Asian Journal of Epidemiology

ISSN 1992-1462

OPEN ACCESS Asian Journal of Epidemiology

ISSN 1992-1462
DOI: 10.3923/aje.2017.63.69

Research Article
Vitamin D Status and its Impact on Body Composition in Elderly Community-dwelling Individuals in Bandung and Sumedang, West Java Province, Indonesia

Vitriana Biben, Irma Ruslina Defi, Gaga Irawan Nugraha and Budi Setiabudiawan

Faculty of Medicine, Universitas Padjadjaran, 40161 Bandung, West Java, Indonesia

Abstract

Background and Objective: Vitamin D status is correlated with musculoskeletal function. Yet, normal cut-off values for vitamin D status vary across studies and remain a subject of debate. This study design to find out a cut-off value of vitamin D in community-dwelling elderly individuals in Indonesia, examine vitamin D status based of the new cut-off value and vitamin D associations with various body characteristics that associate with musculoskeletal function. **Materials and Methods:** A cross-sectional study consisted of 263 community-dwelling elderly individuals in Bandung and Sumedang, West Java province, Indonesia were enrolled between July and December, 2015. Vitamin D status was calculated using adjusted vitamin D cut-off value that determined based on parathyroid hormone suppression through receiver operating characteristic analysis. Correlation analysis then used to found out vitamin D association with muscle mass, muscle strength and body fat. **Results:** Most subjects (94.3%) were vitamin D insufficient-deficient based on the adjusted cut-off value ($\leq$36.0 ng mL^{-1}). Vitamin D status was correlated with muscle mass ($r = 0.239$, $p = 0.000$), muscle strength ($r = 0.324$, $p = 0.000$) and also negatively correlated with percentage of body fat ($r = -0.261$, $p = 0.000$) significantly. **Conclusion:** Hypovitaminosis D is a common condition in community-dwellings elderly individuals in Indonesia. Its association with muscle mass, muscle strength and body fat, highlight the need for comprehensive recommendations, such as education for adequate nutrition intake, sun exposure, physical activity and consideration of vitamin supplementation in rational way should be considered and designed.

Key words: Aging, cut-off value, body fat, community-dwelling, elderly, muscle mass, muscle strength, vitamin D deficiency

Received: February 11, 2017 Accepted: March 10, 2017 Published: March 15, 2017

Citation: Vitriana Biben, Irma Ruslina Defi, Gaga Irawan Nugraha and Budi Setiabudiawan, 2017. Vitamin D status and its impact on body composition in elderly community-dwelling individuals in Bandung and Sumedang, West Java province, Indonesia. Asian J. Epidemiol., 10: 63-69.

Corresponding Author: Vitriana Biben, Department of Physical Medicine and Rehabilitation, Faculty of Medicine, Universitas Padjadjaran, Jl. Eijkman 38, 40161 Bandung, West Java, Indonesia Tel/Fax: +62-22-2034989

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

Hypovitaminosis D was a widespread public health problem that affects all age groups in populations including in Southeast Asian countries where there was sufficient sun exposure for vitamin D synthesis throughout the year. As elderly population increased, that known to be especially vulnerable to hypovitaminosis D, increase in prevalence of hypovitaminosis D was also predicted. However, systematic review conducted in PubMed/Medline in April-June, 2013, showed a lack of information for vitamin D status including in community-dwelling elderly population in Southeast Asian countries, including Indonesia. This study only found one population study in Malaysian elderly individuals that revealed the range of 0-2% elders had <12 ng mL^{-1} and 12-73% elders had <20 ng mL^{-1} vitamin D serum[1].

To date, there was no clear literature consensus regarding the cut-off values for vitamin hypovitaminosis D. Variations in these cut-off values affected the reported prevalence of hypovitaminosis D as well as its diagnosis and treatment. Given that vitamin D function maintains circulating calcium levels to indirectly influence concentrations of parathyroid hormone (PTH), this study also calculated an adjusted cut-off level of 25-hydroxyvitamin D [25(OH)D] that was sufficient for PTH suppression to diagnose hypovitaminosis D in community-dwelling elderly individuals in Indonesia. Many study investigated this relation to find the optimal vitamin D concentration but the result were inconsistent[2].

Vitamin D action affect to many organs, one of them was musculoskeletal system. There was conflicting evidence about whether hypovitaminosis D contributes to the loss of muscle strength and mass; however, several studies have reported an association between vitamin D deficiency and an increased risk of frailty[3-5]. Fact that there was a role of vitamin D in musculoskeletal function through the binding with its receptor and hypovitaminosis D was become a major global public health problem, the prevalence of hypovitaminosis D in community-dwelling elderly was predicted quite high and should be correlate with body characteristics in this study[1,6].

Since a limited number of population-based studies have examined vitamin D status in Indonesia and given the lack of a health policy for hypovitaminosis D, this study conducted to determine the prevalence of hypovitaminosis D based on adjusted cut-off value and subsequently evaluated correlations between vitamin D status and body characteristics in community-dwelling elderly.

MATERIALS AND METHODS

Study participants: A total of 263 healthy community-dwellings elderly individuals (189 women and 74 men) were recruited from Bandung and Sumedang, West Java province, Indonesia between July and December, 2015. All participants were informed about the nature and purpose of the study and provided written informed consent prior to participation. Simple random sampling was used to select elderly (60 years of age or older) individuals who were not living in an institutionalized care facility (i.e., community-dwelling), were able to walk independently, had urea and creatinine levels within normal ranges and had not taken regular vitamin D supplements in the previous 6 months. Subjects were excluded if had an artificial implant that will interfere machine measurement accuracy, in hormonal therapy program, in malnutrition condition, had malignancy, had renal function disturbance and had history of prolonged immobilization.

Method of collecting data: Serum levels of 25(OH)D and PTH were measured using an electrochemiluminescence binding assay (ECLIA) (Elecsys®, Roche Diagnostic, Mannheim, Germany). Vitamin D status was then classified as normal ($\geq$30 ng mL^{-1}), insufficient (21-29 ng mL^{-1}) or deficient ($\leq$20 ng mL^{-1}) based on laboratory reference cut-off points and compared to classifications using cut-off points determined by a Receiver Operating Characteristic (ROC) curve analysis. The adjusted cut-off point of vitamin D was calculated based on PTH suppression in response to the increase of vitamin D level. Cut-off value obtained from the high sensitivity and specificity value that had the widest area under ROC curve, with $p<0.05$. Muscle mass and fat body percentage were measured by portable bioelectrical impedance (BIA, Tanita BC-601, Tokyo, Japan). A hand dynamometer was used to measure muscle strength (Jamar, Lafayette, USA).

Present study was conducted in community setting using portable BIA and hand dynamometer except for blood sample analysis. Blood sample was taken by assistant laboratory at the same day with other variables taken and send to laboratory using cryobox for vitamin D serum level examination in the same day. Body mass index was defined as the weight that measured to the nearest 0.1 kg using BIA and divided by the square of the height in meters that measured to the nearest centimeter by using stadiometer (kg m^{-2}). Body Mass Index (BMI) cut-off point then classified based on WHO classification as underweight (<18.50 kg m^{-2}), normal (18.50-24.99 kg m^{-2}), overweight/pre-obese (25.00-29.99 kg m^{-2}) and obese ($\geq$30 kg m^{-2})[7].

Statistical analysis: All of the variables were checked for normality using chi-square test or Kolmogorov-Smirnov test depend on the type of variables. Numerical data presented in mean, median and range (minimal-maximal), while categorical data were presented in proportions (frequency distributions and percentage). For further data analysis, categorical data then was converted to numeric code (gender: 1 as male and 2 as female and BMI: 1 as underweight, 2 as normal, 3 as overweight, 4 as obese). A Spearman's rank-order correlation was used to test correlations between variables. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 21.0 for Windows (IBM corp., Armonk, NY, USA) and used $p<0.05$ as the threshold for statistical significance.

Ethical approval: The study protocol was approved by the Medical Ethics Board Committee of Faculty of Medicine, Universitas Padjadjaran.

RESULTS

Majority of subjects used in this study were women (71.9%) and overweight (74.5%) (Table 1). Women in this study had significantly lower vitamin D status, muscle mass and muscle strength compared to men (Table 2). It supported the earlier study results showed elder people and female gender was associated with lower vitamin D compare to male gender[8,9]. The mean vitamin D status of women in this study (13.18±9.57 ng mL^{-1}) was deficient (<20 ng mL^{-1}, laboratory reference)[10], whereas the mean vitamin D status of men (23.84±15.95 ng mL^{-1}) was insufficient (21-29 ng mL^{-1}, laboratory reference)[10]. The mean muscle strength of women (15.77±4.48 kg) was classified as low (<18 kg) based on the recommendation of the Asia Working Group for Sarcopenia[11].

There were no significant correlations between vitamin D status and age or Body Mass Index (BMI); however, there was a significant negative correlation between vitamin D-to-PTH ratio and percentage of body fat. Significant positive correlations were also identified between vitamin D status and muscle mass as well as muscle strength (Table 3).

Based on a ROC curve analysis (Fig. 1), PTH level was a good diagnostic reference for determining the hypovitaminosis D cut-off point. The area under the curve was 0.751 (95% confidence interval, 0.649-0.852), indicating that PTH level had good accuracy for distinguishing normal subjects from those with hypovitaminosis D. To obtain a high sensitivity, adjusted cut-off vitamin D level of 36.0 ng mL^{-1} was chosen, with that cut-off point the sensitivity was 81%, the specificity was 56% with p = 0.000. Implementation of this cut-off point increased the proportion of individuals with insufficient-deficient status from 90.9-94.3% (Table 4, 5).

DISCUSSION

The present study revealed an overwhelming prevalence of hypovitaminosis D in a community-dwelling elderly individuals in some region in Indonesia. Hypovitaminosis D reported to affect 6-70% of the population in Southeast Asia using a vitamin D deficiency classification of serum[12]

Table 1: Baseline characteristics of subjects

Variables	n = 263	Mean±SD	Median	Range (min-max)
Age (years)		67.65±6.07	66.00	60-87
Men	74 (28.1%)			
Women	189 (71.9%)			
Height (m)		1.53±0.08	1.53	1.33-1.76
Weight (kg)		59.58±9.79	58.80	37.00-84.70
Body mass index (kg m^{-2})				
Underweight (<18.5 kg m^{-2})	7 (2.7%)			
Normal (18.5-22.9 kg m^{-2})	68 (25.8%)			
Overweight (≥23 kg m^{-2})	188 (71.5%)			
PTH (pg mL^{-1})		53.65±26.4	49.87	11.57-207.20

SD: Standard deviation

Table 2: Baseline characteristics of subjects on different sex

	Women (n = 189)			Men (n = 74)			
Variables	Mean±SD	Median	Range (min-max)	Mean±SD	Median	Range (min-max)	p-value**
Vitamin D (ng mL^{-1})	13.18±9.57	10.58	3.00-72.30	23.84±15.95	20.87	3.48-92.24	0.000
Body fat (%)	37.54±6.63	38.10	19.50-56.40	25.35±7.59	24.50	5.00-47.30	0.000
Muscle mass (kg m^{-2})	7.09±1.80	6.89	4.13-27.31	8.78±3.04	8.29	5.88-25.52	0.000
Muscle strength (kg)	15.77±4.48	16.00	4.00-29.00	23.73±6.35	23.00	8.00-39.00	0.000

SD: Standard deviation, **Significance p<0.05

25(OH)D <50 nmol L^{-1}. Lack of a consensus regarding the cut-off point for vitamin D deficiency as measured in serum, made a need of an adjusted vitamin D cut-off point. In the present study, PTH was used as a parameter of the cut-off point. Studies have demonstrated a small but significant negative correlation between serum 25(OH)D and serum PTH in 25 countries on 5 continents, even in countries near the equator such as Singapore (r = –0.390, p<0.01) and Sri Lanka (r = –0.62, p<0.001)[13,14]. This study also similarly observed a significant negative correlation between serum 25(OH)D and serum PTH (r = –0.324, p<0.001), consistent with the findings of Setiati[15] (r = –0.379, p = 0.001).

The use of different assays for 25(OH)D and PTH may have influenced the apparent threshold 25(OH)D concentration[14]. Most research in the 1990s and early 2000s, used radioimmunoassay to assess serum 25(OH)D. Yet, different radioimmunoassays can yield different results, with variability across laboratories[16]. New methods such as liquid chromatography and mass spectrometry (LC-MS/HPLC) provide more reliable analyses of 25(OH)D concentrations[17]. Moreover, present study showed, the cut-off vitamin D values obtained by ECLIA closer to HPLC values than those obtained by ELISA, with no statistically significant difference between the mean values obtained by ECLIA and HPLC. An earlier study of elderly Indonesian women living in institutionalized care found a serum vitamin D cut-off value 30.4 ng mL^{-1} obtained by ELISA had 93.8% sensitivity and 32.8% specificity[15]. In the present study, determined a higher cut-off value 36.0 ng mL^{-1} obtained by ECLIA had 81% sensitivity and 56% specificity in a population of community-dwelling elderly individuals. This new cut-off point is also higher than the laboratory reference cut-off point (≤29.9 ng mL^{-1}). Taken together, these data suggested ECLIA may be a more useful method for assessing serum vitamin D and that higher cut-off values may be more accurate for determining hypovitaminosis D in elderly populations.

Table 3: Correlation analysis of vitamin D to baseline characteristics

Variables	r	p-value
Correlation vitamin D with age	-0.001	0.982
Correlation vitamin D with BMI	-0.019	0.758
Correlation vitamin D with PTH	-0.324	0.000**
Correlation vitamin D with muscle mass	0.239	0.000**
Correlation vitamin D with muscle strength	0.324	0.000**
Correlation vitamin D with body fat (%)	-0.261	0.000**

In this study, >90% of community-dwelling elderly individuals had hypovitaminosis D. This value was higher than that reported by Setiati[15], who reported a prevalence of 35.1% in institutionalized care elderly Indonesian women. Many factors may have contributed to a discrepancy between these results, such as sun protection behaviors that are influenced by cultural attitudes, religion, daily habits, gender and clothing style. Most of the Indonesian population is Muslim, so women cover almost their entire body, additionally, sun-seeking behavior is uncommon given the hot climate[12,13,15]. The development of urban traffic volume in Indonesia may also be a relevant factor as air pollution can decrease the penetrance of solar UVB rays[18].

High percentage of hypovitaminosis in this study may also influence by age and body composition. Age is essential factor that affects vitamin D levels due to many risk factors as a result of aging that may cause decreasing concentrations of provitamin D_3 (7-dehydrocholesterol) in the epidermis and organs function that involve in controlling level vitamin D serum concentration[9,19]. This study did not identify a correlation between age and vitamin D status, this finding raises possibility that hypovitaminosis D also prevalent in the younger age groups.

Sex difference makes female have higher body fat compared than males and it has been suggested as one of a causal factor to lower 25(OH)D concentration in females[20]. Vitamin D is a fat soluble hormone, thus adipose tissue

Table 4: Comparison of parathyroid hormone (PTH) serum level in different vitamin D status

	Vitamin D status		
Variables	Normal (n = 24)	Insufficient and deficient (n = 239)	p-value
PTH			0.000**
Mean±SD (ng mL^{-1})	36.27±23.83	55.395±26.104	
Median (ng mL^{-1})	33.98	51.37	
Range (min-max) (ng mL^{-1})	11.57-119.44	12.56-207.20	

**p<0.05 was considered statistically significant

Table 5: Proportion comparison in vitamin D status in elderly community-dwelling

Vitamin D status based on laboratory reference[6]		Vitamin D status based on adjusted cut-off point	
Normal (≥30 ng mL^{-1})	Insufficient-deficient (<29 ng mL^{-1})	Normal (>36.2 ng mL^{-1})	Insufficient-deficient (≤36.2 ng mL^{-})
24 (9.1%)	239 (90.9%)	15 (5.7%)	248 (94.3%)

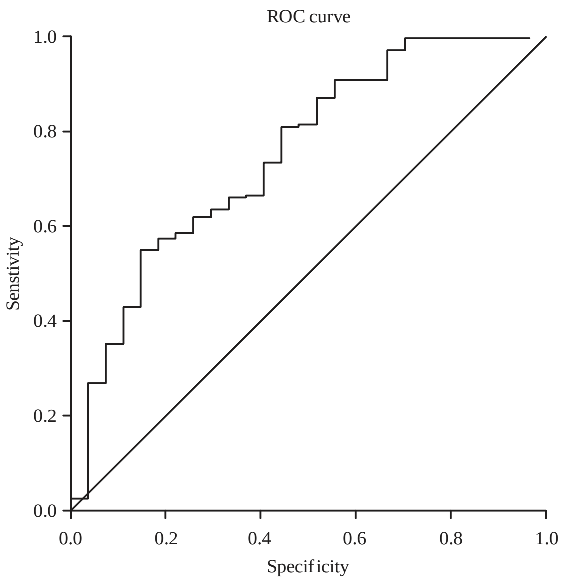

Fig. 1: ROC graphic analysis of vitamin D to PTH

has a correlation to vitamin D level because it might be a site of sequestration of vitamin D, storing and subsequently lowering circulating levels of 25(OH)D[21]. As majority of the subjects were females, it made risk of hypovitaminosis D increase in this study.

Previous studies have reported inconsistent associations between vitamin D level and muscle mass or strength[2,22,23]. This study showed a significant positive association between vitamin D level and muscle mass as well as muscle strength. The interaction of vitamin D and its cognate receptor affects protein synthesis, through anabolic signaling pathways, by sensitizes the Akt/mTOR-dependent pathway through Src, PI3K and p38 MAPK, leading to the stimulatory effects of leucine and insulin on protein synthesis (myogenesis) and can thus influence growth, muscle development and muscle adaptation[6,24-28]. Moreover, vitamin D is necessary to maintain intracellular calcium balance as well as mitochondrial energy metabolism required for muscle contraction. To this end, vitamin D deficiency is associated with low calcium, which can influence muscle contraction and strength[29].

The results of this study suggested that elderly individuals, particularly in Indonesia, require the active prevention of hypovitaminosis D in order to prevent functional disability related to impaired muscle mass and strength. Although vitamin D supplementation for the management of hypovitaminosis D has shown inconsistent effects on elderly function, several trials have indicated its ability to improve strength, function and balance[30].

This study had some limitations. First, unstandardized method made an different cut-off point findings between studies. Future large-scale population-based studies using standardized methods are required to determine more appropriate cut-off points for hypovitaminosis D that will provide a more accurate idea of the exact hypovitaminosis D prevalence and prevent future under-diagnosis. Second, this study did not observe a correlation between vitamin D and BMI, despite the fact that higher BMI has been previously associated with lower serum 25(OH)D[31]. Ability to detect this relationship may have been obscured by a high proportion of women with high body fat in study sample. Future study was required to diminish bias effect because of different of gender proportion and to assess the influence of lowering body fat and vitamin D status in the elderly. Third, this study did not identify a correlation between age and vitamin D status, this result should be confirmed by doing an additional studies to explore more deeply by involving larger sample with various age subjects including younger age populations. Fourth, sample of this study was limited only on elderly in West Java province, that limit of generalization, future population study that can represent general elderly Indonesia population with all contributing factors (including nutrition and lifestyle factors) as well as factors influencing intervention efficacy is needed to give more accurate interpretation of hypovitaminosis D status, its impact to elderly quality of life and to optimize intervention strategies for hypovitaminosis D.

CONCLUSION

It is concluded that most relative healthy community-dwellings elderly in Bandung and Sumedang, West Java province, Indonesia were in hypovitaminosis D. The findings of correlation between muscle mass, muscle strength and also percentage of body fat to vitamin D status made this condition may influence elderly physical functioning in daily activities and at the end elderly quality of life. Comprehensive approach designed based on habituation and cultural background should be underline in public health policy to strengthen all programs to improve vitamin D status.

SIGNIFICANT STATEMENT

Vitamin D status is influenced by many factors and affected many organs function. There was still no consensus for vitamin D cut-off point. By finding the adjusted cut-off point, this study found higher value that made hypovitaminosis D prevalence higher than earlier study in community-dwelling elderly. The possibility that vitamin D have an impact in physical function in elderly was supported by the result of this study that found a correlation of vitamin D to muscle mass, muscle strength and body fat. It leads to an awareness of the importance of adjustment in public health policy to increase the knowledge and improving of the importance of vitamin D adequacy in elderly community dwelling based on cultural and habitual background.

ACKNOWLEDGMENTS

The authors would like to thank the Nurvita Trianasari for her help in processing of data.

REFERENCES

1. Palacios, C. and L. Gonzalez, 2014. Is vitamin D deficiency a major global public health problem? J. Steroid Biochem. Mol. Biol., 144: 138-145.
2. Choi, S.W., S.S. Kweon, J.S. Choi, J.A. Rhee and Y.H. Lee *et al*., 2015. Estimation of the cutoff value of vitamin D: The Dong-gu study. J. Physiol. Anthropol., Vol. 34. 10.1186/s40101-015-0048-4.
3. Marantes, I., S.J. Achenbach, E.J. Atkinson, S. Khosla, L.J. Melton and S. Amin, 2011. Is vitamin D a determinant of muscle mass and strength? J. Bone Mineral Res., 26: 2860-2871.
4. Zhou, J., P. Huang, P. Liu, Q. Hao, S. Chen, B. Dong and J. Wang, 2016. Association of vitamin D deficiency and frailty: A systematic review and meta-analysis. Maturitas, 94: 70-76.
5. Halfon, M., O. Phan and D. Teta, 2015. Vitamin D: A review on its effects on muscle strength, the risk of fall and frailty. BioMed. Res. Int., Vol. 2015. 10.1155/2015/953241.
6. Pojednic, R.M. and L. Ceglia, 2014. The emerging biomolecular role of vitamin D in skeletal muscle. Exercise Sport Sci. Rev., 42: 76-81.
7. Low, S., M.C. Chin, S. Ma, D. Heng and M. Deurenberg-Yap, 2009. Rationale for redefining obesity in Asians. Ann. Acad. Med. Singapore, 38: 66-69.
8. Verdoia, M., A. Schaffer, L. Barbieri, G. di Giovine, P. Marino, H. Suryapranata and G. de Luca, 2015. Impact of gender difference on vitamin D status and its relationship with the extent of coronary artery disease. Nutr. Metab. Cardiovasc. Dis., 25: 464-470.
9. Al-Horani, H., W. Abu Dayyih, E. Mallah, M. Hamad, M. Mima, R. Awad and T. Arafat, 2016. Nationality, gender, age and body mass index influences on vitamin D concentration among elderly patients and young Iraqi and Jordanian in Jordan. Biochem. Res. Int., Vol. 2016. 10.1155/2016/8920503.
10. Holick, M.F., 2009. Vitamin D status: Measurement, interpretation and clinical application. Ann. Epidemiol., 19: 73-78.
11. Chen, L.K., L.K. Liu, J. Woo, P. Assantachai and T.W. Auyeung *et al*., 2014. Sarcopenia in Asia: consensus report of the Asian working group for sarcopenia. J. Am. Med. Directors Assoc., 15: 95-101.
12. Nimitphong, H. and M.F. Holick, 2013. Vitamin D status and sun exposure in Southeast Asia. Dermato-Endocrinol., 5: 34-37.
13. Rodrigo, M., M. Hettiarachchi, C. Liyanage and S. Lekamwasam, 2013. Low serum vitamin D among community-dwelling healthy women in Sri Lanka. Health, 5: 1997-2003.
14. Cashman, K.D., M. Kiely, M. Kinsella, R.A. Durazo-Arvizu and L. Tian *et al*., 2013. Evaluation of vitamin D standardization program protocols for standardizing serum 25-hydroxyvitamin D data: A case study of the program's potential for national nutrition and health surveys. Am. J. Clin. Nutr., 97: 1235-1242.
15. Setiati, S., 2008. Vitamin D status among Indonesian elderly women living in institutionalized care units. Population, 40: 78-83.
16. Holmes, E.W., J. Garbincius and K.M. McKenna, 2013. Analytical variability among methods for the measurement of 25-hydroxyvitamin D: Still adding to the noise. Am. J. Clin. Pathol., 140: 550-560.
17. Vogeser, M., 2010. Quantification of circulating 25-hydroxyvitamin D by liquid chromatography-tandem mass spectrometry. J. Steroid Biochem. Mol. Biol., 121: 565-573.
18. Hosseinpanah, F., S.H. Pour, M. Heibatollahi, N. Moghbel, S. Asefzade and F. Azizi, 2010. The effects of air pollution on vitamin D status in healthy women: A cross sectional study. BMC Public Health, Vol. 10. 10.1186/1471-2458-10-519.
19. Lips, P., 2010. Worldwide status of vitamin D nutrition. J. Steroid Biochem. Mol. Biol., 121: 297-300.
20. Van Dam, R.M., M.B. Snijder, J.M. Dekker, C.D. Stehouwer, L.M. Bouter, R.J. Heine and P. Lips, 2007. Potentially modifiable determinants of vitamin D status in an older population in the Netherlands: The hoorn study. Am. J. Clin. Nutr., 85: 755-761.
21. Pourshahidi, L.K., 2015. Vitamin D and obesity: Current perspectives and future directions. Proc. Nutr. Soc., 74: 115-124.
22. Cipriani, C., J. Pepe, S. Piemonte, L. Colangelo, M. Cilli and S. Minisola, 2014. Vitamin D and its relationship with obesity and muscle. Int. J. Endocrinol., Vol. 2014. 10.1155/2014/841248.
23. Liu, G., L. Lu, Q. Sun, X. Ye and L. Sun *et al*., 2014. Poor vitamin D status is prospectively associated with greater muscle mass loss in middle-aged and elderly Chinese individuals. J. Acad. Nutr. Dietetics, 114: 1544-1551.
24. Ceglia, L., 2008. Vitamin D and skeletal muscle tissue and function. Mol. Aspects Med., 29: 407-414.

25. Garcia, L.A., K.K. King, M.G. Ferrini, K.C. Norris and J.N. Artaza, 2011. 1,25$(OH)_2$ vitamin D_3 stimulates myogenic differentiation by inhibiting cell proliferation and modulating the expression of pro myogenic growth factors and myostatin in C_2C_{12} skeletal muscle cells. Endocrinology, 152: 2976-2986.
26. Ceglia, L., S. Niramitmahapanya, M. da Silva Morais, D.A. Rivas and S.S. Harris *et al*., 2013. A randomized study on the effect of vitamin D3 supplementation on skeletal muscle morphology and vitamin D receptor concentration in older women. J. Clin. Endocrinol. Metab., 98: E1927-E1935.
27. Salles, J., A. Chanet, C. Giraudet, V. Patrac and P. Pierre *et al*., 2013. 1,25$(OH)_2$-vitamin D_3 enhances the stimulating effect of leucine and insulin on protein synthesis rate through Akt/PKB and mTOR mediated pathways in murine C_2C_{12} skeletal myotubes. Mol. Nutr. Food Res., 57: 2137-2146.
28. Buitrago, C.G., N.S. Arango and R.L. Boland, 2012. 1α,25$(OH)_2D_3$-dependent modulation of Akt in proliferating and differentiating C_2C_{12} skeletal muscle cells. J. Cell. Biochem., 113: 1170-1181.
29. Girgis, C.M., 2014. Vitamin D and muscle function in the elderly: The elixir of youth? Curr. Opin. Clin. Nutr. Metab. Care, 17: 546-550.
30. Bruyere, O., E. Cavalier, J.C. Souberbielle, H.A. Bischoff-Ferrari and C. Beaudart, 2014. Effects of vitamin D in the elderly population: Current status and perspectives. Arch. Public Health, Vol. 72. 10.1186/2049-3258-72-32.
31. Vimaleswaran, K.S., D.J. Berry, C. Lu, E. Tikkanen and S. Pilz *et al*., 2013. Causal relationship between obesity and vitamin D status: Bi-directional Mendelian randomization analysis of multiple cohorts. PLoS Med., Vol. 10. 10.1371/journal.pmed.1001383.