ORIGINAL ARTICLE

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Association Between 25-hydroxyvitamin D Levels and Testosterone in Healthy, Non-Obese, Young Adult, Filipino Men

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Abstract

Objective. This study seeks to determine the association between vitamin D and testosterone in healthy, adult Filipino males.

Methodology. This cross-sectional study included 110 healthy, non-obese, male volunteers aged 21–40. History and physical exam were taken, and blood was drawn for vitamin D, total testosterone (TT), sex hormone binding globulin (SHBG), albumin, insulin, fasting plasma glucose, and total cholesterol. Free testosterone (FT) was calculated. Vitamin D data were classified by status and TT, FT, and SHBG levels compared using the Kruskal–Wallis test. The associations of vitamin D levels with TT, FT, and SHBG were explored using multiple regression analysis.

Results. Vitamin D levels were sufficient in 3 (2.7%), insufficient in 17 (15.45%), and deficient in 90 (81.8%) of the sample. There were no significant differences in the mean TT (p = 0.7981), FT (p = 0.8768), nor SHBG (p = 0.1838) across vitamin D status. Vitamin D was not associated with TT nor FT before or after adjustment for age and age plus body mass index (BMI). Vitamin D was associated with SHBG before and after the aforementioned adjustments, but this became insignificant on sensitivity analysis.

Conclusion. There is no association between vitamin D and TT, FT nor SHBG in our cohort with deficient vitamin D levels.

Key words: total testosterone, vitamin D, sex hormone binding globulin

INTRODUCTION

Vitamin D is known for its role in calcium-phosphorus homeostasis and bone mineralization.[1] It is a steroid hormone whose precursor, 7-dehydrocholesterol, is found in the skin and is converted by ultraviolet radiation to pre-vitamin D3 that then isomerizes to cholecalciferol and undergoes sequential hydroxylation in the liver to 25-hydroxyvitamin D (25(OH)D) and then in the kidneys to its active form, 1,25-dihydoxyvitamin D3 or calcitriol.[1] As a stable metabolite, 25(OH)D is used clinically as a biomarker of vitamin D status and the levels are classified as the following: sufficient (≥75 nmol/L or 30 ng/ml), insufficient (50-74.9 nmol/L or 20–29 ng/ml), and deficient (<50 nmol/L or 20 ng/ml).[1] Its biological actions are mediated by the vitamin D receptor (VDR), which has a ubiquitous expression in various tissues throughout the body.[1] In addition to bone health, studies have reported the involvement of hypovitaminosis D in chronic disorders such as depression, hypertension, diabetes, cardiovascular disease, autoimmune diseases, muscle dysfunction, and cancer.[2]

There is an increasing body of evidence from animal and human studies that vitamin D also modulates reproductive processes and androgen levels, specifically testosterone. While the latter plays an established role in spermatogenesis and general health of men, with physiological effects on the brain, muscle, bone, and fat mass,[3] the specific mechanisms by which vitamin D influences male reproduction remain unclear.[4] VDR and vitamin D-metabolizing enzymes are expressed in the entire reproductive male tract, including Leydig cells. This suggests an autocrine as well as paracrine action of vitamin D in the regulation of testicular function.[5]

Leydig cells express the CYP2R1 gene encoding 25-hydroxylase, much like the liver, so cellular dysfunction may influence androgen and 25(OH)D levels. VDR knockout mice also develop hypergonadotropic hypogonadism,[2]

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and human Leydig cells exposed in vitro to 1,25 (OH)D undergo modifications in steroidogenesis genes, resulting in a significant increase in testosterone biosynthesis.[2] It has also been postulated that testosterone secretion could be modulated by vitamin D-induced changes in Leydig cell calcium homeostasis involving calbindin and osteocalcin, highlighting its role in the endocrine system.[2] The negative effect of orchiectomy and testicular dysfunction on circulating 25(OH)D levels also supports their association.[6]

The association between vitamin D and reproductive hormone levels has been under investigation for more than a decade. Observational studies on the status of 25 (OH) D and circulating androgen levels are conflicting. Some did not find an association,[7-17] but others demonstrated a significant independent association of 25(OH)D with total testosterone (TT) levels after adjustment for confounder variables such as age, body mass index (BMI) and muscle mass, season, physical activity, area of residence, smoking, alcohol consumption, and presence of co-morbidities such as diabetes, hypertension, and cardiovascular disease.[18–23]

Regarding hypogonadism, an increase in 25(OH)D quartiles was associated with significantly reduced odds ratios of hypogonadism in three studies,[14,19,21] while another[21] failed to demonstrate an association. One suggested a U-shaped association (nonlinear) of 25(OH)D status and risk of hypogonadism, showing a significantly higher risk of hypogonadism in men within the highest 25(OH)D quintile compared to men in the reference quintile and a trend toward an increased risk of hypogonadism in men within the lowest 25(OH)D quintile.[9]

To date, there have been two systematic reviews and one meta-analysis to address this research question. The systematic reviews are from the same group, with their first publication in 2012,[24] updated subsequently in 2018.[25] Both papers summarized the studies but were unable to combine them into a meta-analysis. A meta-analysis from 2020[26] that included 18 studies with 9892 men with vitamin D deficiency and 10,675 controls, found a slight positive association between 25(OH)D and TT (pooled SMD: −0.23, 95% CI: −0.45 to −0.01; P = 0.04). However, heterogeneity was large (I2 = 98%, P for heterogeneity <0.00001). Subgroup analysis was performed with the studies being divided into those with community-dwelling participants and those with frail participants with a resultant decrease in I^2 (I^2 = 51%, P for heterogeneity = 0.06). A positive association was seen only in frailty states. Both the systematic review and the meta-analysis concluded that the lack of consistency between the studies may be due to different sample sizes, statistical methods, age, comorbidities, and ethnicity.

A study has identified that South Asians are more prone to vitamin D deficiency than those with European descent, presumably because of their darker skin color and excess visceral adipose tissue.[27] Another study found significant differences in 25(OH)D levels between Caucasians and Asians, suggesting a screening program for vitamin D deficiency in the latter population.[28] In this potentially high-risk group, studies on the relationship of vitamin D and testosterone are limited as well. Therefore, this study seeks to determine the association of vitamin D levels (25 (OH) D) with the level of TT, FT, and sex hormone binding globulin (SHBG) in a cohort of Filipino men, seeking to define this relationship specific to this group, most of whom are Malays. To bypass confounding from various comorbidities arising with age, we chose young, healthy, non-obese participants, similar to two previous papers,[11,12] which studied non-obese, young men with a mean age in the early twenties, less than a third of whom were smokers.

METHODOLOGY

Study design

This work is a secondary analysis of the cross-sectional study entitled "Reference Intervals of Total Testosterone in Adult Filipino Men."[29] That study determined the TT reference range among healthy young adult males. The current study received approval from both the Technical Review Board of the Department of Physiology and the Ethics Review Board of the University of the Philippines Manila. Strengthening the Reporting of Observational studies in Epidemiology (STROBE)[30] cross-sectional reporting guidelines was followed in reporting the current study findings.

Study sample

The original study included 110 healthy, Filipino young adult (aged 21-40 years) males who were studying or working at the University of the Philippines Manila between 2016 to 2019. The exclusion criteria were the following: body mass index (BMI) ≥25; hypertension (BP 140/90 mmHg); fasting plasma glucose (FPG)≥126 mg/dl; hypercholesterolemia (total cholesterol ≥240)**;** self-reported history of co-morbidities(diabetes, osteoporosis, chronic lung disease, ulcer, HIV, cancer, cerebrovascular disease, myocardial infarction, stroke, congestive heart failure, bypass, angioplasty, claudication, hyperthyroid or hypothyroid disease, infertility); or family history of hypogonadism or infertility, current use of prescription medication, history and present intake of testosterone, steroids, opioids, anticonvulsants, male fertility agents**;** smoking (present or past); alcohol consumption exceeding 600 ml ethanol(1 ml = 0.786 g) per week, and lastly, if the subject works in shifts. Data on the use of vitamin D supplements were not collected in the original study.

Data collection procedure

Participation in the original study was conditional on consent of the recruited individual. History and physical examination were performed by trained investigators following standardized procedures, as detailed in the previous manuscript. After a 10-hour fast, the blood sample was collected in plain blood collection tubes without additives (red top) within 4 hours of awakening.

Assays

FPG, albumin, and total cholesterol were analyzed using a COBAS Integra 400PLUS clinical chemistry analyzer. TT was measured using the Testosterone [I-125] RIA Kit (RK-61CT), SHBG levels with the SHBG [I-125] IRMA Kit (RK-86CT) while insulin levels were determined using the Insulin [1-125] IRMA Kit (RK-400CT). All kits mentioned were manufactured by the Institute of Isotopes Ltd., Budapest. On the other hand, 25(OH)D levels were tested using the 25-OH vitamin D total [I-125] RIA B46327 kit (Beckman Coulter, Czech Republic). The inter and intra-assay coefficients of variability (CV) for TT, SHBG, Insulin, and 25(OH)D were 12% and 8.9%, 6.04%, and 8.58%,17.1% and 4.4%, and 6.7% and 4.7%, respectively. All assays had a zero calibrator plus 5 calibrators. TT and 25(OH)D had 2 quality control test samples, while the insulin and SHBG assays had one. Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), a standard measure of insulin resistance, was calculated using equation.[31]

$$\frac{\text{Fasting Insulin (microU/L) x Fasting glucose (nmol/L)}}{22.5}$$

FT was calculated using the equation developed by Vermeulen[32] using the online calculator provided by the International Society for the Study of aging Males (ISSAM).[33]

Data analysis

The data were entered into Excel and descriptive statistics were then calculated. Missing data were managed by case deletion. Data on 25(OH)D were categorized based on 25(OH)D serum status. Total testosterone, FT, and SHBG levels were compared across status using the Kruskal–Wallis test. The associations of 25(OH)D with TT and FT levels were assessed using linear regression, while the association between 25(OH)D and SHBG levels was determined using robust regression. Three models were generated for each exposure variable — Model 1 was unadjusted, Model 2 was adjusted for age, and Model 3 was adjusted for age and BMI to eliminate confounding from insulin resistance due to obesity. The adjusted regression coefficients were used to determine the strength of association between the variables of interest. Statistical significance was set at $p<0.05$. Assumptions of the linear regression analysis were tested. It was noted that the normality assumption was violated upon performing the Shapiro-Wilk test. Consequently, sensitivity analysis was performed to check the effects of outliers on the estimates derived from the regression models. Power analysis was conducted to ensure that the adjusted regression models have sufficient power given the study size. Statistical analysis was performed in R version 4.2.2.

RESULTS

All the participants (N = 110) of the original study were included in this secondary data analysis. Vitamin D levels were sufficient in three (2.7%), insufficient in 17 (15.45%), and deficient in 90 (81.8%) of study participants. Baseline characteristics like age, BMI, WHR, FPG, Insulin, and HOMA-IR were similar across vitamin D status (Table 1).

The associations of 25(OH)D with TT and FT were not significant before and after adjustment for age and both age and BMI (Table 2). The association of 25(OH)D with SHBG was significant before and after adjustment for age and both age and BMI. However, the sensitivity analysis (i.e., exclusion of outliers) resulted in non-statistically significant association between 25(OH)D and SHBG. Power analysis revealed that the adjusted regression models had at least 99.5% power when the study size and smallest effect size produced were considered.

DISCUSSION

The proportion of participants with Vitamin D deficiency in our study was high as our sample was drawn from students and office workers who are indoors from 8 AM to 5 PM. This result is similar to data from the 2013 National Nutrition Survey for Filipinos, which showed a 54% prevalence of combined vitamin D insufficiency and deficiency in the National Capital Region of the Philippines.[34] A 2015 study by Chin done in Malaysia, another Southeast Asian country,[15] showed that 23% of the participants were vitamin D deficient.

Table 1. Baseline characteristics of participants according to vitamin D status

Variable mean (SD)	Overall	Vitamin D level		*p*
		<50 nmol/L	≥50 nmol/L	
N	110	90	20	
Age (years)	27.53 (± 5.34)	27.42 (± 5.1)	28.00 (± 6.42)	0.8857
BMI[a] (kg/m^2)	22.31 (± 1.97)	22.40 (± 1.97)	21.89 (± 1.97)	0.2795
Waist-hip ratio	0.92 (± 0.05)	0.92 (± 0.05)	0.92 (± 0.05)	0.6582
Vitamin D (nmol/L)	40.91 (± 13.73)	36.19 (± 8.36)	62.12 (± 13.22)	<0.0001
TT[b] (nmol/L)	21.92 (± 10.83)	21.91 (± 11.02)	21.94 (± 10.19)	0.7981
FT[c] (nmol/L)	0.55 (± 0.33)	0.56 (± 0.33)	0.52 (± 0.31)	0.8768
SHBG[d] (nmol/L)	24.41 (± 10.63)	23.69 (± 9.77)	27.67 (± 13.73)	0.1838
FBS[e] (mg/dl)	86.36 (± 7.46)	85.91 (± 6.95)	88.35 (± 9.37)	0.2562
Insulin (mIU/ml)	10.77 (± 6.93)	10.42 (± 5.61)	12.32 (± 11.19)	0.7099
HOMA – IR[f]	1.37 (± 0.85)	1.32 (± 0.69)	1.56 (± 1.36)	0.7331

[a] Body mass index; [b] Total testosterone; [c] Free testosterone; [d] Sex hormone binding globulin; [e] Fasting blood sugar; [f] Homeostatic Model Assessment of Insulin Resistance

Table 2. The association between 25(OH)D and testosterone, free testosterone and sex hormone binding globulin

Variables	Model 1[a]		Model 2[b]		Model 3[c]	
	β Coefficient (95% CI)	*p*	β Coefficient (95% CI)	*p*	β Coefficient (95% CI)	*p*
Total testosterone	0.03 (-0.12, 0.18)	0.664	0.07 (-0.07, 0.22)	0.3160	0.05 (-0.08, 0.18)	0.469
Free testosterone	-0.00 (-0.01, 0.00)	0.662	0.00 (-0.00, 0.00)	0.9360	-0.00 (-0.00, 0.00)	0.853
Sex hormone binding globulin	0.11 (-0.01, 0.23)	0.076	0.11 (-0.01, 0.24)	0.0705	0.09 (-0.03, 0.21)	0.129

[a]Unadjusted model; [b]Adjusted for age; [c]Adjusted for age and BMI

This is the first study to evaluate the relationship between 25(OH)D levels and TT in Filipinos. There is only one similar study in the same ethnic group by Chin mentioned previously, with Chinese and Malay participants. The Malays were noted to have higher BMI and lower SHBG levels but higher TT than the Chinese. This study showed an association between 25(OH)D and TT as well as SHBG but not with FT. After adjusting for age, ethnicity, and BMI, only the direct relationship between 25(OH)D and SHBG remained significant.[15] In our study, 25(OH)D also had a direct association with SHBG even after correcting for age and BMI but not with TT nor FT. However, the association became insignificant on sensitivity analysis. The difference between this study and ours may be because majority of Filipinos are Malays,[35] while only 39% were such in this study. In addition, BMI was higher at 25 kg/m^2 in Chin's cohort while it was 22 kg/m^2 in ours. Obesity may affect both testosterone and vitamin D levels. First, insulin resistance with increasing adiposity lowers SHBG levels resulting in decreased TT levels but unchanged FT[36] and secondly, obesity has also been associated with lower 25(OH)D levels[37-39]. A 2018 meta-analysis including 55 studies showed that obesity and 25(OH)D levels may be directly related, although the result should be interpreted with caution as heterogeneity was noted to be high.[37] Possible explanations for this relationship would be deposition of vitamin D in adipose tissue[38] or volumetric dilution[39] resulting in lower circulating levels.

We also compared our results with studies with the same age group as age is also being considered as an effect modifier.[22] There are 6 other studies on participants aged less than 40 across varying ethnicities and all showed that TT was not correlated with 25(OH)D levels. These are the studies by Ramlau[7] who studied 347 Danish men aged 18–21 years; Hammoud[8] who looked at American participants with a mean age of 29; Lerchbaum[9] who examined 225 Austrian men with a median age of 35; Blomberg[10] who enrolled 1,427 Danish residents with a mean age of 34; Rudnicka[11] who included data from 198 Spanish students with a mean age of 20; and Ksiazek[12] who recruited Polish men aged 28-35. The first 2 studies had a small number of vitamin D insufficient participants, while the last four had more than 50% of their subjects with vitamin D insufficiency. A significant number of vitamin D insufficient subjects is ideal as a previous paper reported that associations between 25(OH)D and TT are stronger at the lower end of vitamin D concentrations.[20] The mean BMI of the participants in the studies ranged from 22-26 kg/m^2. Five of these studies also looked at SHBG[7-10,12] and two[7,10] showed a positive correlation with 25(OH)D. Four of these papers calculated FT[8-10,12] and one showed an inverse correlation with 25(OH)D.[10] This study had participants drawn from an infertility clinic and may not be representative of the general population. Three of these studies[7,8,10] also evaluated sperm characteristics and found that 25(OH)D levels correlated with sperm quality albeit, with conflicting results. Two studies invited healthy volunteers[7,8] in the community setting, whereas the third study recruited participants from an infertility clinic.[10] These three as well as a study by Ciccone,[18] among others, may imply that the effects of Vitamin D on reproduction are related to sperm parameters instead of hormonal levels.

The published studies with participants older than 40 years are not as concordant, as five studies[13-17] did not show an association, while six others[18-23] showed an association between 25(OH)D and TT even after correcting for confounders. We did not compare these studies with ours as the participants had varying comorbidities related to age, lifestyle, and physical activity, which may affect testosterone levels.

Our study has several strengths. The majority of the subjects had deficient 25(OH)D levels and thus, the relationship between low 25(OH)D levels and TT was well explored. Second, blood was drawn within 4 hours of awakening, thereby minimizing variability due to differing time of collection. Third, a single technician performed the assays using meticulous quality control procedures. Lastly, although the inter-assay variations of TT and insulin were above 10%, all other assays had a coefficient of variation less than 10%.

This study has several limitations. First, we tested healthy volunteers instead of drawing a sample from a population. Moreover, this being a secondary analysis, data on vitamin D supplementation and sun exposure were not available. Third, exclusion of comorbidities was through self-reporting, rather than through diagnostic testing. Fourth, we used radioimmunoassay, which has a lower accuracy and sensitivity than mass spectrometry, the gold standard. Lastly, the cross-sectional nature of the study does not allow the determination of causality.

CONCLUSION

Our study shows 25(OH)D levels are not associated with TT, FT nor SHBG in a vitamin D deficient cohort. Other possible mechanisms should be explored to explain the observed effects of vitamin D on reproductive function.

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Statement of Authorship
The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
MBS: Conceptualization, Methodology, Validation, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition; **RGB:** Conceptualization, Methodology, Validation, Investigation, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization; **DJTD:** Conceptualization, Methodology, Validation, Investigation, Data Curation, Writing – original draft preparation, Writing – review and editing; **MIKC:** Conceptualization, Methodology, Validation, Investigation, Data Curation, Writing – original draft preparation, Visualization, Supervision; **MLT:** Conceptualization, Methodology, Validation, Resources, Writing – review and editing, Supervision, Project administration, Funding acquisition.

Author Disclosure
The authors declared no conflict of interest.

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Data Availability Statement
Data is available from the corresponding author on a reasonable request.

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