Original article

Vitamin D in women with class II/III obesity: Findings from the DieTBra trial

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SUMMARY

Objective: To assess the prevalence of vitamin D deficiency and factors associated with serum vitamin D levels in adult women with class II/III obesity.

Methods: We analysed baseline data from 128 adult women with class II/III obesity i.e. BMI ≥35 kg/m² who participated in the DieTBra clinical trial. Sociodemographic, lifestyle, sun exposure, sunscreen, dietary intake of calcium and vitamin D, menopause, diseases, medication, and body composition data were analysed using multiple linear regression.

Results: 128 women had mean BMI 45.53 ± 6.36, mean age 39.7 ± 8.75 kg/m² and serum vitamin D 30.02 ng/ml ± 9.80. Vitamin D deficiency was 14.01%. There was no association between serum vitamin D levels and BMI, body fat percentage, total body fat and waist circumference. Age group (p = 0.004), sun exposure/day (p = 0.072), use of sunscreen (p = 0.168), inadequate calcium intake (p = 0.030), BMI (p = 0.192), menopause (p = 0.029) and lipid-lowering drugs (p = 0.150) were included in the multiple linear regression. The following remained associated with low serum vitamin D: being 40–49 years (p = 0.003); ≥50 years of age (p = 0.020) and inadequate calcium intake (p = 0.027).

Conclusion: The prevalence of vitamin D deficiency was lower than expected. Lifestyle, sun exposure and body composition were not associated. Age over 40 years and inadequate calcium intake were significantly associated with low serum vitamin D levels.

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Introduction

Severe obesity is a growing public health problem worldwide. It is characterized by a body mass index (BMI) of 35.0 kg/m² or higher and subdivided into class II (BMI 35–39.9 kg/m²) and III (BMI ≥40 kg/m²) [12]. It is estimated that by 2025, the prevalence of severe obesity worldwide will reach 6% in men and 9% in women [1]. Severe obesity increases the risk of non-communicable chronic disease (NCD) and decreases life expectancy compared to individuals with class I obesity [1–3]. Obesity increases the risk of all-cause mortality [4] especially in the context of the SARS-CoV-2 pandemic. Class II/III obesity increases the risk of COVID-19 complication and mortality. Vitamin D also plays a very important role on immunity [5,6]. Given its association with health problems such as bone, endocrine-metabolic and cardiovascular diseases, vitamin D impacts on many health outcomes [7]. Obesity may be associated with a low serum vitamin D concentration [8], as excess adiposity reduces the levels of 25-hydroxyvitamin D [25(OH)D] [9].

Several factors are associated with vitamin D deficiency in the general population, i.e. insufficient food intake [10], low sun exposure, use of sunscreen and darker skin colour [11]. In obese individuals, vitamin D levels have been inversely associated with higher body mass index (BMI) and greater waist circumference [12,13]. In some studies, however, such an association has not been identified [14,15]. The interrelationship between adiposity and vitamin D is very complex and still unclear [8,9,16,17]. The few previous studies looking into the association between vitamin D and morbidity, or severe obesity (without bariatric surgery) have limitations and conflicting findings [16,18]. Such studies used different methods to assess vitamin D serum concentrations and less accurate methods, compared to the dual energy x-ray absorptiometry (DXA), to assess body composition parameters such as total body fat. The variation in climate and sunlight incidence in the country where the study was conducted also affected the vitamin D serum levels found. In our study, DXA was used to assess body composition, enabling us to contribute to research on the association between body composition variables and vitamin D.

The endocrine-hormonal differences between sexes results in a differentiated body composition that can affect women’s health in different ways which justifies studying vitamin D deficiency and obesity only in women. In addition, vitamin D deficiency and obesity are related to infertility problems in women [19]. Furthermore, the prevalence of severe obesity is higher among women [1]. The distribution of body fat is an important cardiometabolic risk factor and abdominal fat is more associated with these complications than gluteofemoral fat. In general, this results from the distinction of sex hormones [20].

Several variables may be involved in the association between obesity level, body composition and vitamin D, although these aspects are still poorly understood [9,21]. The greater excess of body fat in class II/III obesity may be related to either lower or higher levels of vitamin D. A recent systematic review indicated that the relationship between vitamin D and obesity/adiposity remains controversial [16] given the several limitations of the studies. A cohort study showed that obese individuals with deficient 25(OH)D serum levels had greater increase in weight and waist circumference [22]. Therefore, investigating the factors associated with vitamin D in women with class II/III obesity will contribute to elucidate several controversial and unclear aspects mainly related to adiposity and abdominal obesity. The objectives of this study were: (i) to assess the prevalence of serum vitamin D deficiency and (ii) to identify factors associated with serum vitamin D levels, including body composition variables in adult women with class II/III obesity.

Methods

Study population

Data came from the baseline of a randomized clinical trial entitled DietBra Trial [23–25] in which participants were adult women aged 18–65 years with severe obesity (BMI ≥35 kg/m²), that is, class II/III obesity. Exclusion criteria were having undergone bariatric surgery, weight loss >8% in the last trimester, undergoing or having undergone nutritional treatment in the prior two years, using vitamin D supplement, renal or hepatic insufficiency, pregnant women, breastfeeding women, people with physical and/or mental disabilities, having metal rods and implants in the body.

Study procedures

After applying the eligibility criteria (inclusion and exclusion) and signature of the informed consent form, the following procedures were performed: application of questionnaires with sociodemographic, nutritional and health data and 24-h recall (24HR). Participants received guidance on how to prepare for exams and had the accelerometer positioned. Within a period of seven days, the following were performed: blood drawn for laboratory tests; weight and height measurements; DXA assessment; collection of the questionnaire on sun exposure; second application of the 24HR and accelerometer return.

Anthropometric and body composition variables

A 120 kg Welmy platform digital scale with 100 g precision was used for measurements of body mass and height. The BMI was calculated and classified considering class II obesity (BMI: ≥35 to
39.9 kg/m²), class III obesity (BMI: ≥40.0 kg/m²) and super obesity (BMI: ≥50 kg/m²) [3].

To obtain the body composition variables, the participant was positioned in the DXA device in a supine position with arms extended along the body [26]. The following were evaluated: total body mass (kg), total fat mass (kg), total fat free mass (kg), percentage of total fat (%), muscle mass (kg) and waist circumference.

**Sociodemographic and lifestyle variables**

The following variables were collected: age (in years); skin colour (white/mixed race and black); education (<9 years/≥ 9 years); economic class (A-B/C-D-E) [27]; smoking (yes/no); excessive alcohol consumption (yes/no) [28]; sun exposure (yes/no); use of sunscreen (yes/no); sun exposure time per day (<20/≥20 min).

Physical activity was assessed using an ActiGraph WGT3X accelerometer positioned on the non-dominant wrist and fitted with a tape below the head of the ulna on the first day of assessment. The participant was instructed to always keep the device; while sleeping, taking a shower or during any other activity. The accelerometer remained with participants for seven consecutive days. Physical activity time (min/week) was categorized as moderate/intense (≥150 min/week) [29]. Accelerometer data were analysed in minutes/week [30].

**Variables of general health conditions, chronic diseases, and medication use**

The presence and absence of diseases such as diabetes, hypertension, hypothyroidism and if or not the participant was in the menopause period were evaluated.

The use of continuous medication was identified in the medical prescription and/or by reading the package inserts of medications in use. Lipid-lowering drugs, metformin, antidepressants, and diuretics were evaluated (yes/no). Drugs that were used infrequently (anticonvulsants, proton pump inhibitors, H2 blockers, glucocorticoids, laxatives, and antifungals) were grouped into "other drugs" because they have the same pharmacodynamics in relation to vitamin D.

**Biochemical variables**

Serum vitamin D and calcium dosages were performed with fasting participants and by the same laboratory. For classification of participants in terms of serum vitamin D level, the following were used: deficiency <20 ng/ml and sufficiency ≥20 ng/ml [31].

**Food consumption variables**

To calculate vitamin D and calcium intake, three 24HR were considered, one performed by telephone and two in person at seven-day intervals [32]. Home measurements were converted to grams or millilitres according to the conversion tables [33]. Avanutri Online was used for the nutritional analysis of the diet, based on the Brazilian Composition Table (Portuguese acronym: TACO) [34]. Calculations of the mean of the three 24HR values were performed for vitamin D intake (sufficient intake ≥10 mcg/day) and for calcium intake (sufficient intake ≥800 mg/day) [35].

**Ethical aspects**

The main study was approved by the Research Ethics Committee of the Federal University of Goiás under number: 747.792/2014. All participants agreed and signed the informed consent prior to any study procedure.

**Statistical analysis**

The database was structured in EPI DATA® 3.1 with double entry typing for the validation and consistency analysis. The STATA/SE 14.0 Software was used in the analyses. The Kolmogorov Smirnov test was used to assess the normality of continuous variables. The outcome variable - serum vitamin D level had a normal distribution (p = 0.286). Unadjusted Linear Regression and Multiple Linear Regression (MLR) analyses were performed with beta calculation and respective 95% confidence intervals. Variables with p < 0.20 in the unadjusted regression were included in the MLR and those with p-value less than 0.05 were maintained in the MLR (stepwise).

**Results**

In total, 128 women with severe obesity with mean age of 39.71 years (SD ± 8.75) and serum vitamin D mean value of 30.02 ng/ml (SD ± 9.80) were evaluated. The classification of serum vitamin D was 14.66% with deficiency (mean 17.40 ng/ml; SD ± 2.87), while 86% presented sufficiency (mean = 32.08, SD ± 8.94) (Fig. 1). Among those with vitamin D deficiency, the prevalence of obesity classes were class II = 22.22%, class III = 38.89% and super obesity = 38.89% (p = 0.235). We did not observe statistical difference between deficiency and sufficiency by BMI, body fat percentage, and waist circumference (Fig. 1).

In the unadjusted linear regression, the sociodemographic, lifestyle and food intake variables associated with serum vitamin D were age groups 40—49 years (p = 0.005) and ≥50 years (p = 0.014) and inadequate calcium intake (p = 0.030). Sun exposure time per day (p = 0.072) and use of sunscreen (p = 0.168) were also included in the MLR (Table 1).

Among the variables of health conditions and use of medication, the following were considered for inclusion in the MLR: BMI (p = 0.192); menopause (p = 0.029); and use of lipid-lowering drugs (p = 0.150) (Table 2).

Anthropometric and body composition variables were not associated with serum vitamin D. Waist circumference (p = 0.150) was included in the MLR (Table 3).

The variables associated with serum vitamin D in class II and III obese women in the MLR were age groups 40—49 years (β = −5.45, 95% CI: −9.02 to −1.89) and ≥50 years (β = −5.85, 95% CI: −10.79 to −0.93), and inadequate calcium intake (β = −8.78, 95% CI: 16.52 to −1.04) (Table 4).

**Discussion**

Our study provided important evidence on vitamin D serum levels and its associated factors in a population that is still poorly studied i.e., women with class II/III obesity. Underexplored variables such as sun exposure, level of physical activity assessed by an objective method (accelerometer) and body composition (DXA device) variables were investigated as potential factors associated with serum vitamin D levels in individuals with severe obesity. Our key findings showed a lower vitamin D deficiency prevalence than expected in women with severe obesity with no differences between classes II, III and super obesity. Among the factors associated with lower serum vitamin D levels, we highlight age above 40 years and insufficient calcium intake.

In our study, the prevalence of vitamin D deficiency was lower than in morbidly obese individuals in Brazil [18], UK (60.2%) and Abu Dhabi (72%) [36]. Bariatric surgery patients in the South of Brazil have a deficiency prevalence of 55%, although this is the
Brazilian region with the lowest sun incidence [18]. In places such as the United Kingdom, where summers are rarely hot and winters are very cold, there is less cutaneous vitamin D synthesis given the low incidence of ultraviolet rays. Sun exposure is important to reach appropriate vitamin D serum levels, as sunlight stimulates the synthesis of this nutrient through the precursor of vitamin D in the skin [10,11]. Because of the local religious culture in Abu Dhabi, women wear clothing that covers much of their body, which makes it difficult to expose the skin to sunlight. These may be some aspects involved in the high prevalence of vitamin D deficiency in morbidly obese in these two countries.

A possible explanation for the low prevalence of vitamin D deficiency in our study can be the high incidence of sunlight in central Brazil, where this study was conducted. Another reason, although still controversial, is that excess body fat acts as a reservoir of vitamin D in the body. Vitamin D is fat-soluble, that is, vitamin D metabolites are attracted and retained in the adipose tissue. In addition, we found that the mean vitamin D levels in those individuals losing weight vitamin D remained marginally significant [8].

In our study, we did not observe significant differences between the prevalence of deficiency according to BMI class, waist circumference and body composition variables with vitamin D levels. Previous evidence on vitamin D and its association with obesity, central obesity and adiposity, controversial results were found [9,12,18,22,36,37]. We expected a higher prevalence of vitamin D
deficiency in class II/III obese subjects, as well as a negative association between the total fat mass and BMI with serum vitamin D levels. An analysis of 21 cohort studies with 42,024 participants indicated an association between the increase of 1 kg/m² BMI and a 1.15% reduction in vitamin D serum levels [38]. In a systematic review study, a higher prevalence of vitamin D deficiency was observed among obese individuals compared to those of normal weight [37]. The results of these studies were different from ours, where a low occurrence of vitamin D deficiency was observed in classes II and III obesity. It is difficult to compare our results with those of previous studies as these included all levels of obesity and both sexes, while we analyzed only women with class II and III obesity.

We found a reduction of about 5 ng/ml of vitamin D in each year of life between 40 and 49 years of age, and this reduction was greater than 6 ng/ml in those aged 50 years or older. With advancing age, the ability to synthesize vitamin D in the skin decreases because the reduction of the precursor 7-dehydrocholesterol minimizes the ability to produce vitamin D3, which may lead to vitamin D deficiency [39,40]. However, in a study of 118 morbidly obese adults in UK, where 79% were female participants and 90% had vitamin D insufficiency, an association between vitamin D insufficiency and age was not found. Given this result, we suggest that women aged 40 years and older with class II and III obesity should be encouraged to have sun exposure and consumption of foods high in vitamin D to prevent this micronutrient deficiency.

A cross-sectional study with healthy adults in Saudi Arabia identified an association between inadequate calcium intake and a greater chance of vitamin D deficiency [41]. Although we have not found previous studies evaluating this association in patients with severe obesity, in a cross-sectional study of adults in southern Brazil, a significant association between low calcium intake and higher BMI and waist circumference was found [42]. Vitamin D is known to play an important role in bone regulation, along with calcium [40], being considered a steroid hormone, with functions of bone resorption, formation and calcium homeostasis [40]. Long-term calcium deficiency can cause reduced bone mass, resulting in osteoporosis in adults and older adults [40].

We did not identify significant associations between health condition variables such as menopause, hypertension, diabetes, and hypothyroidism with vitamin D deficiency. We also did not find significant associations between sun exposure and use of sunscreen with vitamin D serum levels, as observed in another study with a sample of Brazilian adults [43]. However, in other investigations with an observational design, an association between sun exposure and vitamin D serum levels was found [44].

Potential limitations of our study include the food intake variables that were assessed by three 24-h recalls, since this method depends on participants’ reports and memory. However, applying this instrument on three different days minimizes possible bias and

| Table 1 |

Association of serum vitamin D with sociodemographic variables, lifestyle, and food intake in women with class II/III obesity: unadjusted linear regression.

<table>
<thead>
<tr>
<th>Variables</th>
<th>(n %)</th>
<th>Mean (±SD)</th>
<th>Serum vitamin D (ng/mL)</th>
<th>p</th>
<th>95%CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18–39 years</td>
<td>64 (50.00)</td>
<td>32.80 (±9.92)</td>
<td>1.00</td>
<td>0.004</td>
<td>–8.98 to –1.67</td>
<td></td>
</tr>
<tr>
<td>40–49 years</td>
<td>46 (35.94)</td>
<td>27.51 (±8.73)</td>
<td>–5.29</td>
<td>–6.27</td>
<td>–11.27 to –1.28</td>
<td></td>
</tr>
<tr>
<td>≥50 years</td>
<td>18 (66.14)</td>
<td>26.53 (±9.54)</td>
<td>–6.27</td>
<td>–5.07 to –5.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin color/race</td>
<td>White/mixed race</td>
<td>111 (86.72)</td>
<td>30.02 (±9.94)</td>
<td>1.00</td>
<td>0.997</td>
<td>0.623</td>
</tr>
<tr>
<td>Black</td>
<td>17 (13.28)</td>
<td>30.03 (±9.04)</td>
<td>0.009</td>
<td>–4.50 to –2.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schooling years</td>
<td>&lt;9 years</td>
<td>83 (64.84)</td>
<td>30.33 (±10.04)</td>
<td>1.00</td>
<td>0.292</td>
<td>0.747</td>
</tr>
<tr>
<td>≥9 years</td>
<td>45 (35.16)</td>
<td>29.44 (±9.41)</td>
<td>–0.90</td>
<td>–5.67 to –1.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Socioeconomic class</td>
<td>A-B</td>
<td>26 (20.31)</td>
<td>29.88 (±9.96)</td>
<td>1.00</td>
<td>–4.46 to –6.18</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>78 (60.94)</td>
<td>29.33 (±10.09)</td>
<td>–0.55</td>
<td>0.904</td>
<td>–3.46 to –3.91</td>
<td></td>
</tr>
<tr>
<td>D-E</td>
<td>24 (18.75)</td>
<td>32.40 (±8.58)</td>
<td>2.51</td>
<td>–4.00 to –1.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td>No</td>
<td>36 (53.73)</td>
<td>30.24 (±9.86)</td>
<td>1.00</td>
<td>0.072</td>
<td>0.168</td>
</tr>
<tr>
<td>Yes</td>
<td>41 (66.27)</td>
<td>29.37 (±11.95)</td>
<td>0.86</td>
<td>0.202</td>
<td>–3.46 to –3.91</td>
<td></td>
</tr>
<tr>
<td>Ex-smoker/smoker</td>
<td></td>
<td>40 (31.25)</td>
<td>28.66 (±9.19)</td>
<td>–1.98</td>
<td>0.940</td>
<td>–4.46 to –6.18</td>
</tr>
<tr>
<td>Excessive alcohol consumption (n = 67)</td>
<td>No</td>
<td>16 (18.60)</td>
<td>33.89 (±8.35)</td>
<td>1.00</td>
<td>0.004</td>
<td>–3.46 to –3.91</td>
</tr>
<tr>
<td>Yes</td>
<td>70 (81.40)</td>
<td>29.37 (±9.08)</td>
<td>4.52</td>
<td>–4.96 to –0.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sun exposure</td>
<td>No</td>
<td>68 (79.07)</td>
<td>30.90 (±9.46)</td>
<td>1.00</td>
<td>–8.08 to –1.43</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>18 (20.93)</td>
<td>27.58 (±7.09)</td>
<td>–3.32</td>
<td>0.202</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>Sun time/day (n = 86)</td>
<td>&lt;20 min</td>
<td>89 (91.75)</td>
<td>30.27 (±10.22)</td>
<td>1.00</td>
<td>–4.74</td>
<td>0.030</td>
</tr>
<tr>
<td>≥20 min</td>
<td>87 (67.97)</td>
<td>30.09 (±9.08)</td>
<td>0.23</td>
<td>–12.07 to –2.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sunscreen</td>
<td>No</td>
<td>122 (95.31)</td>
<td>29.60 (±9.60)</td>
<td>1.00</td>
<td>0.328</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>9 (8.08)</td>
<td>38.45 (±10.92)</td>
<td>–8.84</td>
<td>–16.83 to –0.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium intake (mg/day)</td>
<td></td>
<td>35.01 (±6.8)</td>
<td>–4.74</td>
<td>–0.54 to –1.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin D intake (mcg/day)</td>
<td>0.013–9.77</td>
<td>2.00 (±1.60)</td>
<td>0.53</td>
<td>–8.84</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Unadjusted linear regression analysis.
MVP: moderate to vigorous physical activity.
* Smokers: n = 86.
increases reliability. The number of participants in this study may have limited the identification of some associations. The fact that severe obesity affects around 5% of the general population makes it difficult to have a larger sample. Samples from other studies with class II/III obese subjects ranged from 40 to 291 participants [6,18].

We would like to highlight the following strengths of this study: (i) the use of DXA device, which is considered the gold standard for assessing body composition; (ii) methodological rigor during all steps of research and the training of the research team that reflect on the quality of study procedures; and (iii) the simultaneous analysis of several variables that showed plausible association with serum levels of vitamin D; and (iv) the use of an objective physical activity measure i.e. accelerometer.

Obesity is a risk factor for non-communicable chronic diseases, especially cardiovascular diseases, and vitamin D deficiency also contributes to a higher risk for this group of diseases given its association with atherosclerosis of the carotid arteries [45]. Further studies should investigate individuals with class II and III obesity outside the context of bariatric surgery. Future prospective studies should analyze risk and protective factors for vitamin D deficiency in these individuals including the association with cardiovascular diseases. The assessment of body composition with more accurate methods such as DXA is important to clarify the influence of excess adiposity and other body composition variables on vitamin D serum levels.

This study contributed to elucidate some factors associated with vitamin D serum levels in women with class II and III obesity, a population that remains under investigated. We found a low vitamin D deficiency prevalence in women with class II/III obesity. Women with severe obesity aged 40 years or older with inadequate calcium intake from dietary sources are more likely to have vitamin D deficiency also contributes to a higher risk for this group of diseases given its association with atherosclerosis of the carotid arteries [45]. Further studies should investigate individuals with class II and III obesity outside the context of bariatric surgery. Future prospective studies should analyze risk and protective factors for vitamin D deficiency in these individuals including the association with cardiovascular diseases. The assessment of body composition with more accurate methods such as DXA is important to clarify the influence of excess adiposity and other body composition variables on vitamin D serum levels.

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Author contributions

Conceptualization — EAS, PVOV; Data curation — EAS, ASACS, CKSC; Formal analysis — ASACS, AS, PVOV, EAS; Methodology — EAS, PVOV, ASACS, CKSC; Resources — EAS; Investigation — EAS, CKSC, ASACS; Project administration: EAS, ASACS; Supervision — EAS, PVOV; Validation — all authors; Visualization — all authors; Writing (original draft) — EAS, LCS, AS; Writing (review & editing) — all authors; Funding acquisition — EAS.

Declaration of Competing Interest

There are no conflicts of interest to declare.

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