



## A Retrospective Study of 25(OH)D Levels in Children of Different Age, Sex, and Nationality

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**Received:** August 17, 2021

**Published:** February 28, 2022

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### Abstract

**Background and Aims:** The prevalence of vitamin D deficiency—having serum 25-hydroxyvitamin D [25(OH)D] concentrations less than 75 nmol/L (30 ng/mL)—is a common phenomenon worldwide. Regardless of age, vitamin D deficiency is a serious health problem, especially in women and children. Despite plentiful of sunshine, D-deficiency is particularly a major health problem for residents of the Middle Eastern countries. The data presented in this paper are specifically of the juvenile population living in the United Arab Emirates (UAE)—that is, residents younger than 18 years from various countries.

**Methods:** Vitamin D estimation was done by chemiluminescence immunoassay, a quantitative immunoassay method used to determine total 25(OH)D in serum on a fully automated analyzer. Currently, the ideal methods for measuring 25(OH)D<sub>3</sub> are based on high-performance liquid chromatography (HPLC) or liquid chromatography with tandem mass spectrometry detection. This method simultaneously measures 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>. HPLC and mass spectrometry can differentiate between those two forms, yielding distinct results for each fraction.

**Results:** Vitamin D deficiency was highly prevalent among children aged 13-18 years living in the United Arab Emirates. Female sex and ethnicity were the variables most commonly associated with vitamin D deficiency. An inverse relationship exists between the level of 25(OH)D and age of juveniles. Among the study groups, UAE nationals have the lowest level of 25(OH)D. The severe deficiency (<25 nmol/L) was most prevalent in UAE national teenage girls aged 13-18 years (51.0%).

**Conclusion:** Among the age, sex, and nationality variables, age was the most important factor associated with serum 25(OH)D levels. Levels of serum 25(OH)D were inversely correlated with age in children. Severe vitamin D deficiency [25(OH)D < 25 nmol/L] was present in 9.5% of patients aged 1-3 years, 56.4% aged 7-9 years, and 79.9% aged 13-15 years. Therefore, vitamin D deficiency increased with age.

**Keywords:** Vitamin D Deficiency; Children; 25(OH)D; 1,25(OH)<sub>2</sub>D; Age and Sex; UAE

## Introduction

Vitamin D deficiency is common in healthy infants, children, and adolescents. Having prolonged winter seasons and restricted exposure to ultraviolet B (UVB) irradiation aggravates vitamin D deficiency [1], which is higher in people with dark skin, who live at high altitudes, and live farther from the equator. Similarly, deficiency is common in people who avoid sun exposure for various reasons [2]. Although vitamin D deficiency is a global problem, especially for people who live in northern and southern latitudes and high altitude, and among other high-risk groups, few actions have been taken to effectively address the issue [1].

Regardless of age, low vitamin D deficiency is a global problem, especially in women and children. Despite plentiful sunshine, deficiency is particularly a major problem for residents of the Middle Eastern countries [3]. Deficiency of vitamin D is higher in multi-ethnic children, those with increased skin pigmentation, and those who avoid sun exposure for any reason [4]. In addition, vitamin D deficiency is highly prevalent in obese children [5].

Vitamin D deficiency also is common among adolescents with eating disorders [6] and children with developmental disabilities [7]. The classical presentation of severe vitamin D deficiency (<25 nmol/L) is rickets in children and osteomalacia in adults; both are common problems in the Middle East and the Indian subcontinent [8]. In addition, vitamin D has a key role in muscle function in adolescent males [9]. Moreover, emerging evidence supports an association between 25-hydroxyvitamin D (25(OH)D) levels and immune function, respiratory diseases, obesity, metabolic syndrome, insulin resistance, infection, allergy, cancers, and cardiovascular diseases in pediatric and adolescent populations [10-15]. Randomized controlled trials have shown that vitamin D supplementation improves bone mineralization in adolescents. Those studies included trials that address the impact of vitamin D on muscle function [16] and maternal 25(OH)D levels inversely associated with maternal body mass index [17]. High body mass index and limited exposure to the sun increased the risk of developing vitamin D deficiency and its associated complications [18].

## Subjects, study design, and methods

This study's aim was to determine the prevalence of vitamin D deficiency among children (juveniles) of various ethnic groups living in the United Arab Emirates (UAE). Blood samples were ob-

tained for analysis of serum [25(OH)D] levels in children admitted to the Burjeel Hospital in Abu Dhabi, UAE, from October 2012 to September 2014. The study was envisaged to identify common factors affecting levels of 25(OH)D in blood. From the previous dataset, 60,979 patients reported differences in 25(OH)D concentrations in juvenile patients (19). For the present study, we recruited 7883 patients aged 1-18 years; both sexes were equally represented in the study group, with different nationality and six age groups. That group consists of patients, originating from 136 countries, living in the UAE at the time of sampling [19,20].

The measurement of circulating 25(OH)D is the best diagnostic test for determining a person's vitamin D status [21]. Assays used to measure 25(OH)D levels should be able to measure both vitamin D<sub>2</sub> (ergocalciferol) and vitamin D<sub>3</sub> (cholecalciferol) derivatives. Vitamin D estimation was done by chemiluminescence immunoassay, a quantitative immunoassay method used to determine total 25(OH)D in serum on a fully automated analyzer. Currently, the ideal methods for measuring 25(OH)D<sub>3</sub> are based on high-performance liquid chromatography (HPLC) or liquid chromatography with tandem mass spectrometry detection. This method simultaneously measures 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>. HPLC and mass spectrometry can differentiate between those two forms, yielding distinct results for each fraction [22]. In practice, however, automated immunoassays are the methods most commonly used by clinical laboratories [23,24]. Also, we did a comparative study of methods to measure 25(OH)D by a fully automated electrochemiluminescence assay from Roche Diagnostics (Mannheim, Germany) and an HPLC-based method from Chromsystems (Chromsystems Instruments and Chemicals GmbH, Heimbургstrasse, Munich, Germany) [25]. The Roche Diagnostics Total Vitamin D kit has 80% cross-reactivity to vitamin D<sub>2</sub> and 100% cross-reactivity to D<sub>3</sub>. The Roche assay was recently evaluated and its performance was deemed satisfactory in a population in which vitamin D<sub>3</sub> constituted the main form of total vitamin D [25,26]. The variability in analyzed groups of patients was calculated by the coefficient of variation. Correspondence analysis was used to find the structure in objects metric scale. That method gives easily interpreted ordinations [27].

We used a decision tree to determine the most important factor affecting level of serum 25(OH)D. The decision tree is a data mining method to sift through large databases in search of unknown patterns of interest. Decision trees are used to classify an object

or an instance into a predefined set of classes according to values such as age or sex [28]. For each group of patients, custom tables with adjusted residuals were calculated, from which sign schemes were created (as shown in Table 2). Those sign schemes show differences between measured frequencies among analyzed groups and expected frequencies.

**Data analysis**

Age is the most important predictor of serum 25(OH)D. The second-most-important predictor for juveniles aged 1-12 years is sex and for teenagers aged 12-18 years is nationality. Nationality has no role in the level of vitamin D for babies of either sex aged 1-3 years. Because the study has depicted the important predictors by a decision tree, we decided to do a comparative and correspon-

dence analysis by using multiple variables to give more accurate and productive results. The same included a comparative analysis between age and sex, age and nationality, and finally sex and nationality combination groups.

**Results**

**Analysis of serum 25(OH)D for age and sex**

The highest median level of vitamin D was found in the group of baby boys younger than 3 years (88.63 nmol/L), followed by girls younger than 3 years (83.5 nmol/L). Table 1 illustrates that the median level decreases with age for males among all analyzed age groups. The median serum 25(OH)D for females also tends to decrease with age, but the median of serum remains almost similar for ages 13-18 years.

Age and sex	Mean	95% CI for Mean		Median	SD	Min	Max	Coeff.of Variation
		Lower Bound	Upper Bound					
≤3 years old baby girl	86.3	84.0	88.6	83.5	30.8	7.5	175	35.7%
4-6 years old girl	62.4	60.6	64.1	59.5	24.2	7.5	175	38.9%
7-9 years old girl	46.6	45.2	48.0	43.9	18.6	9.7	123	39.9%
10-12 years old girl	36.9	35.4	38.5	32.6	19.7	9.1	128	53.4%
13-15 years old girl	31.6	29.9	33.3	24.6	23.2	7.5	172	73.6%
16-18 years old girl	33.6	32.0	35.2	24.7	26.2	7.5	175	77.8%
≤3 years old baby boy	88.9	86.8	90.9	88.6	29.2	7.5	175	32.8%
4-6 years old boy	65.1	63.4	66.7	61.2	24.7	7.5	174	38.0%
7-9 years old boy	53.9	52.2	55.6	51.2	21.4	9.9	174	39.8%
10-12 years old boy	46.3	44.6	48.0	43.1	19.8	10.0	172	42.9%
13-15 years old boy	41.3	39.3	43.2	37.1	21.0	7.5	131	50.8%
16-18 years old boy	40.2	38.2	42.3	34.9	22.9	7.5	165	56.9%
Age and nationality								
≤3 years old UAE	83.7	81.8	85.5	82.7	26.5	7.5	170	31.7%
4-6 years old UAE	62.6	61.0	64.2	59.3	23.3	7.5	175	37.2%
7-9 years old UAE	47.6	46.3	49.0	44.9	18.6	9.7	144	39.1%
10-12 years old UAE	37.7	36.4	38.9	36.0	17.0	9.1	127	45.0%
13-15 years old UAE	31.1	29.6	32.5	26.5	19.4	7.5	137	62.5%
16-18 years old UAE	32.3	30.8	33.9	25.2	24.1	7.5	175	74.5%
≤ 3 years old other nationality	92.3	89.8	94.7	91.9	32.9	7.5	175	35.7%
4-6 years old other nationality	65.1	63.2	67.0	61.1	25.8	7.5	174	39.6%

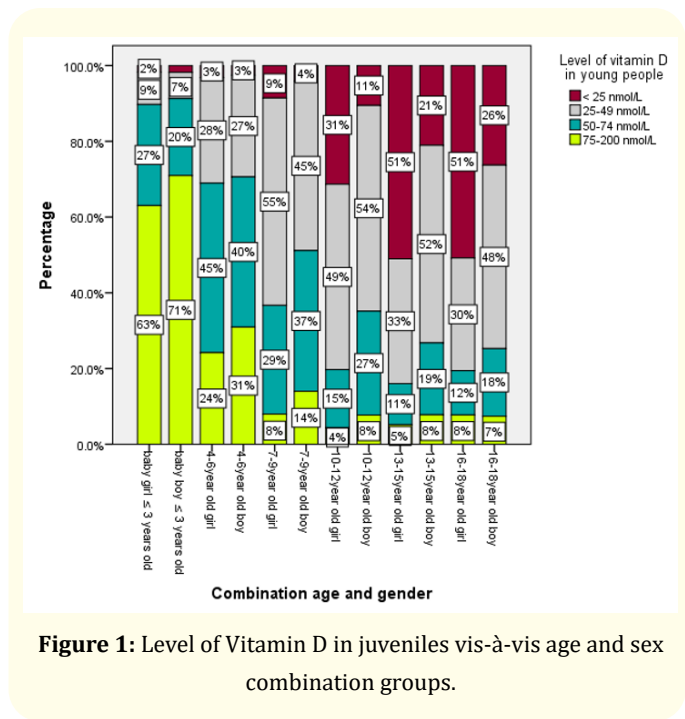
7-9 years old other nationality	53.3	51.4	55.1	50.8	21.94	9.9	174	41.2%
10-12 years old other nationality	46.4	44.3	48.5	41.4	23.4	9.7	172	50.4%
13-15 years old other nationality	41.7	39.3	44.1	34.9	26.1	7.5	171	62.6%
16-18 years old other nationality	41.5	39.3	43.7	35.5	26.3	7.5	165	63.3%
Nationality and sex								
UAE female	44.1	43.0	45.2	37.4	28.5	7.5	175	64.6%
UAE male	56.0	54.8	57.3	50.8	28.4	7.5	172	50.7%
Other nationality female	55.2	53.7	56.7	49.1	33.4	7.5	175	60.4%
Other nationality male	63.8	62.3	65.3	58.4	30.7	7.5	175	48.2%

**Table 1:** Differences in 25(OH)D (nmol/L) concentration in juveniles. 95% CI, 95% confidence interval; SD, standard deviation.

When the coefficient of variation is considered, it can be seen that the highest variability exists in girls aged 13-15 years, at 73.6%, and 16-18 years, at 77.8%. Variability is considered high when it exceeds the 50% threshold. The coefficient of variation is positively associated with both sexes with age; that is, it increases with age, but especially for girls it is generally higher, which means girls have higher variability of vitamin D among patients than boys. The high variability between patients is especially prominent in girls aged 10-18 years (53.4%-77.8%) and for boys aged 13-18 years (50.8%-56.9%) (Table 1).

Figure 1 illustrates that severe deficiency of vitamin D (<25 nmol/L) was most prevalent in the girls aged 13-15 years (51.0%) and then 16-18 years (50.8%). When sexes of the same age group of 13-18 years are compared, it can be seen that females have significantly higher vitamin D deficiency (51%) than males (21%-26%). Moderate vitamin D deficiency (25-49 nmol/L) is most frequent in girls aged 7-9 years (54.7%) and boys aged 10-12 years (54.3%). Optimum 25(OH)D (75-200 nmol/L) is more frequent in both girls (63.1%) and boys (71.0%) aged 1-3 years (Figure 1).

The sign scheme shows that severe deficiency was found significantly more than expected in girls aged 10-18 years and boys aged



**Figure 1:** Level of Vitamin D in juveniles vis-à-vis age and sex combination groups. 16-18 years (Table 2). Significantly fewer patients than expected with optimum vitamin D were found in patients aged 7-18 years (at 99.9% statistical significance; see Table 2).

		Severe Deficiency <25 nmol/L	Moderate Deficiency 25-49 nmol/L	Insufficiency 50-74 nmol/L	Optimum 75-200 nmol/L
Age and sex	≤3 years old baby girl	---	---	o	+++
	4-6 years old girl	---	---	+++	o
	7-9 years old girl	---	+++	+	---
	10-12 years old girl	+++	+++	---	---
	13-15 years old girl	+++	o	---	---
	16-18 years old girl	+++	--	---	---
Age and nationality	≤3 years old baby boy	---	---	--	+++
	4-6 years old boy	---	---	+++	+++
	7-9 years old boy	---	+++	+++	---
	10-12 years old boy	---	+++	o	---
	13-15 years old boy	o	+++	--	---
	16-18 years old boy	+++	+++	---	---
Age and nationality	≤3 years old UAE	---	---	o	+++
	4-6 years old UAE	---	---	+++	o
	7-9 years old UAE	---	+++	+++	---
	10-12 years old UAE	+++	+++	---	---
	13-15 years old UAE	+++	+++	---	---
	16-18 years old UAE	+++	o	---	---
Nationality and sex	≤3 years old Other nationality	---	---	---	+++
	4-6 years old Other nationality	---	---	+++	+++
	7-9 years old Other nationality	---	+++	+++	---
	10-12 years old Other nationality	o	+++	o	---
	13-15 years old Other nationality	+++	+	-	---
	16-18 years old Other nationality	+++	+	---	---
Nationality and sex	UAE female	+++	o	---	---
	UAE male	---	+++	+	+
	Other nationality female	o	-	o	+
	Other nationality male	---	o	+++	+++

**Table 2:** Vitamin D sign scheme vis-à-vis age, nationality, and sex combination groups.

o, no difference between measured frequency and expected frequency; +, measured frequency higher than expected frequency of measurement; -, measured frequency lower than expected frequency of measurement. One sign indicates 95% significance level, two signs indicates 99%, and three signs indicates 99.9%.

### Analysis of serum 25(OH)D for age and nationality

In a comparison study of babies younger than 3 years of other nationalities vis-à-vis UAE nationals, the highest median of vitamin D was found in babies of other nationalities at 91.87 nmol/L versus 82.72 nmol/L in UAE national babies. Table 1 shows that the median of serum 25(OH)D mostly decreases with age for UAE nationals, but the median of serum level for juveniles of other nationalities remains almost similar for ages 13-18. The coefficient of variation for UAE nationals is generally higher for juveniles aged 7-18 years; therefore, the variability of vitamin D is higher among UAE juveniles.

For both UAE nationals and other nationalities, variability increases with age. A higher coefficient of variation is found in juveniles aged 13-18 years: 62.5%-74.5% for UAE nationals and 62.6%-63.3% for other nationalities. The variability of serum 25(OH)D in both compared groups is almost the same for a group of patients aged 13-15 years; but for the age group of 16-18 years, it is visibly higher among UAE nationals (see Table 1). Severe deficiency of vitamin D is most frequent in UAE nationals aged 13-15 years (45.6%) and 16-18 years (49.5%).

Moderate deficiency of vitamin D in UAE nationals was found most often for 7-9 years (55.2%) and 10-12 years (53.8%). Optimum vitamin D is most frequent in babies aged 1-3 years old of other nationality (70.8%) and UAE nationals aged 1-3 years (64.1%). Severe deficiency is most seen in UAE nationals, whereas optimum is seen in both groups of nationalities in babies aged 1-3 years.

The sign scheme (Table 2) shows that significantly more patients than expected with severe deficiency are UAE nationals aged 10-18 years and other nationalities aged 13-18 years. However, significantly fewer patients than expected with optimum vitamin D are in juveniles aged 7-18 years of both nationalities (at 99.9% significance; see Table 2).

### Analysis of serum 25(OH)D for nationality and sex

According to Table 1, the highest median of vitamin D is found in males of other nationalities (58.4 nmol/L) and males of UAE (50.8 nmol/L). Generally, males have a higher median in both groups of nationalities. Females of other nationality (49.1 nmol/L) have a higher median of vitamin D than females of UAE nationals (37.4 nmol/L). High coefficient of variation was found in fe-

males for both groups of nationalities, but in UAE variability is higher among females (64.6%) than females of other nationalities (60.4%). Generally, as a result of the higher coefficient of variation in the group of UAE nationals, UAE nationals have higher variability between juveniles of both sexes than other nationalities. Further, because a higher coefficient of variation is present for females, we can say that they have higher variability of vitamin D than males.

The sign scheme (see Table 2) shows significantly more than expected juveniles with severe deficiency in UAE females and significantly more than expected juveniles with moderate deficiency in UAE males.

### Discussion and Conclusion

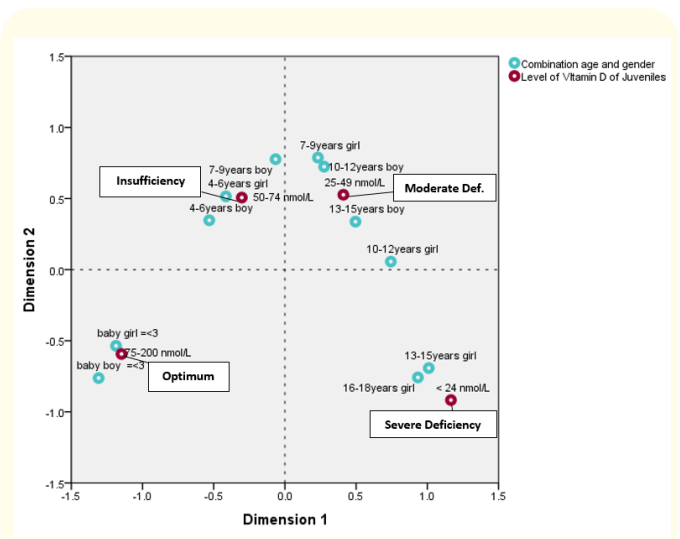
Most boys and girls aged 1-3 years had optimum serum 25(OH)D. By contrast, severe deficiency is the most common problem among the teenage girls aged 13-18 years. Females of that age group had lower 25(OH)D levels because of using abaya or burqa and their limited physical activity and sun exposure—that is, not spending time under the sun as boys do. Also, the lower rate of 25(OH)D-deficient children in our study group might be a result of their sampled age group and increased demand for vitamin D as a result of their accelerated growth. Therefore, it could be recommended for young girls to practice more outside activities. Previous results indicate that the most important predictor of vitamin D level, age, is correlated with 25(OH)D level (Spearman correlation coefficient  $r = 0.62$ ). In a study on children's vitamin D status reported from Korea, the mean serum level of 25(OH)D in 2880 children and adolescents was  $17.4 \pm 9.0$  ng/mL [29]. Another study reported prevalence of vitamin D deficiency in 1212 children, aged 4-15 years, of 58.6% [30]. Our results cannot be compared with those reports because we have divided our study subjects into six subgroups. However, similar results were reported by a Turkish study with children aged 11-18 years: Karagüzel and colleagues [31] used the cutoff value 20 ng/mL for deficiency and found the prevalence of vitamin D deficiency to be 93% during spring and 71% during autumn seasons, with an overall prevalence of 82%.

The moderate deficiency level is common in the age groups 4-6 for both sexes and for boys aged 7-9 years (Figure 1). For age and nationality variables, optimum serum 25(OH)D levels have been detected in those aged 1-3 years in all nationality groups. Babies aged 1-3 years get vitamin D either from supplemental vitamin D

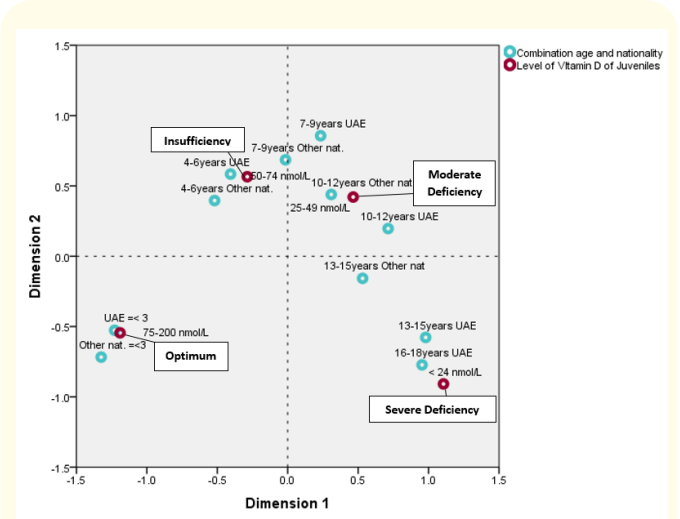
drops, available and popular in the UAE, or through breast-feeding. By contrast, severe deficiency of vitamin D is predominant in UAE teenagers, aged 13-18 years (Figure 3). Teenage girls in the Gulf countries start covering the body, leading to inadequate sun exposure. UVB rays from the sun are fragile and cannot penetrate clothes over the body, so no interaction occurs between UVB rays and 7-dehydrocholesterol (precursor of cholesterol) present in the skin. Therefore, no vitamin D production occurs. Because the weather in the UAE is sunny almost all days of the year, increased sun exposure happens during winter (low season) because temperatures are lower. People avoid sun in the summer (high season) because of excessive heat but increase their outdoor activities significantly in winter.

Our study showed the results of 7883 juveniles divided into six subgroups, offering insight to 25(OH)D levels in a narrow band of age. Most earlier studies, however, have been done either on teenagers or just one or two age ranges. In addition, the present data represent juveniles from 136 countries. Our study has clearly shown that children aged 1-3 years have optimum levels of 25(OH)D. The study does have some limitations. However, it is interesting to consider the seasonal variation of 25(OH)D levels in our studied groups, which is an important aspect. We are further analyzing the data in that respect and will submit a report for publication separately. Reports exist regarding the seasonal effect on variations in serum 25(OH)D levels according to differences in latitude. In the northern hemisphere, serum 25(OH)D levels are reported to gradually decrease from summer to winter [32-35]. According to Foo and colleagues [29], serum 25(OH)D levels of adolescent girls in Beijing, China (latitude 40°N), were 14.4 ng/mL when participating in sports and 12 ng/mL when not participating in sports. UAE is located at a latitude of 24°N and suffers as one of the most vitamin D-deficient countries in the world.

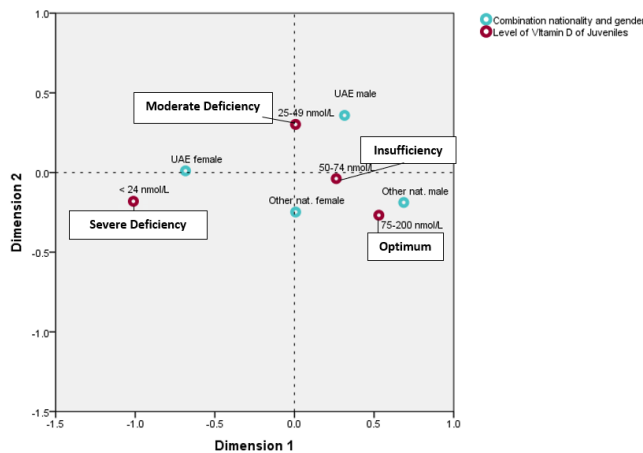
However, from the analysis for the combination of nationality and sex as well (see Figure 4), it can be seen that the problem of deficiency occurs mostly among UAE national juveniles of either sex, but UAE national females are very close to severe deficiency. By contrast, it can be seen that optimum level of vitamin D is more frequent in males of other nationalities, according to the correspondence analysis and two-dimensional biplot diagram (Figure 2).



**Figure 2:** Two-dimensional biplot diagram—symmetrical correspondence analysis for level of vitamin D in juveniles vis-à-vis different age and sex combination groups.



**Figure 3:** Two-dimensional biplot diagram—symmetrical correspondence analysis for level of vitamin D in juveniles vis-à-vis age and nationality combination groups.



**Figure 4:** Two-dimensional biplot diagram—symmetrical correspondence analysis for level of vitamin D in juveniles vis-à-vis nationality and sex combination groups.

Vitamin D status is a major determinant of bone health in infants, children, and adolescents, and supplementation should be considered. In 2011, the U.S. Institute of Medicine proposed a recommended dietary allowance of 400 and 600 IU/day of vitamin D for healthy infants younger than 1 year and for children aged 1-18 years, respectively [36]. Vitamin D deficiency is a preventable public health problem among children of different ages. Public awareness must be increased through health care professionals and by supplying vitamin D supplements that will certainly yield generations with healthy bone structure and growth. Sensible exposure to sunlight and considering time of the day, season, body weight, age, and skin complexion is highly recommended. Adolescents, owing to their habits (indoor activities, computer, mobile phone, social media, TV), are at increased risk of vitamin D deficiency.

### Funding Statement

The work was funded by an IGA research grant (project no. 20151035). We gratefully acknowledge support from the Faculty of Economic and Management, Czech University of Life Sciences Conflict of interest.

### Conflict of Interest

The authors have declared no conflicts of interest.

### Acknowledgments

The work was funded by an IGA research grant (project no. 20151035). We gratefully acknowledge support from the Faculty of Economic and Management, Czech University of Life Sciences.

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