmara, A. B., Souza, I. D., & Dalmolin, R. J .S. (2018). Sunlight incidence, vitamin D deficiency, and Alzheimer's disease. *Journal of Medicinal Food*, *21*(9), 841-848. DOI: 10.1089/jmf.2017.0130

Chen, E. Q., Shi, Y., & Tang, H. (2014). New insight of vitamin D in chronic liver diseases. *Hepatobiliary & Pancreatic Diseases International*, *13*(6), 580-585. DOI: 25475859

Chow, E. C., Magomedova, L., Quach, H. P., Patel, R., Durk, M. R., Fan, J, ... Pang, K. S. (2014). Vitamin D receptor activation down-regulates the small heterodimer partner and increases CYP7A1 to lower cholesterol. *Gastroenterology*, *146*(4), 1048-1059. DOI: 10.1053/j.gastro.2013.12.027

Collongues, N., Patte-Mensah, C., De Seze, J., Mensah-Nyagan, A. G., & Derfuss, T. (2018). Testosterone and estrogen in multiple sclerosis: from pathophysiology to therapeutics. *Expert Review of Neurotherapeutics*, *18*(6), 515-522. DOI: 10.1080/14737175.2018.1481390

Cutillas-Marco, E., Prosper, A. F., Grant, W. B., & Morales-Suárez-Varela, M. M. (2013). Vitamin D status and hypercholesterolemia in Spanish general population. *Dermato-Endocrinology*, *5*(3), 358-362. DOI: 10.4161/derm.27497

Dusso, A., González, E. A., & Martin, K. J. (2011). Vitamin D in chronic kidney disease. *Best Practice & Research Clinical Endocrinology & Metabolism*, *25*(4), 647-655. DOI: 10.1016/j.beem.2011.05.005

Feldman, D., Krishnan, A. V., Swami, S., Giovannucci, E., & Feldman, B. J. (2014). The role of vitamin D in reducing cancer risk and progression. *Nature Reviews Cancer*, *14*(5), 342-357. DOI: 10.1038/nrc3691

Giovannucci, E. (2008). Vitamin D Status and Cancer Incidence and Mortality. In J. Reichrath (Ed.), *Sunlight, Vitamin D and Skin Cancer. Advances in Experimental Medicine and Biology* (Vol. 624, p. 31-42). New York, NY: Springer.

Grant, W. B. (2006). Lower vitamin-D production from solar ultraviolet-B irradiance may explain some differences in cancer survival rates. *Journal of the National Medical Association*, *98*(3), 357-364. DOI: 16573299

Guasch, A., Bulló, M., Rabassa, A., Bonada, A., Del Castillo, D., Sabench, F. & Salas-Salvadó, J. (2012). Plasma vitamin D and parathormone are associated with obesity and atherogenic dyslipidemia: a cross-sectional study. *Cardiovascular Diabetology, 11*, Article 149. DOI: 10.1186/1475-2840-11-149

Holick, M. F. (2007). Vitamin D deficiency. *The New England Journal of Medicine, 357*(3), 266-281. DOI: 10.1056/NEJMra070553

Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P, … Weaver, C. M. (2011). Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *The Journal of Clinical Endocrinology & Metabolism*, *96*(7), 1911-1930. DOI: 10.1210/jc.2011-0385

Hourigan, S. K., Abrams, S., Yates, K., Pfeifer, K., Torbenson, M., Murray, K., … Scheimann. A. (2015). The relationship between vitamin D status and non-alcoholic fatty liver disease in children. *Journal of Pediatric Gastroenterology and Nutrition*, *60*(3), 396-404. DOI: 10.1097/MPG.0000000000000598

Jiang, X., Peng, M., Chen, S., Wu, S., & Zhang, W. (2019). Vitamin D deficiency is associated with dyslipidemia: a cross-sectional study in 3788 subjects. *Current Medical Research and Opinion*, *35*(6), 1059-1063. DOI: 10.1080/03007995.2018.1552849

Karhapää, P., Pihlajamäki, J., Pörsti, I., Kastarinen, M., Mustonen, J., Niemelä, O, … Kuusisto, J. (2012). Glomerular filtration rate and parathyroid hormone are associated with 1, 25-dihydroxyvitamin D in men without chronic kidney disease. *Journal of Internal Medicine*, *271*(6), 573-580. DOI: 10.1111/j.1365-2796.2011.02471.x

Karuwanarint, P., Phonrat, B., Tungtrongchitr, A., Suriyaprom, K., Chuengsamarn, S., & Tungtronchitr, R. (2018). Genetic variations of vitamin D receptor gene in metabolic syndrome and related diseases in the Thai population. *Asia Pacific Journal of Clinical Nutrition*, *27*(4), 935-944. DOI: 10.6133/apjcn.122017.04

Kjalarsdottir, L., Tersey, S. A., Vishwanath, M., Chuang, J. C., Posner, B. A., Mirmira, R. G., & Repa, J. J. (2019). 1, 25-dihydroxyvitamin D3 enhances glucose-stimulated insulin secretion in mouse and human islets: a role for transcriptional regulation of voltage-gated calcium channels by the vitamin D receptor. *The Journal of Steroid Biochemistry and Molecular Biology*, *185*, 17-26. DOI: 10.1016/j.jsbmb.2018.07.004

Lee, S., Lee, E., Maneno, M. K., Johnson, A. A., & Wutoh, A. K. (2019). Predictive factors of vitamin D inadequacy among older adults in the United States. *International Journal for Vitamin and Nutrition Research*, *89*, 55-61. DOI: 10.1024/0300-9831/a000564

Lucas, R. M., Byrne, S. N., Correale, J., Ilschner, S., & Hart, P. H. (2015). Ultraviolet radiation, vitamin D and multiple sclerosis. *Neurodegenerative Disease Management*, *5*(5), 413-424. DOI: 10.2217/nmt.15.33

Mandarino, N. R., Monteiro Júnior, F. C., Salgado, J. V. L., Lages, J. S., & Salgado Filho, N. (2015). Is vitamin D deficiency a new risk factor for cardiovascular disease?. *The Open Cardiovascular Medicine Journal*, *9*, 40-49. DOI: 10.2174/1874192401509010040

Mazzaferro, S., Goldsmith, D., Larsson, T. E., Massy, Z. A., & Cozzolino, M. (2014). Vitamin D metabolites and/or analogs: which D for which patient?. *Current Vascular Pharmacology*, *12*(2), 339-349. DOI: 10.2174/15701611113119990024

Milart, J., Jobs, K., Tłustochowicz, M., Pogonowska, M., & Kalicki, B. (2018). The impact of vitamin D supplementation on vitamin D level, urinary calcium excretion and bone density in patients with hypercalciuria and vitamin D deficiency-preliminary report. *Developmental Period Medicine*, 22(2), 145-152. PMID: 30056401

Mokhtari, Z., Hekmatdoost, A., & Nourian, M. (2017). Antioxidant efficacy of vitamin D. *Journal of Parathyroid Disease*, *5*(1), 11-16. DOI: JPD_20160924145139

Momentti, A. C., Estadella, D., & Pisani, L. P. (2018). Role of vitamin D in pregnancy and Toll-like receptor pathway. *Steroids*, *137*, 22-29. DOI: 10.1016/j.steroids.2018.07.009

Muscogiuri, G., & Bhattoa, H. P. (2018). Vitamin D and endocrine disorders: routine laboratory diagnostic implications. *The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine*, *29*(2), 111-116. PMID: 30050394.

Myszka, M., Mazanowska, O., Kościelska-Kasprzak, K., Bartoszek, D., Żabińska, M., & Klinger, M. (2018). Serum 25-hydroxyvitamin D level is not related to regulatory T cells or kidney function in renal transplant recipients. *Transplantation Proceedings*, *50*(6), 1802-1806. DOI: 10.1016/j.transproceed.2018.03.117

NASA Earth Data. (2019). *Atmospheric Science Data Center: NASA's Surface Meteorology and Solar Energy*

project. Retrieved on Sept. 16, 2019 from https://eosweb.larc.nasa.gov/project/srb/srb_table

Nobili, V., Giorgio, V., Liccardo, D., Bedogni, G., Morino, G., Alisi, A., & Cianfarani, S (2014). Vitamin D levels and liver histological alterations in children with nonalcoholic fatty liver disease. *European Journal of Endocrinology*, *170*(4), 547-553. DOI: 10.1530/EJE-13-0609

Ordóñez-Mena, J. M., & Brenner, H. (2014). Vitamin D and cancer: an overview on epidemiological studies. *Advances in Experimental Medicine and Biology*, *810*, 17-32. PMID: 25207358

Park, H. Y., Lim, Y. H., Kim, J. H., Bae, S., Oh, S. Y., & Hong, Y. C. (2012). Association of serum 25-hydroxyvitamin D levels with markers for metabolic syndrome in the elderly: a repeated measure analysis. *Journal of Korean Medical Science*, *27*(6), 653-660. DOI: 10.3346/jkms.2012.27.6.653

Pilz, S., März, W., Cashman, K. D., Kiely, M. E., Whiting, S. J., Holick, M. F., ... Zittermann, A. (2018). Rationale and plan for vitamin D food fortification: a review and guidance paper. *Frontiers in Endocrinology*, *9*, 373. DOI: 10.3389/fendo.2018.00373

Polachini, C. R. N., Spanevello, R. M., Zanini, D., Baldissarelli, J., Pereira, L. B., Schetinger, M. R. C, ... Morsch, V. M. (2016). Evaluation of delta-aminolevulinic dehydratase activity, oxidative stress biomarkers, and vitamin D levels in patients with multiple sclerosis. *Neurotoxicity Research*, *29*(2), 230-242. DOI: 10.1007/s12640-015-9584-2

Qin, X. F., Zhao, L. S., Chen, W. R., Yin, D. W., & Wang, H. (2015). Effects of vitamin D on plasma lipid profiles in statin-treated patients with hypercholesterolemia: a randomized placebo-controlled trial. *Clinical Nutritrion*, *34*(2), 201-206. DOI: 10.1016/j.clnu.2014.04.017

Reid, I. R., Bolland, M. J., & Grey, A. (2014). Effects of vitamin D supplements on bone mineral density: a systematic review and meta-analysis. *The Lancet*, *383*(9912), 146-155. DOI: 10.1016/S0140-6736(13)61647-5

Rodríguez-Rodríguez, E., Ortega, R. M., González-Rodríguez, L. G., & López-Sobaler, A. M. (2011). Vitamin D deficiency is an independent predictor of elevated triglycerides in Spanish school children. *European Journal of Nutrition*, *50*(5), 373-378. DOI: 10.1007/s00394-010-0145-4

Salerno, G., Ceccarelli, M., de Waure, C., D'Andrea, M., Buonsenso, D., Faccia, V, ... & Valentini, P. (2018). Epidemiology and risk factors of hypovitaminosis D in a cohort of internationally adopted children: a retrospective study. *Italian Journal of Pediatrics*, *44*(1), Article 86. DOI: 10.1186/s13052-018-0527-4

Salzer, J., Biström, M., & Sundström, P. (2014). Vitamin D and multiple sclerosis: where do we go from here?. *Expert Review of Neurotherapeutics*, *14*(1), 9-18. DOI: 10.1586/14737175.2014.864952

Skaaby, T., Husemoen, L. T. N., Borglykke, A., Jørgensen, T., Thuesen, B. H., Pisinger, C., ... Linneberg, A. (2014). Vitamin D status, liver enzymes, and incident liver disease and mortality: a general population study. *Endocrine*, *47*(1), 213-220. DOI: 10.1007/s12020-013-0107-8

Smyk, D. S., Mavropoulos, A., Mieli-Vergani, G., Vergani, D., Lenzi, M., & Bogdanos, D. P. (2018). The role of invariant NKT in autoimmune liver disease: can vitamin D act as an immunomodulator?. *Canadian Journal of Gastroenterology*, *2018*. DOI: 10.1155/2018/8197937

Teixeira, J. S., Campos, A. B. F., Cordeiro, A., Pereira, S. E., Saboya, C., & Ramalho, A. (2018). Vitamin D nutritional status and its relationship with metabolic changes in adolescents and adults with severe obesity. *Nutricion Hospitalaria*, *35*(4), 847-853. DOI: 10.20960/nh.1657

Welsh, J. (2012). Cellular and molecular effects of vitamin D on carcinogenesis. *Archives of Biochemistry and Biophysics*, *523*(1), 107-114. DOI: 10.1016/j.abb.2011.10.019

Yang, C. Y., Leung, P. S. C., Adamopoulos, I. E., & Gershwin, M. E. (2013). The implication of vitamin D and autoimmunity: a comprehensive review. *Clinical Reviews in Allergy & Immunology*, *45*(2), 217-226. DOI: 10.1007/s12016-013-8361-3

Yin, K., You, Y., Swier, V., Tang, L., Radwan, M. M., Pandya, A. N, & Agrawal, D. K. (2015). Vitamin D protects against atherosclerosis via regulation of cholesterol efflux and macrophage polarization in hypercholesterolemic swine significance. *Arteriosclerosis, Thrombosis, and Vascular Biolog*y, *35*(11), 2432-2442. DOI: 10.1161/ATVBAHA.115.306132

Zhang, H. Q., Teng, J. H., Li, Y., Li, X. X., He, Y. H., He, X., & Sun, C. H. (2014). Vitamin D status and its association with adiposity and oxidative stress in schoolchildren. *Nutrition*, *30*(9), 1040-1044. DOI: 10.1016/j.nut.2014.02.024

Supplementary material

a

Supplementary chart S3

53,3%

46,7%

Number of patients with bone diseases

Supplemented   Not supplemented

Vitamin D Supplementation

b

Supplementary table S3. Vitamin D supplementation in patients with bone disease	
Patient	Vitamin D Supplementation
1	Supplemented
2	Not Supplemented
3	Not Supplemented
4	Supplemented
5	Supplemented
6	Not Supplemented
7	Supplemented
8	Not Supplemented
9	Not Supplemented
10	Supplemented
11	Not Supplemented
12	Supplemented
13	Not Supplemented
14	Not Supplemented
15	Supplemented
16	Not Supplemented
17	Supplemented
18	Supplemented
19	Not Supplemented
20	Not Supplemented
21	Supplemented
22	Not Supplemented
23	Supplemented
24	Supplemented
25	Not Supplemented
26	Supplemented
27	Not Supplemented
28	Supplemented
29	Supplemented
30	Supplemented

*The vitamin D supplementation were obtained from the Heart Hospital database (Natal/Brazil)

Supplementary chart . VD Supplementation in 30 patients with bone diseases. The VD supplementation did not affect our results ($p > 0.05$).