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ORIGINAL ARTICLE

Risk factors of low vitamin D status in adolescent females in Kuwait: implications for high peak bone mass attainment

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Abstract
Summary Risks of low vitamin D status in Kuwaiti adolescent girls are high parathyroid hormone (PTH), high waist/hip ratio, veiling and not having a private room. Low vitamin D status is likely to have a negative impact on their bone mass and accrual.
Introduction Low serum 25-hydroxyvitamin D (25OHD) levels are repeatedly found in females in the Middle East, which is a cause for concern particularly for adolescent females. This is because vitamin D has been shown to promote bone mineral accrual in adolescence.
Purpose The aim of this study was to assess the risk factors of low vitamin D status in adolescent females and to assess its impact on their bone mass.
Methods Serum 25OHD and PTH were measured in 232 females. Anthropometric measurements and skin colour were obtained. Bone measurements at the lumbar spine were performed using dual-energy x-ray absorptiometry (DXA). Data on food intake, physical activity (PA) and sun exposure were taken. Binary logistic regression was used to assess the risk factors of serum 25OHD levels <25 nmol/L and multiple linear regression was used to assess the predictors of bone mineral variables.
Results Median 25OHD was 19.4 nmol/L (IQR 16.4–23.68), among which 98.7 % obtained <50 nmol/L. PTH >7 pmol/L (odds ratio (OR) 4.3; 95 % CI 1.8, 10.2), not having a private room (OR 3.7; 95 % CI 1.4, 9.8), veiling (OR 2.4; 95 % CI 1.1, 5.5) and waist/hip ratio >0.75 (OR 2.1; 95 % CI 1.0, 4.3) were risk factors of low vitamin D status, whereas, height, weight, month since menarche, PTH, animal protein intake and PA were independent predictors of bone mineral content ($p < 0.05$).
Conclusion Low vitamin D status is prevalent in Kuwaiti adolescent females, which may have a negative impact on their bone mineralization and accrual. Further investigation is needed to reveal the underlying causes.

Keywords Vitamin D · Kuwait · Bone · Adolescent girls · IDS

Introduction

The populations of the Middle East have shown relatively lower vitamin D status and higher rates of vitamin D deficiency [1], presumably caused by avoidance of sunlight. The risk factors of low vitamin D status and its impact on bone mineral status, however, have not been addressed thoroughly with particular interest in adolescent females in a country like Kuwait.

Kuwait is situated at the north-western corner of the Arabian Gulf (Latitude: 28.30–30.06º N). The population of Kuwait is distributed in six governorates, each of which provides relatively equal services including highly equipped local markets and public healthcare. The sun shines all year round, yet vitamin D deficiency among the population is common [2]. In our preliminary work, 80 % of adolescent

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females recruited in summer had serum 25-hyroxyvitamin D (25OHD) levels <25 nmol/L [3].

A number of factors have been associated with low vitamin D status among which ethnicity, season and obesity are a few. Studies have shown that Black, Hispanic and Asian individuals have a higher risk for low vitamin D status compared to White Caucasian individuals [4, 5]. Low Vitamin D status has also been shown to occur more frequently in winter and spring versus in summer and fall [4, 6]. Higher rates of vitamin D deficiency have been found in obese and overweight individuals compared to normal weight individuals [5], and rare or non milk consumers compared to regular milk consumers [6]. Further, dress type and the amount of skin area covered have also been associated with lower vitamin D status and higher rates of vitamin D deficiency [7, 8].

Adequate vitamin D status is known for its positive impact on bone health as studies continue to show the association between serum 25OHD and bone mineralization [9, 10]. A randomized clinical trial of vitamin D supplementation in healthy Lebanese girls showed significant beneficial effects on bone mineral content [11], whereas, a prospective study reported that vitamin D-deficient Finnish girls had significantly lower bone mineral density values at the lumbar spine [12].

Peak bone mass (PBM) is the highest level of bone mass achieved as a result of normal growth. The time of PBM attainment has been shown to be in late adolescence, provided that more than 90 % of the bone is laid by the age of 18 years [13]. PBM is a key determinant of the lifetime risk of osteoporosis [14]. Maximal PBM attainment is considered a preventive factor of post-menopausal osteoporosis, and thus, the adolescent period is a critical time for maximal bone mineralization. Failure to attain optimal peak bone mass can increase an individual's risk for osteoporotic fractures [15]. Low vitamin D status, thus, may limit PBM and bone mass accretion in young girls.

Further, a number of other health problems such as cancer, heart disease, diabetes and immune related disorders have also been linked to low vitamin D status [16]. In addition, low maternal vitamin D status has been associated with low birth weight and higher risk for developing a number of disorders in their newborns [17].

The Institute of Medicine has set ≥50 nmol/L of circulating 25OHD levels sufficient for good bone health in practically all individuals [18], whereas other recognized bodies prefer higher cut points. Serum levels <25 nmol/L are recognized as deficient [19], and levels falling in between the range 25 and 50 nmol/L are recognized as insufficient.

The aim of our study was to assess the risk factors associated with low vitamin D status among the Kuwaiti adolescent females and to assess its effect on their bone mass.

Methods

This was a cross-sectional study, in which 232 healthy females (10–18 years) were recruited from public schools from each governorate in Kuwait. A total of 122 females were recruited in summer and 110 in winter. Ethical approval to conduct the study was obtained from the Faculty of Medicine, Health Sciences Centre, Kuwait University. Permission to approach female schools was obtained from the Ministry of Education. The response rate was 24.4 % out of the initial number of students who received an invitation letter.

Subject recruitment

Invitation letters were distributed in a total of 15 female schools (both middle and high) located in all governorates. The invitation letter included information about the study, as well as questions designed to screen eligible participants. The students were asked to answer the questions at home with their parents. Those who were interested in participating in the study returned the letters back to school with their parents' signature and telephone numbers. The letters were then collected by the investigator.

Inclusion criteria

The participant was eligible if she was a Kuwaiti citizen or a Saudi citizen who has been resident in Kuwait since birth, aged 10–18 years, nulliparous, preferably Caucasian, clinically healthy without serious medical conditions or use of medication known to affect vitamin D status and bone metabolism and finally not taking any type of vitamin or mineral supplements for the past 6 months.

Data collection

The eligible students were recalled later on to visit the paediatric department in Al-Sabah hospital with at least one of their parents. Written consensus was given after fully understanding the student's role in the study. Accordingly, anthropometric measurements, skin colour assessment, fasting blood collection and bone densitometry of the lumbar spine were all carried out.

Interviewer-administered questionnaires, by a single investigator, were performed to collect data on puberty, dress type, sun exposure, residence, habitual food consumption and physical activity level.

Anthropometric measurements: this was determined by weight measured to the nearest 0.1 kg and height measured to the nearest centimetre using a stadiometer. Both weight and height were recorded with light clothing and without shoes. Body mass index (BMI) was calculated. Waist, hip and wrist circumferences were measured by a measuring tape and were recorded in centimetres. Waist to hip ratio was calculated. All measurements were taken by a single investigator. Chronological age was calculated as the years since birth on the examination date.

Skin colour: this was measured using the Fitzpatrick classification of skin photo-type [20] which classifies the skin according to its susceptibility to sunburn and tanning. People who frequently tan and rarely sunburn are more likely to have darker skin colour. Skin colour type is classified into (I) very fair tone skin that always burns and never tans, (II) fair tone skin that usually burns easily and tans with difficulty, (III) fair to medium skin tone that burns moderately and tans gradually, (IV) medium skin tone that rarely burns and always tans well, (V) olive or dark skin tone that very rarely burns and tans very easily, and (VI) very dark skin tone that never burns and is deeply pigmented. Assessment was performed by a single investigator. Skin colour types were grouped into the following: type II and III which represented a fair skin colour and type V and IV which represented a dark skin colour. Skin colour type I and VI did not occur in this data set.

Puberty assessment: this was performed by determining the time in months since menarche (MSM) on the day of examination. Tanner stage assessment was not applicable due to cultural reasons.

Dress type: this was used as a marker of the area exposed to direct sun light. This marker has been used extensively in the literature. Dress type was determined by the area of skin exposed, and female students were classified as either: (1) unveiled: exposing face, hands, feet, arms and legs, (2) partially veiled: exposing the face, hands and feet and (3) fully veiled: exposing only the hands. The student would be considered veiled if she was veiled for at least the past 6 months. Prior to regression analysis, dress type groups were recombined into two groups, unveiled and veiled.

Sun exposure time: this was measured by asking the student to record the frequency, duration and time of day she was exposed to direct sunlight during the last 2 weeks. This variable was categorized into spending less than 3 min/day, up to 10 min/day and more than 10 min/day of direct sun exposure. Prior to regression analysis, these groups were recombined into: up to 3 min/day and more than 3 min/day. Sunscreen application was reported to be negligible.

Socioeconomic status (SE): this was determined by asking whether the participant has her own room or if she shared her room with others. This question was found more reliable than asking about family income which was inaccurate in many cases. In addition, area of living (defined by the governorate) was reported. The six governorates were further grouped based on their geographical location into three groups (Table 1).

Food frequency questionnaire (FFQ): this was used to assess the habitual dietary intake and was set to determine food intake relative to vitamin D and bone health. Frequency

Table 1 Description of the Kuwaiti adolescent female sample presented in frequency (n=232)

Characteristic	Categories	(n)	Percent
Nationality	Kuwaiti	197	85.0
	Other	35	15.0
Governorate[a]	Asma, Hawali, Farwania	118	51.0
	Mubarak Al-Kabir, Ahmedi	50	21.6
	Jahara	64	27.5
SE marker	Have a private room	88	37.9
	Share room with one member	88	37.9
	Share room with>one	56	24.0
Sun exposure time	≤3 min/day	104	44.8
	≤10 min/day	66	28.4
	>10 min/day	62	26.7
Skin colour type[b]	II and III	82	35.2
	IV	112	48.3
	V	38	16.3
Dress type[c]	Fully veiled	47	20.2
	Partially veiled	133	57.3
	Unveiled	52	22.4
Physical activity	Inactive	197	84.9
	Low activity	13	5.6
	Moderately active	22	9.5
Milk intake	Occasionally[d]	72	31.0
	Weekly[e]	108	46.6
	Daily	56	22.4
Fish intake	Never	76	32.8
	Occasionally[d]	84	36.2
	Weekly[e]	72	31.0
Egg intake	Occasionally[d]	125	53.9
	Weekly[e]	82	35.3
	Daily	25	10.8
Meat intake[f]	Weekly[e]	113	48.7
	Daily	89	38.4
	Twice a day	30	12.9
Carbonated drinks	Occasionally[d]	36	15.5
	Weekly[e]	92	39.7
	Daily	104	44.8

[a] Asma, Hawali and Farwania are governorates located in the centre of Kuwait, Mubarak Al-Kabir and Ahmedi are located South Kuwait, Jahara is in the West

[b] Based on Fitzpatrick classification for skin colour-type [20]: II and III: fair to medium skin tone that usually/moderately burns and difficultly/gradually tans. IV: medium skin tone that rarely burns and always tans well. V:olive to dark skin tone that very rarely or never burns and easily/deeply tans

[c] Dress type; Fully veiled: clothing covering the whole body including face, but may expose hands. Partially veiled: clothing covering the whole body, except face, hands, and sometimes feet. Unveiled: clothing exposing face, hands, feet, arms, legs

[d] Occasionally: ≤once a week

[e] Weekly: three times a week

[f] Meat intake includes lamb, beef, chicken and fish

of consumption of good vitamin D sources such as milk and fish was reported. Frequency of consumption of other food items with special importance to bone health was also reported such as milk, sour-milk, yoghurt, cheese, cream, dairy ice-cream, fruits, vegetables, meat, eggs and carbonated drinks. Frequency of consumption was reported in this study as never, occasionally (<1/week), weekly (3/week), daily and twice a day. Selection of items in the questionnaire was based on common consumable items in Kuwait, frequency of consumption and relative importance as a vitamin D source and for bone health. The detailed results of this part will be reported in a separate article. In the multiple regression models, only associated food items with bone mineral content (BMC) and/or bone mineral density (BMD) were used as independent variables and these were milk, meat, eggs and carbonated drinks.

Physical activity (PA): all students were asked to report the frequency of their physical activity in minutes per week, and whether they participated in any sports at school or gym. The type of sport was reported to classify whether or not the activity was considered of weight-bearing type. Weight-bearing sports included walking, jogging, football, volleyball, basketball, karate and aerobics.

Biochemical analyses: fasting blood samples were drawn to measure serum 25OHD, parathyroid hormone (PTH) and adjusted serum calcium (adCa). The analysis of 25OHD was performed using a RIA kit (IDS Inc, USA), PTH was measured using the Immulite kit with the Immulite 1000 analyzer (Siemens Medical Solutions Diagnostics, USA), and adCa was measured using an automated chemistry analyzer (Dade Dimension RXL, Diamond Diagnostics, USA). Inter-assay and intra-assay CV% were 3.43 and 5.44 % for 25OHD, respectively, 3.43 and 2.59 % for PTH, respectively and 0.6 % for adCa. Biochemical analyses were carried out in Al-Sabah hospital. To verify our 25OHD results, 79 serum samples from the summer group were measured using the gold standard HPLC at the Supra-Regional Assay Services Centre (SAS), Manchester, UK, which is well known for their standardization protocol in measuring vitamin D metabolites in the UK.

Bone measurements: all students underwent bone mineral measurement at the lumbar spine (L1–L4) using dual-energy x-ray absorptiometry (DXA) (Hologic Delphi I, QDR 4500, USA) with Paediatric software (version 11.2:7). Bone area (BA, cm^2), BMC (g), BMD (g/cm^2) and BMD *z* score for the lumbar spine were measured. Precision CV% values for DXA were 1.0 % for BMD.

Statistics

The variables were reported in mean±SD and median and inter-quartile ranges (IQR). Non-parametric tests were used intensively in this dataset because most of the variables were not normally distributed (only bone variables and height were normally distributed). Spearman's correlation was used to detect associations between variables and between possible continuous predictors and serum 25OHD. Whereas Kruskal-Wallis test was used to assess the differences in serum 25OHD across groups of possible categorical predictors, Bland-Altman test [21], Cohen's kappa and Wilcoxon signed rank test were used to compare immunodiagnostic systems (IDS) and HPLC assays.

Binary logistic regression was used to identify the significant predictors of 25OHD with a 25 nmol/L cutoff. This divided serum 25OHD levels into <25 and ≥25 nmol/L. The cutoff was set so low because only 27 % of the samples had levels above 25 nmol/L, and three samples had levels above 50 nmol/L.

Parametric tests were applied to the bone variables as they were normally distributed. Partial correlation was used to assess various associations with BMC and BMD controlling for the following confounders: MSM, height and weight. One way analysis of variance (ANOVA) was performed to compare BMC and BMD across the categories of variables. When a significant difference was detected, Bonferroni test was used to detect which categories were significantly different. The potential predictors were log transformed to obtain approximate normal distribution and then entered as independent variables in the multiple linear regression models. Only height was normally distributed and thus was not log transformed. Data was analyzed using SPSS software, version 17.0 (SPSS Inc, Chicago, USA).

Results

Characteristics of the sample

Mean age was 15.37±1.67 years and puberty had occurred among 96 % of the girls. Mean age at menarche was 12.11± 1.25 years, while height was 159±6.3 cm and weight was 60.6±15.5 kg (Table 2).

Compared to the 2000 CDC values [22], our girls were found slightly shorter occurring in the 25th–50th percentile of height. The exception was in age group 10, 11 to 12 (50th–75th) and 18 (10th–25th). However, the percentage of girls falling in these age groups was 8 % all together. Further, our girls were found heavier in all age groups occurring mostly in the 50th–75th percentile. Girls in age groups 10 to 12, 16 and 18 (29 % of the total) had a higher BMI percentile (75th–97th).

Around 58.4 % had normal weight, while 20.2 % were overweight and 18 % were obese based on the BMI classification by Must [23]. The highest prevalence of normal weight was found among the 13.00–14.49 age group, while the highest percentage of overweight and obesity was found in

Table 2 Clinical characteristics of the Kuwaiti adolescent females in each group and in total

Variables	Summer group[a] (n=122)				Winter group[b] (n=110)				Total (n=232)			
	Mean±SD	Median	25th	75th	Mean±SD	Median	25th	75th	Mean±SD	Median	25th	75th
Age (yrs)	14.99±1.83	14.83	13.67	16.44	15.80±1.37	15.88	15.00	16.54	15.37±1.67	15.58	14.19	16.48
Month since menarche (MSM)	2.77±2.10	2.38	1.17	4.19	3.83±1.79	3.75	2.58	5.08	3.27±2.03	3.42	1.77	4.83
Height (cm)	159.24±6.70	158.50	154.50	164.50	158.97±5.84	159.00	154.25	163.30	159.11±6.29	159.00	154.50	164.00
Weight (kg)	60.65±15.82	56.50	49.00	72.00	60.52±15.13	57.50	49.75	67.55	60.59±15.46	56.50	49.05	69.88
Body mass index (BMI) (kg/m^2)	23.78±5.55	21.85	20.02	27.24	23.81±5.05	22.98	20.02	27.11	23.80±5.31	22.47	20.02	27.17
Waist/hip (w/h) ratio	0.77±0.06	0.76	0.73	0.81	0.76±0.05	0.76	0.73	0.79	0.77±0.05	0.76	0.73	0.81
Vitamin D (25OHD) (nmol/L)	22.69±8.40	20.04	16.88	26.15	20.11±5.76	18.95	16.03	22.33	21.47±7.37	19.40	16.40	23.68
Parathyroid hormone (PTH) (pmol/L)	9.32±10.24	6.80	4.88	9.54	9.11±10.84	6.15	4.28	8.93	9.22±10.51	6.60	4.70	9.20
Adjusted calcium (adCa) (mmol/L)	2.26±0.09	2.26	2.21	2.31	2.34±0.11	2.37	2.30	2.41	2.30±0.11	2.30	2.24	2.38
Bone area (BA) (cm^2)	51.88±5.32	51.76	48.23	55.65	53.78±5.08	54.29	50.57	57.37	52.78±5.29	52.78	49.02	56.34
Bone mineral content (BMC) (g)	46.75±9.34	47.1	40	53.2	48.41±9.77	48.13	41.94	53.38	47.54±9.67	47.69	41.32	53.28
Bone mineral density (BMD) (g/cm^2)	0.89±0.81	0.91	0.81	0.99	0.89±0.12	0.9	0.81	0.96	0.89±0.12	0.90	0.81	0.98
Z score bone mineral density	−0.125±1.11	0.05	−0.9	0.7	−0.464±1.157	−0.5	−1.3	−0.3	−0.29±1.15	−0.30	−1.00	0.50

[a] Summer group recruited between June–August in year 2005 and 2006

[b] Winter group recruited between January–February in 2007

the 17.50–18.99 age group. A positive correlation was found between age and BMI (r= 0.2, p= 0.002).

Associations between 25OHD

The winter group obtained a lower median 25OHD than the summer group (Table 2). Significant associations were found between serum 25OHD and PTH (r=−0.476, p< 0.001) and between 25OHD and adCa (r= 0.22, p< 0.001). However, partial correlation, controlling for the confounders PTH, dress type, SE marker, w/h ratio, season and milk intake, revealed a smaller association between serum 25OHD and PTH (r= −0.244, p< 0.001) and adCa (r= 0.133, p< 0.05). There was no significant association between 25OHD and BMI, age, MSM or BMC before and after controlling for the confounders.

Serum 25OHD levels did not differ significantly between those who were exposed to the sun for up to 3 min/day and those who were exposed for a longer period (Table 3). Significant differences were not found in 25OHD levels between Kuwaiti and non-Kuwaiti girls, or between girls living in different governorates. Further, no significant difference in serum 25OHD levels between different skin colour types was evident.

On the other hand, serum 25OHD was significantly higher in the unveiled girls compared to the partially veiled girls, but not to the fully veiled, and was also significantly higher in the females who consumed milk on a daily basis compared to those who rarely consumed milk. Fish consumption did not show a clear association with 25OHD levels.

There was a significant difference in serum 25OHD between the summer and winter groups only among the unveiled girls (p< 0.05) as the summer group obtained a mean of 25.9± 9.2 nmol/L and the winter group obtained a mean of 20± 6.6 nmol/L. The veiled girls in both the summer and winter groups were not significantly different in their 25OHD levels.

Binary logistic regression analysis showed that PTH, SE marker, dress type and w/h ratio were significant risk factors of serum 25OHD, whereas season and milk intake showed a trend in significance (Table 3). The significant risk factors and those that showed a trend were entered as confounders and covariates in partial correlations and ANCOVA, respectively.

Table 3 Risk factors of low vitamin D status (25OHD <25 nmol/L) in Kuwaiti adolescent females (*n*=232)

Risk factor[a]	Odds ratio	95 % CI:		*p* value
		Lower	Upper	
SE marker				**0.013**
(Private room vs. sharing room with one)	**3.727**	**1.418**	**9.796**	**0.008**
(Private room vs. sharing with more than one)	0.999	0.365	2.730	0.998
Governorate:				0.522
(Asma, Hawalli, Farwania vs. Mubarak Al-kabir and Ahmedi)	1.788	0.595	5.369	0.300
(Asma, Hawalli, Farwania vs. Jahara)	0.920	0.346	2.445	0.868
Summer vs. winter	1.984	0.884	4.455	0.097
Waist/hip ratio (≤0.75 vs. >0.75)	**2.097**	**1.002**	**4.387**	**0.049**
Skin colour type (fair vs. dark)	0.650	0.294	1.438	0.288
Sun exposure time (>3 min/day vs. less)	0.944	0.444	2.009	0.881
Dress type (unveiled vs. veiled)	**2.401**	**1.056**	**5.459**	**0.037**
PTH (<7 pmol/L vs. more)	**4.259**	**1.781**	**10.189**	**0.001**
Milk intake:				0.093
(Daily vs. occasionally)	**3.302**	**1.100**	**9.908**	**0.033**
(Daily vs. weekly)	1.503	0.617	3.660	0.370
Fish intake:				0.439
(Weekly vs. never)	1.680	0.669	4.221	0.270
(Weekly vs. occasionally)	1.662	0.684	4.038	0.262

Bold indicates significance at *p*< *0.05*

[a] Binary logistic regression model applied

Vitamin D assay verification

Mean 25OHD of 79 serum samples measured by HPLC was 20.7±9.8 nmol/L compared to 22.03±7.22 nmol/L using our IDS kit. Serum 25OHD$_2$ was measured separately using HPLC and was found to be negligible (mean±SD 1.06± 1.32 nmol/L).

Cohen's kappa test showed that there was up to 0.31 agreement between the assays and Wilcoxon signed rank test revealed a significant difference between the two ($p< 0.05$). The Bland and Altman plot (Fig. 1) showed that values <20 nmol/L were likely to be overestimated by IDS, while values >20 nmol/L were likely to be underestimated. IDS was constantly underestimating 25OHD with values >28 nmol/L. In total, IDS overestimated 65 % of the values. Only 3.8 % of the values by IDS fell below 14 nmol/L compared to 29 % of those by HPLC. Also, 30.4 % of the values in the lowest quartile of HPLC were not recognized as being among the

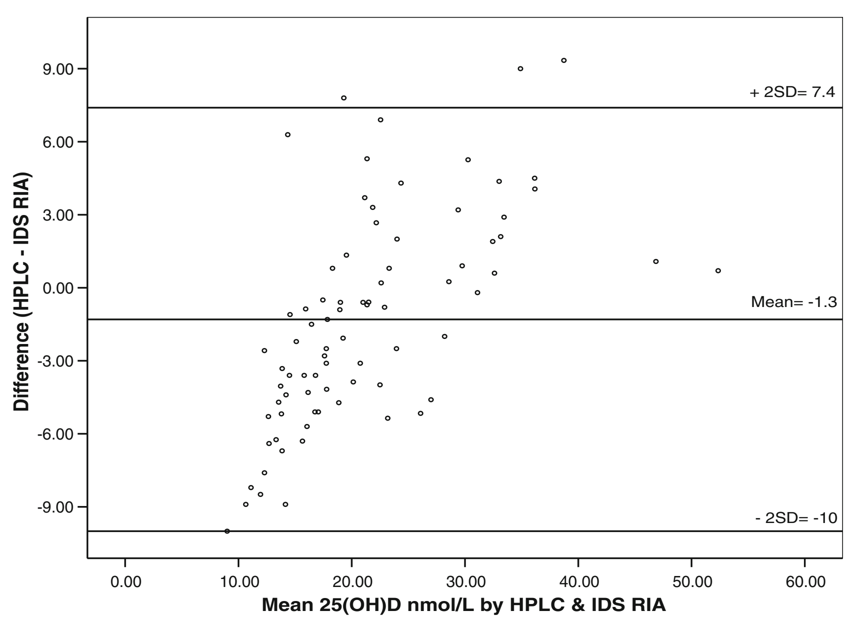

Fig. 1 Bland and Altman plot showing the level of agreement between 25OHD values using HPLC and IDS RIA (*n*=79). The limits of agreement are shown by the +2SD and −2SD. The mean difference was below zero (−1.3 nmol/L). This indicates that IDS RIA tends to overestimate 25OHD with lower values

lowest quartile of IDS. In 95 % of the cases, overestimation occurred by up to 10 nmol/L and underestimation occurred by up to 7.4 nmol/L.

BMC and BMD associations and potential predictors

Mean BMC was 47.54±9.67 g, whereas, mean BMD was 0.89±0.12 g/c^2 (Table 2). After controlling for height, weight and MSM, significant associations were found between BMC and serum 25OHD, PTH and adCa ($r=0.136$, $p<0.05$, $r=-0.266$, $p<0.001$ and $r=0.134$, $p<0.05$, respectively), and between BMC and weight, wrist, waist and hip circumferences ($r=0.315$, $p<0.001$, $r=0.272$, $p<0.001$, $r=0.212$, $p<0.001$, and $r=0.272$, $p<0.001$, respectively). No association was found with age ($p>0.05$). No significant difference in mean BMC between the summer and winter groups was found ($p>0.05$). Associations between BMD and serum 25OHD, PTH and adCa were ($r=0.178$, $p<0.01$, $r=-0.29$, $p<0.001$ and $r=0.113$, $p=0.083$, respectively) controlling for height, weight and MSM.

Further, 85 % were physically inactive, having no participation in weight-bearing activity or any type of exercise (Table 1). Mean BMC of this group was 46.78±9.73 g compared to 54.1±6.74 g obtained by the moderately active group (9.5 %) showing a significant difference ($p<0.01$).

Using a multiple linear regression model with the independent variables height, weight, w/h ratio, wrist circumference, MSM, 25OHD, PTH, adCa, milk intake, meat intake, egg intake, carbonated drinks intake and PA, only height, weight, MSM, PTH, meat intake and PA were significant predictors for BMC. They contributed to 49.7 % of its variation (Table 4).

Discussion

Extremely low vitamin D status

Despite ample sunshine year-round, the highest serum 25OHD level obtained was 61 nmol/L. The females were recruited from all around the country, and thus, it is likely to expect that the low 25OHD levels are common among the adolescent female population in Kuwait.

Low 25OHD levels have been reported from many parts of the Middle East such as Lebanon, Jordan and Iran, with much lower levels found in populations of the Arabian Peninsula [1]. In a recent study, 81 % of Saudi adolescent females had levels <25 nmol/L [24], and 51.4 % of Qatari adolescent females were reported to be vitamin D deficient [25].

Earlier studies in Kuwait have shown similar results in women in which mean 25OHD levels were reported to be <16 nmol/L [2] and 86 % of the women in an early study obtained levels <25 nmol/L [7].

Although 25OHD levels ≥50 nmol/L has been set for the general population to support good bone health [18], further studies are needed to determine whether children and adolescents need a different cutoff.

Risk factors of low vitamin D status

The reported time of sun exposure was subject to inaccuracy and overestimation. It was found common among the females to have a sun-avoiding attitude for cosmetic reasons. However, none had reported the use of sunscreen. Whereas, it was shown in a previous study that the use of sunscreen among adults in Kuwait is prevalent [26].

Temperatures are extremely high during the months of June–August, and are tolerable during the rest of the year. However, most of the daily activities are preferred to be carried out indoors to avoid the hot breeze, direct sunlight and dust. It is mainly in spring when people tend to increase outdoor activities such as camping in the desert. Private cars are the main transportation for families in Kuwait. Walking is seldom practiced as a method of transportation.

The literature shows that fair coloured skin produces more serum 25OHD levels when exposed to direct sunlight [27]. However, Kruskall-Wallis test revealed a significantly higher serum 25OHD mean in the darker group (type V) compared to the fairer groups (type II, III and IV), which disagrees with the general finding.

A logical explanation would be that dark skin might indicate prolonged sun exposure, and this might partly explain why girls of type V obtained significantly higher levels. Sixty one percent of them spent up to 20 min/day under direct sun exposure compared to 54 % of the girls with fairer types (II, III, IV). Also, 40 % of them were unveiled compared to only 19 % of types II, III, IV.

Fewer residences, nowadays, have an open area exposed to direct sunlight. This is because small and young families are living in apartments, whereas wealthy families can afford big houses with open areas and many may afford a beach house as well to spend the weekends and holidays.

Thus, a simple way of determining SE status was by asking whether the participant had a private room, which would likely indicate a higher SE status, and perhaps, owning a bigger house and/or a beach house. We have found that the girls who had a private room had significantly higher 25OHD levels probably due to more frequent exposure time under direct sunlight.

Schools, therefore, might be one of the few places where the majority of female students, especially veiled females, can spend enough time under sunlight. It is unfortunate, however, that although schools are designed to have an open ground for daily assembly, most tend to cover it.

Obesity is another risk factor for a low vitamin D status and has been associated in many studies with lower 25OHD levels

Table 4 Predictors of Lumbar spine bone indices in Kuwaiti adolescent females (n=232)

Dependent variable	LS-BMC				LS-BA				LS-BMD				LS-BMD *z* score			
Predictors	Beta[a]	*p* value	95 % CI		Beta[a]	*p* value	95 % CI		Beta[a]	*p* value	95 % CI		Beta[a]	*p* value	95 % CI	
			Lower	Upper			Lower	Upper			Lower	Upper			Lower	Upper
MSM[b]	**0.206**	**0.000**	3.074	8.983	**0.242**	**0.000**	2.117	5.526	**0.137**	**0.013**	0.012	0.096	**−0.140**	**0.018**	−0.950	−0.090
Height (cm)	**0.317**	**0.000**	0.313	0.664	**0.573**	**0.000**	0.376	0.578	0.052	0.399	−0.001	0.004	0.021	0.752	−0.021	0.030
Weight (kg)[b]	**0.254**	**0.005**	7.024	37.848	**−0.290**	**0.002**	−22.725	−4.942	**0.553**	**0.000**	0.434	0.876	**0.510**	**0.000**	3.477	7.962
Wrist (cm)[b]	0.145	0.084	−6.349	99.559	**0.280**	**0.002**	18.042	79.144	0.015	0.863	−0.692	0.825	0.077	0.422	−4.558	10.853
W/H ratio[b]	−0.064	0.253	−54.583	14.465	0.011	0.857	−18.089	21.746	−0.102	0.090	−0.922	0.067	−0.096	0.136	−8.841	1.207
Serum 25OHD (nmol/L)[b]	0.015	0.784	−6.633	8.776	−0.074	0.212	−7.270	1.620	0.074	0.215	−0.041	0.180	0.075	0.240	−0.452	1.790
Serum PTH (pmol/L)[b]	**−0.160**	**0.010**	−7.971	−1.073	**−0.153**	**0.022**	−4.318	−0.338	−0.123	0.064	−0.096	0.003	−0.136	0.057	−0.989	0.015
Serum adCa (mmol/L)[b]	0.026	0.651	−36.054	57.528	0.005	0.936	−25.897	28.093	0.021	0.729	−0.552	0.788	0.034	0.604	−5.015	8.602
Physical activity (min/week)	**0.102**	**0.045**	0.000	0.033	0.100	0.066	−0.001	0.018	0.071	0.193	0.000	0.000	0.072	0.218	−0.001	0.004
Egg intake	0.086	0.095	−0.428	5.321	**0.115**	**0.038**	0.101	3.418	0.046	0.407	−0.024	0.059	−0.020	0.740	−0.489	0.348
Milk intake	0.022	0.670	−1.007	1.564	−0.021	0.709	−0.882	0.601	0.025	0.652	−0.014	0.023	0.058	0.335	−0.095	0.279
Meat intake	**−0.114**	**0.029**	−2.852	−0.159	−0.065	0.243	−1.238	0.316	−0.108	0.052	−0.038	0.000	−0.058	0.330	−0.293	0.099
Carbonated drinks intake	0.044	0.386	−0.699	1.800	0.041	0.446	−0.442	1.000	0.018	0.738	−0.015	0.021	0.018	0.757	−0.153	0.210
Adjusted R^2	**0.497**				**0.424**				**0.425**				**0.339**			

Bold indicates significance at $p < 0.05$

[a] Standardized beta

[b] Log transformed

[5, 28]. We have found that females who had a waist/hip ratio of more than 0.75 were twice as likely to have 25OHD levels <25 nmol/L. With the rise in obesity in the population [29], low vitamin D status in Kuwait would be very common.

Veiling as a risk factor of low vitamin D status has been reported previously in Kuwait [7] and in other regions [8]. The veiled females, in this data set, had a relatively lower 25OHD level compared to the unveiled females and they were twice as likely to obtain 25OHD levels <25 nmol/L compared to the unveiled females.

Further, we have found that the partially veiled females had lower 25OHD levels compared to the fully veiled females. This was unexpected because the fully veiled females generally exposed less skin area, they did not report spending more time under the sun and did not report consuming more milk or fish. However, their skin was relatively fairer. Only 8.5 % of them were dark (V and VI) compared to 14.3 % of the partially veiled group and 29 % of the unveiled group.

Milk consumption has been previously associated with higher levels of serum 25OHD [4]. We have found that rare consumption of milk increases the risk of 25OHD levels <25 nmol/L three times higher than those who consume milk on a daily basis. The vast majority of milk brands available in Kuwait claim to be fortified with 400 IU/L vitamin D, and this perhaps has contributed to the variation in serum 25OHD levels.

Serum 25OHD levels have shown to fall dramatically in winter [30], due to the decrease in UV-B. In Kuwait, however, the decrease in UV-B from sun rays is not significant and sun light continues to shine all year round [31].

Interestingly, the fluctuation in 25OHD levels between the seasonal groups was mostly found among the unveiled girls, perhaps because those girls wear lighter clothing in summer and heavy clothing in winter, resembling their veiled peers.

IDS RIA verification

We found that the IDS RIA method overestimated 25OHD levels <28 nmol/L. This finding would suggest that the percentage of females in our study with low serum 25OHD levels is greater than that found. RIA has previously shown a similar trend [32]. With very low 25OHD values being common in Kuwait, IDS assay would, therefore, frequently overestimate the values. Further, IDS is unable to detect $25OHD_2$ with the same efficiency as $25OHD_3$ [33]. This test, thus, would be unreliable if the population depends on vitamin D supplementation from plant sources. In Kuwait, cholecalciferol (vitamin D_3) remains the main source for vitamin D fortification and supplementation, and our results showed negligible serum $25OHD_2$ levels.

However, despite its tendency to overestimate 25OHD levels, the IDS method agrees overall with HPLC when classifying values <25, 25–50 or ≥50 nmol/L. Using HPLC methodology, 74.4 % were <25 nmol/L and 97.6 % were between 25 and 50 nmol/L, whereas using the IDS method, 72.5 % were <26 nmol/L and 97.6 % were between 25 and 50 nmol/L, respectively. Only nine samples were misplaced.

Bone status among the Kuwaiti adolescent girls

Compared to the Canadian normative data for paediatrics, mean *z* score for BMD were found to be low. The values are also lower than their Lebanese counterparts [34]. An earlier study had shown that the spinal and femoral BMD values of 623 healthy Kuwaiti women were not significantly different from the normative Caucasian (US/North European) female reference data used, and that the values were higher than the Arab female reference data (Lebanese/Saudi) [35].

However, a more recent study found that the spinal and femoral BMD of 805 Kuwaiti women were significantly lower than their US counterparts, except for the femoral BMD at ages 40 through 55 years where the Kuwaiti values significantly exceeded [36]. The Kuwaiti BMD values were found significantly higher than their Lebanese counterparts and comparative to their Saudi and Qatari counterparts [37].

Given that most of the bone mass of the proximal femur and vertebral body is accumulated by late adolescence [13], the relatively low values found in the females might increase their risk of developing osteoporosis even earlier than their mothers.

Dietary and physical impact on BMC

Generally, bone mass has been associated with certain foods, such as dairy, protein, carbonated drinks, vegetables and fruit consumption. However, studies have shown controversial and inconsistent results. For example, some studies have shown that the risk of hip fracture is reduced by an increased consumption of animal protein [38]; others have found that high animal protein intake is associated with lower BMD [39]. Animal protein intake, in our results, was negatively associated with BMC and had predicted 11 % of its variation. Meat consumption is very common among the population, and teenagers frequently depend on fast food restaurants for their meat-dense meals. Thus, it is worth assessing the meat intake-bone mass relation further to determine the possible effect of meat intake on PBM.

Milk, vegetable and fruit consumption have been positively associated with BMC and BMD. In this study, however, we found no significant association.

Physical activity has shown to be a promising way to increase bone mass in children [40]. Adolescent female athletes and gymnasts have shown a significant increase in BMC and BMD values in various skeletal sites and, accordingly, greater peak bone mass attainment [41]. In our study, physical activity was positively associated with BMC and predicted

10.2 % of its variation. The fact that 85 % of the girls had not participated in any form of sports or regular exercise was likely to contribute to their low BMC values.

The PTH, 25OHD and bone connection

Adolescent females who had serum PTH ≥7 pmol/L were four times more likely to have 25OHD <25 nmol/L. The significant inverse association between PTH and 25OHD is in concordance with the current literature [42]. The association between PTH and bone indices, however, is not consistent. Whereas some studies have shown that elevated serum PTH levels result in significantly lower BMD values [43], others have found no association [44]. In a study on elderly men and women from the UK and China, a significant association between PTH and hip BMC was found in the UK group, but not in the Chinese group despite the latter having significantly lower 25OHD and higher PTH values [44]. No significant difference in rates of bone loss was found between African American women who had 25OHD levels below and above 40 nmol/L [45].

Thus, the relation between PTH and bone mass is more likely influenced by a number of factors including ethnicity. Similarly, the relation between 25OHD and bone indices may be subject to confounders and is not consistently demonstrated in studies. In our study, serum 25OHD had no direct association with BMC or any of the bone indices, whereas PTH was an independent predictor of BMC. This demonstrates the impact of PTH on bone mass, and thus, the impact of 25OHD on bone health. The fundamental, well recognized, role of vitamin D is to increase intestinal calcium absorption, which, in turn, increases serum calcium for its physiological functions including depositing in the bone matrix. PTH works synergistically with 25OHD and calcium in the body and plays an important role in the regulation of both serum levels of calcium and phosphate, in addition to its role in activating 25OHD into 1,25OHD in the kidneys. When either calcium or 25OHD is insufficient, PTH acts by stimulating the process of dissolving the bone matrix to liberate minerals, hence, increasing serum calcium as needed. Thus, insufficient 25OHD and/or calcium levels are associated with an increase in PTH.

Conclusion

Low vitamin D status is prevalent in Kuwaiti adolescent girls in whom 78.4 % obtained levels <25 nmol/L. Risk factors for low vitamin D status were serum PTH level >7 pmol/L, not having a private room, rarely consuming milk, being veiled and having a waist/hip ratio of >0.75.

Maintaining a normal serum PTH level, an adequate 25OHD level, regular exercise, in addition to a healthy diet are essential factors to increase PBM.

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Conflicts of interest Susan Lanham-New is Co-Director of D3Tex Ltd which holds the UK Patent (GCC pending) on the application of UV-B transparent material for prevention of vitamin D deficiency in women who dress for cultural style. Khulood Alyahya, Warren Lee, Zaidan Al-Mazeedi and Jane Morgan declare no conflict of interest.

References

1. Bassil D, Rahme M, Hoteit M, El-Hajj Fuleihan G (2013) Hypovitaminosis D in the Middle East and North Africa. Dermatol Endocrinol 5(2):274–298
2. Molla A, Al Badawi M, Hammoud M, Shukkur M, Thalib L, Eliwa M (2005) Vitamin D status of mothers and their neonates in Kuwait. Pediatr Int 47(6):649–652
3. Alyahya K, Almazidi Z, Morgan J, Lee W, Berry J, Lanham-New SA (2009) Extensive vitamin D deficiency among the Kuwaiti adolescent females during the summer period: effects of lifestyle habits and implications for PBM attainment. In: 7th International symposium on nutritional aspects of osteoporosis, Tyne & Wear, Lauzanne, Switzerland, p 73
4. Gordon C, DePeter K, Feldman H, Grace E, Emans S (2004) Prevalence of vitamin D deficiency among healthy adolescents. Arch Pediatr Adolesc Med 158(6):531
5. Elizondo-Montemayor L, Ugalde-Casas PA, Serrano-Gonzalez M, Cuello-Garcia CA, Borbolla-Escoboza JR (2010) Serum 25-hydroxyvitamin D concentration, life factors and obesity in Mexican children. Obesity (Silver Spring) 18(9):1805–1811
6. Weng FL, Shults J, Leonard MB, Stallings VA, Zemel BS (2007) Risk factors for low serum 25-hydroxyvitamin D concentrations in otherwise healthy children and adolescents. Am J Clin Nutr 86(1):150–158
7. El-Sonbaty M, Abdul-Ghaffar N (1996) Vitamin D deficiency in veiled Kuwaiti women. Eur J Clin Nutr 50(5):315
8. Mishal A (2001) Effects of different dress styles on vitamin D levels in healthy young Jordanian women. Osteoporos Int 12(11):931–935
9. Bischoff-Ferrari HA, Dietrich T, Orav EJ, Dawson-Hughes B (2004) Positive association between 25-hydroxy vitamin D levels and bone mineral density: a population-based study of younger and older adults. Am J Med 116(9):634–639
10. Pekkinen M, Viljakainen H, Saarnio E, Lamberg-Allardt C, Makitie O (2012) Vitamin D is a major determinant of bone mineral density at school age. PLoS One 7(7):e40090
11. El-Hajj Fuleihan G (2006) Effect of vitamin D replacement on musculoskeletal parameters in school children: a randomized controlled trial. J Clin Endocrinol Metab 91(2):405
12. Lehtonen-Veromaa MK, Mottonen TT, Nuotio IO, Irjala KM, Leino AE, Viikari JS (2002) Vitamin D and attainment of peak bone mass among peripubertal Finnish girls: a 3-y prospective study. Am J Clin Nutr 76(6):1446–1453
13. Matkovic V, Jelic T, Wardlaw G, Ilich J, Goel P, Wright J, Andon M, Smith K, Heaney R (1994) Timing of peak bone mass in Caucasian females and its implication for the prevention of osteoporosis. Inference from a cross-sectional model. J Clin Investig 93(2):799
14. Bachrach LK (2001) Acquisition of optimal bone mass in childhood and adolescence. Trends Endocrinol Metab 12(1):22–28

15. Pettinato A, Loud K, Bristol S, Feldman H, Gordon C (2006) Effects of nutrition, puberty, and gender on bone ultrasound measurements in adolescents and young adults. J Adolesc Health 39(6):828–834
16. Holick MF (2007) Vitamin D deficiency. N Engl J Med 357(3):266–281
17. Javaid M, Crozier S, Harvey N, Gale C, Dennison E, Boucher B, Arden N, Godfrey K, Cooper C (2006) Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. Lancet 367(9504):36–43
18. Ross AC (2011) The 2011 report on dietary reference intakes for calcium and vitamin D. Public Health Nutr 14(5):938–939
19. Heaney RP (2005) The vitamin D requirement in health and disease. J Steroid Biochem Mol Biol 97(1–2):13–19
20. Pathak MA (2004) In memory of Thomas Bernhard Fitzpatrick. J Investig Dermatol 122:20–21
21. Bland J, Altman D (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 327(8476):307–310
22. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, Flegal KM, Guo SS, Wei R, Mei Z, Curtin LR, Roche AF, Johnson CL (2000) CDC growth charts: United States. Adv Data 314:1–27
23. Must A, Dallal GE, Dietz WH (1991) Reference data for obesity: 85th and 95th percentiles of body mass index (wt/ht2) and triceps skinfold thickness. Am J Clin Nutr 53(4):839–846
24. Siddiqui A, Kamfar H (2007) Prevalence of vitamin D deficiency rickets in adolescent school girls in Western region, Saudi Arabia. Saudi Med J 28(3):441
25. Bener A, Al-Ali M, Hoffmann G (2009) High prevalence of vitamin D deficiency in young children in a highly sunny humid country: a global health problem. Minerva Pediatr 61(1):15
26. Al-Mutairi N, Issa BI, Nair V (2012) Photoprotection and vitamin D status: a study on awareness, knowledge and attitude towards sun protection in general population from Kuwait, and its relation with vitamin D levels. Indian J Dermatol Venereol Leprol 78(3):342–349
27. Clemens TL, Adams JS, Henderson SL, Holick MF (1982) Increased skin pigment reduces the capacity of skin to synthesise vitamin D3. Lancet 1(8263):74–76
28. Absoud M, Cummins C, Lim M, Wassmer E, Shaw N (2011) Prevalence and predictors of vitamin D insufficiency in children: a Great Briain population based study. PLoS One 6(7):e22179
29. Ng SW, Zaghloul S, Ali HI, Harrison G, Popkin BM (2011) The prevalence and trends of overweight, obesity and nutrition-related non-communicable diseases in the Arabian Gulf States. Obes Rev 12(1):1–13
30. Levis S, Gomez A, Jimenez C, Veras L, Ma F, Lai S, Hollis B, Roos BA (2005) Vitamin d deficiency and seasonal variation in an adult South Florida population. J Clin Endocrinol Metab 90(3):1557–1562
31. Kollias N, Baqer A (1984) Measurements of solar middle ultraviolet radiation in Kuwait. Sol Wind Technol 1(1):59–62
32. Binkley N, Novotny R, Krueger D, Kawahara T, Daida Y, Lensmeyer G, Hollis B, Drezner M (2007) Low vitamin D status despite abundant sun exposure. J Clin Endocrinol Metab 92(6):2130
33. Carter GD, Carter CR, Gunter E, Jones J, Jones G, Makin HLJ, Sufi S (2004) Measurement of vitamin D metabolites: an international perspective on methodology and clinical interpretation. J Steroid Biochem Mol Biol 89–90:467–471
34. Arabi A, Nabulsi M, Maalouf J, Choucair M, Khalifé H, Vieth R, El-Hajj Fuleihan G (2004) Bone mineral density by age, gender, pubertal stages, and socioeconomic status in healthy Lebanese children and adolescents. Bone 35(5):1169–1179
35. Dougherty G, Al-Marzouk N (2001) Bone density measured by dual-energy X-ray absorptiometry in healthy Kuwaiti women. Calcif Tissue Int 68(4):225–229
36. Mahussain S, Badr H, Al-Zaabi K, Mohammad M, Alnafisi N (2006) Bone mineral density in healthy Kuwaiti women. Arch Osteoporos 1(1):51–57
37. Hammoudeh M, Mohammed A-K, Mahmoud Z, Abdulbari B (2005) Bone density measured by dual energy X-ray absorptiometry in Qatari women. Maturitas 52(3):319–327
38. Promislow JHE, Goodman-Gruen D, Slymen DJ, Barrett-Connor E (2002) Protein consumption and bone mineral density in the elderly. Am J Epidemiol 155(7):636–644
39. Weikert C, Walter D, Hoffmann K, Kroke A, Bergmann MM, Boeing H (2005) The relation between dietary protein, calcium and bone health in women: results from the EPIC-Potsdam cohort. Ann Nutr Metab 49(5):312–318
40. Stear SJ, Prentice A, Jones SC, Cole TJ (2003) Effect of a calcium and exercise intervention on the bone mineral status of 16–18-y-old adolescent girls. Am J Clin Nutr 77(4):985–992
41. Barkai H-S, Jeanne FN, Mitchell JR, Michelle TB, Mandra JL, Susan SL (2007) Influence of sports participation and menarche on bone mineral density of female high school athletes. J Sci Med Sport Sport Med Aust 10(3):170–179
42. Arabi A, Baddoura R, El-Rassi R, El-Hajj Fuleihan G (2010) Age but not gender modulates the relationship between PTH and vitamin D. Bone 47(2):408–412
43. Tylavsky FA, Ryder KM, Li R, Park V, Womack C, Norwood J, Carbone LD, Cheng S (2007) Preliminary findings: 25(OH)D levels and PTH are indicators of rapid bone accrual in pubertal children. J Am Coll Nutr 26(5):462–470
44. Yan L, Zhou B, Wang X, Ath SD, Laidlaw A, Laskey MA, Prentice A (2003) Older people in China and the United Kingdom differ in the relationships among parathyroid hormone, vitamin D, and bone mineral status. Bone 33(4):620–627
45. Aloia JF, Talwar SA, Pollack S, Feuerman M, Yeh JK (2006) Optimal vitamin D status and serum parathyroid hormone concentrations in African American women. Am J Clin Nutr 84(3):602–609