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## Research Article

# Relationship Between Body Mass Index, Calcium Intake and Vitamin D Status with Bone Mineral Density among Young Adults: A Preliminary Investigation

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## Abstract

**Background and Objective:** Body mass index (BMI), calcium intake and vitamin D status have been suggested to be contributing factors to bone mineral density (BMD). However, there are very few studies on BMD and its associated factors among young adults in Malaysia. The present study examined BMI, calcium intake and vitamin D status and their relationship with BMD among young Malay adults. **Materials and Methods:** A total of 100 Malay students (50 males and 50 females) with a mean age of  $23.7 \pm 3.9$  years were recruited for this cross-sectional study. The BMI was calculated from weight and height, while calcium intake was assessed using a food frequency questionnaire. Vitamin D status and calcaneal BMD were measured using serum 25-Hydroxyvitamin D analysis and quantitative ultrasound, respectively. **Results:** The mean BMI of subjects was  $24.4 \pm 5.9$  kg m<sup>-2</sup> and mean calcium intake was  $520.6 \pm 371.5$  mg/day. Male subjects had a significantly higher ( $p < 0.05$ ) serum 25-Hydroxyvitamin D ( $65.8 \pm 20.2$  nmol L<sup>-1</sup>) and BMD T-score ( $1.32 \pm 1.52$ ) compared to females ( $42.9 \pm 13.3$  nmol L<sup>-1</sup> and T-score  $0.75 \pm 1.22$ , respectively). The BMI significantly correlated with BMD ( $r = 0.233$ ,  $p = 0.020$ ), but not with calcium intake ( $r = 0.140$ ,  $p = 0.166$ ) or serum 25-Hydroxyvitamin D levels ( $r = 0.102$ ,  $p = 0.330$ ). **Conclusion:** The results suggested that BMI has a major influence on BMD. Low calcium and vitamin D intake is a definite concern and should be increased as recommended to maintain a healthy BMD later in life.

**Key words:** Body mass index, calcium intake, vitamin D status, bone mineral density, 25-Hydroxyvitamin D

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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 characterized by low bone mass and micro-architectural deterioration of bone tissue which enhances the reduction of bone strength and predisposes individuals to an increased risk of fractures<sup>1</sup>. Lee and Khir<sup>2</sup> reported the incidence of hip fracture in Malaysian elderly over 50 years of age to be 90 per 100,000 individuals, with fracture rates being highest among Chinese (63%), Malay (20%) and Indians (13%). The prevalence of osteoporosis in Malaysia is reportedly the highest (24.1%)<sup>3</sup> compared with other Asian countries, including Philippines (19.8%)<sup>4</sup>, China (16.1%)<sup>5</sup>, Thailand (12.6%)<sup>6</sup> and Taiwan (10.1%)<sup>7</sup>.

One of the major risk factors for osteoporosis is a low bone mineral density (BMD)<sup>8</sup>. Previous studies have reported that body weight and body mass index (BMI) showed a significant relationship with BMD<sup>9,10</sup>. The study by Salamat *et al.*<sup>9</sup> revealed that BMI was a significant predictor of osteoporosis, whereby individuals with a low BMI (<25 kg m<sup>-2</sup>) have a higher risk of osteoporosis compared to individuals with a higher BMI (>25 kg m<sup>-2</sup>). Moreover, having a heavier mechanical load (i.e., BMI or body weight) on bones can result in subsequent bone remodeling in order to support such a load<sup>10</sup>.

Modifiable risk factors, such as calcium intake, have been shown to have a positive effect on BMD, especially in achieving the higher peak bone mass during young adulthood<sup>11</sup>. An adequate amount of calcium may increase BMD through the bone remodelling process by reducing the rate of bone resorption and increasing the rate of bone formation<sup>11</sup>. Similarly, a previous study showed that calcium intake positively correlates with BMD<sup>12</sup>. In their study, women with a lower BMD (T-score<-2.5) reportedly consumed significantly lower ( $p = 0.002$ ) amounts of calcium ( $360.4 \pm 226.1$  mg/day) compared to women with a higher BMD (T-score>-2.5,  $431.1 \pm 263.1$  mg/day)<sup>12</sup>.

However, insufficient intake of vitamin D, either from dietary or ultraviolet light, may interrupt the intestinal absorption of calcium. Insufficiency of this vitamin can lead to secretion of parathyroid hormone, which stimulates the bone resorption process, thereby reducing the BMD of an individual<sup>13</sup>. Vitamin D aids in the bone mineralization process and can also help avoid rickets in children and osteomalacia in adults<sup>14</sup>. Previous studies have shown a significant correlation ( $p < 0.001$ ) between vitamin D status and BMD<sup>15,16</sup>. Unfortunately, a local study reported that Malaysians have a high prevalence of vitamin D insufficiency,

especially among female Malay adults (86.9%) compared to their male counterparts (41.1%)<sup>17</sup>.

In Malaysia, published reports on BMI, calcium intake and vitamin D status are still scarce among the adult population. To date, research on the association between BMD with these parameters has primarily focused on postmenopausal women<sup>18,19</sup>. However, research focusing on the young adult population is extremely important as peak bone mass is achieved during this phase of life (20-35 years-old)<sup>20</sup>. Therefore, the present study investigated the BMI, calcium intake and vitamin D status among young adults in Bangi, Selangor, Malaysia, in order to identify any relationship with BMD.

## MATERIALS AND METHODS

**Subjects:** The present cross-sectional study was carried out among 100 Malay students (50 males and 50 females) at Universiti Kebangsaan Malaysia (UKM). Subjects were between 18-30 years old, selected based on convenient sampling and recruited through advertisements (posters and flyers) and referrals. Subjects were included if they were healthy and non-smokers. Subjects were excluded if they had taken calcium or vitamin D supplements or any medications known to affect bone metabolism, were pregnant or lactating mothers and those diagnosed with a disease related to bone health. Written informed consent was obtained from all subjects and the study was approved by the UKM Research Ethics Committee in 2014 (UKM1.5.3.5/244/GGPM-2013-077).

**Anthropometry measurement:** The body weight and height (without shoes) of subjects were recorded using a combined digital weight and height scales (SECA 220, Hamburg, Germany) to the nearest 0.1 kg and 0.1 cm, respectively. The BMI was calculated by dividing body weight (kg) by the square of body height (m<sup>2</sup>). The classification of BMI was based on the World Health Organization (WHO)<sup>21</sup>.

**Assessment of calcium intake:** Calcium intake was assessed using a validated semi-quantitative food frequency questionnaire (FFQ)<sup>22</sup>. The FFQ consisted of 80 food items categorized into 11 groups. The subjects were asked about the frequency with which they ingested each food item (per day, week, or month) and the serving size. The FFQ was administered by a trained researcher through one-on-one interviews. Various sizes of food portions were referenced based on a food photo album, which was used as a guide during the interview<sup>23</sup>. The frequency of intake was based on habitual intake in the previous month. Formula

of the conversion of food frequency to the amount of intake was calculated as follow<sup>24</sup>:

$$\text{Amount of food (g) per day} = \frac{[\text{Frequency of intake (conversion factor)}] \times (\text{Serving size}) \times (\text{Total number of servings})}{(\text{Weight of food in 1 serving})}$$

**Assessment of serum 25-Hydroxyvitamin D:** Fasting venous blood (3 mL) was taken from respondents to analyze serum 25-Hydroxyvitamin D [25(OH)D] levels. Only 94 blood samples were taken from consenting subjects for this assessment. Serum was extracted immediately from the blood and stored at -20°C before the analysis. The serum 25(OH)D concentration was determined by the electrochemiluminescence protein binding assay method on a Cobas e411 analyzer (Roche Diagnostics, Mannheim, Germany). Then, blood samples were outsourced to the University Malaya Medical Centre for biochemical analyses. Vitamin D deficiency was defined as a serum 25(OH)D level less than 30 nmol L<sup>-1</sup>, vitamin D insufficiency was defined as a serum 25(OH)D level between 30 and 50 nmol L<sup>-1</sup>, while a serum 25(OH)D level above 50 nmol L<sup>-1</sup> was considered a normal vitamin D status<sup>14</sup>.

**Measurement of BMD:** Calcaneal BMD was estimated by broadband ultrasound of attenuation (BUA) via quantitative ultrasound (QUS), using QUS-2 ultrasound densitometer (Quidel Corporation, CA, USA) operated by a single trained technician. BUA was expressed in units of dB/MHz. Using the manufacturers standard guidelines, quality control and calibration were conducted before each data collection session began. According to current WHO recommendations, the ultrasound-derived T-scores were automatically calculated by the built-in software and classified according to the WHO criteria<sup>1</sup>. T-scores above -1 were defined as a normal BMD, T-scores between -1 and -2.5 were defined as

osteopenia, while osteoporosis was considered for T-scores of -2.5 or less. Each T-score was compared with a healthy young adult reference population.

**Statistical analysis:** Data were analyzed using Statistical Package for Social Science version 22.0 software (SPSS Inc., Chicago, IL, USA). The data were presented as means ± standard deviations unless specified otherwise. Prior to the analyses, the data were checked for normal distribution using the Kolmogorov–Smirnov test. All parameters were normally distributed, hence, the parametric test was used. An independent t-test was used to compare mean BMI, calcium intake, serum 25(OH)D levels, calcaneal BUA and BMD T-scores between genders. Vitamin D and BMD status were also compared between genders using the Chi-square test. The Pearson correlation was used to investigate the relationship between bone-related variables and BMD. Statistical significance was defined as a p-value of 0.05 or less and all statistical analyses were performed using Statistical Package for Social Science version 22.0 software (SPSS Inc., Chicago, IL, USA).

## RESULTS

**Subject characteristics:** Table 1 shows the general characteristics of studied subjects. The mean age of subjects was 23.7 ± 3.9 years old and anthropometrical data showed that male subjects had significantly higher (p = 0.007) BMI than their female counterparts (26.0 ± 6.9 versus 22.9 ± 4.1 kg m<sup>-2</sup>). Generally, more male subjects were overweight or obese (42%) than female subjects (26%). Weight and height of male subjects (74.3 ± 21.0 kg and 1.69 ± 0.07 m) was significantly (p < 0.001) higher compared to female subjects (54.6 ± 10.5 kg and 1.54 ± 0.05 m).

**Calcium intake:** Table 2 shows that the average calcium intake of subjects (520.6 ± 371.5 mg/day) was only 65% of

Table 1: General characteristics of subjects

Characteristics	Mean ± standard deviation, n (%)			
	Male (n = 50)	Female (n = 50)	p-value	Total (n = 100)
Age	23.7 ± 4.70	23.6 ± 3.10	0.960	23.7 ± 3.90
Weight (kg)	74.3 ± 21.0	54.6 ± 10.5	<0.001**	64.47 ± 19.24
Height (m)	1.69 ± 0.07	1.54 ± 0.05	<0.001**	1.62 ± 0.09
BMI (kg m <sup>-2</sup> )	26.0 ± 6.90	22.9 ± 4.10	0.007**	24.4 ± 5.90
<b>Category of BMI</b>				
Underweight (<18.5 kg m <sup>-2</sup> )	4 (8%)	4 (8%)	1.000	8 (8%)
Normal (18.5-24.9 kg m <sup>-2</sup> )	25 (50%)	33 (66%)	0.294	58 (58%)
Overweight (25.0-29.9 kg m <sup>-2</sup> )	8 (16%)	10 (20%)	0.637	18 (18%)
Obese (>30.0 kg m <sup>-2</sup> )	13 (26%)	3 (6%)	0.012*	16 (16%)

\*p < 0.05 by Chi-square test, \*\*p < 0.01 by independent t-test, BMI: Body mass index

Table 2: Calcium intake among subjects

Variables	Mean $\pm$ standard deviation, n (%)				RNI	RNI (%)
	Male (n = 50)	Female (n = 50)	p-value	Total (n = 100)		
Calcium (mg/day)	533.5 $\pm$ 384.4	507.7 $\pm$ 355.8	0.730	520.6 $\pm$ 371.5	800	65
<b>Calcium intake</b>						
<800 (mg/day)	40 (80 %)	43 (86 %)	0.742	83 (83 %)		
>800 (mg/day)	10 (20 %)	7 (14 %)	0.467	17 (17 %)		

\*p<0.05 by independent t- and Chi-square tests, RNI: Malaysian recommended nutrient intake

Table 3: Status of vitamin D among subjects

Variables	Mean $\pm$ standard deviation, n (%)			
	Male (n = 49)	Female (n = 45)	p-value	Total (n = 94)
Serum 25(OH)D (nmol L <sup>-1</sup> )	65.8 $\pm$ 20.2	42.9 $\pm$ 13.3	<0.001**	54.8 $\pm$ 20.8
<b>Status of vitamin D</b>				
Vitamin D deficiency (<30 nmol L <sup>-1</sup> )	0 (0%)	6 (13%)	-	6 (6%)
Vitamin D insufficiency (30-49 nmol L <sup>-1</sup> )	9 (19%)	28 (62%)	0.002**	37 (40%)
Vitamin D normal (>50 nmol L <sup>-1</sup> )	40 (82%)	11 (24%)	<0.001**	51 (54%)

\*\*p<0.01 by independent t- and Chi-square tests, 25(OH)D = 25-Hydroxyvitamin D

Table 4: Status of BMD among subjects

Variables	Mean $\pm$ standard deviation, n (%)			
	Male (n = 50)	Female (n = 50)	p-value	Total (n = 100)
Calcaneal BUA <sup>a</sup> (dB/MHz)	105.4 $\pm$ 18.9	98.3 $\pm$ 15.3	0.041*	101.8 $\pm$ 17.5
BMD <sup>c</sup> (T-score <sup>b</sup> )	1.32 $\pm$ 1.52	0.75 $\pm$ 1.22	0.042*	1.03 $\pm$ 1.40
<b>BMD status</b>				
Normal (T-score $\geq$ -1.0)	48 (96%)	46 (92%)	0.400	94 (94%)
Osteopenia (-2.5 < T-score < -1.0)	2 (4%)	4 (8%)	0.400	6 (6%)
Osteoporosis (T-score $\leq$ -2.5)	0	0	-	0

\*p<0.05 by independent t- and Chi-square tests, <sup>a</sup>BUA: Broadband ultrasound attenuation, <sup>b</sup>Ultrasound-derived T-score, compared with a younger reference population, <sup>c</sup>BMD: Bone mineral density

the Malaysian recommended nutrient intake (RNI) (800 mg/day)<sup>25</sup>. Percentage of male subjects (20%) who achieved RNI for calcium were higher when compared to females (14%). While male subjects had a higher mean calcium intake (533.5  $\pm$  384.4 mg/day) than females (507.7  $\pm$  355.8 mg/day), but this difference was not significant ( $p = 0.730$ ).

**Vitamin D status:** Table 3 shows that male subjects had significantly higher ( $p < 0.001$ ) mean serum 25(OH)D levels (65.8  $\pm$  20.2 nmol L<sup>-1</sup>) and were categorized as having a normal vitamin D status compared to females (42.9  $\pm$  13.3 nmol L<sup>-1</sup>), which were categorized with vitamin D insufficiency. In fact, significantly more ( $p < 0.001$ ) male subjects (82%) had a normal vitamin D status than females (24%), while the percentage of females with vitamin D insufficient (62%) was significantly higher ( $p = 0.002$ ) than their male counterparts (19%). Only 13% of female subjects were categorized as vitamin D deficient and 0% of males.

**BMD status:** According to Table 4, QUS showed the mean calcaneal BUA among males (105.4  $\pm$  18.9 dB/MHz) which was significantly higher ( $p = 0.041$ ) than that of females

(98.3  $\pm$  15.3 dB/MHz). While the overall T-score of all subjects was associated with a normal BMD (1.03  $\pm$  1.40), the mean T-score among males (1.32  $\pm$  1.52) was significantly higher ( $p = 0.042$ ) than that of their female counterparts (0.75  $\pm$  1.22). The distribution of BMD classifications illustrates that most of the male and female subjects had a normal BMD (96 and 92%, respectively), while only a few of them were categorized as having osteopenia (4 and 8%, respectively). None of the subjects were classified with osteoporosis.

**Correlation of bone-related variables with BMD:** Table 5 shows the correlation between bone-related variables and BMD. The overall BMI of all subjects was found to have a significant correlation with the overall BMD ( $r = 0.233$ ,  $p = 0.020$ ). This indicates that subjects with a higher BMI also had a higher BMD, indicating that a low BMI is an important risk factor for future bone fractures. While calcium intake ( $r = 0.140$ ,  $p = 0.166$ ) and serum 25(OH)D levels ( $r = 0.102$ ,  $p = 0.330$ ) did not show a significant correlation with BMD. A significant relationship between calcium intake and BMD was observed only in male subjects ( $r = 0.323$ ,  $p = 0.022$ ) while female subjects did not show any relationship ( $r = -0.119$ ,  $p = 0.411$ ).

Table 5: Correlation of bone-related variables with bone mineral density

Variables	Bone mineral density (Calcaneal BUA <sup>a</sup> , dB/MHz)					
	Male		Female		Total	
	r-value	p-value	r-value	p-value	r-value	p-value
Body mass index (kg m <sup>-2</sup> )	0.177	0.219	0.219	0.126	0.233	0.020**
Calcium (mg/day)	0.323	0.022**	-0.119	0.411	0.140	0.166
Serum 25(OH)D <sup>b</sup> (nmol L <sup>-1</sup> )	0.052	0.723	-0.144	0.345	0.102	0.330

\*\*p<0.01 by Pearson correlation, <sup>a</sup>BUA: Broadband ultrasound attenuation, <sup>b</sup>25(OH)D: 25-Hydroxyvitamin D

## DISCUSSION

Preliminary results of the current study suggest that BMI has a significant relationship with BMD ( $r = 0.233$ ,  $p = 0.020$ ) among young adults. These results are consistent with those of Montazerifar *et al.*<sup>26</sup> who also showed a significant positive correlation ( $p < 0.005$ ) between BMI and BMD. This can largely be explained by a higher BMI contributing to an increase mechanical load on bones leading to remodelling<sup>27</sup>. The higher BMI among male subjects in the present study was likely contributed to their significantly higher ( $p < 0.005$ ) BMD compared to females. However, the naturally smaller size of the female body structure relative to males could also be a factor contributing to the low female BMD found herein, which would subsequently increase the risk of bone fractures (44%) in females compared to males (25%)<sup>28</sup>. In contrast, Greco *et al.*<sup>29</sup> reported that there was no significant relationship ( $p > 0.05$ ) between BMI and BMD. According to their study, individuals who were obese had a low BMD and were categorized as having osteoporosis<sup>29</sup>.

Calcium intake in the present study did not show a significant ( $p = 0.140$ ) correlation with BMD. This may be due to the low calcium intake found among subjects ( $520.6 \pm 371.5$  mg/day) which did not meet Malaysian RNI (800 mg/day), may contribute to non significant effect towards BMD. A previous study conducted by Skowronska-Jozwiak *et al.*<sup>30</sup> in Poland, involving 277 women aged between 20-80 years old, also showed a low mean calcium intake ( $797 \pm 43$  mg/day) which did not meet recommended levels (1000 mg/day<sup>14</sup>) and had no significant relationship with BMD. One factor that may be contributed to the low calcium intake found herein is the high price of milk and dairy products in Malaysian markets which were mostly imported from pastoralism countries<sup>31</sup>.

Male subjects showed a significant relationship ( $r = 0.323$ ,  $p = 0.022$ ) between calcium intake and BMD. This significant relationship was explained by the higher intake of calcium among male subjects ( $533.5 \pm 384.4$  mg/day), thus give higher result of BMD ( $105.4 \pm 18.9$  dB/MHz) compared to female

subjects ( $507.7 \pm 355.8$  mg/day and  $98.3 \pm 15.3$  dB/MHz, respectively). This result was supported by Shin *et al.*<sup>32</sup>, who studied 1351 Korean male young adult (aged 10-25 years) and reported that subjects with high intake of dairy food had positive significant correlation with BMD at lumbar spine ( $r = 0.066$ ,  $p < 0.05$ ) and sub-total body (whole body less head) ( $r = 0.084$ ,  $p < 0.001$ ). Therefore, an adequate calcium intake is important especially during adulthood in order to maximize bone mass and prevent the risk of osteoporosis<sup>11</sup>.

Apart from calcium intake, vitamin D status was also reported to influence the BMD of an individual<sup>16</sup>. However, the present study did not find a significant relationship ( $p = 0.330$ ) between vitamin D status and BMD. Similarly, Hosseinpanah *et al.*<sup>33</sup>, reported that there was no significant relationship ( $p > 0.05$ ) between serum 25 (OH)D levels with femoral, trochanteric and lumbar spine BMD among postmenopausal women. A study of Lanzhou population (2942 men and 7158 women aged 40-75 years) in China also showed non-significant relationship between serum 25(OH)D and BMD ( $p > 0.05$ )<sup>34</sup>. This lack of significant relationship among the young adult in the current study represent that role of vitamin D in bone health is less significant among young adult.

Surprisingly, the mean level of serum 25(OH)D among female subjects was significantly lower ( $42.9 \pm 13.3$  nmol L<sup>-1</sup>,  $p < 0.001$ ) compared to males ( $65.8 \pm 20.2$  nmol L<sup>-1</sup>). Similar results were reported by Moy and Bulgiba<sup>17</sup>, who reported a significantly lower ( $p < 0.001$ ) mean serum 25(OH)D level among female Malay adults ( $36.2 \pm 13.4$  nmol L<sup>-1</sup>) in Kuala Lumpur compared to their male counterparts ( $56.2 \pm 19.0$  nmol L<sup>-1</sup>). Many Malaysian adults tend to avoid doing activities in the midday sun, which could be a factor contributing to the decreased level of vitamin D absorption<sup>35</sup>.

Research limitations were also considered throughout the current study. A study population consisting only of subjects of Malay ethnicity does not represent the general population in Malaysia. Furthermore, other factors that have been related to BMD, such as physical activity and lactose intolerance, were not investigated in the present study. Thus, inclusion of other major ethnic groups in Malaysia, such as Chinese and Indian,

should be prioritized in future. Besides, further studies are also needed to investigate multiple risk factors that may influence BMD. Nonetheless, the information obtained from the present study will be useful for future studies as data on serum 25(OH)D levels and its relationship with BMD among adults are fairly limited in Malaysia at present.

### **CONCLUSION**

The present study demonstrated that BMI is a significant factor contributing to BMD. Mechanical load factors related to a higher BMI may enable individuals with the capacity to withstand larger mechanical load to reduce bone resorption and stimulate bone formation. Therefore, achieving an ideal BMI is crucial in maintaining a healthy BMD. Majority of young adults in the present study had low calcium intake compared to the Malaysian dietary recommendation and only half of them had normal vitamin D status. Thus, awareness of the importance of calcium and vitamin D intake should be a concern in order to increase the daily consumption of nutrients that help optimize BMD.

### **SIGNIFICANCE STATEMENT**

This study discovers that BMI has a positive significant effect on BMD. Besides, the prevalence of low calcium intake and low vitamin D status is unexpectedly high among young adult's university students. This study will help the researchers to uncover the critical area of risk factors that contribute to low BMD among young adults that many researchers were not able to explore. In addition, this study is also expected to benefit the public health policy makers to refocus the nutrition education especially on milk and dairy products intake.

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