Abstract

Aims: hypovitaminosis D has frequently been identified in patients with heart failure (HF). However, few studies have been conducted in regions with high solar incidence. Therefore, this study aimed to evaluate vitamin D status and predictors of 25-hydroxyvitamin D (25(OH)D) levels in patients with HF living in a sunny region (5 °- 6 °S).

Methods: this cross-sectional study enrolled 70 patients with HF. Biodemographic, clinical, biochemical, dietary, and sun exposure data were collected, and 25(OH)D levels were measured.

Results: the mean 25(OH)D level was 40.1 (12.4) ng/mL, and 24.3 % (95 % CI: 14.2-33.8) of patients with HF had hypovitaminosis D (25(OH)D < 30 ng/mL). Female patients (p = 0.001), those with ischemic etiology (p = 0.03) and those with high parathyroid hormone levels (> 67 pg/mL) (p = 0.034) were more likely to present hypovitaminosis D. Higher 25(OH)D levels were observed in men than in women (β = 7.78, p = 0.005) and in patients with HF in New York Heart Association (NYHA) functional class I when compared to those in class III/IV (β = 8.23, p = 0.032).

Conclusions: the majority of patients with HF had sufficient 25(OH)D levels. Sex and functional classification were identified as independent predictors of 25(OH)D levels. These results highlight the need for increased monitoring of vitamin D status among female patients with heart failure and those with more severe symptoms.

Keywords: Heart failure. Cardiovascular disease. Vitamin D. Hypovitaminosis D. Nutritional status.
INTRODUCTION

Heart failure (HF) is a clinical syndrome characterized by structural and/or functional cardiac abnormalities that impair ventricular filling or blood ejection, resulting in reduced cardiac output and/or elevated intracardiac pressure at rest and during stress. Furthermore, HF involves complex metabolic, immunological, and neuroendocrine interactions that can negatively affect muscle, fat, and bone metabolism (1).

Vitamin D has been recognized for its key role as a mediator of calcium and phosphorus homeostasis in bones (2). Additionally, vitamin D is also involved in extra-skeletal tissues (3); in particular, the specific nuclear vitamin D receptor (VDR) and enzymes associated with vitamin D metabolism have been observed in tissues other than bones, including in the cardiovascular system (4). 1,25-dihydroxy-vitamin D [1,25(OH)2D], the active vitamin D metabolite, exerts effects on the heart since it binds to the vitamin D receptor (VDR), and has potential modulatory effects on cardiac hypertrophy and failure (5); it also has a regulatory role in: a) the renin-angiotensin-aldosterone system; b) the remodeling and mediation of collagen deposition, fibrosis, and matrix metalloproteinase levels in the cardiac system; c) immediate and non-genomic effects on signal transduction mediators; and d) the modulation of the inflammatory process (3-5).

Low concentrations of vitamin D are frequently found in patients with HF, and they have been associated with increased plasma renin activity, inflammation profile, and high concentrations of natriuretic peptides and parathormone (PTH). These changes impair renal function, thereby worsening the functional capacity and severity of HF (6-9). In most cases, hypovitaminosis D is attributed to low dietary intake of vitamin D and reduced sun exposure, which itself is attributable to low tolerance for exercise (10,11). However, most studies evaluating vitamin D status were conducted in geographical regions with higher latitudes and lower solar incidence (7-9,12,13), and failed to consider factors directly related to vitamin D uptake, including sun exposure, skin type, and dietary intake (2). To address this gap, our study aimed to evaluate vitamin D status and predictors of 25(OH)D levels in patients with HF living in a sunny region of north-eastern Brazil.

MATERIAL AND METHODS

STUDY POPULATION AND ETHICAL ASPECTS

This analytical cross-sectional study included adult and elderly subjects of both sexes aged ≥ 18 years who were diagnosed with HF at Onofre Lopes University Hospital in Natal (5th 45° 54” S), Rio Grande do Norte, Brazil. Natal, a city in north-eastern Brazil, has a high solar radiation intensity, with an ultraviolet index > 7 year-round and levels > 10 between October and February (14).

Clinical diagnoses of HF were confirmed by a team of cardiologists, according to the Framingham and Boston criteria, as well as Doppler echocardiography (15). We excluded patients with a previous diagnosis of cancer, autoimmune disease and/or osteoporosis; haemodialysis or peritoneal dialysis; history of bariatric surgery; hepatic transaminase levels greater than three times the reference values; and patients who were pregnant or lactating. Patients who used calcium, phosphorus, or vitamin D supplements, glucocorticoids, and/or antiepileptics during the last 3 months were also excluded.

Patients were selected by convenience sampling from the outpatient patient care flow between January and December 2017. At data collection, 120 patients were enrolled, of whom 40 were excluded because they did not meet the previously defined criteria. Additionally, five of the remaining 80 recruited patients refused to participate. Four patients dropped out and one died during the study. Finally, data were collected from 70 participants, who were stratified into two groups: “hypovitaminosis D” (n = 17) and “sufficient vitamin D” (n = 53).

The research proposal was approved by the institutional review board (protocol CAAE 59827516.2.0.0.0.5292). All participants were provided information about the study and signed an informed consent form.

CLINICAL, BIODEMOGRAPHIC AND ANTHROPOMETRIC DATA

Clinical data regarding the etiology of HF, New York Heart Association (NYHA) functional classification, presence of comorbidities,
and left ventricular ejection fraction (LVEF) value as assessed by Doppler echocardiography were obtained from electronic medical records. Patients with a LVEF < 40 %, 40-49 %, or ≥ 50 % were classified as having HF with a reduced ejection fraction (HFrEF), a mid-range ejection fraction (HFmrEF), or a preserved ejection fraction (HFpEF), respectively (1).

Physical activity practice was evaluated as previously reported (16). Skin type was defined according to Fitzpatrick (17), who classified human skin color into six categories, ranging from type I (white) to type VI (black). Sun exposure was assessed using a score corresponding to the last 7-day period prior to blood collection. First, a daily score that considered the time of sun exposure and the amount of exposed skin was calculated. The weekly score was obtained from the sum of daily scores and ranged from 0 to 56 hours (18). Considering a half-life of 3 weeks for 25(OH)D (19), each participant was allocated to the season of the year that corresponded to the date 21 days before blood collection.

Weight and height measurements were obtained using a digital scale with a capacity of 200 kg and a precision of 0.1 kg (Leader Balanças®, São Paulo, Brazil, LD-1050 Line, Model P-200 C). Height was measured using a stadiometer attached to the scale. The body mass index (BMI) was used as anthropometric assessment. BMI classifications for adults and elderly individuals were performed according to the World Health Organization (20) and Lipschitz (21), respectively.

**BIOCHEMICAL ANALYSIS**

Blood samples were collected via venipuncture after a 12-hour overnight fast. Blood glucose, triglycerides, HDL-c, albumin, and total calcium levels were determined via colorimetric-enzymatic assays. Phosphorus, glutamic-oxaloacetic transaminase (GOT), and glutamic-pyruvic transaminase (GPT) levels were measured using enzymatic ultraviolet (UV) assays. Glycated hemoglobin (HbA1c) and high-sensitivity C-reactive protein (hs-CRP) levels were analyzed by immunoturbidimetry. Creatinine was measured by the kinetic method, and urea by the UV kinetic method. All analyses were performed using the Wiener Lab® test kit (Wiener Laboratory Group, Santa Fe, Argentina) and COBAS 6000 Modular Equipment automated device (Diamond Diagnostics, Holliston, MA, USA). LDL-c levels were determined using Friedewald’s formula (22). A hemoglobin analysis was performed using the cyanide-free colorimetric method and the Advia® 2120i Haematology System (Siemens Healthcare, Erlangen, Germany). Serum intact PTH (iPTH) levels were determined by chimeriluminescence using the commercially available Unicel® DxI800 immunoassay system (Beckman Coulter, Brea, CA, USA).

Serum 25(OH)D levels were evaluated using the COBAS 6000 Modular Equipment automated system (Roche®, Basel, Switzerland) and the Elecsys® Vitamin D total electrochemiluminescence immunoassay kit (Roche®, Basel, Switzerland). A cut-off point of 25(OH)D ≥ 30 ng/mL was used to define vitamin D sufficiency. Vitamin D deficiency was defined as serum 25(OH)D < 20 ng/mL (19). For this study, patients with 25(OH)D < 30 ng/mL were allocated to the hypovitaminosis D group (23). iPTH levels were also categorized; here, a level > 67 pg/mL was considered high. The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, and a reduced eGFR was defined as a CKD-EPI result of < 60 mL/min/1.73 m² (1).

**DIETARY INTAKE ASSESSMENT**

The dietary intakes of vitamin D, calcium, and phosphorus were determined in triplicate by trained researchers using the 24-hour food recall method. A dietary analysis was performed using Virtual Nutri Plus® 2.0 (Keeple Company, São Paulo, Brazil). The Multiple Source Method was used to estimate the habitual intakes of nutrients and remove intrapersonal variability.

Subsequently, nutrients were adjusted by energy levels according to the method proposed by Willett and Stampfer (24). The prevalence of inadequate vitamin D intake was estimated using the equation below (25), with reference to the Estimated Average Requirement (EAR) (26).

\[ z = \frac{(\text{mean of intake})}{\text{standard deviation}} \]

**STATISTICAL ANALYSIS**

Continuous variables are expressed as means (standard deviation) for normally distributed data, and by medians (quartiles 1-3) for asymmetrical distributed data. Categorical variables are expressed as absolute frequencies (percentages). The Shapiro–Wilk test was used to evaluate the distributions of data.

Data were missing for the following variables: sun exposure, total calcium, total cholesterol, HDL-c, LDL-c, fasting glucose, hemoglobin, HbA1c, iPTH, triglycerides, urea, dietary vitamin D, dietary calcium, and dietary phosphorus. In such cases, a simple imputation method was applied to replace the missing value with the central trend value of the variable. Imputation could not be performed for albumin, phosphorus, and hs-CRP levels because the amount of missing data exceeded the maximum allowable level.

Student’s t-test and the Mann–Whitney test were used for comparisons of variables between groups according to 25(OH)D cut-off points as appropriate. The chi-square test was used to evaluate associations between categorical variables. Correlations between 25(OH)D levels and continuous variables were evaluated using Pearson’s coefficient (r) for normally distributed data, and Spearman’s coefficient for abnormally distributed data. The generalized linear regression model was adjusted for the variable 25(OH)D according to the stepwise method for the selection of model variables. We considered the independent variables to be clinically relevant for predicting 25(OH)D. The variables whose p-value was less than 0.10 in the bivariate analysis were included in the model. The criteria used to assess the permanence of the independent variable in the model were: 1) absence of...
multicollinearity; 2) the Wald chi-squared test, and 3) the ability to improve the quality of the model using the Akaike information criterion (AIC). The initial predictors were sex, etiology, HF functional class, parathormone, and TGP (AIC = 572.69). However, after adjustment criteria were applied, the model selected to predict 25(OH)D levels was composed of sex, parathormone, and HF functional class (AIC = 565.24). For all analyses, a p-value < 0.05 was considered statistically significant. The software package SPSS Statistics® version 22.0 was used for data storage and analysis.

RESULTS

Most of the participants were male (64.3 %) with a mean age of 53(15) years, and 57.1 % of them were overweight. Furthermore, 60 % of the participants had HFrEF, and most met the criteria for NYHA functional class I. Female patients and those with an ischemic etiology were more likely to present with hypovitaminosis D, as well as patients who did not use angiotensin II receptor antagonists or angiotensin-converting enzyme inhibitors (ARA/ACEI), and those who did use antiplatelet medications (Table I).

| Variables                  | Total n = 70 | 25(OH)D | p-value |
|----------------------------|--------------|---------|---------|
|                            | Hypovitaminosis D | Sufficient |        |
|                            | < 30 ng/mL n = 17 | ≥ 30 ng/mL n = 53 |        |
| Age (years)a               | 53 (15) | 58 (15) | 52 (15) | 0.119 |
| Sexb                       |         |         |         | 0.001*|
| Female                     | 25 (35.7) | 12 (70.6) | 13 (24.5) |         |
| Male                       | 45 (64.3) | 5 (29.4) | 40 (75.5) |         |
| BMI (kg/m²)a               | 26.63 (5.13) | 26.29 (5.06) | 26.73 (5.19) | 0.762 |
| Malnutrition               | 6 (8.6) | 1 (1.4) | 5 (7.1) |         |
| Eutrophy                   | 24 (34.3) | 8 (11.4) | 16 (22.9) | 0.437 |
| Overweight                 | 40 (57.1) | 8 (11.4) | 32 (45.8) |         |
| Skin typeb                 |         |         |         |         |
| II                         | 7 (10) | 2 (11.8) | 5 (9.4) |         |
| III                        | 26 (37.1) | 7 (41.2) | 19 (35.8) |         |
| IV                         | 15 (21.4) | 5 (29.4) | 10 (18.9) | 0.611 |
| V                          | 18 (25.7) | 3 (17.6) | 15 (28.3) |         |
| VI                         | 4 (5.7) | 0 (0.0) | 4 (7.5) |         |
| Seasonsb                   |         |         |         |         |
| Summer                     | 13 (18.6) | 1 (5.9) | 12 (22.6) |         |
| Autumn                     | 25 (35.7) | 4 (23.5) | 21 (39.6) | 0.110 |
| Winter                     | 23 (32.9) | 9 (52.9) | 14 (26.4) |         |
| Spring                     | 9 (12.9) | 3 (17.6) | 6 (11.3) |         |
| Solar exposure scorea      | 15 (6-28) | 10.0 (4.0-23.5) | 16.0 (6.5-28.0) | 0.232 |
| Sunscreen                  | 12 (17.1) | 3 (17.6) | 9 (17.0) | 0.949 |
| Exercise time (min/week)c  | 37.5 (0.0-150.0) | 0.0 (0.0-175.0) | 75.0 (0.0-150.0) | 0.389 |
| Etiologyb                  |         |         |         |         |
| Ischemic                   | 29 (41.4) | 11 (64.7) | 18 (34.0) | 0.030**|
| Non-ischemic               | 30 (42.9) | 4 (23.5) | 26 (49.1) |         |
| Undefined                  | 11 (15.7) | 2 (11.8) | 9 (17.0) |         |
| LVEF (%)c                  | 36 (26-50) | 41 (28-54) | 34.5 (26-47) | 0.255 |

(Continuation in the next page)
Table I (Cont.). Biodemographic, anthropometric, and clinical characteristics of patients with heart failure according to 25(OH)D levels

| Variables                        | Total n = 70 | 25(OH)D |
|----------------------------------|--------------|---------|
|                                 |              | Hypovitaminosis D | Sufficient |
|                                 |              | ≤ 30 ng/mL | ≥ 30 ng/mL |
| HF definition†                   |              |          |          |
| HFrEF                            | 42 (60.0)    | 8 (47.1) | 34 (64.2) |
| HFmrEF                           | 10 (14.3)    | 3 (17.6) | 7 (13.2)  |
| HFrEF                            | 18 (25.7)    | 6 (35.3) | 12 (22.6) |
| NYHA functional classification‡  |              |          |          |
| I                                | 46 (65.7)    | 8 (47.1) | 38 (71.7) |
| II                               | 14 (20.0)    | 4 (23.5) | 10 (18.9) |
| III/IV                           | 10 (14.3)    | 5 (29.4) | 5 (9.4)   |
| Comorbidity§                     |              |          |          |
| Arterial hypertension            | 34 (48.6)    | 8 (47.1) | 26 (49.1) |
| Diabetes mellitus                | 19 (27.1)    | 6 (35.3) | 13 (24.5) |
| eGFR < 60 mL/min/1.73 m²         | 21 (30)      | 5 (29.4) | 16 (30.2) |
| Medication‡                      |              |          |          |
| ACEI/ARA                         | 66 (94.3)    | 14 (82.4) | 52 (98.1) |
| Diuretics                        | 57 (81.4)    | 16 (94.1) | 41 (77.4) |
| β-blockers                       | 65 (92.9)    | 15 (88.2) | 50 (94.3) |
| Vasodilators                     | 17 (24.3)    | 3 (17.6)  | 14 (26.4) |
| Digoxin                          | 15 (21.4)    | 3 (17.6)  | 12 (22.6) |
| Antiplatelet agents              | 32 (45.7)    | 13 (76.5) | 19 (35.8) |

Overall, the mean serum 25(OH)D level was 40.1 (12.4) ng/mL, and the frequency of hypovitaminosis D was 24.3 % (95 % CI: 14.2-33.8). Vitamin D deficiency was recorded in 7.1 % (95 % CI: 1.1-13.2) of participants with HF. In the total group, 19 patients (27.1 %) had a high iPTH level (> 67 pg/mL), and those individuals were more likely to present hypovitaminosis D (p = 0.034). The prevalence of inadequate vitamin D intake was 100 %, with no difference in mean intake between the groups (p ≥ 0.05). No significant inter-group differences were observed in other analyses of biochemical and dietary variables (all p ≥ 0.05) (Table II).

Significant positive correlations were observed between 25(OH) D levels and albumin (r = 0.365, p = 0.015), total calcium (r = 0.266, p = 0.026), hemoglobin (r = 0.249, p = 0.037), and GPT (r = 0.366, p = 0.002) levels. Furthermore, a significant, negative correlation was observed between 25(OH)D and iPTH levels (r = -0.255; p = 0.033) (Table III).

In a regression model adjusted for 25(OH)D, the NYHA functional classification, iPTH category, and sex were identified as variables with a better adjustment quality. This analysis indicated that men with HF had significantly higher 25(OH)D levels as compared to women (β = 7.78, p = 0.005). Additionally, participants in the NYHA class I had significantly higher 25(OH)D levels when compared to those in NYHA class III/IV (β = 8.23, p = 0.032). Although a high iPTH level (> 67 pg/mL) was correlated inversely with 25(OH)D level (β = -5.52, p = 0.062), this association was not statistically significant (Fig. 1, Table IV).

**DISCUSSION**

This is the first study to assess vitamin D status in patients with HF living in a region of north-eastern Brazil with high solar radiation intensity. Most patients with HF were clinically stable (NYHA I) and had a sufficient 25(OH)D status. However, we observed significant associations of 25(OH)D levels with clinical and biochemical parameters. We identified sex and functional classifi-
cation as independent predictors of 25(OH)D levels; specifically, we observed lower vitamin D levels in female participants and in those with worse functional NYHA classification.

Our findings regarding the 25(OH)D status are in contrast with those studies of HF patients from various populations. Generally, studies conducted in higher-latitude regions reported a high prevalence of vitamin D deficiency (7–9,12). Additionally, a previous study in a sample of patients with HF living in Rio de Janeiro (22 °S) in Brazil reported that 47% of patients had hypovitaminosis D (< 30 ng/mL) (27); another study in Porto Alegre (30 °S) reported a rate of 70.5% (13). Although these studies were also conducted in Brazil, the authors used different methods of analysis for the quantification of 25(OH)D; for example, radioimmunoassay (13) and liquid chromatography (27), thus limiting the comparability of studies. However, the Elecsys Vitamin D Total assay used in our study demonstrated a good overall performance, and was very suitable for automated measurement of 25(OH)D (28).

In addition, these Brazilian studies carried out in patients with HF were conducted in southeastern and southern regions of Brazil, which are different from the north-eastern region where this study was performed. The angle of solar radiation increases with increasing latitude; therefore, in regions at latitudes distant from the equator, UV-B rays are absorbed to a greater extent by the ozone layer and little radiation reaches the earth’s surface, reducing the efficiency of vitamin D production (2). Likely, this factor best explains the relatively lower frequency of hypovitaminosis D

Table II. Biochemical and dietary data of patients with heart failure according to 25(OH)D levels

| Variables                  | Total n = 70 | Hypovitaminosis D < 30 ng/mL n = 17 | Sufficient ≥ 30 ng/mL n = 53 | p-value |
|----------------------------|--------------|--------------------------------------|-------------------------------|---------|
| 25(OH)D (ng/mL)            | 40.1 (12.4)  | 22.85 (4.1)                          | 45.62 (8.5)                   | < 0.001* |
| iPTH (pg/mL)               | 49.15 (35.9-75.2) | 66.5 (44.0-112.1)                   | 46.9 (34.9-64.9)              | 0.020*  |
| iPTH > 67 pg/mL            | 19 (27.1)    | 8 (47.1)                             | 11 (20.8)                     | 0.034*  |
| iPTH ≤ 67 pg/mL           | 51 (72.9)    | 9 (52.9)                             | 42 (79.2)                     |         |
| Total calcium (mg/dL)     | 10.0 (0.6)   | 9.8 (0.8)                            | 10.1 (0.6)                    | 0.175   |
| Phosphorus (mg/dL)        | 3.8 (0.6)    | 3.6 (0.5)                            | 3.84 (0.6)                    | 0.208   |
| Hemoglobin (g/dL)         | 13.2 (1.7)   | 12.8 (2.0)                           | 13.4 (1.5)                    | 0.258   |
| Fasting glycemia (mg/dL)  | 103.0 (95.8-120.3) | 109 (94-155)                        | 103 (96.0-117.5)              | 0.279   |
| HbA1c (%)                 | 6.3 (6.0-6.7)   | 6.3 (5.9-7.35)                      | 6.3 (6.0-6.6)                 | 0.721   |
| Total cholesterol (mg/dL) | 160.5 (1323-182.3) | 165.0 (125.5-191.5)                  | 159 (131.5-180.5)             | 0.529   |
| HDL-c (mg/dL)             | 35.4 (8.0)   | 35.7 (7.8)                           | 35.3 (8.1)                    | 0.860   |
| LDL-c (mg/dL)             | 89.0 (70.8-110.8) | 94 (74-113.5)                       | 89 (69.5-1100)                | 0.661   |
| Triglycerides (mg/dL)     | 119.0 (76.5-185.5) | 129.0 (53.0-217.0)                  | 119.0 (77.0-163.5)            | 0.701   |
| GOT (IU/L)                | 22.5 (18.0-29.0) | 22.00 (17.5-33.0)                   | 23.00 (19.0-29.0)             | 0.995   |
| GPT (IU/L)                | 23.0 (18.0-29.5) | 21.0 (15.0-25.5)                    | 23.0 (19.0-31.5)              | 0.236   |
| Albumin (g/dL)            | 4.4 (4.2-4.7)  | 4.4 (3.6-4.5)                        | 4.4 (4.3-4.75)                | 0.104   |
| Creatinine (mg/dL)        | 1.1 (0.9-1.3)  | 1.0 (0.9-1.3)                        | 1.1 (0.9-1.3)                 | 0.699   |
| Urea (mg/dL)              | 38.0 (31.5-55.0) | 38.0 (33-56.5)                      | 38.00 (30.0-54.5)             | 0.722   |
| hs-CRP (mg/L)             | 1.0 (0.3-6.7)  | 2.0 (0.5-13.9)                       | 0.8 (0.3-5.6)                 | 0.189   |
| eGFR (mL/min/1.73 m²)     | 73.8 (24.9)   | 65.5 (21.3)                          | 76.5 (25.5)                   | 0.115   |
| Vitamin D intake (µg/d)   | 2.6 (1.2)     | 2.7 (1.2)                            | 2.6 (1.2)                     | 0.725   |
| Calcium intake (mg/d)     | 354.7 (313.5-487.1) | 383.2 (325.7-483.0)             | 349.19 (308.9-487.5)         | 0.493   |
| Phosphorus intake (mg/d)  | 766.7 (147.9) | 766.05 (92.9)                        | 766.96 (162.3)                | 0.983   |

aData are presented as means (standard deviation) with p-value determined using the t-test; bData are presented as medians (quartiles 1-3) with p-values determined using the Mann-Whitney test; cData are presented as n (%) with p-values determined using the χ² test; 1Phosphorus, n = 54 (12/42); albumin, n = 44 (15/29); hs-CRP, n = 58 (15/43); 2Statistically significant comparison between the groups (p < 0.05); 25(OH)D: 25-hydroxyvitamin D; iPTH: intact parathyroid hormone; HbA1Ac: glycated hemoglobin; GOT: glutamic-oxaloacetic transaminase; GPT: glutamic-pyruvic transaminase; hs-CRP: high-sensitivity C-reactive protein; eGFR: estimated glomerular filtration rate.
and vitamin D deficiency observed in our study, considering that Natal has a latitude between 5° and 6° south of the equator, resulting in high solar radiation levels throughout the year (14).

Although the associations observed between sun exposure, dietary vitamin D intake, and 25(OH)D levels were not significant in this study, vitamin D status has been discussed not only by dietary intake or endogenous production, but also by the body’s ability to store and release the vitamin (29). Lifelong exposure to a high ultraviolet index may provide adequate endogenous vitamin D production and storage. The mechanisms underlying 25(OH)D storage and bioavailability are still unclear, but cell culture studies suggest that PTH (30) and 1,25-dihydroxyvitamin D [1,25(OH)2D] (31) regulate the uptake and release of vitamin D by skeletal and adipose muscle cells. Thus, it is suggested that the high frequency of vitamin D sufficiency observed in our study possibly reflected the bioavailability of 25(OH)D stored in the patients’ tissues.

We observed a higher likelihood of hypovitaminosis D among patients with HF of ischemic etiology as compared to those with non-ischemic etiology. Vitamin D deficiency appears to contribute to an imbalance in vascular homeostasis and compromises arterial functioning, affecting the progression of atherosclerosis (32).

The observation that women displayed lower 25(OH)D levels and faced a greater risk of hypovitaminosis D relative to men should be emphasised; notably, these findings agree with the results of other studies (7,33). Sex steroid hormones regulate the synthesis of hepatic vitamin D-binding protein, the major vitamin D carrier protein. In postmenopausal women reduced estrogen levels appear to imply a lower total vitamin D level (34). We note that 64 % of the women in our study were menopausal. In addition, fat mass significantly increased between premenopausal and postmenopausal women when evaluated by BMI. Body mass can reduce proportionally circulating vitamin D levels (35). Although there was no significant correlation between BMI and 25(OH)D in our study, the body composition changes of menopause may explain the identification of sex as an independent predictor of 25(OH)D levels.

We identified the variable of functional classification as a predictor of vitamin D status. Previous reports regarding the associations of low 25(OH)D levels with highest symptom severity, determined by the functional classification, have been conflicting (7,11,36,37). However, this relationship is supported by evidence from other parameters that indicate clinical severity, such as associations between low 25(OH)D levels and elevated natriuretic peptide levels (7,36), reduced functional capacity (9), and the occurrence of poor outcomes in HF (6,7,37).

A previous meta-analysis confirmed an association of the most serious functional classification with increased bone loss, and an imbalance in calcium and phosphorus homeostasis, as well as in the hormones involved in this balance, have been identified.

### Table III. Correlations between 25(OH)D concentrations and clinical parameters in all patients with heart failure

| Variables                     | 25(OH)D |
|-------------------------------|---------|
|                               | R       | p-value |
| Age                           | -0.108  | 0.372   |
| BMI                           | -0.004  | 0.972   |
| LVEF                          | -0.125  | 0.302   |
| Total calcium                 | 0.266   | 0.026*  |
| iPTH                          | -0.255  | 0.033*  |
| Phosphorus                    | 0.158   | 0.253   |
| Hemoglobin                    | 0.249   | 0.037*  |
| Albumin                       | 0.365   | 0.015*  |
| GOT                           | -0.232  | 0.054   |
| GPT                           | 0.366   | 0.002*  |
| hs-CRP                        | -0.131  | 0.328   |
| eGFR                          | 0.157   | 0.195   |
| Solar exposure score          | 0.174   | 0.150   |
| Vitamin D intake              | 0.088   | 0.466   |
| Calcium intake                | 0.046   | 0.704   |
| Phosphorus intake             | 0.090   | 0.436   |

*Pearson’s correlation; †Spearman’s correlation; *Statistically significant at a level of p < 0.05; Phosphorus, n = 54; albumin, n = 44; hs-CRP, n = 58; 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; LVEF, left ventricular ejection fraction; iPTH, intact parathyroid hormone; GOT, glutamic-oxaloacetic transaminase; GPT, glutamic-pyruvic transaminase; hs-CRP, high-sensitivity C-reactive protein; eGFR, estimated glomerular filtration rate.

### Table IV. Stepwise forward regression analysis of 25(OH)D predictors

| Variable                          | β      | Standard error | p-value |
|-----------------------------------|--------|----------------|---------|
| Intercept                         | 30.55  | 3.90           | < 0.000 |
| Sex                               |        |                |         |
| Male                              | 7.78   | 2.75           | 0.005*  |
| Female                            | 0      |                |         |
| PTH                               |        |                |         |
| > 67 pg/mL                        | -5.52  | 2.96           | 0.062   |
| ≤ 67 pg/mL                        | 0      |                |         |
| NYHA functional classification    |        |                |         |
| I                                 | 8.23   | 3.83           | 0.032*  |
| II                                | 3.09   | 4.54           | 0.495   |
| III/IV                            | 0      |                |         |

*Statistically significant at a level of p < 0.05; 25(OH)D: 25-hydroxyvitamin D; NYHA: New York Heart Association.
evaluate 25(OH)D may have cross-reacted with other vitamin D metabolites (40), and the sun exposure instrument can only evaluate sun exposure in the 7 previous days.

CONCLUSION

In conclusion, a majority of patients with HF residing in a sunny region had sufficient 25(OH)D levels. Sex and functional classification were identified as independent predictors of 25(OH)D levels, with low 25(OH)D levels among women and patients with HF in NYHA class III/IV. Our results reinforce the importance of monitoring female patients with HF, and those with worse symptomatic severity to prevent poor outcomes related to hypovitaminosis D.
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