Article

Bariatric Surgery and Vitamin D: Trends in Older Women and Association with Clinical Features and *VDR* Gene Polymorphisms.

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Abstract: (1) Background: Obesity and its comorbidities can cause burdens and limitations. Bariatric surgery (BS) is indicated as a safe procedure to reduce body mass and improve present comorbidities. Yet, several complications were reported, like vitamin D [25(OH)D] deficiency. We evaluated if 25(OH)D serum levels relate to clinical characteristics, symptoms, or habits in women after their BS and whether the vitamin D receptor (*VDR*) gene's *Taq*I and *Fok*I polymorphisms affected 25(OH)D levels and the total body bone mineral density (TBBMD). (2) Methods: This cohort cross-sectional comparative analytical prospective study consisted of 27 women, 61.6 ± 5.0 years, submitted to BS one year prior at a public reference hospital, DF-Brazil. All participants were asked to follow the physical and dietary activity recommendations and received vitamin D3 supplements. Their anthropometric, biochemical, and immunological measurements and blood samples were obtained. (3) Results: 73.3% of participants had low 25(OH)D levels, and their levels correlated positively with TBBMD and negatively with systolic pressure. *VDR Taq*I did not affect 25(OH)D levels, whereas *VDR FokI*'s allele f presence correlated to a median rise in 25(OH)D levels. Neither polymorphism correlated to TBBMD. (4) Conclusions: 25(OH)D levels were positively correlated with TBBMD, negatively with systolic blood pressure, and were higher in those with the *VDR Fok*I allele *f*.

Keywords: Roux-en-Y gastric bypass bariatric surgery; vitamin D; Vitamin D Receptor; FokI (rs2228570); TaqI (rs731236)

1. Introduction

The quantitative increase in the population's older adult ratio worldwide is strongly known internationally [1,2]. Some demographic projections argue that by the year 2050, about 21% of the world's population will consist of people in their sixties or older (>60 years) [1,2]and that approximately 80% of these will be living in low- and middle-income classified nations [3]. This increase, however, in life expectancy has not necessarily been in healthy work-capable older adults' portion that could justify an increase in the state pension age, but mainly in illness-prone, chronically ill portion. Even though some major

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risk factors have declined with age (e.g., smoking), others have increased - particularly Chronic Non-Communicable Diseases (CNCD), such as obesity. In older adults, obesity and morbid obesity are dangerous organic conditions [4,5]identified in both industrialized and post-industrialized nations [6] The increase in obesity in the past 40 years has been staggering, representing approximately two billion people worldwide [2], and might configure the most significant global risk public health has ever met [6–8].

For some researchers, obesity is characterized as a systemic inflammatory disorder [9], complex and multifactorial [7,9], highly prevalent [6,7,9], and correlated with several chronic and cardiovascular diseases [7–10]. Physical inactivity [10,11], adipose tissue accumulation [10–12], consequences related to dietary factors [12–14], psychosocial aspects [12–14], genetic susceptibility [15,16], and obesogenic coefficients [17] are some of the phenomena related to obesity that negatively impact health [12–14,18]and quality of life [13,15].

For other researchers, obesity and its comorbidities can cause complex commitments and limitations in older adults [11,14]regarding their life and existence, for example, in work performance [7,11,17], financial and economic activities, and participatory citizenship in society [11,17]. Among the various diseases and comorbidities related to obesity, we can mention arterial hypertension [10,19], coronary artery disease [19], diabetes mellitus [10,19] dyslipidemia [7,10], obstructive sleep apnea syndrome [7,10], steatohepatitis[17], various types of cancer [10,19], deterioration of lower limb torque [20], in addition to an increased risk of death on account of the comorbidities mentioned above [19,21,22].

This way, bariatric surgery (BS) is indicated as a safety procedure [21,23,24] to combat obesity that significantly reduces body mass and improves present comorbidities [21–26]. Nonetheless, there are still controversies about its effectiveness in older adults [25,26] and their postoperative recovery [25]. According to some researchers, older patients are less frequently submitted to BS compared to patients in a lower age group [22,25], although their numbers have increased in the last decades to approximately 5-6% of the total BS [22].

In contrast, several BS-related complications have been documented, such as malnutrition, chronic nausea and vomiting, acid reflux, inability to eat certain foods, and esophageal dilation [10,11]. Among these, malnourishment significantly threatens the health of patients subjected to bariatric surgery [12,18], as even purely restrictive surgeries can render about a 20–50% insufficiency of micronutrients[18]. The gap in knowledge on the potential health problems linked to each type of bariatric procedure must be considered in both pre-surgery and post-surgery stages to manage patients' long-term weight loss and health. For instance, all types of bariatric operations reported vitamin D [25-hydroxyvitamin D - 25(OH)D, with D representing either D2 or D3] deficiency [14], which could result in secondary hyperparathyroidism (SHPT), especially in patients subjected to malabsorptive surgical interventions [13].

Hence, human BS studies should also assess the presence of genetic polymorphisms in vitamin D nutritional metabolic processes, such as vitamin D's receptor (VDR) and its metabolizing enzymes. This receptor's gene, located on chromosome 12 (12q13.11), is expressed in most of the immune system’s cells, including CD4+ and CD8+ T lymphocytes, as well as antigen-presenting cells, such as macrophages and dendritic cells[27]. VDR belongs to the nuclear receptor superfamily of the transcription regulatory factors for steroid hormones, retinoic acid, thyroid hormone, and vitamin D and consists of eleven exons [28]. The VDR protein is encoded by exons II to IX, with exons VII to IX involved in the binding of VDR to its ligand, vitamin D [29] .After 1,25(OH)2D [1,25-dihydroxy vitamin D] binds with the VDR, the receptor interacts with the retinoic acid receptor (retinoid X receptor), forming a heterodimeric complex (RXR-VDR) which, in turn, binds to specific DNA sequences known as the vitamin D responsive element (VDRE) [30]. The main target organs for 1,25(OH)2D are the intestine, bone, parathyroid glands, and kidney. However, several other tissues also have VDR [31].

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Genetic alterations in the *VDR* gene can lead to significant defects in gene activation, affecting calcium metabolism, cell proliferation, and immune function that changes in receptors' protein conformation could explain [32]. Interestingly, despite the increasing number of studies scrutinizing associations between genetic variations and several diseases, only a few studies have considered *VDR* polymorphisms [33]. *VDR* gene contains several different genetic polymorphisms, including *Apa*I (rs7975232, intron 8, +64978 C>A), *Bsm*I (rs1544410, intron 8, +63980 G>A), *Taq*I (rs731236, exon 9, +65058 T>C), and *Fok*I (rs2228570, formerly rs10735810, exon 2, +30920 C>T) [34,35], all of which been reported to be associated with several diseases[32,33].

The *Taq*I (rs731236) polymorphism is a single nucleotide polymorphism (SNP) that leads to a synonymous change (T>C), as both encode the amino acid isoleucine [36]. Although generating a silent mutation, this transition changes some functional characteristics of the protein, with the T allele associated with increased transcriptional activity, mRNA stability, and a high vitamin D serum level [36,37]. Another functional SNP is the *Fok*I (rs2228570) that modifies the translation initiation codon (C>T) and produces a peptide shorter by three amino acids (424<427) with a higher transcriptional activity compared to the original, leading to a change in the VDR protein activity [38,39]. However, this effect appears to be gene-specific and cell-type-specific.

In this context, this study evaluated if 25(OH)D serum levels relate to anthropometric, biochemical, and immunological characteristics and other remarkable characteristics in women after twelve months of their bariatric surgery. Furthermore, this study assessed whether there is a difference between 25(OH)D serum level and the total body bone mineral density according to the presence of the *VDR* gene's *Taq*I (rs731236) and *FokI* (rs2228570) polymorphisms.

2. Materials and Methods

2.1. Study design and Research Participants

This research is a cohort, cross-sectional, comparative, analytical, prospective study with a quantitative and qualitative approach. The research participant sample consisted of 27 older female adults, aged fifty (50) years or more (61.6 ± 5.0 years), submitted to Roux-en-Y gastric bypass bariatric surgery (BS) one (01) year prior at a reference public hospital of the Federal District's Secretary of State of Health (SES-DF, Brazil), and able to understand, verbalize, and answer the proposed questions (inclusion criteria). Participants were excluded from the study if they had a mental illness, were under the age of fifty (50), did not undergo BS, had their BS performed procedure in less than one (01) year, if BS was not performed with the Hospital Regional da Asa Norte (HRAN), or if they not fit the inclusion criteria established by this research. The Federal District State Department of Health (SES-DF)'s Health Sciences Teaching and Research Foundation (FEPECS)'s Research Ethics Committee (CEP), under opinion number 1.910.166, approved this study. All participants signed the Informed Consent Form (ICF).

2.2 Clinical and Laboratory Evaluation

For their Clinical and Laboratory Evaluation, all the participants were asked to follow the physical and dietary activity recommendations detailed in the clinical protocols for identifying, assessing, and treating obesity and overweight in adults [21] and received vitamin D3 supplements (1000 IU/day). The age, height, weight, and body mass index (BMI) were obtained from the nursing consultation's medical record and responses to the collection instruments.

The clinical data and the blood samples were collected at 12 months post-surgery. All the biochemical parameters, i.e., triglycerides (TG), fasting blood sugar (FBS), and minerals, were assessed in the same laboratory using standard commercial methodolo-

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gies. The serum levels of the vitamin D [25-hydroxyvitamin D - 25(OH)D, with D representing either D2 or D3] were also measured using a standard commercial chemiluminescence immunoassay in the same laboratory of the other biochemical analysis.

TNF-α, IL-6, IL-10, and IL-2 serum levels were measured by enzyme-linked immunosorbent assay (ELISA) technique - Human ELISA Kit (Invitrogen, San Diego, CA; Thermo Fisher Scientific, Schwerte, Germany) - these assays detect only human cytokines. The minimum detectable concentrations considered in our laboratory were 4.8pg/mL for TNF-α, 1.1pg/mL for IL-6, 2.0pg/mL for IL-10, and 1.0pg/mL for IL-2.

2.3 Genotype Analysis

For genotyping, deoxyribonucleic acid (DNA) was extracted from the participants' collected blood using Invitrogen's PureLink® Genomic DNA Mini (catalog #K1820-02, lot #19339891), with a 20 ng/μL average concentration.

*VDR Taq*I (rs731236, exon 9, +65058 T>C) polymorphism was genotyped using the polymerase chain reaction combined with the restriction fragment length polymorphism (PCR-RFLP) based analysis. The primers used were forward/sense 5'-CAG AGC ATG GAC AGG GAG CAA G -3 'and reverse/antisense 5'-GCA ACT CCT CAT GGG CTG AGG TCT CA -3'[40]. The DNA's amplification was performed using the following thermocycling conditions: 95˚C for 5 minutes (initial denaturation), followed by 35 cycles of denaturation at 94˚C for 30 seconds, annealing at 65˚C for 30 seconds, and extension at 72˚C for 30 seconds. The final extension occurred at 72˚C for 10 minutes. The PCR product is a 740 bp fragment amplified from the *VDR* gene's exon 9 region. After its 3h digestion with *Taq*I restriction enzyme (Jena, Germany), the polymorphism is cleaved into three bands of 290, 245, and 205 bp, defined as mutant *t* (C) allele, while the appearance of two 490 and 245 bp fragments indicates the presence of the ancestral allele *T* (T). Therefore, the TT (TT) genotype is defined by 490 and 245 bp presence, the *Tt* (TC) genotype by 490, 290, 245, and 205 bp, and the tt (CC) genotype by 290, 245, and 205 bp.

*VDR Fok*I (rs2228570, formerly rs10735810, exon 2, +30920 C>T) SNP analysis was also performed by PCR-RFLP. The primers' sequence were as follows: forward/sense 5'-AGCTGGCCCTGGCACTGACTCTGCTCT-3' and reverse/antisense 5'-ATGGAAACAC-CTTGCTTCTTCTCCCTC-3'[41]; with thermocycling parameters as follows: 95˚C for 5 minutes (initial denaturation), followed by 35 cycles of denaturation at 94˚C for 30 seconds, annealing at 61˚C for 30 seconds, and extension at 72˚C for 30 seconds, and one final extension cycle at 72 °C for 5 minutes. The 280 bp PCR product was then digested with FokI restriction enzyme (cat# FD2144, Thermo Scientific) and incubated at 37 °C for 15 minutes. The digested products were 265 bp for the C (*F*) allele and 169 and 96 bp for T (*f*) allele. Whereas the *FF* (CC) genotype is defined by 265 bp presence, the *Ff* (CT) genotype by 265, 169, and 96 bp, and the *ff* (TT) genotype by 169 and 96 bp.

The *VDR Taq*I (rs721236) and VDR *Fok*I (rs2228570) polymorphism genotypic patterns were determined by running the digested products on a 3% agarose gel.

2.4 Body composition assessment

Each set (two repetitions per participant) of individual's dual-energy X-ray absorptiometry (DXA) measurements were performed on the same day by the same operator, with the participants under at least four hours of fasting and 24 hours of exercise abstinence to ensure adequate hydration conditions.

A Lunar Prodigy Advance equipment (General Electric Systems, Madison, WI) was employed to determine Fat Mass (FM, g), Lean Mass (LM, g), and Total Body Bone Mineral Density (TBBMD, g). The DXA device was calibrated with phantoms before each set of measurements. Our laboratory's variability coefficient was 1.03, 1.35, and 0.83% for FM, LM, and TBBMD, respectively.

2.5 Statistical Analysis

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For statistical analysis, absolute and relative frequency distribution was applied for categorical variables and quartiles for continuous variables — with continuous data expressed as mean ± standard error (SE) or Percentiles. Spearman's coefficient was used to test the correlation between the continuous data of anthropometric, biochemical, and immunological measures and 25(OH)D levels. For the clinical characteristics expressed as categorical data or genotypic frequency, the evaluation of the difference in 25(OH)D serum levels/Total body bone mineral density (TBBMD, g) ratio between the groups was evaluated by non-parametric Mann-Whitney U test or Kruskal-Wallis test, because the assumptions of normality were not observed. The chi-square test with one degree of freedom analyzed Hardy-Weinberg equilibrium adherence to the genotypic frequency in controls. The tests were performed with SPSS software version 28.0 (SPSS Inc., Chicago, IL, USA), adopting a significance level of 5.0%.

3. Results

3.1. Vitamin D association with Anthropometric and Biochemical Measurements

Vitamin D [25-hydroxyvitamin D, 25(OH)D] serum levels in women after 12 months of bariatric surgery averaged 27.31 ±7.71 ng/mL. In this group, 11 women (40.0%) had dangerously low levels, up to 25.0 ng/mL, while 20 women (73.3%) had up to 30 ng/mL (standard range in literature). The minimum value found was 12.50 ng/mL, and the maximum was 40.70 ng/mL.

25(OH)D's possible associations with anthropometric, immunology or biochemical parameters were assessed by calculating the association's Spearman correlation coefficients. Table 1 displays 25(OH)D correlations with the selected variables. 25(OH)D serum levels significantly correlated with Total Body Bone Mineral Density (TBBMD, g) (ϱ = 0.514*, P = 0.010) and were negatively associated with systolic pressure (ϱ = -0.711*, P = 0.049) at 12 months (r = −0.219, P = 0.041) after surgery.

On the other hand, 25(OH)D serum levels correlations with diastolic pressure (mmHg), Body Mass Index (BMI, kg/m-2), Magnesium, Vitamin B12, TSH, T3, T4 total, insulin, fasting blood glucose, Total cholesterol, Triglycerides, HDL, LDL, VLDL, Non-HDL cholesterol, Total Lipids, Uric Acid, Sodium, Potassium, Chlorine, Calcium, IL-2 (pg/mL), TNF-A (pg/mL), IL-6 (pg/mL), IL-10 (pg/mL), Fat Mass (FM, g), Lean Body Mass (LBM, g) were not significant (Table 2).

Table 1. Correlation between vitamin D [25(OH)D] serum levels and biochemical, immunological, and anthropometric parameters.

25(OH)D (ng/ml) x	ϱ	P	Parameters
Magnesium (mg/dL)	-0.182	0.516	Biochemical parameters
Vitamin B12 (pg/mL)	0.125	0.657	
TSH (mUI/L)	-0.057	0.84	
T3 (ng/dL)	-0.008	0.977	
T4 total (μg/dL)	-0.097	0.732	
Insulin (mU/L)	0.057	0.841	
Fasting blood glucose (mg/dL)	0.081	0.776	
Total cholesterol (mg/dL)	-0.171	0.545	
Triglycerides (mg/dL)	-0.397	0.143	
HDL (mg/dL)	-0.297	0.283	
LDL (mg/dL)	0.068	0.811	
VLDL (mg/dL)	-0.397	0.143	
Non-HDL Cholesterol (mg/dL)	-0.054	0.849	

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Total lipids (mg/dL)	-0.296	0.283
Uric acid (mg/dL)	-0.106	0.707
Sodium (mEq/L)	0.089	0.751
Potassium (mEq/L)	0.102	0.718
Chlorine (mEq/L)	-0.068	0.811
Calcium (mg/dL)	0.368	0.177
IL-2 (pg/mL)	0.189	0.499
TNF-α (pg/mL)	-0.051	0.861
IL-6 (pg/mL)	0.368	0.177
IL-10 (pg/mL)	0.404	0.136
Fat Mass (FM, g)	0.343	0.211
Lean Body Mass (LBM, g)	0.136	0.631
Total body bone mineral density (TBBMD, g)	0.514	0.049*
Systolic pressure (mmHg)	-0.711	0.010*
Diastolic pressure (mmHg)	-0.311	0.327
Body mass index (BMI, kg/m-2)	-0.051	0.861

Immunological parameters

Anthropometric parameters

*P<0,05 – Spearman correlation coefficient; ϱ: Spearman's rank correlation coefficient; TSH = Thyroid-stimulating hormone; T3 = Triiodothyronine; T4 = Thyroxine; HDL = High-density lipoprotein; LDL = Low-density lipoprotein; VLDL = Very low-density lipoprotein; IL-2 = interleukin 2; TNF-α = Tumor necrosis factor alpha; IL-6 = interleukin 6; IL-10 = interleukin 10.

3.2. Participants' vitamin D serum levels and other clinical signs and symptoms

The participants were also evaluated regarding the median difference in 25(OH)D serum levels and the presence/absence of other clinical characteristics, symptoms, or habits, such as smoking, alcoholic, hypertension, depression/anxiety, fibromyalgia, neuropathy, arthralgia or myalgia, frequent thirst, d getting up at night to drink water, loss or alteration of taste, xerostomia (dry mouth), photophobia, itching or rash (pruritus), tingling or numbness, pain in the lower limbs, decreased sweating (perspiration) and altered sexual performance. None of these characteristics were related to changes in 25(OH)D serum levels (Table 2).

Table 2. Participants' vitamin D [25(OH)D] serum levels according to the presence/absence of other clinical characteristics, symptoms, or habits.

Clinical characteristics, symptoms, or habits		**25(OH)D (ng/mL)**				
		P25	**Median**	**P75**	**N**	**P**
Smoker	yes	20.20	27.10	34.00	2	
	no	20.65	26.70	35.15	18	0.983
	ex-smoker	25.20	26.30	27.00	7	
Alcoholic	yes	23.70	28.85	34.00	2	0.999
	no	22.30	26.30	30.60	25	
Hypertension	yes	22.30	26.25	34.00	23	0.933
	no	27.00	27.00	27.00	4	
Depression/anxiety	yes	20.20	26.65	30.60	11	0.864
	no	23.70	26.20	34.00	16	
Fibromyalgia	yes	22.30	39.70	40.70	4	0.233

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	no	21.95	26.25	28.90	23	
Dyslipidemia	yes	19.00	21.35	23.70	5	0.171
	no	25.20	27.00	34.00	22	
Vaginal dryness	yes	24.80	32.80	37.35	6	0.343
	no	22.30	26.20	27.20	21	
Retinopathy	yes	22.30	26.20	27.20	6	0.734
	no	21.95	26.65	34.50	21	
Nephropathy	yes	--	--	--	0	NA
	no	22.30	26.30	34.00	27	
Neuropathy	yes	20.20	20.20	20.20	3	0.401
	no	23.70	26.65	34.00	24	
Arthralgia or myalgia	yes	22.30	27.00	35.00	10	0.779
	no	21.95	26.25	30.60	17	
Dysphagia or dyspepsia	yes	25.20	32.45	39.70	3	0.571
	no	22.30	26.30	30.60	24	
Frequent thirst	yes	23.70	26.20	30.60	11	0.867
	no	21.25	26.75	34.50	16	
Difficulty chewing dry food	yes	27.00	30.60	39.70	8	0.206
	no	22.30	25.70	27.20	19	
Difficulty speaking	yes	25.20	27.20	30.60	5	0.734
	no	21.25	26.25	34.50	22	
Gets up at night to drink water	yes	23.70	26.65	35.00	13	0.513
	no	20.20	26.20	30.60	14	
Loss or alteration of taste	yes	25.20	27.10	34.00	10	0.607
	no	22.30	26.20	30.60	17	
Xerostomia (dry mouth)	yes	25.20	26.30	34.00	13	0.607
	no	20.20	24.65	30.60	14	
Dry eyes	yes	23.70	26.30	34.00	13	0.776
	no	20.20	26.60	30.60	14	
Eye irritations	yes	23.70	26.30	34.00	12	0.779
	no	19.60	26.60	32.80	15	
Photophobia	yes	21.95	25.70	30.50	15	0.463
	no	22.30	27.20	39.70	12	
Myopia/hyperopia	no	20.20	27.10	34.00	4	0.999
	yes	23.70	26.30	30.60	23	
Eye drops	yes	23.00	25.00	26.75	5	0.571
	no	20.20	27.00	35.00	22	
Skin dryness	yes	25.20	27.20	35.00	17	0.145
	no	19.00	24.95	26.30	10	
Itch or rash (pruritus)	yes	22.70	27.90	32.30	6	0.999
	no	22.30	26.30	35.00	21	
Cracks (fissures) or red spots	yes	20.20	25.20	39.70	4	0.945

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	no	23.00	26.65	32.30	23	
Tingling or numbness	yes	23.70	26.25	30.60	17	0.859
	no	20.20	27.00	34.00	10	
Lower limb pain	yes	23.70	26.30	35.00	19	0.851
	no	21.25	26.45	32.30	8	
Decrease perspiration	yes	22.30	23.70	30.60	4	0.633
	no	22.70	26.65	34.50	23	
Altered sexual performance	yes	19.00	22.30	27.20	8	0.295
	no	24.45	26.65	34.50	19	

Note: P25: 25th percentile; P75: 75th percentile.

3.3. Vitamin D receptor (VDR) gene polymorphisms and their relationship with vitamin D [25(OH)D] serum levels and total body bone mineral density

After determining that the *VDR Taq*I and *VDR Fok*I polymorphisms' genotypic distribution obeyed the Hardy-Weinberg equilibrium (P > 0.05), we ultimately verified whether the participants' vitamin D receptor genetic polymorphism altered their 25(OH)D serum levels. For VDR FokI polymorphism, the mutant allele f presence correlated to a median rise in 25(OH)D serum level both in the genotypic distribution (P = 0.005) and in the dominant model (*FF* versus *Ff+ff*, P = 0.001) evaluation. In comparison, no differences were found regarding the *VDR Taq*I polymorphism. Furthermore, neither of their presences was related to total body bone mineral density (TBBMD). All these analyzes are presented in Table 3.

Table 3. VDR TaqI and VDR FokI polymorphisms' genotypic distribution according to the participants' vitamin D [25(OH)D] serum level distribution and total body bone mineral density (TBBMD).

VDR polymorphism		N	P (HW)	25(OH)D (ng/mL)			P	Total body bone mineral density (TBBMD, g)			P
				P25	Median	P75		P25	Median	P75	
TaqI	*TT*	12	0.266	22.30	27.20	35.00	0.592	1926.5	2112.5	2388.0	0.835
	Tt	13		12.50	26.20	27.00		1903.0	1990.0	2336.0	
	tt	2		26.30	26.30	26.30		1757.0	2064.5	2372.0	
	TT	12	NA	22.30	27.20	35.00	0.412	1926.5	2112.5	2388.0	0.581
	Tt+tt	15		19.35	26.25	26.65		1849.0	1990.0	2372.0	
FokI	*FF*	10	0.257	15.75a	19.60	21.25	0.005*	1733.0	2141.0	2501.0	0.217
	Ff	15		26.20b	27.00	30.60		1903.0	1953.0	2336.0	
	ff	2		39.70c	40.20	40.70		2370.0	2437.5	2505.0	
	FF	10	NA	15.75a	19.60	21.25	0.001#	1733.0	2141.0	2501.0	0.711
	Ff+ff	17		26.20b	27.20	35.00		1913.0	1973.0	2370.0	

Note: P25 - 25th percentile; P75 - 75th percentile; HW - Hardy-Weinberg equilibrium. Different letters denote statistical differences. *P < 0.005, Kruskal Wallis H Test. #P < 0.005, Mann-Whitney U test.

4. Discussion

Many nutrients are co-dependent and simultaneously influenced by genetic and hormonal factors, reciprocal interaction with various lifestyle modifiers, or a combination of these. Due to these interactions' complexity and the biological factors' dominant influence,

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nutrients' effects might be masked and hard to distinguish. Notably, some statements about the potential role of micronutrients (minerals and vitamins) in body health (bones, muscles, among others) are either based on animal studies or just theoretical presumptions, either untested or unproven in human studies. All these factors are probable reasons why many studies have controversial or inconsistent findings regarding the contribution of a single or a group of nutrients to body health[42].

The present study found that 73.3% of patients who underwent Roux-en-Y gastric bypass a year prior, even under vitamin D3 supplements (1000 IU/day), had low vitamin D [25(OH)D] serum levels (up to 30 ng/mL) and that their 25(OH)D serum levels positively correlated with their Total Body Bone Mineral Density (TBBMD, g).

Vitamin D deficiency, considered one of the main determinants of senile osteoporosis, is much more frequent than imagined in the older adult population, making minimalizing these neuromuscular effects relevant in preventing osteoporotic fracture. 25(OH)D serum levels lower than 80 nmol/L (approx. <32ng/mL; 1 ng/mL = 2,5 nmol/L) [43], the vitamin D's current functional status indicator, are associated with reduced calcium absorption, osteoporosis, and increased fracture risk, with the classic histological alterations of osteomalacia and rickets are already evident, with deficient mineralization of the osteoid matrix[44–46]. In this situation, hypocalcemia and hypophosphatemia may be manifest [47].

The high vitamin D deficiency prevalence in older adults could have several causes. For instance, after sun exposure, the synthesis of cholecalciferol (vitamin D3) in the skin is less effective in old age due to a decline in cutaneous 7-dehydrocholesterol levels, roughly 25% lesser in a 70-year-old than in young people[48–50]. This reduction worsens by the decreased exposure to sunlight due to immobility, lack of transport, and social isolation usually associated with aging. Another contributing factor is the increase in body fat with aging, which leads to a larger distribution volume for the fat-soluble 25(OH)D3, decreasing the 25(OH)D3 bioavailability[51].

Regarding vitamin D3 supplementation, older adults typically need a supplemental oral intake of approximately 1300 IU/d to reach the lower end of the 25(OH)D optimal range. Multiple preparations of vitamin D and its metabolites are commercially available for supplement use, with the two most common being ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3) - both absorbed similarly [52]. However, as a large single dose (50'000 IU), vitamin D3 maintains 25(OH)D serum levels more efficiently than vitamin D2 as 25(OH)D blood levels last three days before dropping rapidly after vitamin D2 dose compared to two weeks before gradually declining after vitamin D3 dose [53,54]. On the other hand, when considering daily doses of 1000 IU, vitamin D2 was as effective as vitamin D3 in maintaining 25(OH)D serum levels [53,54]

Interestingly, Compher et al. (2008)[55], in a systematic review, analyzed the relationship between the vitamin D, severe obesity, and the impact of obesity surgery on vitamin D status and described that the mean 25(OH)D serum level was low (<80 nmol/L) in most patients preoperatively and remained unrestored to the optimal concentration (>80 nmol/L) postoperatively. Notably, secondary hyperparathyroidism and bone loss were likewise typical in these patients, especially when the obesity surgery included a malabsorptive component. This systematic review also noted that the periodic postsurgical vitamin D supplementation has been unsatisfactory in overcoming secondary hyperparathyroidism or reestablishing the optimal vitamin D range. This gap in understanding the mechanisms behind vitamin D deficiency in severe obesity complicates establishing well-defined evidence-based corrective actions.

Moreover, our study found that after 12 months of bariatric surgery, 25(OH)D serum levels correlated negatively with systolic pressure (ϱ = - 0.711*, P = 0.049). However, the median 25(OH)D level difference between patients with hypertension reports and those without was insignificant, despite the high frequency of hypertensive patients in the sample (85.2%). A possible limitation might be that participants' blood pressure and 25(OH)D levels were measured only at a single time point.

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This observation of high blood pressure association to 25(OH)D serum levels started the research into vitamin D involvement in cardiovascular disease pathogenesis [56,57]. Vitamin D may protect against hypertension development as seen by the seasonal variation in blood pressure - lower values in the summer while higher in the winter (an inverse correlation with UV light exposure and circulating 25(OH)D levels) [58]. Similarly, Judd et al. [59]study with mostly non-hypertensive participants reported a decrease in the age-related increase in systolic blood pressure in patients with 25(OH)D levels >80 nmol/L, i.e., the systolic blood pressure was lower (0.40 mmHg/year) in 25(OH)D-sufficient participants compared to deficient and insufficient ones. Wang et al. [60] also reported that participants with low 25(OH)D serum levels had a higher risk of developing cardiovascular events, including incident hypertension, than 25(OH)D sufficient ones. This inverse correlation was maintained even after adjusting for age, sex, ethnicity, and physical activity [61], although further adjusting for body mass index (BMI) and PTH reduced this effect, implying that PTH might mediate most of the 25(OH)D and blood pressure association [62].

However, Snijder et al. [63]observed no positive effect of vitamin D supplementation on blood pressure in the general older adult population but noted that PTH might be a potentially modifiable determinant. On the other hand, in Rei et al. [64]'s cohort study with over 1000 participants (>40 years), neither PTH in women nor 25(OH)D levels in either sex was related to metabolic syndrome, including hypertension.

Unfortunately, observational studies cannot prove causality. Therefore, to test for causality, many intervention studies investigated how dietary vitamin D supplementation affects hypertension. Short-term high vitamin D3 (4000 IU) doses combined with calcium supplements also lowered blood pressure in older German women more than calcium alone [65]. However, Forman et al. [66] reported significantly higher rates of hypertension and lower circulating 25(OH)D levels in Blacks than in Whites - each 1 ng/mL of vitamin D3 (cholecalciferol) supplementation increased in plasma 25(OH)D significantly reduced 0.2 mmHg in systolic pressure but had no effect on diastolic pressure. In contrast, Kunutsor et al. [67]performed a pooled random effects meta-analysis of weighted mean differences across 16 trials of vitamin D supplementation and showed a non-significant reduction in both systolic and diastolic blood pressure. Interestingly, the diastolic blood pressure was significantly reduced in participants with pre-existing cardiometabolic disease.

Our study found no difference in 25(OH)D serum levels of the participants diagnosed with anxiety/depression (40.7%; N = 11) compared to those without anxiety or depression.

Although growing evidence points to a vitamin D role in depression's pathobiology and treatment, this evidence is inconsistent in many aspects needing more randomized controlled trials to determine whether this association is causal. Menon et al.'s [68]narrative review found an inverse association between vitamin D levels and clinical depression that appears driven by vitamin D's homeostatic, trophic, and immunomodulatory effects, though this association directionality remains unclear. Furthermore, a systematic review and meta-analysis of randomized controlled trials (all published before January 2019) reported in ten studies (total participants = 3336; median duration = 12 months) an association between high vitamin D supplementation (≥4000 IU) and reduced depressive symptoms, but not in the case of lower vitamin D supplementation levels (<4000 IU). Baseline 25(OH)D serum levels before supplementation and the depression-scoring scales did not affect this association, grading the overall quality of evidence as 'moderate´[69]. In contrast, Penckofer et al. [70] reported no difference in the dosing effect of vitamin D3 supplementation in treating depressive symptoms in a double-blind, randomized, active comparator-controlled trial conducted in women with type 2 diabetes (T2DM), significant depressive symptoms, and low 25(OH)D levels that received weekly oral vitamin D3 supplementation (50,000 IU) or an active comparator (5,000 IU) for six months.

In our study, 40.7% (N=11) of the women reported frequent thirst, 29.6% (N =8) difficulty chewing dry foods, 48.2% (N = 13) xerostomia (dry mouth), and also 48.2% (N = 13)

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reported getting up at night to drink water, but these reports were also unrelated to 25(OH)D serum levels.

Nonetheless, Kong et al. 's experimental study[71] investigated vitamin D's association with water and electrolyte homeostasis. Vitamin D receptor (VDR)-null mice had polyuria but with normal urine osmolarity due to high salt excretion. This polyuria is not caused by impaired renal fluid handling (similar urinary responses to water restriction and vasopressin as wild-type) or increased intestinal salt absorption (maintained increased water intake and urinary output despite a salt-deficient diet) but rather by increased water intake induced by the increase in systemic and brain angiotensin II (dramatically upregulated in the kidney and brain of VDR-null mice compared to wild-type). On the other hand, researchers identified that 1,25 dihydroxy vitamin D3 downregulates renin expression; thus, vitamin D deficiency or defects in the VDR signaling might lead to renin overexpression and renin-angiotensin system (RAS) activation that might cause renal and cardiovascular injuries and other detrimental effects (RAS activation in other tissues) [72]. Rephrasing vitamin D may play a physiological role in maintaining the renal and cardiovascular systems' homeostasis via suppressing the RAS.

Regarding *VDR Fok*I and *VDR Taq*I polymorphisms, we found that *VDR Fok*I polymorphism's mutant allele *f* presence correlated to a median rise in 25(OH)D serum level, both in the genotypic distribution (P =0.005) and in the dominant model (*FF* versus *Ff+ff*, P = 0.001) evaluation. In contrast, *VDR Taq*I polymorphism did not affect 25(OH)D serum levels. Neither polymorphism correlated to total body bone mineral density (TBBMD).

Studying genetic backgrounds is vital to understand the context of obesity [73,74]. For instance, the *VDR* gene is highly polymorphic with many SNPs that might affect its functionality by altering its gene expression, mRNA stability, protein translation efficiency, and protein sequence [75]. These changes might alter VDR binding pattern with vitamin D or its analogs, thus, changing its related signaling pathways. VDR expression and nuclear activation are necessary for vitamin D effects. In this respect, many epidemiological studies have compared case and control groups to test possible linkages between *VDR* polymorphisms and several diseases, including its role in bone biology, renal diseases, diabetes, and other conditions, such as obesity [76]. To illustrate, in fat cells (adipocytes), vitamin D or its analog binds to VDR proteins and acts as a regulator agent in adipocytes' differentiation and metabolism, and, consequently, alterations in this binding might influence the context of obesity [77].

In the context of obesity/metabolic syndrome/diabetes, some studies have evaluated the 25(OH)D serum level relationship with *VDR* gene polymorphisms. An inquiry included 201 obese Egyptian women with vitamin D deficiency and 249 obese age-matched healthy controls with sufficient 25(OH) levels (ages 25 and 30). Women with *VDR* mutant alleles for *Apa*I (*Aa+aa*), *Fok*I (*Ff+ff*), and *Taq*I (*Tt+tt*) showed significantly lower 25(OH)D serum levels and higher HOMA-IR and blood pressure than those with *VDR* wild genotypes: *Apa*I (AA), *Fok*I (*FF*) and *Taq*I (*TT*), respectively [78]. Another study (cross-sectional with 277 patients) assessed the associations between vitamin D deficiency, *VDR* gene polymorphisms (*Fok*I, *Bsm*I, *Apa*I, and *Taq*I), and cardiovascular risk factors in T2DM Caribbean patients. They reported that the rate of vitamin D deficiency was higher in T2DM patients and was associated with the *VDR Fok*I and *VDR Apa*I polymorphisms and cardiovascular risk profile. So *VDR* polymorphisms might explain why vitamin D deficiency is more frequently present in some T2DM patients [79]. In another cross-sectional study with 697 middle-aged Russian women, *VDR Bsm*I and *VDR Apa*I polymorphisms and vitamin D deficiency correlated with metabolic syndrome parameters [80].

Other research examines the same *VDR* polymorphisms and 25(OH)D serum levels in a population but aims to understand distinct illness processes. Hossein-Nezhad et al. (2014)'s survey determined that vitamin D deficiency correlated with the *VDR Fok*I polymorphism in 760 Iranian patients who underwent angiography due to suspected coronary artery disease (CAD), as vitamin D deficiency was more prevalent in CAD patients might be a result from FokI polymorphism [81]. At the same time, Rashedi et al. (2014)'s study

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observed that increases in 25(OH)D serum levels in individuals with *VDR Fok*I's *ff* genotype and low 25(OH)D serum levels might protect them against active tuberculosis [82].

Our study involves older Brazilian women living in Brazil's central-western region. In Brazil's southern region, investigators determined the vitamin D deficiency prevalence in girls and investigated whether the genotypic distribution of the *VDR* gene's *Bsm*I, *Apa*I, and *Taq*I polymorphisms and their haplotypes were associated with vitamin D levels. They verified that vitamin D deficiency and insufficiency were highly prevalent in that sample, and the *VDR Bsm*I, *VDR Apa*I, and *VDR Taq*I wild variants and the GGT (*BAT*) haplotype were associated with lower 25(OH)D serum levels. These results suggest that VDR gene polymorphisms could be linked to higher susceptibility to vitamin D deficiency in a subpopulation of children and adolescents [83]. Pereira-Santos et al. (2019) [83]cohort study with 270 pregnant women living in northeastern Brazil evaluated the associations between *VDR* gene polymorphisms, maternal 25(OH)D concentration, and gestational outcomes. They found that participants with *VDR Taq*I's *tt* genotype had a higher 25(OH)D concentration during gestation; the children of women with *VDR Apa*I SNP's Aa genotype were born with a lower weight; women with *VDR Taq*I SNP's *Tt* genotype decreased the risk of a shorter gestation duration; while women with *VDR Apa*I SNP's aa genotype were negatively affected and had decreased gestation duration. Another case-control study (101 T2DM patients and 62 sex-, age-, and BMI- matched non-diabetic controls) from Brazil's southeastern region evaluated the association between the *VDR* gene's *Bsm*I, *Apa*I, *Fok*I, and *Taq*I polymorphisms in T2M patients and 25(OH)D serum levels. They suggested that Brazilian T2DM patients presented lower 25(OH)D serum levels unrelated to obesity and *VDR* polymorphisms [84].

Nevertheless, VDR expression and role in transactivating target genes is determined not only by genetics but also by ethnicity and environment involving complex interactions which may confound disease association. O'Neill et al. (2013) [85] hypothesized that VDR expression, VDR level, and transactivation of target genes, *CAMP* and *CYP24A1*, depend on vitamin D, ethnicity, and FokI genotype. The mean 25(OH)D3 serum level was normal and not significantly different between ethnicities; neither 25(OH)D3 serum level nor 1,25(OH)2D3 supplementation significantly influenced VDR's expression or level. Africans had significantly higher mean VDR protein levels, nonetheless transactivated less CAMP expression than Whites. *Fok*I polymorphism genotyping showed a significantly higher *ff* genotype frequency in Africans than Whites. *Fok*I genotype did not influence VDR's expression or level but influenced the overall CAMP transactivation and 1,25(OH)2D3-elicited CYP24A1 induction; the latter interacted with ethnicity. In conclusion, differential VDR expression relates to ethnicity rather than 25(OH)D3 serum level and FokI genotype. Instead, VDR transactivation of CAMP is influenced by the *Fok*I genotype and, together with ethnicity, affects 1,25(OH)2D3-elicited CYP24A1 expression.

For instance, the *Fok*I polymorphism also correlated with differences in TBBMD, but whereas some papers linked the longer protein form presence with lower TBBMD [86,87], some others liked the shorter form [88,89].

This study considered a sample of older women who underwent bariatric surgery and looked at changes in vitamin D levels in these patients. However, a series of new questions have arisen that researchers should investigate: Are these hormone levels maintained after other years? Are the possible biological alterations due to this intervention, in terms of vitamin D metabolism, more impactful in this age group compared to the others?

5. Conclusions

In older Brazilian women who underwent bariatric Roux-en-Y gastric bypass surgery, after twelve months, 25(OH)D3 serum levels were positively correlated with total body bone mineral density, negatively with systolic blood pressure measurement, and their levels´ production was higher in those with the VDR *Fok*I polymorphism's C (*f*) allele.

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