

# Low 25(OH)D Serum May Not Reflect at Risk Skeletal Health but Not with Body Mass Index in Women

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**Abstract.** Background: The mechanism of low circulating 25(OH)D serum in obese people may be due to high 25(OH)D level in adipocyte resulting low in circulation or low vitamin D-binding protein (DBP). However, obese people have a higher bone mineral density (BMD), which suggests that low 25(OH)D may not associate with other bone health parameters. Objective: We sought to determine whether there is association between 25(OH)D *1*) with bone health parameter differ by body weight; 2) Body mass index and BMD. Design: We conducted a cross-sectional observational study of 132 women aged 20-50 years in North Sumatera, Indonesia. Results: Serum total 25(OH)D was no different in normal, overweight, and obese women subjects ( 15.2±4.9 ng/mL). There was 77.3% categorized in deficiency and insufficiency (22.7%). There was no association among bone health parameters and body weight, but there is an association between BMD and body weight ( $p<0.05$ ). Conclusions: Serum total 25(OH)D lower at all categorize body mass index, which cannot be explained by higher in adipocyte and lower in circulation or low DBP or shorter half-life of 25(OH)D. Lower 25(OH)D may not reflect at risk skeletal health and higher BMI should be considered as a marker of higher BMD.

## 1. Introduction

Osteoporosis is a metabolic bone disorder characterized by low bone mass and microarchitectural deterioration, with a subsequent increase in bone fragility and susceptibility to fracture [1]. Micronutrient that associated with bone health is calcium, but not only calcium, vitamin D also playing an important role in osteoporosis. Vitamin D deficiency and insufficiency is associated with increased risk of osteoporosis and possibly poorer muscle function and other adverse health outcomes [2,3]. Vitamin D is an essential factor for the intestinal absorption of dietary calcium and skeletal mineralization. Vitamin D deficiency causes undermineralization, increased bone resorption, osteomalacia, and rickets [4].



Biomarker for vitamin D status is serum total 25-hydroxyvitamin D [25(OH)D], which it has a long plasma half-life and reflects both skin synthesis, natural sources oral and supplement intake [5]. The Institute of Medicine recommends that 20 ng/mL is sufficient for most of the population[6], but The Endocrine Society recommends that <20 ng/mL is deficient and that 20-30 ng/mL is insufficient [7] and Grant and Hollick recommends that 54-90 ng/mL is normal value in tropical countries [8]. Vitamin D deficiency leads to secondary hyperparathyroids, increase bone turnover, boneloss, and is also implicated as a cause of hip fracture [9].

Tropical countries such as Indonesia and Malaysia should be less of vitamin D deficiency in the population, but the fact is vitamin D deficiency also occurs in children and adult population especially in women in those country[9-11]. Lifestyle of a women such as avoiding sunlight exposure, less vitamin D intake, less physical activity, and higher body mass index (BMI) directed to vitamin D deficiency [10]. Serum total 25(OH)D is lower in obese people and inversely correlated with BMI. These findings have been reported in adults and children of different ethnic groups all over the world [12-18]. Based on the previous research, the cause and clinical significance of low 25(OH)D serum and hence, the value of total 25(OH)D as biomarker of vitamin D status in different body weights are not clear. Possible cause of low serum 25(OH)D in obesity are a lower vitamin D supply, greater volume of distribution, reduced biological availability, or more rapid clearance [19,20].

There is contradictive research results in body weight, vitamin D, and bone health: low 25(OH)D would be expected to be associated with higher bone turn over and lower bone mineral density (BMD), but BMI and fat mass are positively correlated with BMD, and higher body weight is generally protective against fracture [21,22]. Previous studies also showed that low 25(OH)D serum did not associated with BMI in tropical country women [9,10].

The aims of this study wereto determine whether there is association between 25(OH)D1) with bone health parameter differ by body weight; 2) Body mass index and BMD. To our knowledge, this is the first to study to find out the association between 25(OH)D serum with bone health parameters in tropical country women.

## 2. Method

We conducted a cross-sectional study of 132 women aged 20-50 years in North Sumatera, Indonesia July to October 2016, during the dry season (dry season in Indonesia is between April and October, when there was abundant sunlight exposure). The location of recruitment was in Sumatera Island (Sumatera Utara, Medan) with latitude: 3.57 N and longitude 98.65 E, average temperature:  $\pm 32^{\circ}\text{C}$  ( $90^{\circ}\text{F}$ ). This study was carried out after ethical approval was obtained from the Health Research Ethics Committee of Sumatera Utara University Medical School (No. 560/TGL/KEPK FK USU-RSUP HAM/2016) and all participants were given written informed consent to the study procedures.

### 2.1. Study participants

The subjects of this study consisted healthy adult women with various occupations, and divided into three groups for normal, overweight, and obese based on Body Mass Index (BMI), and taken purposively. There were 45 subjects in normal group, 21 in overweight group, and 66 subjects in obese group. The inclusion criteria were women within the range of 20-50 years old. Exclusion criteria were subjects with history of diabetes mellitus, myocardial infarction, renal or liver dysfunction. In addition to those exclusion criteria, subjects who were pregnant, lactating, or using medications that may alter lipid profile were also excluded.

### 2.2. Anthropometry, status body fat, and nutrient intake

Anthropometry included height (to the nearest 0.5 cm), weight (to the nearest 0.1 kg), waist circumference using a standardized measuring tape in centimetres, systolic and diastolic blood pressure measurement, and body mass index (calculated as  $\text{kg}/\text{m}^2$ ). Categorized BMI was based on Asia Pacific (6), <18.5 classified as underweight, 18.5-22.9 classified as normoweight, 23-24.9 classified as

overweight/at risk, 25-29.9 classified as obese I, and >30 classified as obese II. Assessment of body fat percentage were using Body Composition Monitor with Scale (*HBF-362, KaradaScan-Omron*). Body fat percentage referred to the amount of body fat mass in regards to the total body weight expressed as a percentage, the following classified: normal  $\leq 29.9\%$  and high  $>30.0\%$  based on Bioelectrical Impedance. Assessment of nutrient intake was based on food recall for two days (one day for weekday and one day for weekend), including energy, protein, fat, carbohydrate, calcium, phosphor, cholesterol, vitamin D, and percentage of fulfilment. Calculation were using *Nutrisurvey2005*, which included Indonesian foods.

### 2.3. Laboratory analysis

We measured 25(OH)D serum concentration by chemiluminescent immunoassay (CLIA) technology (Diasorin, Stillwater, MN), measures were between 4.0 and 150 ng/mL. The lowest value was 4.0 ng/mL which is based on an inter-assay precision 3.90% CV. Reference range were  $<20$  ng/mL categorized deficiency, 20-30ng/mL (insufficiency), 30-100 ng/mL (suficiency). To convert ng/mL to nmol/L is multiply with 2.496 [5]. Calcium serum was measured by ADVIA Bayer Assayed Chemistry Controls, with principle procedure: calcium ions form a violet complex with *o*-cresolphthalein complexone in an alkaline medium. The reaction is measured at 545/658 nm, and normal concentration of calcium was 8.3-10.6mg/dL. C-terminal telopeptide of type I collagen (CTX), which is a bone-resorption marker, was measured with the use of an automated immunoassay (Cobas e-411; Roche Diagnostics), interassay was 4.0%. Bone mineral density and T-score were assessed with the use of DXA Scan, whole body, lumbar spine, and hip DXA were performed with a Discovery densitometer (precision of the BMD measurements was 0.2-5.5%).

### 2.4. Statistical analysis

Continuous variables were expressed as continuous variables as means $\pm$ SDs. The unpaired *t* test was used to compare continuous values of the study groups if the data normally distributed and Mann Whitney test was used for non-normally distributed data. The *p* values  $<0.05$  were considered statistically significant. Categorical variables were expressed as percentage proportions and using chi-square to expressed significancy difference between three groups,  $p < 0.05$ . We used SPSS program (version 11.5; SPSS Inc, Chicago, IL) to perform the analysis.

## 3. Results and Discussions

The results will be discussed in 4 subsections, they are characteristic of study participants, body mass index, factors to low vitamin D, and bone health.

### 3.1. Characteristic of study participants

The aim of this study was to determine whether there is association between 25(OH)D with bone health parameter differ by body weight and also its association between body mass index and BMD especially in tropical country healthy women, who lived in abundant sunlight exposures area such as Indonesia, Many study found vitamin D deficiency only found in the country with four seasons, some of the study showed vitamin D deficiency was found in obese subject [24]. This study showed that even in abundant sun exposure such tropical country with two season (Indonesia), vitamin D deficiency could be found in women not only in higher adiposity (obese subjects), but also in normal adiposity (overweight and obese) as report in the other study [9,10].

Latitude has influenced to vitamin D deficiency, number of solar UV B photon (280-320nm) reaching the earth depending on zenith angle of the sun, above about 35 degrees north latitude, little or no vitamin D<sub>3</sub> can be produced. Prospective and retrospective epidemiologic studies indicated that hypertension and cardiovascular disease found higher in people living at higher latitudes compared with people living at lower latitudes [25]. Age in this study showed the age that most women productive in Indonesia, they had a job and all of them had to leave their house to stay at work place. They spent all day long in the building which less sunlight exposure.

**Table 1.** Characteristics of all subjects

Parameters	Mean $\pm$ Standard Deviation
Age (years)	35.3 $\pm$ 8.4
Height (cm)	156.6 $\pm$ 5.1
Body Mass Index (kg/m <sup>2</sup> )	24.9 $\pm$ 3.7
Fat mass (%)	33.3 $\pm$ 4.1
Lean mass (%)	25.4 $\pm$ 1.9
25(OH)D serum levels (ng/mL)	15.2 $\pm$ 4.9
Calcium serum levels (mg/dL)	8.8 $\pm$ 0.3
CTX levels (ng/L)	0.3 $\pm$ 0.1
Bone mineral density (g/cm <sup>2</sup> )	0.9 $\pm$ 0.1
Vitamin D intake per day (mcg)	7.2 $\pm$ 6.1
Calcium intake per day (mg)	310.5 $\pm$ 271.7
Sunlight exposure per day (minute)	12.9 $\pm$ 1.9
Physical activity	4.6 $\pm$ 0.5

### 3.2. Body mass index

Adiposity could be assessed from BMI that showed a higher mean of BMI, and also body fat percentage showed a higher result according to BMI categorized. This was come along with 25-hydroxyvitamin D serum concentration that showed lower concentration, but even adiposity showed higher, when all the subjects separated into three groups, vitamin D deficiency could be found in normal, overweight, and obese groups.

**Table 2.** Characteristic of subjects based on body mass index categorized

BMI group	Age (y)	Height (m)	BMI (kg/m <sup>2</sup> )	Fat mass (kg)	Lean mass (kg)
Normal (45)	32.8 $\pm$ 7.8	157.1 $\pm$ 4.1	21.03 $\pm$ 1	29.2 $\pm$ 3.1	27.3 $\pm$ 1.8
Overweight (21)	35.7 $\pm$ 9.5	161.1 $\pm$ 7.6	24.2 $\pm$ 0.7	34.7 $\pm$ 1.6	25.3 $\pm$ 0.7
Obese (66)	36.6 $\pm$ 8.3	155.2 $\pm$ 3.9	27.7 $\pm$ 2.8	35.5 $\pm$ 3.3	24.3 $\pm$ 1.2

### 3.3. Factors to low vitamin D

We investigated several possible mechanisms for the effects of body weight on vitamin D status. Dietary vitamin D intake and sunlight exposure were not significant across BMI groups. A previous study also showed that sunlight exposure did not vary with BMI [10,26]. Vitamin D intake was shown to be below the recommended dietary allowance in all subjects. Vitamin D<sub>3</sub> has 5 times the activity of vitamin D<sub>2</sub>; and dietary food sources may not supply enough for adequate health. Cholecalciferol (D<sub>3</sub>) is found mainly in salmon, sardine, mackerel, tuna and cod fish oil. It is also found in limited quantities in milk, egg yolk, butter and margarine. Supplements commonly contain ergocalciferol (D<sub>2</sub>) extracted from mushroom or D<sub>3</sub> extracted from lanolin. Ordinary dietary sources of vitamin D<sub>3</sub> evidently do not supply enough for adequate health (around 250–300 IU/day in USA). Individuals with low vitamin D intake are advised to take supplements that are safe and reliable sources of vitamin D<sub>3</sub>.

However, according to other study, neither vitamin D supplements nor food sources of vitamin D are consumed on a daily basis [27]. Alarmingly, this study revealed that working women consumed very limited amounts of vitamin D food sources (egg yolk, fish, meat and mushroom). Women also seldom consume vitamin D supplements. They tend to consume vitamin C or E supplements because of their antioxidant effects on the skin, even though low dietary intake of vitamin D and low sunlight exposure can have detrimental effects on health.

According to a study, the inability to buy vitamin D food sources was because of vitamin D deficiency [28]. Our study showed that salmon and fish oil were expensive food sources in the market in North Sumatra. Mushroom can be easily obtained, but for some regions, such as Sumatra, mushroom is not

a food that is regularly consumed. Mushroom is more oftenly found as herbal medicine especially on Chinese ethnic group, and not as a regularly consumed food.

**Table 3.** Possible factors to low vitamin D in body mass index categorized

	BMI group			<i>p</i> value
	Normal (n=45)	Overweight (n=21)	Obese (n=66)	
Dietary vitamin D intake (µg/day)	6.4±5.4	8.2±5.4	7.1±6.7	0.82
Dietary calcium intake (mg/day)	275.5±259.9	295.6±186.3	337.3±301.9	0.79
Sunlight exposure (minute/day)	12.7±1.9	12.9±2.3	13.0±1.9	0.88
Physical activity	4.7±0.5	4.4±0.5	4.5±0.5	0.28

Continues variable: mean ± SD; SD=standard deviation

### 3.4. Bone health

If the low 25(OH)D serum in obese women was negatively affecting bone health, this result showed an expected increased of bone turnover, and BMD would have been decreased. However, bone resorption markers were lower in the obese subjects than in normal-weight subjects, formation markers were similar across BMI categorized. Although higher BMI is generally protective factor against fracture and osteoporosis, there is an excess of some limb fractures in obesity, which suggests that the changes in bone density and structure may not be adequate for the increase in body weight [29]

Previous study showed that 25(OH)D serum are lower at higher BMI, they speculated that low 25(OH)D serum may not reflect at-risk skeletal health in obese people, and BMI should be considered when interpreting serum 25(OH)D as a marker vitamin D status[4]. In our result showed that there were no difference low 25(OH)D serum between all three BMI groups, deficiency or insufficiency could occur in normal, overweight, and obese group.

**Table 4.** Bone health and its association with body mass index categorized

	BMI group			<i>p</i> -value
	Normal (n=45)	Overweight (n=21)	Obese (n=66)	
25(OH)D serum (ng/mL)	14.8±5.5	17.3±6.2	15.0±4.3	0.53
Vitamin D categorized				
Deficiency (n%)	30 (66.7)	12 (57.1)	60 (90.9)	0.09
Insufficiency (n%)	15 (33.3)	9 (42.9)	6 (9.1)	
Calcium serum	8.8±0.3	8.8±0.3	8.9±0.3	0.84
CTX (ng/L)	0.4±0.2	0.4±0.2	0.3±0.2	0.18
Bone Mineral Density (g/cm <sup>2</sup> )	0.8±0.1	0.8±0.1	0.9±0.1	0.02*
T-Score				
Normal(n%)	30(66.7)	15 (71.4)	48 (72.7)	0.92
Osteopenia(n%)	15 (33.3)	6 (28.6)	18 (27.3)	

\**p*<0.05=significant; Continues variable: mean ± SD; categorical variable: n (%); SD=standard deviation

Possible causes of low 25(OH)D serum level in all BMI groups may be due to low vitamin D-binding protein (DBP) or faster metabolic clearance, however, obese people have a higher bone mineral density which suggests that low 25(OH)D may not be associated with other bone health markers such as calcium serum and T-score. Greater than 99% of circulating 25(OH)D is bound to vitamin D-binding protein and albumin, and remaining free fraction is the most biologically available. There is a paradox in body weight, vitamin D, and bone health: low 25(OH)D would be expected to be associated with higher bone turnover, however, this result study showed a negative fact that low 25(OH)D serum occurs in all groups. Higher BMI and fat mass are positively correlated with BMD and higher body weight is generally protective against osteoporosis.

#### 4. Conclusions

It is well recognized that serum total 25(OH)D is low in obesity, but we have shown that 25(OH)D serum are also lower in normal and overweight. Which it cannot be explained by higher in body mass index and lower in circulation or low DBP or shorter half-life of 25(OH)D. Lower 25(OH)D may not reflect at risk skeletal health and higher BMI should be considered as a marker of higher BMD.

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