The relationship between Vitamin D levels and the body mass index (BMI) in a population cohort.

Relación de la Vitamina D con el IMC en una cohorte poblacional.

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Santander, Junio 2017
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Background: Low serum vitamin D (25(OH)D) concentrations, have been associated with the development of obesity, metabolic syndrome, diabetes and cardiovascular disease. Objective: The aim of this study is to evaluate the possible relationship between serum 25(OH)D levels and the body mass index (BMI) in postmenopausal women and determinate if any differences are observed throughout the year. Design: This is a cross-sectional study of 1826 women from Camargo (Cantabria, Spain). All participants are Caucasian postmenopausal women. Study variables are weight, height, serum 25(OH)D concentrations in all seasons, general laboratory parameters and some bone parameters. Results: Mean serum 25(OH)D levels were lower at a higher body weight in community-dwelling postmenopausal women of our area. It is also observed that lower levels of 25(OH)D in obese and overweight women are uniform throughout the year, and always are lower than normal weighted women.
INTRODUCTION

Obesity

Obesity is a chronic disease that is increasing in prevalence in adults, adolescents and children around the world, and is now considered to be a global epidemic(1). Obesity has become a serious health problem in most developed countries. The World Health Organization estimated that in 2008 the global prevalence of over-weight and obesity was around 1.5 billion and 500 million adults, respectively. This condition is also well known to be associated with an increase in the prevalence of type 2 diabetes, cardiovascular diseases, certain types of cancer and total mortality(2). Overweight refers to a weight above the “normal” range, with normal defined on the basis of actuarial data. This is determined by calculating the body mass index (BMI, defined as the weight in kilograms divided by height in meters squared). Overweight is defined as a BMI of 25 to 29.9 kg/m²; obesity is defined as a BMI of ≥ 30 kg/m². Severe obesity is defined as a BMI of ≥ 40 kg/m² or ≥ 35 kg/m² in the presence of comorbidities. These cutoffs apply to Caucasian, Hispanic and Black individuals. They underestimate risk in the Asian and South Asian population.(1,3)

People can become overweight at any age. However, there are certain times when weight gain tends to occur, which vary between men and women. Most overweight women gain their excess weight after the onset of puberty. This weight gain may be precipitated by a number of events, including pregnancy, treatment with oral contraceptives or menopause.

In addition, another thing to consider is the lifestyle: obesity is more prevalent in adults with physical, sensory or mobility health disabilities. Those with impaired lower extremity mobility are the highest risk. The role of physical activity in the prevention and treatment of obesity is paramount.

The morbidity and mortality associated with being overweight or obese have been known to the medical profession for more than 2000 years. Mean BMI is increasing worldwide. In the United States, one-third of the population is affected by obesity, according to the National Health and Nutrition Examination survey.(4)Although there is evidence that the obesity epidemic is levelling off in some populations,(5) the prevalence of excess weight remains high in many countries of the world, including Spain(6). In developed countries, obesity rates in 2013 were approximately 18 and 20 percent in men and women, respectively. In 2013, reported prevalence rates of obesity by country included 21 percent of men and 23 percent of women in Belgium, 25 percent of men and 33 percent of women in the UK, 20.6 percent of men and 33 percent of women in Mexico, 12.3 percent of men and 31 percent in women in South Africa, and 14 percent of men and women in Pakistan. Despite the wide range across countries, data suggest that the percentage of obesity has increased in most populations over the past 30 years.

The ENPE study, published in the Spanish Cardiology Journal by Javier Aranceta-Bartrinaet. Al. in June 2016, estimated prevalence of overweight of 39.3% and general
obesity of 21.6% in the Spanish adult population aged 25 to 64 years. These data are consistent with the estimates obtained in the ENRICA study for 2008-2010, a study which estimated a prevalence of 22.9% for obesity in the Spanish population older than 18 years(7). The frequency of obesity and abdominal obesity increased with age and affected, respectively, 35 and 62% of persons aged 65 and over (ENRICA)(6). The prevalence of obesity in the population in Spain is lower than the rates estimated for the United States, a country where the prevalence of obesity (BMI ≥ 30) in the population older than 20 years (2011-2012) is 35.1%. They observed that the prevalence of general obesity and abdominal obesity in Spain is high but its distribution changes among different autonomous communities(7).

**Vitamin D**

Vitamin D or calciferol, is a fat- soluble vitamin. Dermal synthesis is the predominant natural source of Vitamin D, with smaller quantities coming from a few foods of the diet. Those vitamins, Vitamin D from diet and cutaneous synthesis is biologically inactive and requires enzymatic conversion to active metabolites.(8)

Cholecalciferol and ergocalciferol from these sources are converted in the liver to 25-hydroxyvitamin D (25-(OH)D), and circulating 25-(OH)D reflects cutaneous and dietary vitamin D intake. 25-(OH)D is filtered at the glomerulus and actively reabsorbed into renal tubular cells via megalin and cubulin, where it is converted to the potent hormone 1,25-dihydroxyvitamin D (calcitriol) by the enzyme 1-α-hydroxylase.(9)
Vitamin D metabolites are lipophilic molecules with low aqueous solubility that must be transported in the circulation bound to plasma proteins. The most important of these carrier proteins is the vitamin D binding protein (DBP), which binds the metabolites with high affinity in the order $25-(OH)_2D = 24,25(OH)_2D > 1,25(OH)_2D >$ Vitamin D. Plasma levels of DBP are 20 times higher than the total amount of vitamin D metabolites, and $>99\%$ of circulating vitamin D compounds are protein bound, mostly to DBP, although albumin and lipoproteins contribute to lesser degrees. This has a major impact on their pharmacokinetics. DBP-bound vitamin D metabolites have limited access to target cells.(10) Once inside the cells, DBP is degraded, apparently by legumain(11), releasing $25-(OH)D$ which is metabolized by 1-α-hydroxylase or 24-hydroxylase; however, $25-(OH)D$ translocation to the mitochondria may also be facilitated rather than passive.

Dietary vitamin D is incorporated into micelles, absorbed by enterocytes, and then packaged into chylomicrons. Disorders associated with fat malabsorption, such as celiac disease, Crohn’s disease, pancreatic insufficiency, cystic fibrosis, short gut syndrome, and cholestatic liver disease, are associated with low serum 25-hydroxyvitamin D ($25(OH)D$) levels.(8)

Vitamin D receptors are present throughout at the body in different tissues, and hundreds of human genes contain Vitamin D response elements(10). Pleiotropic actions have been described for vitamin D, beyond those traditionally described for maintenance of bone health. These include suppression of the renin-angiotensin-aldosterone system and blood pressure reduction as well as modulation of immune function and cellular proliferation. Additional beneficial effects may include podocyte survival, albuminuria reduction, and prevention of glomerulosclerosis(9). Vitamin D also acts at intestine level, stimulating the synthesis of the protein that constitutes calcium channels, the calbindin and the synthesis of the ATPase enzyme, that is, participates in the calcium absorption by the cell and the absorption of phosphate and magnesium. It also has its function in the muscle where increases calcium transport and its uptake by the sarcoplasmic reticulum favoring muscle traction and relaxation.

Vitamin D

![Figure 1. Functions of vitamin D (9)](image-url)
**Vitamin D deficiency**

As well as obesity, vitamin D deficiency is a public health concern, because its prevalence is increasing, particularly among elderly subjects (12).

Talking about the optimal serum 25-hydroxyvitamin D concentration, there is not a consensus. The best laboratory indicator of vitamin D adequacy is the serum 25(OH)D concentration. The lower limit of normal levels varies depending on the geographic location and sunlight exposure of the reference population (range 8 to 15 ng/mL). The Institute of Medicine concluded that a serum 25(OH)D concentration of 20 ng/mL is sufficient for most individuals, but other experts (Endocrine Society, National Osteoporosis Foundation or American Geriatrics Society) suggest that a minimum level of 30 ng/mL is necessary in older adults to minimize the risk of falls and fracture. Furthermore, the weight could also influence seasonal changes observed in serum concentration of 25(OH)D.

The serum PTH level typically is inversely related to 25(OH)D levels in adults, and may be a useful secondary indicator of vitamin D insufficiency (8).

There are several causes of vitamin D deficiency (Table I) and it appears to be common in some special groups of people: older persons, dark skinned, obese people, people who take medications that accelerate the metabolism of vitamin D, hospitalized on a general medical service or institutionalized and those who have limited effective sun exposure, osteoporosis or malabsorption (13).

Practically all the literature supports the idea that obese and overweight people have a vitamin D deficiency. This vitamin D deficiency can have serious consequences, it is associated with an increased risk of cardiovascular-, cancer-, infectious disease-, and trauma-related mortality. Eaton et Al (14) examined whether low serum concentrations of 25(OH) predicted an increased risk of cardiovascular, cancer, and all-cause mortality in a prospective cohort of ethnically diverse postmenopausal woman. Participants with low vitamin D levels were older, had higher BMIs, higher waist circumferences, were less educated. They were also more likely to be current or past alcohol drinkers, current smokers, sedentary and diabetics, but less likely to be white or to receive vitamin D supplements.

A lack of vitamin D can also cause myopathy (15), which tends to be more marked in the proximal muscles. In addition, vitamin D levels have been shown to be significantly associated with muscle strength in healthy postmenarchical girls, suggesting that muscle contractility may be affected by vitamin D status. The mechanisms underlying the effect of vitamin D on muscle strength are not fully understood but could be related to an independent effect on muscle mass, or alternatively, to enhancement of muscle function.
mediated through the effect of vitamin D. In a study, Gilsanz et Al(16), proposed that 25(OH)D concentrations are reciprocally related to adipose tissue infiltration in muscle independently of muscle mass. They examined the relation between vitamin D and skeletal muscle lipid content in a sample of 90 postpubertal healthy females who have been more thoroughly described in a previous investigation on the relation of vitamin D to body fat and bone. Thirty seven women (41%) had 25(OH)D concentrations ≥30ng/ml, whereas 57 women (59%) had insufficient 25(OH)D levels (<29ng/ml), of which 22 women (24%) were vitamin D deficient (≤20 ng/ml). Compared with women with normal 25(OH)D values, vitamin D-insufficient subjects were significantly shorter and heavier and had greater BMI and abdominal subcutaneous and visceral fat.

Vitamin D deficiency is also causes different problems on the bone health. Vitamin D takes part in the process of bone mineralization, that is, lack of this vitamin causes under-mineralization, increased bone resorption, osteomalacia and rickets. That deficiency is also associated with increased risk of osteoporosis and possibly poorer muscle function and other adverse health outcomes.(17)

Bone remodeling markers: P1NP and CTX

The commonly used bone resorption markers are degradation products of type I collagen, but noncollagenousproteins such as the enzyme of osteoclast origin tartrate-resistant acid phosphatase 5b (TRACP) have also been investigated as resorption markers. The pyridinium cross-links, pyridinoline (PYD) and deoxypyridinoline(DPD) are formed during the maturation of bone collagen, present in significant amounts in bone and dentine, released during resorption of bone and excreted in urine in the free and peptide-bound forms without being metabolised. The peptide-bound forms of PYD and DPD include the C-terminal and N-terminal cross-linking telopeptides(CTX, NTX) of the type I collagen molecule, and these are also released into the circulation and subsequently excreted in urine(18,19).

It is interesting to note that the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) recommend that a marker of bone formation (serum procollagen type I N-propeptide, s-PINP) and a marker of bone resorption (serum C-terminal telopeptide of type I collagen, s-CTX) are used as reference analytes for bone turnover markers in clinical studies(18).

Those bone remodeling markers could be very useful like as osteoporosis and osteomalacia markers.
Relation between Vitamin D and BMI: Review about the present situation

Factors known to influence 25(OH)D concentrations include race, Vitamin D intake, sun exposure, adiposity, age and physical activity(2). Serum 25(OH)D levels are inversely associated with total body fat and have been found to be decreased in obese patients. The reason for this is not completely understood, although a higher storage in adipose tissue has been suggested as a plausible explanation. In particular, visceral fat obesity compared with overall obesity might pay a major role in the development of vitamin D deficiency.

Serum 25(OH)D level is the most widely accepted biomarker to estimate short-term vitamin D status, since it reflects both the dermal Vitamin D synthesis and vitamin D obtained from foods and supplements.(20)

Over the years, different experts have studied the relation between the Vitamin D and other agents like the parathormone, some gene polymorphisms with BMI, and it has been studied in different groups of people considering the age, race, sex, the diseases, the physical characteristics, etc.

In an article in 2012, Guasch et al. (2) evaluated the associations between 25(OH)D or PTH concentrations and the risk of obesity, and Metabolic syndrome and its individuals components in a large sample of individuals with a wide range of adiposity. In obese people, low levels of 25(OH)D can be attributed mainly to: a) the lower bioavailability of the vitamin, due to its sequestration by adipose tissue; b) the dilution of ingested or cutaneously synthesized vitamin D in the enlarged fat mass; c) the low sun exposure of large areas of the body; or d) a low intake of calcium and vitamin D.

They did a cross-sectional study with 316 patients, where 240 of these were women. They measured a lot of data, among others things, weight, height, BMI, blood pressure, HDL and LDL cholesterol and triglycerides. They observed that the prevalence of vitamin D deficiency and insufficiency increased with obesity. When vitamin D deficiency and insufficiency were merged, only 38% of individuals with a BMI lower than 30 kg/m² had vitamin D insufficiency or deficiency, compared to 88-95% of those with a BMI higher than 35 kg/m². The conclusion is that BMI is the variable that is most strongly associated with plasma 25(OH)D and PTH concentration, but they are not associated with metabolic syndrome(2). Another study in 2010 got the same conclusion. Pitroda et al. demonstrated a positive association of increased adiposity with serum PTH in a generally healthy cohort of older American Adults(21).

The relation between Vitamin D and the obesity has also been studied from a genetic point of view. Ochs-Balcom et al. did a study to evaluate the association of Vitamin D Receptors (VDR) gene variants and adiposity phenotypes. The active form of vitamin D, 1,25(OH)₂D₃, binds the vitamin D receptor on chr12q13.1 and a member of the steroid hormone receptor superfamily. The VDR is a key mediator of the action of 1,25(OH)₂D₃ in adipocyte differentiation. They selected haplotype tagging SNPs, specially, two functional SNPs (Cdx-2 and Folk I) were selected for genotyping on the basis of the
literature for a total of 14 SNPs. 2115 women participated in the study, and it was studied the waist circumference, the abdominal height, BMI, fasting glucose concentrations, HDL and LDL cholesterol, blood pressure and triglycerides. They identified a positive association of one VDR SNP (rs2782905) with BMI, waist circumference and abdominal height, a positive association of Cdx-2 with waist circumference and abdominal height and a borderline positive association with BMI, but they did not observe significant associations for Folk I.(22)

In the literature that we know about this matter, it has been studied that relationship in different population, people from United States, Europe, South Asia, Puerto Rico and Korea, and also the different of the association between Hispanic and African Americans. In all of those studies they obtain a similar conclusion: The vitamin D levels are lower in those people who have de BMI higher.

In august 2012, Dian C. Sulistyonigrum et Al.(23) published a study with 182 European people and 188 South Asian people, where 49 and 47% were women respectively. Previously, a small number of studies prone to low circulating 25(OH)D concentrations than individuals of European descent, presumably because of their darker skin colour, but in this study they pretended to investigated the relationship between plasma 25(OH)D, adiposity and body fat distribution in both populations. They supported what the before studies quoted had published, that is, South Asian had lower plasma 25(OH)D concentrations than the Europeans, as well as they proved that in both ethnicities, plasma 25(OH)D concentrations of women were negatively associated with BMI (p<0.001), waist circumference (p=0.015), total abdominal adipose tissue (p<0.001), Visceral adipose tissue (p<0.001), subcutaneous adipose tissue (p<0.001), and total body fat (p<0.001).

The association between obesity and 25(OH)D concentrations appeared stronger for populations in North America compared to Europe, possibly reflecting differences in the distribution of BMI across the continents (24,25).

Another study was done with Korean adults and a different one was done with overweight and obese Puerto Rican Adults. This last study(20) evaluated 98 subjects, 66% females, to determine the nutritional status of vitamin D in overweight and obese persons living at latitude 18º and to understand the association of serum 25(OH) levels with vitamin D intake, sun exposure and body composition. They took their anthropometric measurements (weight, height, percent body fat and waist and hip circumference), they also did a food frequency questionnaire to the subjects, ask about the sun exposure time and about the physical activity, all of that in males and females. They concluded that vitamin D intake from foods and supplements and total vitamin D intake were similar between females and males. Serum 25(OH)25D levels were similar in both sexes and they stratified this serum levels by BMI classification and ability to tan and tendency to burn: the levels were lower among dark-skinned people, as expected, but the difference between overweight and obese individuals was contrary to expectations, that is, 25(OH)D levels were higher in subjects with light skin and within these, obese people had higher levels
than overweight people. They correlated all the things they were studying with serum vitamin D levels, which were negatively correlated with percent body fat ($r=-0.24$) and positively associated with total vitamin D intake and with sun exposure ($r=0.23$ and $r=0.32$ respectively). These significant correlations were observed mainly in females. In the Korean study, they pretended to clarify, once again, the role of adiposity in the relationship between serum vitamin D level and insulin resistance among middle-age elderly Korean adults. They used data from 2710 individuals aged ≥ 50 years, and they confirmed that BMI was significantly associated with 25(OH)D ($p=0.008$), however, waist circumference was not significantly associated with vitamin D. They support that endogenously-produced vitamin D might be stored in subcutaneous fat deposits.
Our objective is to evaluate the possible existence of a relation between Vitamin D levels and the body mass index (BMI) in a population cohort of our area, and in particular:

i) to describe the 25(OH)D status in Spanish obese postmenopausal women

ii) to compare their results with those of the overweight or normal weight people

iii) to determine whether any differences are observed throughout the year
SUBJECTS AND METHODS

Participants and study design

Our study is an observational cross-sectional study. The study population consisted of 1826 women attending a primary care center in Northern Spain (Camargo, Cantabria). This is a community-based study designed to evaluate the prevalence and incidence of metabolic bone diseases and disorders of mineral metabolism, specially, the relation between Vitamin D levels and BMI. The study was approved by the local Ethics Committee (Comité Ético de Investigación Clínica de Cantabria – IDIVAL) and all subjects gave written informed consent.

In this study, it has used the same population cohort that the investigators had used in a previous study they have already published in 2010(27). Camargo is a town of more than 30,000 inhabitants, situated near the Cantabrian coast. The population of Camargo is more than 95% white, and its age and sex distributions closely resemble those of the entire population of our region (Cantabria, Spain. 43° N Latitude).

All participants in this study are white, postmenopausal women. Exclusion criteria were either having the principal residence outside the region or being unable to attend the recruiting Primary Care Center.

At the baseline visit, participants were interviewed by investigators and provided data regarding the risk factors for osteoporosis and fractures using a structured questionnaire including age, race, age at menarche, age at menopause, type of menopause, weight, height, body mass index, personal history of fractures after 40 years of age, history of osteoporotic fractures among first-degree relatives, tobacco use, consumption of dairy products, alcohol consumption, physical exercise, existence of sensory problems, number of falls in the previous year, presence of chronic general diseases or disorders affecting bone health, and present or past consumption of medications with influence on bone metabolism. Any personal history of hypertension, diabetes, or dyslipidemia was also recorded.

To avoid alterations in the study because the effect of antiosteoporotic therapy and corticosteroid drugs on bone mineral metabolism, participants whose baseline assessment revealed the presence of disease or treatments know to affect bone metabolism, such as osteoporosis, primary hyperparathyroidism, hyperthyroidism or use of bisphosphonates, estrogens or glucocorticoids among other things, in the previous one year, were excluded from the study as well as those participants on calcium and/or Vitamin D supplements.

Height and weight were measured with participants wearing light indoor clothing but without shoes. BMI was defined as weight (Kg) divided by squared height (m²). Waist perimeter was measured in centimeters at a level midway between the lower rib margin and iliac crest after breathing out, with a flexible tape all around the body in an erect position with feet together.
In order to evaluate the seasonal variation of serum 25(OH)D, the period of March-May, represented spring; June-August, summer; September-November, autumn; and December-February, winter.

It was also collected information about dairy calcium consumption, the level of education by asking for the highest educational level completed, ranging from none to university, tobacco smoking routine (current smoker or never smoker), alcohol consumption, the habitual physical activity (high, moderate and sedentary) and the presence of chronic diseases like cardiovascular, disease stroke, chronic obstructive pulmonary disease, diabetes mellitus, chronic liver diseases, malignant neoplasms, and rheumatoid arthritis.

**Biochemical determinations**

For each participant, blood samples were obtained from an antecubital vein in the morning, between 9:00 and 10:30 h, after a requested 12-hour overnight fast. Serum was divided into 0.5 mL aliquots and stored at -40ºC. Serum total calcium, phosphate, glucose, creatinine, total cholesterol, HDL-c, LDL-c (calculated using the Friedewakd formula), triglycerides, albumin, and total alkaline phosphatase were measured by standard automated methods in an ADVIA 2400 Chemistry System auto-analyzer (Siemens, Germany)(27).

Total calcium measurements were corrected for albumin concentration in accordance with a previously published formula. Serum concentration of animo-terminal P1NP, CTX, 25(OH)D and intact PTH were determined by a fully automated Roche electrochemiluminescence system (Elecsys 2010, Roche Diagnostics, GmbH, Mannheim, Germany). The P1NP limit of detection was 5 ng/mL (reference range, 15-78 ng/mL), and its intra-assay and interassay coefficients of variation (CVs) were 3.9% and 4.1% respectively(28). Intra-assay and interassay CVs for β-CTX were 4.2% and 4.7% respectively (reference range, 0.100-1.000 ng/mL). The detection limit of serum 25(OH)D was 4ng/mL, the intraassay CV was 5% and the interassay CV was 7.5%. Regarding intact PTH, the detection limit was 6 pg/mL (reference range, 15-65 pg/mL). Intra-assay and interassay CVs were 3.4% and 5.9% respectively.

**Statistical analysis**

Results are expressed as mean ± SD, median [interquartile range] or percentages, as appropriate. Student’s *t* test or Mann-Whitney *U* test was used to determine the differences between groups for continuous variables, and the *X*² test was used for categorical variables. All continuous variables were tested for normality and non-variables non-normally distributed underwent logarithmic transformation before statistical analyses.
Participants were divided according to BMI groups:

- Normal: 18.5-24.9 kg/m².
- Overweight: 25.0-29.9 kg/m².
- Obese: > 30 kg/m².

Analysis of variance was used to compare the outcome variables between groups, applying the Bonferroni method for multiple comparisons.

A $P$ value of less than 0.05 was considered statistically significant in all the calculations. All analyses were conducted using SPSS.
Baseline characteristics

A total of 1826 women were entered into the study. Table 1 shows the general epidemiological characteristics of the participants in the study according to their obesity status. The obese people, in comparison with the non-obese group, were older and had a higher prevalence of hypertension, diabetes mellitus, dyslipemia, and ischemic heart disease. Diary calcium intake and educational level was lower in obese people and they were also more frequent statin-users.

Table 1. Epidemiological features

<table>
<thead>
<tr>
<th></th>
<th>Normal (n=416)</th>
<th>Overweight (n=773)</th>
<th>Obese (n=637)</th>
<th>p¹</th>
<th>p²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>59.7±10.1</td>
<td>63.6±9.9</td>
<td>65.1±9.9</td>
<td>&lt;0.0001</td>
<td>0.011</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.3±1.3</td>
<td>27.5±1.4</td>
<td>34.3±3.7</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>82.7±8.1</td>
<td>93.4±8.6</td>
<td>106.8±10.7</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dairy calcium (mg/d)</td>
<td>650 [450-900]</td>
<td>650 [450-900]</td>
<td>600[450-850]</td>
<td>0.06</td>
<td>0.008</td>
</tr>
<tr>
<td>Current drinkers (%)</td>
<td>14.7</td>
<td>12.2</td>
<td>10.0</td>
<td>0.024</td>
<td>0.21</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>22.8</td>
<td>11.8</td>
<td>8.2</td>
<td>&lt;0.0001</td>
<td>0.025</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>23.3</td>
<td>37.9</td>
<td>61.9</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>5.0</td>
<td>10.1</td>
<td>21.2</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Education level (yrs.)</td>
<td>8 [8-12]</td>
<td>8 [8-8]</td>
<td>8 [8-8]</td>
<td>&lt;0.0001</td>
<td>0.03</td>
</tr>
<tr>
<td>Active exercise (%)</td>
<td>97.6</td>
<td>97.5</td>
<td>95.4</td>
<td>0.07</td>
<td>0.035</td>
</tr>
<tr>
<td>Dyslipidemia (%)</td>
<td>18.5</td>
<td>30.1</td>
<td>33.9</td>
<td>&lt;0.0001</td>
<td>0.13</td>
</tr>
<tr>
<td>Ischemic heart disease (%)</td>
<td>2.4</td>
<td>2.8</td>
<td>5.2</td>
<td>0.026</td>
<td>0.024</td>
</tr>
</tbody>
</table>

p¹: Normal weight vs. obese subjects. p²: overweight vs obese individuals. Values non-normally distributed were expressed as median [IQR]

Obese people had also higher fasting glucose and triglyceride levels, but lower total cholesterol, HDL-c and LDL-c concentrations (p< 0.0001) like table 2 shows, nevertheless, triglycerides concentrations are higher in obese women than in normal weight or overweight women (p< 0.0001). In addition, in this table it can be seen others general laboratory parameters and its difference between non-obese and obese women.
Table 2. General laboratory parameters

<table>
<thead>
<tr>
<th></th>
<th>Normal (n=416)</th>
<th>Overweight (n=773)</th>
<th>Obese (n=637)</th>
<th>p¹</th>
<th>p²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>229.8±35.6</td>
<td>226.2±35.4</td>
<td>217.9±39.1</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL colesterol (mg/dl)</td>
<td>68.5±15.6</td>
<td>61.5±15.2</td>
<td>56.0±13.8</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL colesterol (mg/dl)</td>
<td>143.7±31.5</td>
<td>143.9±32.9</td>
<td>136.4±32.8</td>
<td>0.002</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>76 [61-105]</td>
<td>94 [72-128]</td>
<td>109 [82-147]</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>88.5±14.5</td>
<td>93.6±18.3</td>
<td>103.1±28.9</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.90 [0.80-0.99]</td>
<td>0.90 [0.80-1.00]</td>
<td>0.90 [0.80-1.00]</td>
<td>0.003</td>
<td>0.61</td>
</tr>
<tr>
<td>GFR (ml/min/1.73 m²)</td>
<td>70 [61-80]</td>
<td>79 [59-78]</td>
<td>66 [58-77]</td>
<td>&lt;0.0001</td>
<td>0.31</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/l)</td>
<td>71.6±20.4</td>
<td>74.1±23.3</td>
<td>76.9±25.9</td>
<td>0.001</td>
<td>0.09</td>
</tr>
<tr>
<td>Phosphate (mg/dl)</td>
<td>3.6±0.5</td>
<td>3.5±0.4</td>
<td>3.5±0.5</td>
<td>0.007</td>
<td>0.99</td>
</tr>
<tr>
<td>Total calcium (mg/dl)</td>
<td>9.66±0.37</td>
<td>9.61±0.38</td>
<td>9.59±0.53</td>
<td>0.13</td>
<td>0.99</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.5±0.3</td>
<td>4.4±0.3</td>
<td>4.4±0.3</td>
<td>&lt;0.0001</td>
<td>0.99</td>
</tr>
<tr>
<td>C-reactive protein (mg/dl)</td>
<td>0.10 [0.10-0.30]</td>
<td>0.20 [0.10-0.40]</td>
<td>0.40 [0.20-0.63]</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

p¹: Normal weight vs. obese subjects. p²: overweight vs obese individuals. Values non-normally distributed were expressed as median [IQR]

Table 3 shows all bone parameters which have been measured in the study. The difference in serum concentration of 25(OH)D (Figure 2): obesity: 20.4 ± 8.3 ng/ml; overweight: 23.4 ± 9.0 ng/ml; p< 0.0001; and normal weight 25.1 ± 8.9 ng/ml; p< 0.0001. P1NP and CTX were lower in both obese and overweight women, all of them statistically significant. However, in the case of PTH levels, the difference were: obese women 55 [45-69] pg/ml; overweight women: 50 [40-64]; p < 0.0001 and with normal weight women: 46 [37-58] pg/ml; p < 0.0001.

Table 3. Bone parameters

<table>
<thead>
<tr>
<th></th>
<th>Normal (n=416)</th>
<th>Overweight (n=773)</th>
<th>Obese (n=637)</th>
<th>p¹</th>
<th>p²</th>
</tr>
</thead>
<tbody>
<tr>
<td>iPTH (pg/ml)</td>
<td>46 [37-58]</td>
<td>50 [40-64]</td>
<td>55 [45-69]</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>25(OH)D (ng/ml)</td>
<td>25.1±8.9</td>
<td>23.4±9.0</td>
<td>20.4±8.3</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>P1NP (ng/ml)</td>
<td>48.7 [36.7-64.3]</td>
<td>44.6 [34.3-60.2]</td>
<td>42.9[30.9-56.2]</td>
<td>&lt;0.0001</td>
<td>0.003</td>
</tr>
<tr>
<td>CTX (ng/ml)</td>
<td>0.41 [0.28-0.57]</td>
<td>0.38 [0.26-0.51]</td>
<td>0.32 [021-0.43]</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

p¹: Normal weight vs. obese subjects. p²: overweight vs obese individuals. Values non-normally distributed were expressed as median [IQR]
BMI and Waist Perimeter

The BMI and waist perimeter was also measured to compare with 25(OH)D levels. In this study, it was found that BMI and waist perimeter are inversely associated with 25(OH)D levels (r = -0.214; p < 0.0001 and r = -0.174; p < 0.0001).

Bone remodeling markers, both P1NP and CTX, were also inversely correlated with BMI (P1NP: r = -0.101; p < 0.0001; CTX: r = -0.202; p < 0.0001, respectively), whereas it has been observed a positive correlation between PTH and BMI (r = 0.151, p < 0.0001)

### Relations between 25(OH)D, BMI and WP and correlation between BMI and bone remodeling markers and PTH

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>-0.214</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Waist perimeter</td>
<td>-0.174</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1NP</td>
<td>-0.101</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>CTX</td>
<td>-0.202</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>PTH</td>
<td>0.151</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
Season differences

As it has been mentioned before, weight could also influence seasonal changes observed in serum concentration of 25(OH)D. There was a significant seasonal difference in mean serum 25(OH)D concentrations, with higher level in summer than in winter (25.6 ± 9.1 ng/ml vs. 21.4 ± 8.9 ng/ml; p< 0.0001).

Lower levels of 25(OH)D in obese and overweight women was uniform throughout the year (Figure 2). In the winter and spring, 43.5% of overweight and 58.3% of obese women compared with 35.9% of normal weight women had 25(OH)D concentrations <20 ng/ml (p= 0.0002). In the autumn and summer 25.3% of overweight and 42.2% of obese women compared with 19.2% of normal weight women had 25(OH)D concentrations <20 ng/ml (p= 0.0005). That is, insufficient levels of vitamin D is more frequent in obese women than in overweight women and more than normal weight women, in all season, besides this study shows that that vitamin D deficiency is more prevalent in sprint and winter than in summer and autumn.

<table>
<thead>
<tr>
<th></th>
<th>Spring + Winter</th>
<th>Summer + Autumn</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal weight</td>
<td>84 (35.9%)</td>
<td>35 (19.2)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Overweight</td>
<td>190 (43.5%)</td>
<td>85 (25.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Obese</td>
<td>204 (58.3%)</td>
<td>121 (42.2%)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Percentage of normal, overweight and obese women with serum concentrations of 25(OH)D lower than 20 ng/ml.
The following figure (Figure 3), shows that lower levels of 25(OH)D in obese and overweight women are uniform throughout the year, and always are lower than normal weight women.

Figure 3: Seasonal variations of serum 25(OH)D in normal, overweight and obese women.
DISCUSSION

Results of this survey show that mean serum 25(OH)D concentration was lower at a higher body weight in community-dwelling postmenopausal women of our area. In women, 25(OH)D was inversely correlated with BMI and waist perimeter, and obese women had lower serum 25OHD values than overweight or normal weight women. Conversely, obese and overweight women have higher levels of PTH that were directly correlated with BMI. Our results are consistent with previous studies (20,23) reporting an inverse relationship between vitamin D levels and increased adiposity.

As it has been mentioned previously, the causes of the decrease of 25(OH)D levels in overweight and obese subjects are no known. Increased storage of 25(OH)D in adipose tissue, reduced biological availability, or more rapid clearance is a plausible explanation for increased rates of vitamin D deficiency in obese individuals (17,29). However, obese individuals may be at increased risk for Vitamin D deficiency due to decreased sun exposure for increased clothing, limited mobility (17,30,31), or lower dietary intake (32). In our study, de dairy calcium intake has been measured and for all subjects were similar (650 mg/d for normal weight and overweight women and 600 mg/d for obese women). About active exercise, obese people obtained a less percentage in relation to overweight and normal weight women.

Alternatively, obesity might also be the consequence of low vitamin D levels because it has been hypothesized that low vitamin D status, by causing PTH excess and calcium influx into adipocytes, may promote weight gain (33). In addition, remembering the vitamin D metabolisms, 1,25-dihydroxyvitamin D, which is derived from 25(OH)D, might inhibit adipogenesis (34). Recently, Walsh et al showed that biological available free serum 25(OH)D and the active hormone, 1, 25-dihydroxyvitamin D were also lower at higher body weight, suggesting that likely cause of lower 25(OH)D in obesity was the greater volume of distribution (17).

Different experts have studied the relation between the Vitamin D and others agents with BMI, and it has been studied in different groups of people considering the age, race, sex, the diseases, the physical characteristics. The association between obesity and 25(OH)D concentrations appeared stronger for populations in North America compared to Europe, possibly reflecting differences in the distribution of BMI across the continents (24,25). On the other hand, Dian C. Sulistyonoigrum et Al (23) demonstrated that South Asian had lower plasma 25(OH)D concentrations than the Europeans, as well as they proved that in both ethnicities, plasma 25(OH)D concentrations of women were negatively associated with BMI, waist circumference, total abdominal adipose tissue, Visceral adipose tissue, subcutaneous adipose tissue, and total body fat. Since in our study we did not measured visceral (VAT) nor subcutaneous adipose tissue (SAT), it seems possible that we have not been able to detect the possible 25(OH)D variations associated with VAT. Conversely, because subcutaneous adipose tissue and BMI are closely correlated, it is possible that
most of the association between BMI and 25(OH)D that we observed in women could be attributable to variation in SAT and body size that is also captured by BMI (35).

In addition to BMI and vitamin D levels, other parameters has been measured in our study and they has showed that between women who had higher BMI, the prevalence of hypertension, diabetes mellitus, and ischemic heart disease is higher too. Nevertheless, cholesterol, HDL-c and LDL-c levels are inversely proportional to the BMI, nevertheless, triglycerides levels increase as body mass index increases.

In our study, obese and overweight women also have higher PTH that was related positively with BMI and waist perimeter. These results were in accordance with previous studies (2,21,33). If the low vitamin D in obesity was negatively affecting bone health, we would have expected bone-turnover markers would have been increased, and bone mineral density would have been decreased. However, because we have showed that these women have an increase in bone mineral density, the lower and higher than normal levels of 25(OH)D and PTH, respectively, do not seem to have any great effect on bone homeostasis.

In our study, another thing we have evaluated is the levels of bone remodeling markers, specifically P1NP and CTX markers. Despite higher levels of PTH, these bone remodeling markers showed lower values in obese and overweight women than in controls, it has been demonstrated that both of them were inversely correlated with BMI. A decrease in bone markers has been previously reported in obesity (17,36), and could be related with the higher mechanical load that it entails, estrogen synthesis from adipocyte aromatase, or adipocyte hormones such us leptin (27).

We have observed that serum 25(OH)D levels difference between obese, overweight and normal weight woman are constant throughout the year. In others studies, like Walsh et al (17), the difference among normal weight and obese people was grater in the autumn and spring than in winter. Bolland et al (37) also showed the grater fat mass was associated with lower peak 25(OH)D concentrations and smaller seasonal variations in 25(OH)D that could be related with an effect of both reduced sunlight exposure in heavier individuals and the role of adipose tissue as a reservoir of vitamin D and its metabolites. However, the seasonal difference we have found could be explained with the latitude (our city is at 43°N latitude), ethnicity, visceral and abdominal fat distribution in woman from each area, and UV light exposition.

When exposed to UVB normal-weight and obese people have a similar cutaneous synthesis of vitamin D (38), but the serum 25(OH)D rise is attenuated in obese people. Therefore obesity may alter the release of vitamin D3 from the skin into the circulation levels but not affect the capacity of the skin to produce vitamin D3 (39).

Our study has several limitations. Firstly, the design is that of a cross-sectional study, and therefore no causal relationship may be inferred. Moreover, as an observational study, it is therefore subject to some possible bias due to confounding factors. Secondly, we did
not measured visceral or subcutaneous fat, so we cannot know if there is any association between 25(OH)D levels and visceral adipose tissue. Among the strengths we want to emphasize, it is worth mentioning that the participants were well-characterized and all postmenopausal women were carefully studied from the mineral and bone metabolism point of view, and excluded if any disease of treatment known to affect this were present. In addition, participants on calcium and/or vitamin D supplements were also excluded. Finally, all samples were obtained at the same time of the day and in a fasting state and all bone mineral density measurements were performed with the same device. Thus factors to minimize biological variability were controlled.
CONCLUSIONS

To sum up, we found a lower serum concentrations of 25(OH)D in obese and overweight community-dwelling postmenopausal women of our area that persisted throughout the year and were inversely correlated with Body Mass Index and waist perimeter. Obese and overweight had higher bone mineral density at all levels despite higher levels of PTH. Bone remodeling markers were lower than in controls, suggesting that low 25(OH)D was not negatively affecting bone health.


