Journal of Taibah University Medical Sciences (2016) 11(5), 456–463

Taibah University

Journal of Taibah University Medical Sciences

www.sciencedirect.com

Original Article

Assessment of plasma level of 25(OH)D and its correlation with cardiorespiratory fitness in young females of Dammam City, KSA

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Received 4 May 2016; revised 27 June 2016; accepted 3 July 2016; Available online 18 August 2016

الملخص

أهداف البحث: يعد نقص فيتامين د مشكلة صحية في تزايد في جميع أنحاء العالم. فهو يعتبر عامل خطورة للإصابة بمتلازمة الأيض، وارتفاع الضغط، داء السكري. تهدف هذه الدراسة لتقدير نسبة الإصابة بنقص فيتامين د لدى الشابات السعوديات بمدينة الدمام والبحث عن العلاقة المحتملة بين فيتامين د ومستوى اللياقة القلبية التنفسية.

طرق البحث: في هذه الدراسة المستعرضة، شارك ٨٧ شابة سعودية بمتوسط عمر= ٢.٣٧± ٢٠.٧٨ عاما. وأُخذت موافقة خطية مسبقة من كل مشاركة، وعمل فحص الجهد البدني. كما تم تحليل مستوى فيتامين د في الدم لجميع المشاركات. لم تتناول أي مشاركة مكملات غذائية تحوي فيتامين د ولم يكن لديهن أي من أمراض الغدد، أو داء السكري أو أي أمراض أخرى تتعارض مع ممارسة تمرين الجهد البدني.

النتائج: أظهرت النتائج أن نقص فيتامين د لدى العينة المشاركة بلغ ٩٢٪. بمتوسط فيتامين د في الدم يصل إلى ٧.١٩ ± ١٥.١٩نغ/مل. ومستوى اللياقة القلبية التنفسية

ممثلة بحجم الأكسجين الأقصى = ٨.٧٢± ٣٠.٦٧ مل/كغ. دقيقة. هناك علاقة إيجابية وثيقة بين مستوى فيتامين د/وزن الجسم ومستوى حجم الأكسجين الأقصى.

الاستنتاجات: أظهرت هذه الدراسة أن هناك تهديدا كبيرا ومتواصلا لدى الشابات

السعوديات بالدمام، وضح جليا من ارتفاع حالات الإصابة بنقص فيتامين د. كما أظهرت هذه الدراسة أيضا وجود علاقة إيجابية وثيقة بين مستوى فيتامين د/وزن الجسم ومستوى اللياقة القلبية التنفسية في المجموعة المشاركة. كما يمكن استخدام هذه النتيجة الحاسمة في رسم خطة استراتيجية وطنية تستهدف التركيز على الإناث.

الكلمات المفتاحية: فيتامين د؛ فحص الجهد القلبي التنفسي؛ حجم الأكسجين الأقصى؛ الشابات؛ مدينة؛ الدمام

Abstract

Objectives: Vitamin D deficiency is a growing health problem worldwide. This vitamin is considered a risk factor for metabolic syndrome, hypertension, and diabetes. The current study aimed to estimate vitamin D deficiency incidence in young Saudi females in Dammam City and to examine the possible correlation between vitamin D level and cardiorespiratory fitness.

Methods: In this cross-sectional study, 87 young Saudi females were recruited with mean age = 20.78 ± 2.37 years. Each participant signed a written informed consent and performed a treadmill exercise stress test. Blood analysis for 25(OH)D was performed on all participants. None of the participants was taking a vitamin D supplement, and they were not suffering from endocrine diseases, diabetes or any contraindications for the exercise stress test.

Results: As much as 92% of the recruited sample demonstrated vitamin D deficiency with mean value = 15.19 ± 7.19 ng/ml. Cardiorespiratory fitness was represented by VO_2 peak with a mean value of 30.67 ± 8.72 ml/kg/min. A positive significant correlation was demonstrated between 25(OH)D/body weight and VO_2 peak.

Conclusions: The current study showed a magnificent and persistent health threat to the young Saudi female population of Dammam, as demonstrated by the high incidence of vitamin D deficiency. This study also demonstrated a positive correlation between vitamin D/body weight with cardiorespiratory fitness (VO_2 peak) in the studied group. This crucial finding could be used in designing an optimal national strategic plan focusing on the female population.

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Peer review under responsibility of Taibah University.

ELSEVIER Production and hosting by Elsevier

1658-3612 http://dx.doi.org/10.1016/j.jtumed.2016.07.002

Keywords: 25(OH)D; Cardiorespiratory exercise testing; Dammam City; HPLC; VO_2 peak; Young females

Introduction

Several health problems have emerged in the last three decades as a result of modernization of life in KSA. A new lifestyle has gradually invaded rural communities near urban areas and is characterized by a great reduction in physical activity, a diet that includes increased fat intake, and intensification of social stresses. These factors, individually or combined, have had an adverse effect on health.[1,2]

Vitamin D deficiency has recently been identified as a worldwide epidemic with growing incidence.[3,4] It is currently known as a risk factor for multiple diseases. In addition to the role of vitamin D deficiency in increasing bone resorption and precipitation of osteomalacia and osteoporosis,[5] it has been observed that vitamin D deficiency contributes to metabolic syndrome, hypertension, and diabetes[6,7] and contributes independently to heart failure.[8] Several recent studies observed a correlation between vitamin D deficiency and certain types of malignancy, such as breast cancer.[8]

Studies performed in Arab countries reveal the existence of vitamin D deficiency among the Arab population. One such study was performed in the United Arab Emirates (UAE) and included 259 women with demonstrated vitamin D deficiency.[9] A study involving 316 Lebanese volunteers (men and women) showed that 72.8% suffered from hypovitaminosis D.[10] Other studies were performed in KSA in scattered regions and demonstrated hypovitaminosis D in different sectors of the population with incidence ranges from 65 to 100%.[11–13]

Several precipitating factors have been suggested for the growing incidence of vitamin D deficiency, including lack of exposure to the sun,[14] a vitamin D-deficient diet, reduced physical activity, and obesity.[15] Most of the studies performed in KSA draw attention to these factors and try to find significant correlations between these factors and vitamin D deficiency. All of these studies and others assessed the dietary intake of vitamin D and Ca^{++}, sun exposure, and physical activity using only a structured questionnaire.[16]

Diminished physical activity was also reported in our population. Using a self-reported questionnaire, Taha et al. found that approximately 45% of male and 66% of female school students perform physical activity less than three times per week.[17] Hazzaa et al. studied the physical activity of Saudi children based on heart rate telemetry and concluded that 60% of the individuals in his sample were inactive.[18]

The correlation between vitamin D and physical inactivity was demonstrated in several studies. A positive correlation was found between reduced physical activity and low vitamin D in elderly subjects as assessed by three tests: a short walk, rising from a kitchen chair five times, and standing with one leg raised for five minutes.[19] Ethnic variation of this correlation was also demonstrated. Ellis et al. found a positive correlation of 25-hydroxyvitamin D (25(OH)D) and cardiopulmonary fitness in an African American group only and not in European Americans.[20]

Identification of evidence of correlation between vitamin D level and physical fitness in KSA is essential to help the health authorities build new strategies for fighting metabolic and cardiovascular diseases.

To the best of our knowledge, there are no previous studies performed in KSA that assess physical activity in any segment of the population using a standard exercise fitness test and then correlate the findings with the level of plasma 25(OH)D. Cardiopulmonary exercise testing is considered the gold standard method for assessing cardiorespiratory fitness and providing a reliable and valid reflection of the general physical activity of a subject.[21] Females in KSA might be more vulnerable to both vitamin D deficiency and reduced physical fitness than males as a result of multiple environmental and social factors. The hot and humid weather of the region extensively limits outdoor activities.

Therefore, in the current study, we aim to assess the level of 25(OH)D in young Saudi females with the best described method of analysis, high performance liquid chromatography (HPLC),[11] and to correlate the test results with cardiorespiratory fitness using cardiopulmonary exercise testing (CPET) and VO_2 max estimation.

Materials and Methods

The current cross-sectional study was approved by the Institutional Review Board at the University of Dammam (IRB certificate number 2015-01-065), Dammam, KSA and follows the principles of the Helsinki Declaration.[22]

A total of 87 young Saudi female college students were recruited from different University of Dammam colleges in the period from October to April 2015, excluding exam periods.

All recruited subjects were apparently healthy young Saudi females with no history of any of the contraindications of exercise stress testing such cardiovascular, respiratory, haematological, or musculoskeletal disorders. None of the participants had been pregnant or lactating during the previous two years. None of the participants had taken vitamin D or any multivitamin supplement within the previous six weeks and none were suffering from an endocrine disease or receiving hormones.

The inclusion and exclusion criteria adopted in this study were designed to limit the effect of the confounding factors.

Each subject was interviewed, a full medical history was obtained, and a vitamin D questionnaire was completed. Next, all study procedures were explained (CPET, blood extraction), and written informed consent was obtained.

Vitamin D questionnaire

Subjects were interviewed and a vitamin D questionnaire was completed. The questionnaire assessed the probable related sources and factors influencing vitamin D level including diet, sun exposure, and physical activity. The questionnaire is a modified version of the vitamin D questionnaire used by Bolek-Berquit et al.[23] See the questionnaire in Appendix 1. The subjects were not aware of their scores.

Cardiopulmonary exercise testing (CPET)

The CPET procedure was clearly explained to the subject before starting the test. The subject was also encouraged to exert maximum effort during the test. However, the participant had the right to stop the test if she was unable to continue.

All CPET procedures were performed in the morning. Baseline pulse rate, blood pressure, and electrocardiogram (ECG) were recorded.

The subject's pulse rate, percentage haemoglobin (Hb) saturation, arterial blood pressure, and ECG were monitored continuously during the exercise procedure and the subject was carefully observed by an expert.

Each subject performed the Bruce treadmill protocol[24] in an exercise physiology laboratory (AD Instruments, Australia). In the Bruce protocol, the subject begins at a speed of 1.7 mph and at a 10% grade. Both speed and incline are increased every 3 min until exhaustion.

All participants were encouraged to continue exercise until exhaustion and the increment in pulse rate was observed and compared to the calculated maximal heart rate of the subject as predicted by age using the formula: Predicted maximum heart rate = 208−0.7 × age.[25]

The Pollock formula was used to calculate the VO_2 peak for active and sedentary women using the total run time (T): Pollock VO_2 peak $= 4.38 \times T - 3.9$.[26]

Vitamin D determination

Blood samples were collected by a nurse through venipuncture between 12:00 and 1:00 p.m. (1200−1300) for all subjects. Blood was collected in ethylenediaminetetraacetic acid (EDTA) vacutainers, centrifuged, and the plasma kept at −80 °C for a maximum period of one week before analysis.

Plasma 25(OH)D was measured using the ClinRep® high-performance liquid chromatography (HPLC) complete kit (Recipe, Germany).[27] HPLC parameters include isocratic pump with flow rate: 1.0 ml/min; injection volume: 50 μl; injection interval: 10 min; UV detector: 264 nm. The inter-assay and intra-assay coefficients of variation are 3.1−4.7 and 2.3−4.9, respectively.

Statistical analysis

IBM SPSS Statistics version 20 was used to analyse the data. All data were expressed as the mean ± SD and analysed as normally distributed data according to the result of the Kolmogorov−Smirnov Z test. The frequency of 25(OH)D deficiency and deficiency categories were determined according to the Endocrine Society clinical practice guidelines for vitamin D deficiency.[28] Analysis of variation (ANOVA) and least significant difference (LSD) post hoc tests were used to compare data from the questionnaire and parameters of the exercise stress test, including time to exhaustion, maximum heart rate, and VO_2 peak between vitamin D categorical groups.

Pearson bivariate correlation was used to determine possible correlation between the plasma 25(OH)D level and the questionnaire data, VO_2 peak, time to exhaustion, and maximum heart rate. The level of significance was set at $p < 0.05$.

Results

The general characteristics, questionnaire data, plasma vitamin D values, and exercise test parameters of all participants are presented in Table 1.

Plasma vitamin D levels

The mean plasma value of 25(OH)D in 87 young Saudi females was 15.19 ± 7.19 ng/ml (normal and sufficient vitamin D level is ≥30 ng/ml).[28] The current mean value of vitamin D is low. However, for a better understanding of the vitamin D level in this group, all values were classified according to the Endocrine Society clinical practice guidelines for vitamin D deficiency (Table 2).[28]

Based on this classification, only 8% of our sample is considered to have a normal and sufficient level of vitamin D.

Table 1: General characteristics, questionnaire data, 25(OH)D levels and exercise test parameters of all subjects.

	Value
Number of subjects	87
Age (years)	20.78 ± 2.37
Weight (Kg)	58.14 ± 14.76
Height (cm)	158.30 ± 6.26
BMI	23.04 ± 4.82
Milk amount	2.48 ± 1.36
Milk score	7.94 ± 2.90
Yoghurt	2.22 ± 1.27
Oil and butter	2.80 ± 1.32
Cheese	3.00 ± 0.89
Bread	3.56 ± 0.60
Meat	3.05 ± 0.62
Eggs	2.43 ± 0.93
Total diet score	24.95 ± 4.35
Vitamin D fortified drinks	1.14 ± 1.30
Carbonated drinks	1.16 ± 1.11
Sun exposure	2.84 ± 1.06
Sunscreen	1.18 ± 1.72
Indoor tan	0.07 ± 0.45
TV or computer screen time	8.80 ± 4.48
Stair climbing	3.36 ± 2.47
Home physical activities	8.28 ± 1.92
Exercise activity	5.42 ± 1.93
Plasma 25(OH)D (ng/ml)	15.19 ± 7.19
Maximum heart rate (HRmax) beats/min	146.81 ± 22.34
Time of exercise until exhaustion (min)	7.73 ± 1.99
Pollock VO_2 peak ml/kg/min	30.67 ± 8.72

Table 2: Classification of plasma 25(OH)D level in 87 young Saudi females according to the endocrine society guidelines.

Class No.	Class Description		Percentage of the studied group
1	Class range	Normal and sufficient ≥30 ng/ml	7 (8%)
	Range in the studied group	(30.26–39.65 ng/ml)	
2	Class range	Normal and insufficient ≥20–<30 ng/ml	8 (9%)
	Range in the studied group	(20.46–27.91 ng/ml)	
3	Class range	Mild deficiency ≥10–<20 ng/ml	52 (59%)
	Range in the studied group	(10.40–19.87 ng/ml)	
4	Class range	Moderate deficiency ≥5–<10 ng/ml	18 (20%)
	Range in the studied group	(5.52–9.86 ng/ml)	
5	Class range	Severe deficiency < 5 ng/ml	2 (2%)
	Range in the studied group	(4.43–4.61 ng/ml)	
		Total	87 (100%)

Questionnaire data

Vitamin D questionnaire data included milk amount, milk score, yogurt, oil and butter, cheese, bread, meat, eggs, total diet score, vitamin D-fortified drinks, carbonated drinks, sun exposure time and extent, use of sunscreen, frequency of indoor tanning, computer or TV screen usage time, stair climbing, home physical activity, and exercise activity. These data were compared between the five groups of vitamin D classification using ANOVA and the LSD post-hoc test. No significant difference was found among any of the mentioned parameters between any of the concerned groups (Table 3).

Parameters of the exercise test

All participants performed the exercise stress test to exhaustion safely without any complaints or complications. All participants were cooperative and followed the instructions.

The mean value of calculated VO_2 peak using the Pollock formula was 30.67 ± 8.72 ml/kg/min.

Parameters reflecting exercise fitness including maximal heart rate, time to exhaustion, and Pollock VO_2 peak were compared between all 25(OH)D categorical groups using ANOVA and the LSD post hoc test and revealed no significant differences between the groups in any of the listed parameters (Table 4).

Pearson bivariate correlation

Pearson bivariate correlation was run between plasma 25(OH)D and 25(OH)D normalized to body weight and all other parameters. There was no significant correlation between plasma 25(OH)D and 25(OH)D normalized to body weight and the data of the questionnaire (data not shown). There was also no correlation between plasma 25(OH)D level and exercise test parameters (Table 5). However, 25(OH)D normalized to body weight showed a positive correlation with time to exhaustion and Pollock VO_2 peak, using Pearson bivariate correlation ($p = 0.012$, 0.015, $r = 0.267$, 0.259, respectively) (Table 5).

Table 3: Comparison of parameters extrapolated from vitamin D questionnaires among the classes of vitamin D levels using ANOVA and LSD post hoc tests.

	Class 1	Class 2	Class 3	Class 4	Class 5
Milk amount	2.42 ± 1.72	3.00 ± 1.51	2.37 ± 1.39	2.44 ± 1.15	3.50 ± 0.71
Milk score	8.29 ± 2.69	8.88 ± 3.09	7.63 ± 3.02	7.89 ± 2.65	11.00 ± 1.41
Yoghurt	2.71 ± 0.49	2.25 ± 1.28	2.00 ± 1.37	2.50 ± 1.20	3.00 ± 0.00
Oil and butter	2.57 ± 1.62	3.63 ± 0.52	2.75 ± 1.28	2.89 ± 1.49	1.50 ± 0.71
Cheese	3.14 ± 0.69	3.38 ± 0.92	2.85 ± 1.00	3.06 ± 0.64	3.00 ± 0.00
Bread	3.86 ± 0.38	3.75 ± 0.46	3.50 ± 0.67	3.61 ± 0.50	3.00 ± 0.00
Meat	3.14 ± 0.38	2.75 ± 0.71	3.04 ± 0.68	3.17 ± 0.38	3.00 ± 1.41
Eggs	2.71 ± 0.49	2.63 ± 0.92	2.38 ± 1.00	2.39 ± 0.98	2.00 ± 0.00
Total diet score	26.43 ± 3.21	27.25 ± 4.92	24.15 ± 4.30	25.50 ± 4.64	26.50 ± 2.12
VD fortified drinks	1.14 ± 1.35	1.38 ± 1.30	1.15 ± 1.33	1.11 ± 1.32	0.50 ± 0.70
Carbonated drinks	1.43 ± 0.98	1.38 ± 1.06	1.00 ± 1.19	1.33 ± 1.03	1.50 ± 0.71
Sun exposure	2.57 ± 2.23	3.88 ± 1.56	2.69 ± 2.15	2.89 ± 2.11	3.00 ± 1.41
Sunscreen	0.71 ± 1.11	1.00 ± 1.60	1.19 ± 1.66	1.22 ± 2.10	3.50 ± 2.12
Indoor tan	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.14	0.28 ± 0.96	0.00 ± 0.00
TV or computer screen time	7.71 ± 4.54	8.26 ± 5.44	8.61 ± 4.65	9.83 ± 3.94	9.00 ± 2.83
Stair climbing	2.43 ± 1.28	3.63 ± 2.20	3.44 ± 2.28	3.78 ± 3.37	1.50 ± 0.71
Home physical activities	9.86 ± 3.02	9.88 ± 3.72	7.77 ± 3.65	8.00 ± 3.43	1.00 ± 4.24
Exercise activity	5.00 ± 1.91	5.50 ± 1.31	5.33 ± 1.82	5.56 ± 2.06	8.50 ± 4.95

Table 4: Comparison of parameters extrapolated from the exercise stress test among the classes of 25(OH)D levels using ANOVA and LSD post hoc tests.

	Class 1	Class 2	Class 3	Class 4	Class 5
Maximum heart rate (HRmax) beats/min	131.3 ± 23.3	141.0 ± 14.4	148.9 ± 22.6	150.9 ± 23.0	128.5 ± 23.3
Time of exercise until exhaustion (min)	7.9 ± 1.9	7.9 ± 2.1	7.8 ± 2.1	7.8 ± 1.9	6.2 ± 0.3
Pollock VO_2 peak ml/kg/min	32.5 ± 8.7	31.3 ± 9.2	31.0 ± 9.1	30.8 ± 8.4	23.7 ± 8.8

Class 1 Normal and sufficient ≥30 ng/ml, class 2 Normal and insufficient ≥20–<30 ng/ml, class 3 Mild deficiency ≥10–<20 ng/ml, class 4 Moderate deficiency ≥5–<10 ng/ml, class 5 Severe deficiency < 5 ng/ml.

Table 5: The results of Pearson bivariate correlations between plasma 25(OH)D levels and parameters of exercise testing.

Parameter 1	Parameter 2	r	p-Value
25(OH)D	Maximum heart rate (HRmax) beats/min	−0.195	0.071
	Time of exercise until exhaustion	0.032	0.772
	Pollock VO_2 peak ml/kg/min	0.019	0.861
Adjusted 25(OH)D level[a]	Maximum heart rate (HRmax) beats/min	−0.148	0.168
	Time of exercise until exhaustion	0.267	0.012*
	Pollock VO_2 peak ml/kg/min	0.259	0.015*

*$p < 0.05$.
[a] Adjusted 25(OH)D = plasma 25(OH)D/body weight.

Linear regression

A linear regression of 25(OH)D per body weight, VO_2 peak, and time to exhaustion was obtained and revealed a significant relationship with p = 0.012 and 0.015 and r = 0.259 and 0.267, respectively (Figure 1).

Discussion

The main finding of the current study is the high incidence of vitamin D deficiency in young Saudi females, which was apparent in approximately 92% of the recruited sample. The deficient group included 9% of participants with normal and insufficient vitamin D, 59% with a mild deficiency, 20% with a moderate deficiency, and 2% with a severe deficiency. Upon exploring the effect of diet, sun exposure, and physical

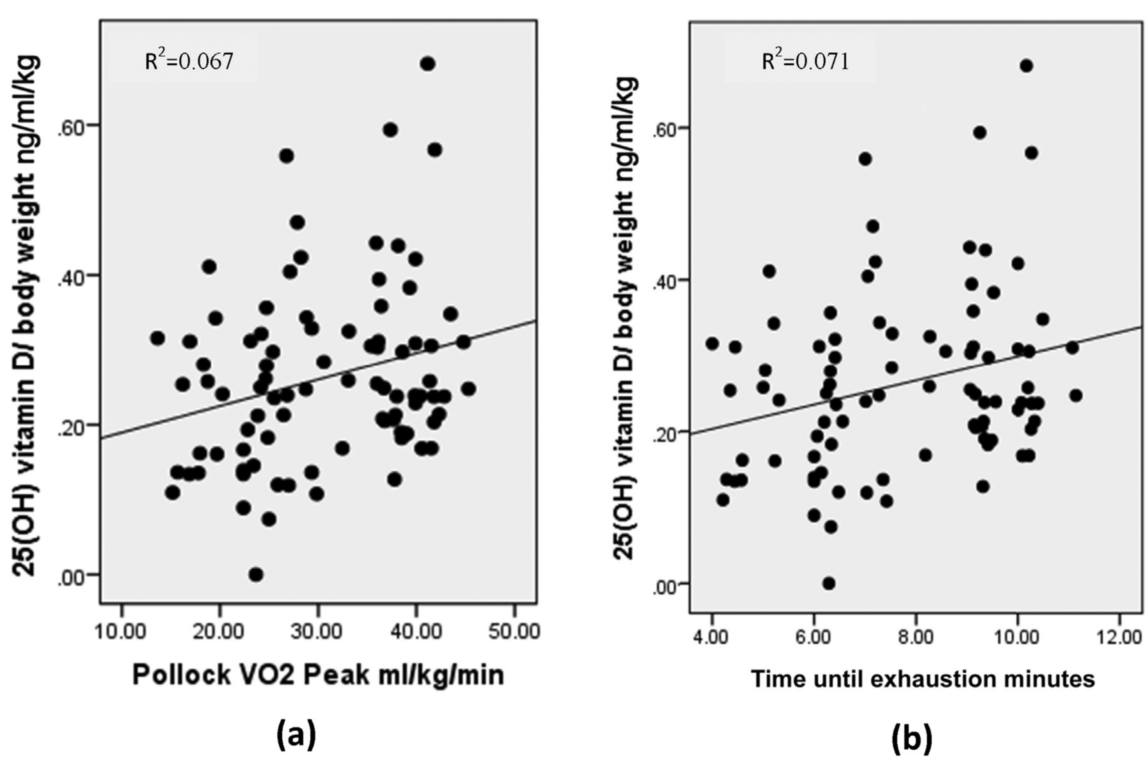

Figure 1: Association between cardiorespiratory fitness as represented by VO_2 peak and vitamin D level in young Saudi females. Linear regression between vitamin D level/body weight and VO_2 peak (a) and time until exhaustion (b).

activity on the vitamin D level using the structured questionnaire, none of these factors showed a correlation with the level of vitamin D or difference between categories of vitamin D levels.

In this study, the level of vitamin D in the blood was based on the plasma level of 25(OH)D. The latter is internationally considered as a reliable estimate of vitamin D status in the body. HPLC was the technique used in this study to estimate 25(OH)D. This technique is considered the best adopted method for measuring 25(OH)D, when compared with chemiluminescence immunoassay (CLIA) and radioimmunoassay (RIA), and yields the highest values.[11]

The current reported value of vitamin D deficiency in young Saudi females appears comparable to the previously reported values in Arab countries and KSA.[29,30] However, this value represents a profound warning for two reasons. First, the findings concern a young and educated female population, and second, these findings reflect a persistent high incidence of vitamin D deficiency in the Saudi population despite the cumulative efforts to increase awareness of this major health threat.

Data collected by the questionnaires failed to demonstrate a causative link to the reported level of vitamin D despite the detailed description of diet, sun exposure, and physical activity. Tangpricha et al. also failed to demonstrate an association between vitamin D level and milk intake in healthy young individuals.[31] Although we considered our questionnaire superior to that of Tangpricha et al. because we included other dairy product items and other vitamin D food sources, we could not show a correlation of vitamin D deficiency with any of these items. Davila et al.,[32] on the other hand, demonstrated a weak correlation with sun exposure, physical activity, and dietary intake of vitamin D-rich foods in medical residents in San Juan, Puerto Rico. The use of questionnaires in reporting dietary intake of vitamin D-rich food elements, sun exposure, and physical activity is vulnerable to overestimation and variability.[33] In addition, we believe that the relative homogeneousness of the questionnaire responses and the overall low intake of vitamin D-rich food, low sun exposure, and low physical activity in our sample were the main reasons for the lack of association between vitamin D and the parameters of the questionnaire.

The second main finding is the significant positive correlation between 25(OH)D level normalized to body weight and cardiorespiratory fitness represented by VO_2 peak and time to exhaustion, with no significant difference between the parameters used to describe exercise fitness including maximum heart rate (HR max), time to exhaustion, and Pollock VO_2 peak among the different classes of vitamin D deficiency. A similar association between vitamin D and VO_2 peak was shown in a study performed by Mowry et al.[34] on 59 young white females, in which the mean value of vitamin D was normal and sufficient (46.19 ng ml).[34] Another positive association between vitamin D and VO_2 peak obtained from a standard incremental exercise test was reported in the large study of Health in Pomerania (SHIP) of a combined 2127 male and female participants.[35] Ethnic differences in the expression of the association of vitamin D and VO_2 peak was reported in a study comparing African Americans and European Americans, where a positive correlation was shown only in the former group.[20] The simultaneous existence of low vitamin D and the association of vitamin D and cardiorespiratory fitness indicator (VO_2 peak) might help the health sector authority in orchestrating the best strategic solution for the growing incidence of vitamin D deficiency and reduced physical fitness.[36] These factors are strongly related and considered threatening risk factors for cardiometabolic disorders.[37,38]

In conclusion, the current study revealed a high incidence of vitamin D deficiency in young Saudi females in Dammam City. In addition, to the best of our knowledge, this report describes the first study in KSA to observe a significant positive correlation between vitamin D level as reliably measured by the metabolite 25(OH)D using the HPLC method and cardiorespiratory fitness expressed by VO_2 peak as estimated by the standard exercise stress test in vitamin D-deficient young Saudi females.

Further prospective studies are recommended to explore the underlying mechanisms of the positive correlation between vitamin D and cardiorespiratory fitness and to adopt these findings to establish new strategies for fighting two important risk factors in Saudi Arabian society—vitamin D deficiency and physical inactivity.

This study might be limited by its cross-sectional design and the relatively small sample size. However, the apparently small sample size might be considered adequate owing to the recruitment and focus on one gender. Another strong point of the current study is that it reflects the high incidence of vitamin D deficiency in young educated females, thus resolving the misconception that the aging population is the only group that is vulnerable to this condition. Other strong points of this study include its use of the HPLC method for vitamin D analysis (the gold standard for vitamin D estimation), its period of study in the best sun exposure season, and its inclusion of physical fitness assessment via the exercise stress test. Finally, a scoring system was applied in this study to estimate the effects of diet including vitamin D-rich foods other than milk and dairy products.

Conflict of interest

The author has no conflict of interest to declare.

Author's contribution

LIA is the sole author for this manuscript and was responsible for the design of the current study, data collection, analysis, and manuscript preparation.

Acknowledgment

The project was executed by grant number 2013183 provided by the Deanship of Scientific Research at University of Dammam, Dammam, KSA.

Appendix B. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jtumed.2016.07.002.

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How to cite this article: Al Asoom LI. Assessment of plasma level of 25(OH)D and its correlation with cardiorespiratory fitness in young females of Dammam City, KSA. J Taibah Univ Med Sc 2016;11(5):456–463.