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Abstract

Background: Despite a remarkable reduction in the occurrence of many micronutrient deficiencies in most countries, vitamin D deficiency has remained a global problem. Age-adjusted disability-adjusted life years lost due to cardiovascular disease in the Eastern Mediterranean Region, including in the Islamic Republic of Iran, are higher than the global average.

Aims: To assess the effects of latitude and season on vitamin D status in the Iranian population and the association between vitamin D status and certain cardiometabolic risk factors.

Methods: A sample of 1111 participants aged 19–65 years was randomly selected from 6 regions with latitudes ranging from 29º.0 N to 37.5º N. All anthropometric and biochemical assessments were performed twice a year, summer and winter during 2013 to 2014.

Results: Overall mean 25(OH)D concentration was 26.9 [standard deviation (SD) 17.8] nmol/L in winter and 43.4 (SD 32.9 nmol/L in summer (P < 0.001). Poor vitamin D status was noticeable in both seasons (90.1% and 69.2%, respectively). Being male (B, 7.6; 95% CI: 4.3 to 10.8; P < 0.001) and living at a latitude higher than 33 ° were positive predictors, and serum 25(OH)D concentration in winter (B, –0.2; 95% CI: –2.9 to –0.11; P < 0.001) was a negative predictor of changes of 25(OH)D concentrations.
Conclusion: We found a high prevalence of suboptimal vitamin D status in Iranian adults throughout the year, irrespective of latitude and season. Improvement of mean circulating 25(OH)D concentrations in the community to 50+ nmol/L through a fortification programme is likely to engender healthy cardiometabolic changes.

Keywords: vitamin D, seasonal variation, cardiometabolic risk factor, latitude

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Introduction

Despite a remarkable reduction in the occurrence of many micronutrient deficiencies in most, if not all, countries, vitamin D deficiency has remained a global nutritional problem (1). The importance of this single nutrient deficiency lies in its association with a wide range of human morbidities including cardiovascular disease (CVD), some types of cancers, autoimmune disorders like multiple sclerosis, diabetes and infectious diseases (2,3).

Vitamin D is synthesized in skin from the conversion of the precursor 7-dehydrocholesterol under the influence of solar ultraviolet (UV) light. Several extrinsic and intrinsic factors, including clothing, latitude, season, time of day, socioeconomic status, age, skin type, use of sunscreen and amount of body fat, are important determinants of coetaneous vitamin D biosynthesis (4). Most common diets do not contain natural sources of vitamin D in appreciable amounts. Direct exposure to sunlight is, therefore, the main natural source (5).
Previous studies have demonstrated a seasonal variation in blood levels of 25(OH)D, with lower concentrations in the late winter and early spring than in summer and early fall (6). In the northern hemisphere, decreased serum concentrations of 25(OH)D are seen in winter owing to inefficient UVB light. Consequently, from October to March, spending most of the daytime exposed to the sun does not guarantee optimal vitamin D status. The importance of this cold season fall in serum 25(OH)D would be more sizeable when considering that even in summer time, dermal biosynthesis of vitamin D might not be adequate due to such lifestyle factors as using sunscreens, reduced outdoor activity and abstinence of sun exposure due to fear of its potential health hazards (7).

Recently it has been reported that age-adjusted disability-adjusted life years due to CVD in the Eastern Mediterranean Region are higher than global average (8) and the Islamic Republic of Iran is no exception (9). Most of risk factors of CVD are modifiable and it has been estimated that by reducing CVD risk factors, some 100 million lives could be saved globally (10). Evidence obtained from prospective cohort studies indicates an increased risk of CVD in people with hypovitaminosis D as compared with those with sufficient status of vitamin D (11). Association between blood concentration of 25(OH)D and some cardiometabolic risk factors including obesity, hypertension, dyslipidaemia and inflammatory cytokines may suggest an explanation for the effect of vitamin D status on the development of CVD (12).

The prevalence of poor vitamin D status in the Islamic Republic of Iran, on the other hand, is alarming enough for the stakeholders to take an urgent action (13). Although the supplementation programme for school children and pregnant women has been implemented since a few years ago, several subpopulations including adults remained out of coverage umbrella. Sustainability of supplementation is another challenging issue. As a result, policy-makers at the Ministry of Health started evaluating different aspects of mass fortification of a staple food. To do this, some initial data were needed such as: <list>

- Is vitamin D status affected by latitude, i.e. how does the prevalence of hypovitaminosis D vary in different latitudes?

- Is there a seasonal variation in vitamin D status?

- If yes, what is the range of circulating calcidiol concentration in different latitudes and overall?
Is there any association between vitamin D status and certain cardiometabolic risk factors?

The answer to the last question was especially important as it implied the contribution of the vitamin D fortification programme in reducing the burden of CVD and probably many other related diseases.

To answer these questions, we longitudinally studied a sample of adult volunteers across a broad latitudinal range in the Islamic Republic of Iran.

**Methods**

**Participants**

The participants were part of the National Food and Nutrition Surveillance programme, a population-based study conducted during 2013–2014 by the Ministry of Health and the National Nutrition and Food Technology Research Institute and supported by UNICEF to examine and monitor the nutritional status of the Iranian population. This programme used a 2-stage cluster sampling design: there were 1683 clusters in West Azarbaijan, 1703 in Khoozestan, 1209 in South Khorasan, 1343 in Semnan, 1645 in Fars and 14,212 in Lorestan. From each province, 12 clusters were selected randomly using probability-proportional-to-size, based on the population data from the 2012 census. After the selection of clusters, 20 households were chosen per cluster. All eligible people in the selected households were included in the programme. A sample of 1,111 adults aged 19–65 years from both sexes were randomly selected from 6 regions of the country at different latitudes: West Azarbaijan (37.5° N, 45.0° E), Semnan (35.5° N, 53.3° E), Lorestan (33.4° N, 48.3° E), South Khorasan (32.8° N, 59.2° E), Khoozestan (31.3° N, 48.6° E) and Fars (29.6° N, 52.5° E).

The exclusion criteria were: intake of vitamin D or omega-3 supplements within the past 3 months; use of medications that could potentially influence vitamin D metabolism within the past 3 months; any other concomitant clinical disease that could influence vitamin D metabolism (e.g. renal, hepatic, other endocrine disorders). The assessments of participants were done twice a year: summer (August–September) and winter (February–March) during 2013–2014.

**Questionnaire**

A general questionnaire comprising demographic data and sun exposure habits was completed for all participants via face-to-face interview carried out in the clinic by health workers; the
average duration of the interview was 10–15 minutes. Sun exposure habits were evaluated based on duration of outdoor activity in a typical day and the usual time of outdoor activity (10.00 to 15.00, i.e. peak UV period in the day, and other times of day) (14). These questions were itemized in the questionnaire so that the duration of exposure (< 10 mins, 10–59 mins, 60–120 mins and > 120 mins) as well as time of exposure (before 10.00, 10.00–15.00 and after 15.00) were specified by a checkmark in the relevant checkbox. However, in this study, the duration of exposure was divided into 2 categories, < 1 and ≥ 1 hr/day.

**Anthropometry**

Weight and height were measured by using a digital scale to the nearest 0.1 kg and a stadiometer to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight (kg)/height (m)2. Overweight and obesity were categorized as BMI 25–30 and > 30 kg/m2, respectively (15).

**Blood sampling and handling**

Blood samples were drawn in early morning following an overnight fast (12–14 hr). After 30–60 minutes at room temperature, sera were immediately recovered, aliquoted and stored at –80 °C until the day of analysis.

**Biochemical analyses**

Blood lipids: components of blood lipid profile [triglycerides (TG), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C) and high-density lipoprotein-cholesterol (HDL-C)] were determined using commercial enzyme kits (Pars-Azmoon, Tehran, Islamic Republic of Iran) and an autoanalyser (Selecta E, Vitalab, Holliston, Netherlands).

Serum 25(OH)D: Serum concentrations of 25(OH)D, calcidiol, were measured using a direct enzyme immunoassay (EIA, Diasource, Louvain-la-Neuve, Belgium). The EIA 25(OH)D assay results were checked by high performance liquid chromatography to minimize between-method variation (16). The Laboratory of Nutrition Research has been participating in the Vitamin D External Quality Assessment Scheme (DEQAS) since 2008 and achieved the performance targets set by DEQAS.

In this study, vitamin D status was defined according to serum 25(OH)D concentrations as: deficiency < 25 nmol/L, insufficiency 25–50 nmol/L and sufficiency > 50 nmol/L (3).
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The combination of above normal BMI (> 25 kg/m²), suboptimal HDL-C concentration (< 40 mg/dL in males and < 50 mg/dL in females) and high serum TG concentration (> 150 mg/dL) was defined as a cardiometabolic risk factor (CMRF) (17).

**Statistical analyses**

Mean and standard deviation were used to summarize continuous variables and frequencies were used for categorical variables. The Shapiro–Wilk test was used to check normality of distribution. Tests for differences in the continuous variables among latitudes were performed using analysis of variance (ANOVA) or Kruskal–Wallis. Significant associations for categorical analyses were determined by chi-squared. The comparisons for changes in variables between the 2 seasons were made using the t-test for paired data or Wilcoxon’s test, as appropriate. The effects of latitude (≥ 33° vs < 33°) and sex on changes in 25(OH)D between summer and winter were examined using 2-way ANOVA. We also used 2-way multivariate ANOVA to assess the effect of latitude and sex on the combined changes in BMI and lipid profile variables. Pearson’s correlation coefficient and multiple linear and logistic regression analyses were used to assess relationships between variables. Results were considered statistically significant at P < 0·05. Statistical analyses were performed using SPSS, version 21.0.

**Results**

**Characteristics of the study population**

The distribution of serum 25(OH)D, lipid profile and duration of sun exposure among the study sample according to sex and seasons are presented in Table 1. At the beginning of the study, the mean age of participants was 38.8 [standard deviation (SD) 8.1] years. No statistically significant difference was found for mean age between males and females [males: 39.1 (SD 7.9) years (n = 497); females: 38.5 (SD 8.2) years (n = 614) (P = 0.200)].

**Vitamin D status and seasonal variation**

The overall mean 25(OH)D concentration was 26.9 (SD 17.8) nmol/L in winter and 43.4 (SD 32.9) nmol/L in summer (P < 0.001). Only in summer was there a statistically significant difference in the 25(OH)D concentration among latitudes with the lowest mean in Semnan and the highest in West Azarbaijan (P < 0.001) (Figure 1). For all latitudes, the occurrence of poor vitamin D status was remarkable in both seasons. Thus, in winter 90.1% of participants and in summer 69.2% had serum 25(OH)D concentrations below 50 nmol/L. Using serum 25(OH)D concentrations below 75 nmol/L to describe undesirable vitamin D status, only 3.4% of the participants had sufficient status in winter and only 10.6% in summer.

About 23% of those with hypovitaminosis D (< 50 nmol/L) in winter were in the sufficient category (> 50 nmol/L) in summer. Prevalence of hypovitaminosis D showed a significant
association with latitude only in summer, thus, people who resided in regions at latitude < 33° N had statistically significantly lower 25(OH)D concentrations compared with those living at ≥ 33° N latitude (P = 0.010) (*Table 2*).

In winter, the range of circulating concentrations of 25(OH)D was from 24.5 (SD 14.7) nmol/L in Semnan, 35.5° N with a cold semi-desert climate, to 29.5 (SD 20.7) nmol/L in West Azarbaijan, 37° N with a Mediterranean climate and spring rains. However, the difference between these values was not statistically significant (P = 0.106). In summer, on the other hand, the lowest 25(OH)D concentrations were found in Khoozestan, latitude 31.3° N with a warm dry desert climate, and the highest concentrations in West Azarbaijan [37.1 (SD 34.5) vs 51.2 (SD 27.7) nmol/L; P < 0.001]. Nevertheless, the prevalence of suboptimal vitamin D status was more or less similar for all latitudes during winter. The mean serum 25(OH)D concentration across the latitudinal gradient ranged from 24.5 nmol/L in winter to 51.2 nmol/L in summer.

The seasonal rise in circulating 25(OH)D showed no significant difference between provinces located at < 33° N (+14.2 (SD 28.6) nmol/L) and ≥ 33° N (+17.3 (SD 25.3) nmol/L) (P = 0.061). Although in summer males had slightly, but statistically significantly, higher concentrations of 25(OH)D than females (46.9 (SD 25.5) vs 40.4 (SD 36.3) nmol/L; P = 0.001), this difference was not observed in winter (27.4 (SD 15.8) vs 26.5 (SD 19.3); P = 0.370). Moreover, the seasonal increase in 25(OH)D was statistically significantly greater in males than in females [+19.5 (SD 24.4) vs +12.4 (SD 28.9) nmol/L; P < 0.001]. Accordingly, the duration of direct sun exposure in both seasons was greater in males than in females (P < 0.001) (*Table 1*). The serum concentrations of 25(OH)D in those who spent ≥ 1 hour in sunlight were higher than in those who had shorter sun exposure (< 1 hour) [45.6 (SD 31.6) vs 41.3 (SD 32.4) nmol/L; P = 0.029]. However, these differences were not observed in winter [26.9 (SD 15.9) vs 26.7 (SD 18.4) nmol/L; P = 0.888].

**Cardiometabolic risk factors.**

There were significant seasonal changes in BMI (P < 0.001) and the components of blood lipids except for LDL-C. Prevalence of overweight/obesity were slightly but significantly higher in winter than in summer (P < 0.001). The occurrence rates of overweight/obesity in summer and winter were 62.5% (overweight: 39.1%; obese: 23.4%) and 66.4% (overweight: 40.9%; obese: 25.5%), respectively. Similarly, the prevalence of dyslipidaemia was higher in winter (*Figure 2*).

Two-way multivariate ANOVA showed that latitude (Wilks’ Lambda = 0.987; P < 0.001) and sex (Wilks’ Lambda = 0.916; P = 0.003) were both related to the combined changes of BMI and lipid profile components, but there was no interaction effect (Wilks’ Lambda = 0.998; P = 0.084). In summer, the combination of dependent variables was associated with sex (Wilks’ Lambda =
0.901; \( P < 0.001 \) and vitamin D status (Wilks' Lambda = 0.979; \( P = 0.001 \)) and the post hoc analysis showed that those with undesirable vitamin D status (< 50 nmol/L) had higher BMI, serum TG, total cholesterol and LDL-C than those with desirable vitamin D status (≥ 50 nmol/L).

The predefined CMRF was found in 4.1% of the participants in winter and 2.7% in summer. In winter, 36.4% of participants had at least 3 components of CMRF. However, this decreased to 26.0% in summer. In summer, the proportion of participants who had ≥ 3 components of CMRF was higher among those with serum 25(OH)D ≤ 50 nmol/L than among those with serum calcidiol concentrations ≥ 50 nmol/L (29.5% vs 18.5%; \( P < 0.001 \)).

**Associations and predictors of 25-hydroxyvitamin D concentration**

Serum 25(OH)D was negatively associated with BMI in both winter and summer but only in summer was this association statistically significant \( (r = -0.052; P = 0.086 \text{ vs } r = -0.092; P = 0.002 \text{, respectively}) \).

There were weak but significant inverse correlations between seasonal changes in serum concentrations of 25(OH)D and those of BMI \( (r = -0.088; P = 0.004) \), TG \( (r = -0.095; P = 0.002) \), TC \( (r = -0.130; P < 0.001) \) and LDL-C \( (r = -0.097; P = 0.002) \). Summer rise in serum 25(OH)D did not differ between normal weight and overweight/obese people [16.2 (SD 25.3) vs 15.1 (SD 28.6) nmol/L; \( P = 0.533 \)].

In multiple regression analysis, the most important predictors of serum 25(OH)D concentration in winter were 25(OH)D level in summer (\( B = 0.3, 95\% \text{ CI: 0.2 to 0.3; } P < 0.001 \)), age (\( B = 0.2; 95\% \text{ CI: 0.1 to 0.3; } P = 0.001 \)) and daily sun exposure between 10.00 and 15.00 (\( B = 2.3; 95\% \text{ CI: 0.4 to 4.1; } P = 0.017 \)). Sex, duration of sun exposure and BMI did not remain in the final model.

Two-way ANOVA revealed that changes in 25(OH)D were significantly associated with both latitude and sex \( (P < 0.001 \text{ for both}) \). No significant interaction between latitude and sex was found \( (P = 0.193) \). The logistic regression model (vitamin D status as dependent variable and BMI categories and sun exposure status as independent variables) after adjusting for age and sex, showed that in summer, odds of undesirable vitamin D status was 33% greater in people with BMI > 25 compared with normal weight participants \( (\text{OR: 1.33, 95\% CI: 1.02 to 1.7; } P = 0.035 \text{)} \) and 39% less in those with ≥ 1 hour a day solar exposure than those with < 1 hour exposure \( (\text{OR: 0.61, 95\% CI: 0.47 to 0.84; } P < 0.001) \).
To determine the predictors of changes of 25(OH)D concentration in more detail, we carried out multivariate analyses. Being male (B, 7.6; 95% 95% CI: 4.3 to 10.8; P < 0.001) and living in an area at latitude higher than 33 °N (B, 3.8; 95% 95% CI: 0.5 to 7.0; P = 0.020) were positive predictors. Serum 25(OH)D concentration in winter (B, –0.2; 95% 95% CI: –2.9 to –0.11; P < 0.001) was a negative predictor of changes of 25(OH)D concentrations.

Discussion

To our knowledge, this is the first reported study of seasonality in the vitamin D status of adults in a broad latitudinal range and its relation to CMRF in the Islamic Republic of Iran. Our findings showed a widespread prevalence of hypovitaminosis D in both sexes residing in latitudes 29–37 °N, all year round. Despite a high prevalence of sub-desirable vitamin D status in both winter and summer and irrespective of latitude, there was a seasonal variation in concentration of circulating calcidiol.

Seasonal changes of circulating calcidiol concentrations have already been reported from many regions around the world including North Greenland with long winters and high solar zenith angle during summer (18,19). A study of adolescent girls from Finland reported that mean 25(OH)D concentrations were highest in September (59.5 (SD 13.4) nmol/L) and lowest in February (37.3 (SD 15.5) nmol/L) (20). A study from Estonia (latitude 59 °N) also reported that the mean 25(OH)D in winter was 43.7 (SD 15.0) nmol/L, with only a third of the Estonian population showing sufficient vitamin D levels. A statistically significant increase in 25(OH)D concentration was observed in summer to 59.3 (SD 18.0) nmol/L (18). This variation may be accompanied by associated changes in other health-related variables such as inflammatory biomarkers (21) and thyroid stimulating hormone (22). Even the outcome of communicable disease may somehow be influenced by this seasonal changes in concentrations of circulating calcitriol (23).

We found that exposure to sun for more than 1 hour per typical summer day was a predictor for sufficient vitamin D status. Nevertheless, in multiple linear regression, variation in sun exposure was a predictor of 25(OH)D concentration only in males not in females, who are mostly veiled in the Islamic Republic of Iran. We also found that suboptimal vitamin D status was common, even among people residing in a sunny climate like Khoozestan (31.3 °N, 48.6 °E) and Fars (29.6 °N, 52.5 °E). It is possibly due to “sunshine getaway” behaviour of people in those provinces due to hot weather at most times of the year. Hence, it seems that living in a sunny climate might be unconnected with the prevalence of hypovitaminosis D.
Latitude affected 25(OH)D concentrations were mainly observed in summer months, during which vitamin D status was better in provinces located above 33 °N. The associations observed between latitude and vitamin D status have differed among studies. A meta-analysis of 394 studies demonstrated a significant decline in 25(OH)D concentrations with increasing latitude only in healthy white subjects but not in other ethnicities (24). It seems diversities in living conditions, including outdoor activities, clothing, sun-seeking behaviours and vitamin D intake (diet, fortified foods, supplements), can outweigh the effect of latitude (1).

We found a significant association between the changes in 25(OH)D levels and those of certain CMRF including BMI. However, the seasonal variation in serum 25(OH)D concentrations did not differ between normal weight people and overweight/obese people. The association of circulating 25(OH)D concentrations and CMRF has been already reported by some recent studies (25–27). Data from NHANES revealed that serum 25(OH)D concentrations and percentage of body fat or BMI were negatively related (28). Recently it was reported that serum calcidiol concentrations below 50 nmol/L were associated with death from cardiometabolic factors in both normal weight and obese people (25). The Framingham study revealed that vitamin D status was associated with subcutaneous as well as visceral adiposity (29). A meta-analysis reported that the desirable serum 25(OH)D concentrations were associated with 43% reduction in cardiometabolic diseases, with a remarkable decrease in the occurrence of metabolic syndrome, type 2 diabetes and CVD, especially in the middle-aged and the elderly (30). However, another meta-analysis study conducted at almost the same time did not reach the same conclusion (31).

We found the serum 25(OH)D concentrations above 50 nmol/L may be associated with healthy changes in CMRF. However, another study proposed that the protective effects of vitamin D against cardiometabolic outcomes appear in serum 25(OH)D concentrations above 27.5–35 nmol/L (32), concentrations considered as insufficient.

Of very special interest was the concomitant seasonality of vitamin D status and CMRF. We found that physiological elevation in circulating 25(OH)D during summer was negatively paralleled by changes in serum concentrations of TG, LDL-C, TC and BMI. Seasonal changes in serum lipid profile (33) and glycaemic markers (34) have already been documented. Our findings boost the possibility of healthy changes of CMRF due to summer improvement in vitamin D status.

Despite there being several studies on the relationship between vitamin D and CVD, understanding of the connection is still lacking. Recent evidence suggests some pathways for the beneficial effect of vitamin D on the cardiovascular system, including decrease in
renin–angiotensin–aldosterone system activity and antihypertensive, antiinflammatory, antiproliferative, antihypertrophic, and antithrombotic effects. It has also been shown that vitamin D may modify lipid profiles via increasing the activity of lipoprotein lipase in adipose tissue (35). Although some human studies have reported the beneficial effects of vitamin D on blood lipids and lipoproteins (36,37), the current evidence is still insufficient and further well-designed studies are warranted (38,39).

Vitamin D seems to play a role in modulating adipogenesis by inhibiting such critical molecular components as peroxisome proliferator-activated receptor gamma 2 (PPAR-γ2). Therefore, undesirable vitamin D status may cause additional differentiation of pre-adipocytes to adipocytes (40). Animal studies have reported the function of the vitamin in energy regulation (41). However, there is limited evidence to support this role in humans (42).

Some limitations of this study are acknowledged. We were not able to measure sun exposure directly. The sun exposure habits questionnaire was not validated in our country. However, we used this questionnaire in our previous studies with consistent results (3,43). Additionally, actual intake of vitamin D from foods was not measured. However, previous studies have revealed that the typical Iranian diet is limited in natural sources of vitamin D (3).

**Conclusion**

In conclusion, we found firstly a high prevalence of suboptimal vitamin D status in Iranian adults throughout the year, irrespective of latitude and despite significant increase in circulating 25(OH)D concentrations in the warm season. Secondly, the summer increase in vitamin D status offered healthy changes in CMRF. Therefore, improvement in mean circulating 25(OH) concentration in the community to ≥ 50 nmol/L throughout the year via a national mass fortification programme is likely to bring about some healthy cardiometabolic changes. The expected increase in 25(OH)D due to consumption of fortified foods and drinks has been already evaluated (3,43,44).

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