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Vitamin D status and determinants of deficiency among non-pregnant Jordanian women of reproductive age

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Abstract

BACKGROUND/OBJECTIVES: Vitamin D deficiency, a risk factor for osteomalacia and osteoporosis, is a re-emerging health problem globally. While sunlight is an important vitamin D source, previous investigations among women whose culture encourages skin covering have been small, not nationally representative, or both. We investigated serum 25–hydroxyvitamin D ($25(OH)D_3$) status and factors associated with deficiency in a nationally representative survey of 2013 Jordanian women of reproductive age in Spring 2010.

SUBJECTS/METHODS: We measured $25(OH)D_3$ concentrations by liquid chromatography-tandem mass spectrometry and calculated prevalence ratios for deficiency associated with skin covering and other factors.

RESULTS: Results showed 60.3% (95% CI: 57.1–63.4%) deficiency (<12 ng/ml) and 95.7% (95% CI: 94.4–96.8%) insufficiency (<20 ng/ml) among women. Prevalence of deficiency was 1.60 times higher for women who covered with a scarf/*hijab* (95% CI: 1.06–2.40, $P = 0.024$) and 1.87 times higher for women who wore full cover, or a *niqab* (95% CI: 1.20–2.93, $P = 0.006$), compared with the women who did not wear a scarf/*hijab* or *niqab*. Compared with rural women completing at least secondary education, prevalence of deficiency was 1.30 times higher for urban women of the same education level (95% CI: 1.08–1.57, $P = 0.006$), 1.18 times higher for urban women completing less than secondary education (95% CI: 0.98–1.43, $P = 0.09$), and 0.66 times lower for rural women completing less than secondary education (95% CI: 0.52–0.84, $P = 0.001$).

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

CONCLUSION: Vitamin D deficiency and insufficiency pose significant public health problems in Jordanian women. Prevalence of deficiency is significantly higher among urban women and among women who cover with a scarf/*hijab* or *niqab*.

Keywords

vitamin D; deficiency; women; Jordan

INTRODUCTION

Vitamin D deficiency is a widespread health problem globally.[1] Vitamin D facilitates regulation of calcium and phosphorus levels, which support cellular processes, bone ossification and neuro-muscular function.[2 – 4] Proper levels of vitamin D help prevent multiple bone disorders, including rickets in children and osteomalacia and osteoporosis in adults. It has also been suggested that vitamin D-deficient women of reproductive age may be at increased risk for negative reproductive outcomes, including pre-eclampsia, pregnancy-induced hypertension, obstructed labor, vaginosis and low infant birthweight.[5] The greatest source of vitamin D in the body is synthesis triggered by exposure to ultraviolet B radiation from sunlight.[3]

Vitamin D status is frequently assessed by measuring serum concentration of 25–hydroxyvitamin D (25(OH)D), which reflects total vitamin D from both dietary intake and sunlight exposure.[6] A recent Institute of Medicine (IOM) committee review of data suggested that individuals are considered to be deficient in vitamin D at serum 25(OH)D levels <12 ng/ml, the level below which rickets and osteomalacia may be expected to occur. Individuals with serum 25(OH) levels <5 ng/ml are considered to be severely deficient, and serum 25(OH)D levels ⩾20 ng/ml are considered sufficient.[5]

Prevalence of vitamin D deficiency is particularly high among persons living in urban areas where air pollution leads to a decrease in ultraviolet B photons,[7 – 9] persons with dark skin pigmentation that absorbs lesser amounts of ultraviolet B radiation,[10] older adults whose ability to convert previtamin D_3 to 25(OH)D_3 is diminished and persons with limited exposure to the sun.[11] Education and parity have also been associated with deficiency among women. Meddeb *et al.*[12] found a statistically significant lower prevalence of hypovitaminosis D among Tunisian women with higher educational status. Some studies have found positive associations between the number of pregnancies and higher prevalence of vitamin D deficiency.[12,13] However, two studies have noted no association between parity and vitamin D status.[14,15]

Researchers have investigated vitamin D status and associated factors among women in Middle Eastern countries, where religious and cultural tradition encourages wearing clothing that covers the majority of the skin, including the head, arms and legs, and sometimes the face and hands. Among urban women in Jordan, Shilbayeh[16] reported a 29.6% prevalence of osteoporosis and 43.8% prevalence of osteopenia. However, previous studies used convenience samples that were small, non-representative, or both, and reported mixed results on associations between vitamin D status and associated factors.[8,12,14,17–25] The present analysis aimed to use high-quality laboratory methods to assess vitamin D status and factors associated with vitamin D deficiency, including clothing cover, among a nationally representative sample of non-pregnant Jordanian women of reproductive age.

SUBJECTS AND METHODS

Survey population and sampling design

The survey team collected data for this analysis in March and April 2010 during a national micronutrient survey in Jordan. Jordan is situated at 31°00′ North of the Equator; historical UV Index averages for the months of March and April range from 7 to 10 (high to very high UV exposure levels).[26] Participants provided informed consent verbally, and the Al Basheer Hospital Human Resources Committee approved the procedures. The CDC Institutional Review Board considered this survey public health practice. The survey gathered micronutrient-related data on Jordanian women between 15 and 49 years of age. Field personnel asked participants to complete a questionnaire and provide a blood sample.

We calculated sample size estimates for multiple objectives. Estimating an average of 1.4 women per household, a participation rate of 80% and a design effect of 2.0, we determined that a survey with 166 clusters and 12 households per cluster (1992 households) would provide a sufficient sample size to meet the primary objectives. The largest sample size needed to estimate the levels of vitamin D deficiency would be that for an estimated prevalence of 50.0% deficiency with a precision of 5.0% (1090 participants). Thus, the survey sample size exceeded that needed to estimate vitamin D deficiency levels.

The statistical team selected households through complex multistage stratified cluster sampling, using a sampling frame based on the 2004 Jordan Population and Housing Census. This frame excluded the population living in remote areas (most of whom are nomads), non-Jordanian households and those living in collective dwellings, such as hotels, hospitals, work camps and prisons. The team used probability proportionate to size sampling to randomly select 166 clusters from 30 strata. Within each cluster, the team enumerated and mapped Jordanian households and systematically selected 12 households for participation. The team defined households as Jordanian if the head of household considered himself/herself Jordanian. Field personnel invited all women from 15 to 49 years of age from the participating households to participate.

Factors associated with deficiency

We investigated the factors of self-reported age in years, parity (number of live births), residence (urban or rural) and level of education completed (less than secondary versus secondary or higher) to determine the level of association of these variables with vitamin D deficiency. We included marital status (ever married versus never married) in the present analysis because of its potential confounding relation to cover status and vitamin D status. For reasons of cultural sensitivity, interviewers did not ask women who reported that they were single about parity; instead, they were treated as having zero live births.

The questionnaire also captured information on multivitamin use and cover status. Interviewers asked each respondent whether she was currently taking any supplement that contained iron or any vitamins to ‘improve the blood’ or ‘make her strong’. For any respondent who answered 'yes', the interviewer asked to see the supplement or vitamin package and indicated the contents of the package(s) on the questionnaire. Information was captured on multivitamin use but not specifically vitamin D supplement use. Cover status was determined by asking women whether they cover their hands or arms (yes/no) and how they cover their head when they leave the house or go outside (no cover; scarf or *hijab*, a scarf-like cover of the head but not face; or *niqab*, a gown covering the full body, including the face). We observed no consistent patterns between head covering practices and reports of covering hands or arms. Thus, for the present analysis, we categorized women only according to how they reported covering their head: 'no cover', 'scarf or *hijab*', or '*niqab*'.

Biochemical testing

A trained phlebotomist collected venous blood samples that were transported (4–10 °C in a cold box containing frozen gel packs) to a central laboratory and processed within 24 h; samples were kept frozen until analysis at the Jordan University of Science and Technology. We assessed vitamin D status by measuring serum 25(OH)D_3 concentrations. The Jordan University of Science and Technology analyzed blood specimens using the gold-standard liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for measurement of 25(OH)D_3 status.[27] The interassay coefficient of variation was 2% and the limit of detection was 1.0 ng/ml. Results from participation in the CDC Vitamin A Laboratory–-External Quality Assurance (VITAL-EQA) program, an external quality assurance program for a number of micronutrients including 25(OH)D,[28] showed excellent precision and minimal bias.

Statistical analysis

We calculated sample weights for stratification and the response rate for each cluster to account for non-response. All analyses presented take into account the complex sample design with sample weights. We calculated percent deficiency (<12 ng/ml) and insufficiency (<20 ng/ml) with 95% CI.

We examined factors associated with vitamin D deficiency in non-pregnant Jordanian women of reproductive age by calculating prevalence, prevalence ratios, and 95% CI for cover status and other covariates. We used binomial regression to identify factors with an independent effect on vitamin D deficiency; we retained covariates with a moderately significant univariate relationship with vitamin D deficiency ($P<0.25$) after univariable analysis.[29] In a multivariable model, we first included all covariates that were moderately significant in the univariable model and then used a backward elimination method to remove covariates that were non-significant ($P>0.10$) and not confounders of other effect measures in the model (<15% change to other effect measures).[29] We used liberal significance levels ($P<0.25$ and $P<0.10$) in initial models to retain variables with potential associations for further consideration in the final model. We examined all the two-way interactions and defined significance of factors in the final model using a standard statistical significance of $P<0.05$. We assessed collinearity in the final model by calculating Cramer's V for categorical variables and phi coefficients. We conducted all statistical analysis using Stata 10.1 SE (StataCorp., College Station, TX, USA).

RESULTS

Field personnel invited 1992 households to participate. Among those, 1741 (87.4%) households agreed to participate, 157 (7.9%) refused and 94 (4.7%) were not available. From the households that agreed to participate, field personnel asked 2607 eligible women to become survey participants. Of these, 2473 (94.9%) agreed to complete the questionnaire and 2039 (78.2%) also provided a blood specimen. Complete data for assessing vitamin D status were available for 2032 women and for assessing factors associated with deficiency for 2013 women (Figure 1).

Among women living in an urban area, 5.9% refused any participation compared with 1.9% of women who resided in a rural area ($P = 0.001$). Table 1 compares characteristics of participants who provided a blood sample with characteristics of those who refused to give a blood sample but completed a questionnaire. A greater percentage of women who refused to give a blood sample wore a *niqab*, were younger, were more educated, had fewer live births and were more likely to take a multivitamin compared with women who gave a blood sample.

All observed 25(OH)D_3 values were within the range of physiologically plausible values, thus, we did not drop any observations from analysis. Figure 2 depicts the unweighted frequency distribution of serum 25(OH)D_3 concentration among women. The overall median 25(OH)D_3 concentration was 11.0 ng/ml with an IQR of 9.1–13.5 ng/ml. Almost all women (95.7%, 95% CI: 94.4–96.8%) were vitamin D insufficient, having 25(OH)D_3 levels <20.0 ng/ml; 60.3% (95% CI: 57.1–63.4%) of women were deficient, having 25(OH)D_3 levels <12.0 ng/ml. No women were severely deficient, with 25(OH)D_3 levels <5.0 ng/ml.

A comparison of prevalence of deficiency across factors (Table 2) showed statistically significant differences in the prevalence of vitamin D deficiency among women who covered with a scarf/*hijab* (61.7%, 95% CI: 59.0–64.3%), women who covered with a *niqab* (68.0%, 95% CI: 57.2–77.2%) and women who did not cover with a scarf/*hijab* or *niqab* (39.7%, 95% CI: 24.0–57.9%) (overall $P = 0.010$). Differences were also seen between participants living in rural areas (42.0%, 95% CI: 36.3–47.9%) compared with the participants living in urban areas (64.5%, 95% CI: 60.6–68.2%) ($P<0.001$).

After we adjusted for all other variables, all variables in the final model showed a statistically significant relationship with vitamin D deficiency (Table 3). We observed no collinearity between the factors in the final regression model. Prevalence of vitamin D deficiency was 1.60 times higher for women who covered with a scarf/*hijab* (95% CI: 1.06–2.40) and 1.87 times higher for women who covered with a *niqab* (95% CI: 1.20–2.93) than for women who did not cover with a scarf or *niqab*. A test for trend showed a statistically significant linear trend with increasing degree of cover ($P<0.001$). Having one or more live births (prevalence ratio = 0.81–0.89) compared with having no live births was protective from deficiency ($P = 0.003$–0.094), though no linear trend was observed with higher parity ($P = 0.420$). Because of the observed interaction between residence and education, we stratified results for education by residence. Compared with rural women who completed at least secondary education, urban women who completed at least secondary education had a

1.30 times higher prevalence of deficiency (95% CI: 1.08–1.57), urban women who completed less than secondary education had a 1.18 times higher prevalences of deficiency (95% CI: 0.98–1.43) and rural women who completed less than secondary education had a 0.66 times lower prevalence of deficiency (95% CI: 0.52–0.84).

We found the percentage of deficiency was approximately the same among women living in urban areas, regardless of education level, with 66.0% deficiency among women who completed at least secondary or higher education (95% CI: 58.2–73.0%) compared with 63.1% deficiency among women who completed less than secondary education (95% CI: 58.7–67.2%). However, among rural residents, the percentage of deficiency was significantly lower in women who completed less than secondary education (34.8%, 95% CI: 28.7–41.5%) compared with women who completed secondary education or higher (52.5%, 95% CI: 43.7–61.3%) (data not shown).

DISCUSSION

Results of this analysis must be interpreted noting that serum 25(OH)D_3 concentrations were analyzed using the gold-standard LC-MS/MS method for measuring 25(OH)D_3 status; no studies reviewed used LC-MS/MS methodology. Accordingly, interassay differences must be considered when results are compared with those of other studies.[8,25]

To the extent that results can be compared, the high prevalence of deficiency observed in this survey is comparable to that found in various subpopulations of Middle Eastern women.[8,12,30] Our findings of 95.7% vitamin D insufficiency contrast with those of a recent, non-representative national survey conducted in Jordan that reported insufficiency (25[OH]D<20 ng/ml) among only 16.1% of women 19–39 years old and among 12.5% of women 40–59 years old.[18] Although Batieha *et al.*[18] used the same cutpoint for insufficiency that was used for the present survey, the radioimmunoassay method used by Batieha *et al.* may have yielded overestimated concentrations compared with most other methods.[31] While other studies have observed an association between skin covering and lower vitamin D concentration,[8,12,14,17–25] to our knowledge, the present analysis provides the first nationally representative findings on this association.

Jordanian women show generally higher levels of deficiency and insufficiency than women in non-Middle Eastern countries. Mithal *et al.*[1] found that about 2–30% of European adults are deficient (<10 ng/ml), 35% of US women aged 20–49 years are insufficient (<20 ng/ml) and 21–32% of women in Canada have inadequate levels (<16 ng/ml). In Australia, estimates show 37–67% of women <60 years of age are insufficient (⩽20 ng/ml). Estimates of insufficiency in Asia were among the highest outside the Middle East, with up to 80% of women insufficient (<20 ng/ml).

We observed nulliparity as a factor associated with deficiency. Subsequent analysis of the present data showed an association between age and parity (Cramér’s V = 0.55). However, given the lack of association between age and vitamin D status and the lack of linear trend in greater parity and vitamin D deficiency in the present analysis, age does not appear to explain the observed relationship between parity and vitamin D deficiency. Previous Middle

East studies reported an association between higher parity and deficiency, citing associations between fewer pregnancies and being less housebound in younger women in northern Jordan[13] and associations among wearing a veil, older age and higher parity.[12] Our findings are consistent with Islam *et al.*,[32] who observed increased 25(OH)D concentration with increased parity and attributed their findings to increased time outdoors among women with more children. No information on sun exposure was available in the present analysis, and these findings warrant further investigation.

Our findings that vitamin D deficiency was higher in urban versus rural areas are consistent with those of others[7,8] and support the observed interaction between residence and education, where having less than secondary education was protective against deficiency in rural but not urban areas. In contrast with the present analysis, other studies have reported a protective effect of higher education against vitamin D deficiency.[12,23] This effect has been attributed to the western clothing worn by women with higher education status, compared with full covering worn by Turkish women with lower education status.[23] We suspect that the protective effect of lower education among women in rural areas is likely explained by occupational differences; however, no employment information was collected in this survey.

The negative association of age with vitamin D status has most often been observed in women older than 50 years.[13,14,33–35] Thus, it is not surprising that no association between age and vitamin D status was observed in this survey of women between 15 and 49 years. The lack of association between multivitamin use and vitamin D deficiency in this analysis is likely due to the small number of women who reported currently taking a multivitamin (n = 37) (Table 2).

Data for this analysis were collected during Jordan's spring when the UV index historical averages fall between 7 and 10.[26] Multiple studies have suggested that vitamin D status improves during seasons of increased sun exposure.[25,36,37] While a lag of 2 months has been demonstrated between sunlight exposure and improved 25(OH)D status,[38] a greater magnitude of difference between those who did and did not use a scarf/*hijab* or *niqab* may have been detected if vitamin D status had been assessed during the summer months of June to August,[8] when UV index averages in Jordan reach a maximum level of 11 (extreme exposure).[26]

Limitations include potential selection bias due to differences in participation status. Also, the survey lacked information on individual sun exposure, occupation, dietary intake, lactation, medication use and skin color-–factors known to affect vitamin D status.

In June 2010, after completion of the micronutrient survey, the Ministry of Health added vitamin D to its mandatory wheat flour fortification program. Hence, 2 µg or 80 IU of dry vitamin D_3 is added per 200 g serving of flour (or 300 g serving of bread) to meet 40% of the WHO recommended nutrient intake of 200 IU for vitamin D.[39] Flour fortification with vitamin D is a new process, but one study has confirmed the suitability of fortified bread as a safe, feasible and effective way to improve vitamin D status.[40] Jordan and Palestine[41] are currently the only areas with a vitamin D wheat flour fortification program and thus may serve as a model for countries where vitamin D deficiency and insufficiency pose significant public health problems.

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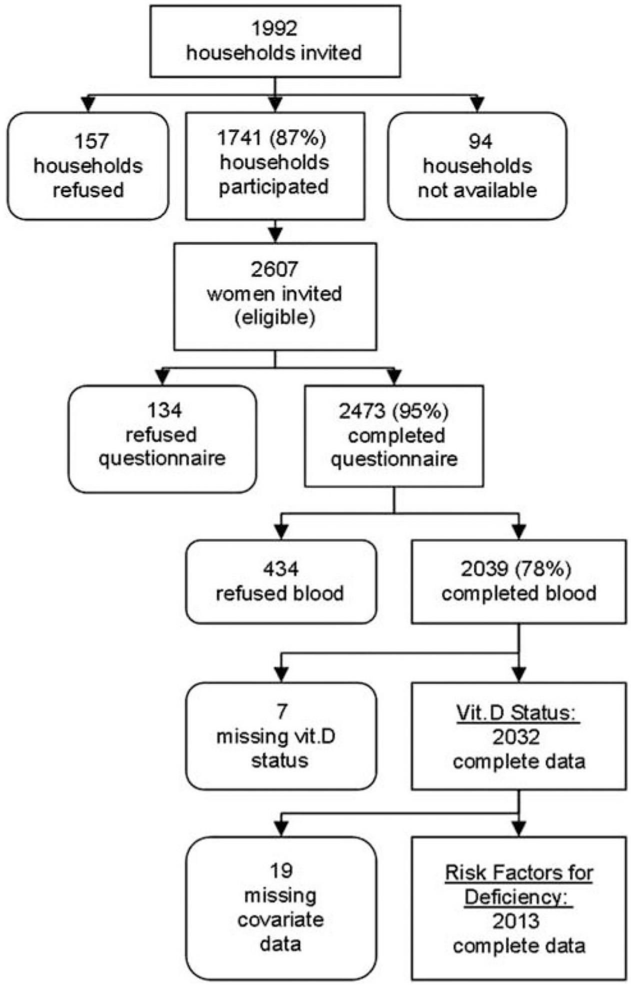

Figure 1.
Flow chart of participation in the 2010 Jordan National Micronutrient Survey.

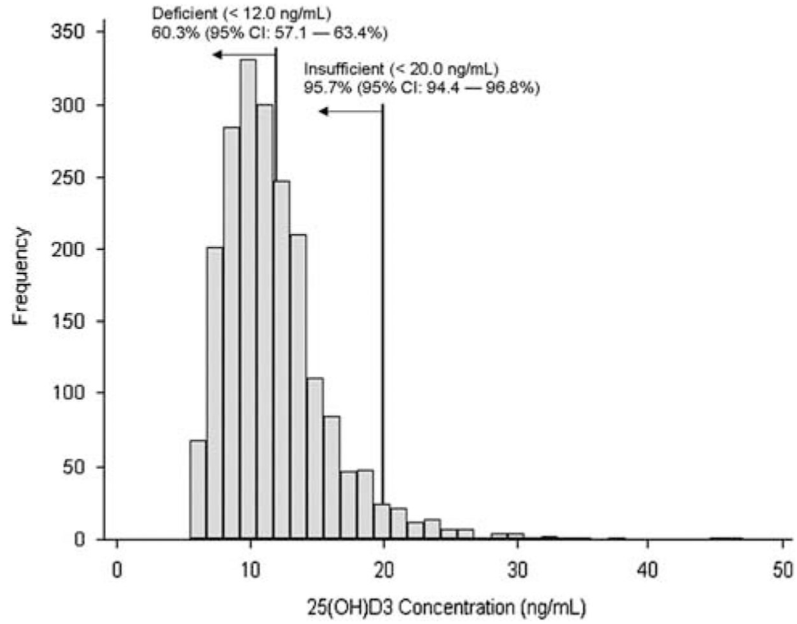

Figure 2.

Frequency distribution of serum $25(OH)D_3$ concentration among non-pregnant Jordanian women (15–49 years old), 2010.

Table 1.

Demographic characteristics of non-pregnant Jordanian women participants aged 15–49 years by whether they provided a blood sample, 2010

Characteristic	Provided blood (%) *n*=2032	Refused blood (%) *n*=434	χ^2 *P*-value[a]
Cover status			< 0.001
No cover	4.8	6.5	
Scarf/hijab	91.6	84.1	
Niqab (full)	3.6	9.4	
Marital status			0.41
Never married	39.1	41.2	
Ever married	60.9	58.8	
Parity			< 0.001
No live births	42.8	50.0	
1 – 2 Live births	13.2	19.3	
3 – 5 Live births	31.3	25.6	
6+ Live births	12.7	5.1	
Residence			0.24
Urban	79.3	81.8	
Rural	20.7	18.2	
Age (years)			< 0.001
15–24	36.0	39.9	
25 – 34	25.6	35.9	
35 – 49	38.4	24.2	
Education completed			< 0.001
Below secondary	53.3	43.1	
Secondary and above	46.7	56.9	
Multivitamin use			< 0.001
No	98.2	95.3	
Yes	1.8	4.7	

[a]Adjusted for cluster survey design.

NOTE: unweighted.

Table 2.

Prevalence and unadjusted PR for vitamin D deficiency (25[OH]D_3<12.0 ng/ml) by select characteristics for non-pregnant women (n = 2,013) aged 15–49 years, Jordan 2010

Characteristic	***n***	**% Low 25(OH)D_3 (95% CI)**	**Unadjusted/univariate**	
			PR (95% CI)	***P*-value**
Total	2013	60.3 (57.1 – 63.4)	-	
Cover status				
No cover	98	39.7 (24.0 – 57.9)	1.00	
Scarf/hijab	1842	61.7 (59.0 – 64.3)	1.55 (1.00 – 2.40)	0.048
Niqab (full)	73	68.0 (57.2 – 77.2)	1.71 (1.08 – 2.71)	0.021
		P = 0.010		
Marital status				
Never married	792	63.6 (59.1 – 68.0)	1.00	
Ever married	1221	58.2 (53.3 – 62.9)	0.91 (0.81–1.03)	0.13
		P = 0.127		
Parity				
No live births	865	63.7 (59.4 – 67.8)	1.00	
1 –2 Live births	265	53.6 (46.2 – 60.8)	0.84 (0.72 – 0.98)	0.025
3–5 Live births	631	59.7 (51.5 – 67.4)	0.94 (0.80–1.10)	0.42
6+ Live births	252	57.0 (50.7–63.1)	0.89 (0.79–1.01)	0.07
		P = 0.196		
Residence				
Rural	416	42.0 (36.3 – 47.9)	1.00	
Urban	1597	64.5 (60.6 – 68.2)	1.54 (1.32–1.78)	0.000
		P <0.001		
Age (years)				
15 – 24	730	62.3 (57.6 – 66.8)	1.00	
25 – 34	514	60.0 (55.3 – 64.6)	0.96 (0.87–1.07)	0.48
35 – 49	769	58.7 (51.6 – 65.5)	0.94 (0.81–1.10)	0.44
		P = 0.607		
Education completed				
Below secondary	1097	57.1 (53.3 – 60.9)	0.89 (0.78–1.02)	0.10
Secondary and above	916	63.9 (57.3 – 69.9)	1.00	
		P = 0.106		
Multivitamin use				
No	1976	60.5 (57.2 – 63.7)	1.00	
Yes	37[a]	54.0 (38.2 – 69.0)	0.89 (0.66–1.21)	0.46
		P = 0.425		

Abbreviations: CI, confidence interval; PR, prevalence ratio.

[a]Estimates are based on <50 observations and therefore should be interpreted cautiously.
NOTE: prevalences and PR weighted and CI adjusted for sample design.

Table 3.

Adjusted PR for low serum 25-hydroxyvitamin D_3 [25(OH)D_3] concentration (<12.0 ng/ml) by select characteristics for women (n = 2,013) aged 15–49 years, Jordan 2010

Characteristic	***n***	**Adjusted PR[a]**	
		PR (95% CI)	***P*-value**
Total	2013	-	-
Cover status			
No cover	98	1.00	
Scarf/hijab	1842	1.60 (1.06–2.40)	0.024
Niqab (full)	73	1.87 (1.20 – 2.93)	0.006
Parity			
No live births	865	1.00	
1 – 2 Live births	265	0.80 (0.70 – 0.93)	0.003
3 – 5 Live births	631	0.89 (0.79–1.02)	0.09
6+ Live births	252	0.89 (0.80–1.00)	0.06
Residence			
Rural			
Below secondary	257	0.66 (0.52 – 0.84)	0.001
Secondary and above	159	1.00	
Urban			
Below secondary	840	1.18 (0.98–1.43)	0.09
Secondary and above	757	1.30 (1.08–1.57)	0.006

Abbreviations: CI, confidence interval; PR, prevalence ratio.

[a]PR adjusted for all characteristics shown.

NOTE: PR weighted and CI adjusted for sample design.