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Research Article Body Mass Index but not 25(OH)D Serum is Associated with Bone Mineral Density Among Indonesian Women in North Sumatera: A Cross Sectional Study

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Abstract

Background and Objective: 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 such as calcium, C-terminal telopeptide, bone mineral density and T-score. This study aimed to determine whether there is association between 25(OH)D, (1) With bone health parameter differ by body weight and (2) Body mass index and BMD. **Methodology:** The study design was a cross-sectional 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 ($15.2 \pm 4.9 \text{ ng mL}^{-1}$). There was 77.3% categorized in deficiency and insufficiency (22.7%). There was no association among bone health parameters (calcium serum, C-terminal telopeptide, T-score) and body weight but there is an association between BMI and BMD (p<0.05). **Conclusion:** Serum total 25(OH)D lower at all categorize body mass index, which cannot be explained by higher in adipocyte and lower in circulation. Lower 25(OH)D may not reflect at risk skeletal health and higher BMI should be considered as a marker of higher BMD.

Key words: Adipocyte, body weight, bone mineral density, calcium serum, C-terminal telopeptide, marker, obese, observational, T-score, vitamin D deficiency

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

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 fracture¹. 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 adcerrse health outcomes^{2,3}. Vitamin D is an essential factor for the intestinal absorption of dietary calcium and skeletal Vitamin mineralization. D deficiency causes undermineralization, increased bone resorption, osteomalacia and rickets⁴.

Biomarker for vitamin D status is serum total 25-hydroxy vitamin D [25(OH)D], which it has a long plasma half-life and reflects both skin synthesis, natural sources oral and supplement intake⁵. The institute of medicine recommends that 20 ng mL⁻¹ is sufficient for most of the population⁶ but the endocrine society recommends that $<20 \text{ ng mL}^{-1}$ is deficient and that 20-30 ng mL⁻¹ is insufficient⁷ and Grant and Holick⁸ recommended that 54-90 ng mL⁻¹ is normal value in tropical countries. Vitamin D deficiency leads to secondary hyperparathyroids, increase bone turnover, boneloss and is also implicated as a cause of hip fracture⁹.

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 country⁹⁻¹¹. 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 deficiency¹⁰. 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 world¹²⁻¹⁸. Based on the previous study, 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 which stated that 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}. However,

previous studies showed that low 25(OH)D serum did not associated with BMI in tropical country women^{1,9}. Therefore these hypotheses could be further knowledge to understand about bone health and factors that contributing its regulation. The aims of this study were 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. 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.

MATERIALS AND METHODS

The study were completed, design of the study was a cross-sectional study and included 132 women aged 20-50 years in North Sumatera, Indonesia, from July-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 (North Sumatera, Medan, Indonesia) with latitude: 3.57 N and longitude 98.65 E, average temperature: $\pm 32^{\circ}C$ (90°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.

Study participants: The subjects of this study consisted healthy adult women with various occupations and devided 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.

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 kg m⁻²). Categorized BMI was based on Asia Pacific (6), <18.5 classified as underweight, 18.5-22.9 classified as normal/normal weight, 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 refered 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 analysis. Assessment of nutrient intake was based on food recall for 2 days (1 day for weekday and 1 day for weekend), including vitamin D and calcium intake. Calculation were using Nutrisurvey 2005, which included Indonesian foods.

Laboratory analysis: This study measured 25(OH)D serum concentration by chemiluminescent immunoassay (CLIA) technology (Diasorin, Stillwater, MN), measures were between 4.0 and 150 ng mL⁻¹. The lowest value was 4.0 ng mL⁻¹ which is based on an inter-assay precision 3.90% CV. Reference range were <20 ng mL⁻¹ categorized deficiency, 20-30 ng mL⁻¹ insuficiency and 30-100 ng mL⁻¹ suficiency. To convert ng mL⁻¹ to nmol L⁻¹ is multiply with 2.496⁵. Calcium serum was measured by ADVIA Bayer Assayed Chemistry Controls, with principle procedure: Calcium ions form a violet complex with o-cresolphthaleincomplexone in an alkaline medium. The reaction is measured at 545/658 nm and normal concentration of calcium was 8.3-10.6 mg dL⁻¹. 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

Table 1: Characteristics of all subjects

Parameters	Mean ± Standard Deviation
Age (years)	35.3±8.4
Height (cm)	156.6±5.1
Body mass index (kg m ⁻²)	24.9±3.7
Fat mass (%)	33.3±4.1
Lean mass (%)	25.4±1.9
25(OH)D serum levels (ng mL ⁻¹)	15.2±4.9
Calcium serum levels (mg dL ⁻¹)	8.8±0.3
CTX levels (ng L ⁻¹)	0.3±0.1
Bone mineral density (g cm ⁻²)	0.9±0.1
Vitamin D intake per day (mcg)	7.2±6.1
Calcium intake per day (mg)	310.5±271.7
Sunlight exposure per day (min)	12.9±1.9
Physical activity	4.6±0.5

Continues variable: Mean±SD, SD: Standard deviation

Table 2: Characteristic of subjects based on body mass index categorized

spine and hip DXA were performed with a discovery densitometer (precision of the BMD measurements was 0.2-5.5%).

Statistical analysis: Continuous variables were expressed as continuous variables as Means \pm SDs. The ANOVA test was used to compare continuous values of the study groups (more than two groups) if the data normally distributed and Kruskal Wallis test was used for non-normally distributed data. The p<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. SPSS program (version 11.5; SPSS Inc, Chicago, IL) was used to perform the analysis.

RESULTS AND DISCUSSION

Characteristic of study participants are shown in Table 1, including mean of age, height, body mass index, fat mass and lean mass. Besides 25(OH)D serum level, bone health parameters were calcium serum, CTX levels and bone mineral density. This study included 132 women and overall subjects fulfilled the examinations. Mean values for calcium level was 8.8 ± 0.3 mg dL⁻¹, classified as normal range. The mean of 25(OH)D serum concentration was 15.2 ± 4.9 ng mL⁻¹, all the subjects had low 25(OH)D serum concentration (vitamin D deficiency). All the subjects did not reached normal 25(OH)D concentration level, average 54-90 ng mL⁻¹ which is normal in sunny countries.

Table 2 showed mean of age, height, BMI, fat mass and lean mass of all subjects. Age ranged within 32.4 ± 7.8 years in normal weight but along with higher BMI, also showed higher age ranged. Mean BMI values in normal weight were found to be 21.0 ± 1 kg m⁻² and higher along with higher BMI.

Table 3 showed that there were no significant difference between three groups in body mass index categorized, in dietary vitamin D intake, dietary calcium intake, sunlight exposure and physical activity. Based on Table 3, there are no different among this BMI group in lifestyles, such as vitamin D and calcium intake, sunlight exposure and physical activity. Based on nutrient intake, all the subjects did not reach recommended daily allowance. They had lower vitamin D and calcium intake than they should consume everyday. For daily

BMI group	Age (y)	Height (m)	BMI (kg m ⁻²)	Fat mass (kg)	Lean mass (kg)
Normal (45)	32.8±7.8	157.1±4.1	21.03±1.0	29.2±3.1	27.3±1.8
Overweight (21)	35.7±9.5	161.1±7.6	24.20±0.7	34.7±1.6	25.3±0.7
Obese (66)	36.6±8.3	155.2±3.9	27.70±2.8	35.5±3.3	24.3±1.2

Continues variable: Mean±SD, SD: Standard deviation

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

	BMI group				
	Normal (n = 45)	Overweight (n = 21)	Obese (n = 66)	p-value	
Dietary vitamin D intake (µg day ⁻¹)	6.4±5.400	8.2±5.400	7.1±6.700	0.82	
Dietary calcium intake (mg day ⁻¹)	275.5±259.9	295.6±186.3	337.3±301.9	0.79	
Sunlight exposure (min day ⁻¹)	12.7±1.900	12.9±2.300	13.0±1.900	0.88	
Physical activity	4.7±0.500	4.4±0.500	4.5±0.500	0.28	

Continues variable: Mean±SD, SD: Standard deviation

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

Parameters	BMI group			
	Normal (n = 45)	Overweight (n = 21)	Obese (n = 66)	p-value
25(OH)D serum (ng mL ⁻¹)	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
Insuficiency (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 ⁻¹)	0.4±0.2	0.4±0.2	0.3±0.2	0.18
Bone mineral density (g cm ⁻²)	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)	

Significant at *p<0.05, continues variable: Mean±SD, categorical variable: n (%), SD: Standard deviation

allowance calcium intake, women should consume 800 mg daily but they reported lower calcium intake. All these recommended based on recommended daily allowance for Indonesian women. Vitamin D food sources were not usual food to consume based on their food recall reports for these subjects. For vitamin D intake, subjects had lower vitamin D intake from food sources or supplement, than daily recommended (10-15 mg day⁻¹), they consumed only about less vitamin D sources per day.

Table 4 showed the significant difference only found in bone mineral density but not with other bone health parameters such as CTX as osteoporotic parameter and T-score. Adiposity could assessed from BMI that showed a higher mean of BMI and also body fat percentage showed a higher results 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 group, vitamin D deficiency could be found in normal, overweight and obese group.

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 subject²³. 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 (280-320 nm) reaching the earth depending on zenith angle of the sun, above about 35° North latitude, little or no vitamin D3 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 latitudes²⁴. Age in this study showed 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.

Adiposity could assessed from BMI that showed a higher mean of BMI, waist circumference that showed a higher mean too and also body fat percentage showed a higher results. This was come along with 25-hydroxy vitamin 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 non-obese group. This study showed that even there are significant different in adiposity such as BMI and body fat percentage, it showed that there is no significant different in 25-hydroxy vitamin D concentration between three groups. The BMI and body fat percentage presenting adiposity.

Factors to low vitamin D: The study investigated several possibel mechanism 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^{10,25}. Vitamin D intake was shown to be below the recommended dietary allowance in all subjects. vitamin D3 has 5 times the activity of vitamin D2 and dietary food sources may not supply enough for adequate health. Cholecalciferol (D3) is found mainly in salmon, sardine, mackerel, tuna and cod fish oil. It is also found in limited quantities in milk, eggyolk, butter and margarine. Supplements commonly contain ergocalciferol (D2) extracted from mushroom or D3 extracted from lanolin. Ordinary dietary sources of vitamin D3 evidently do not supply enough for adequate health (around 250-300 IU day⁻¹ in USA). Individuals with low vitamin D intake are advised to take supplements that are safe and reliable sources of vitamin D3.

However, according to other study, neither vitamin D supplements nor food sources of vitamin D are consumed on daily basis²⁶. 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 acause of vitamin D deficiency²⁷. This study showed that salmon and fish oil were expensive food sources in the market in North Sumatera. Mushroom can be easily obtained but for some region, such as Sumatera, 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.

Bone health: If the low 25(OH)D serum in obese women was negatively affecting bone health, this result showed an expected increased of burn 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 exces 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 weight²⁸.

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 status⁴. Our result showed that there were no differ low 25(OH)D serum between all three BMI groups, deficiency or insufficiency could occurs in normal, overweight and obese group.

This study result assumed that the low vitamin D in obesity was negatively affecting bone health, we would have expected that bone-turnover markers would have been increased and BMD would have been decreased. However, these results showed that there were no significant different among three group of BMI classification. This result also showed that bone-resorption markers were no different between in the obese, overweight and normal subjects.

The researchers speculated that several possible mechanisms for the effects of body weight on vitamin D status. Dietary vitamin D intake and sunlight exposure were similar across BMI groups, so this lifestyles parameters such as vitamin D intake and sunlight exposure could not become a reason of vitamin D deficiency. 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, CTX 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 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 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.

CONCLUSION AND LIMITATIONS

It is well recognized that serum total 25(OH))D is low in obesity but the author speculated that 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 higher in adipocyte 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.

There are some limitations to this study. The study was not assessed vitamin D receptor gene polymorphism and the relation between obesity and bone may differ between children and adults and thus, these results cannot be extrapolated to children as study of Khor *et al.*¹¹. This study also did not assess the effects of low 25(OH)D beyond the musculoskeletal system. Vitamin D deficiency has been associated with diseases such as metabolic syndrome, for which obesity is also a risk factor. However, there is not yet evidence for a causative role of vitamin D deficiency.

SIGNIFICANCE STATEMENTS

- Higher body mass index is a marker of bone mineral density
- Vitamin D deficiency has no association with bone health parameters
- There were no different between 25(OH)D serum levels with body mass index

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