ORIGINAL ARTICLE

Vitamin D inadequacy is widespread in Tunisian active boys and is related to diet but not to adiposity or insulin resistance

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Background: Vitamin D inadequacy is widespread in children and adolescents worldwide. The present study was undertaken to assess the vitamin D status in active children living in a sunny climate and to identify the main determinants of the serum concentration of 25-hydroxyvitamin D (25-OHD).

Methods: This cross-sectional study included 225 children aged 7–15 years practicing sports in a football academy. Anthropometric measures were performed to calculate body mass index (BMI), fat mass, and maturity status. A nutritional enquiry was performed including 3-day food records and food frequency questionnaire. Plasma 25-OHD and insulin were assessed by immunoenzymatic methods ensuring categorization of vitamin D status and calculation of insulin sensitivity/resistance indexes. A logistic regression model was applied to identify predictors for vitamin D inadequacy.

Results: Vitamin D deficiency (25-OHD < 12 μg/L) was observed in 40.9% of children and insufficiency (12 < 25-OHD < 20 μg/L) was observed in 44% of children. In a multivariate analysis, vitamin D deficiency and insufficiency were associated with a lower dietary intake of vitamin D, proteins, milk, red meat, fish, and eggs. However, no significant relationship was observed with maturation status, adiposity, or insulin resistance.

Conclusions: Tunisian children and adolescents are exposed to a high risk of vitamin D inadequacy despite living in a sunny climate. Circulating 25-OHD concentrations are related to the intake of vitamin D food sources but not to maturation status or body composition. Ensuring sufficient and safe sun exposure and adequate vitamin D intake may prevent vitamin D inadequacy in children from sunny environments.

Keywords: *25-hydroxyvitamin D*; *adiposity*; *child*; *insulin resistance*; *vitamin D deficiency*

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Accumulating evidence suggests that optimal vitamin D status throughout the life span may be important in maintaining bone health, and protecting against many chronic conditions, such as infectious, autoimmune and cardiovascular diseases, diabetes, and cancer (1, 2). Vitamin D derives from skin synthesis under the influence of ultraviolet light and from food. Its status in the body depends on sun exposure, which varies according to latitude, season, skin color, and clothing, as well as on vitamin D provided by diet or supplements. Plasma 25-hydroxyvitamin D (25-OHD) reflects both skin synthesis and the supply from the diet or supplements. It is regarded as the best indicator of vitamin D status (3).

A high prevalence of vitamin D inadequacy was observed in children and adolescents in several regions of the world (4–10). This high prevalence is mainly attributed to insufficient sun exposure and low intake of vitamin D-rich foods. It was also suggested that the pandemic increase in obesity in children contributes to this high frequency. Adiposity is supposed to be a risk factor for vitamin D deficiency (9, 11, 12). Nevertheless, the relationship between adiposity and low 25-OHD during childhood remains uncertain and the underlining mechanisms are still hypothetic (7, 8, 13, 14). There was also a suggestion of a relationship between vitamin D deficiency and insulin resistance, a recognized feature of obesity (15, 16). But the evidence of such association is still inconclusive (17, 18).

In sunny environments, most vitamin D in the body would be provided from skin synthesis and minimally derived from food (3). Children living in sunny areas are expected to achieve adequate vitamin D status on the assumption that ample sun exposure covers the needs for this vitamin (3). However, this condition could be influenced by factors such as time spent outdoors, exposed area of the skin, skin color, and body composition, as well as dietary intake. The present study was undertaken to examine plasma 25-OHD in active children who undergo reasonably outdoor activities and to test the effect of adiposity, insulin sensitivity, and diet on vitamin D status in these children.

Materials and methods

Subjects
The study included 225 boys aged 7–16 years, recruited from two centers of a football academy in the area of Tunis (latitude, 35°N). Children with liver, renal, or bone disease; intestinal malabsorption; or cancer; and those taking vitamin D supplements, anticonvulsant drugs, or systemic corticosteroids were not included. The study was carried out from January to March 2014, a period during which temperatures varied between 10°C and 22°C and the humidity ranged from 70 to 75%. Besides 2 h per week of outdoor physical activity as part of the school program, each child attends three weekly outdoor sessions in the football academy. The training sessions are scheduled from 5 PM to 6 PM on Friday and Saturday, and from 9 AM to 10 AM on Sunday. No child declared having used sun screen during the 3 months of observation. Skin color of each participant was determined by two investigators (IB and MF), together with one parent of the child, and classified as fair, corresponding to Fitzpatrick skin types 1 and 2, or dark, corresponding to Fitzpatrick skin types 3 and 4 (19). Written parental permission was obtained for each participant. The Ethics Committee of Rabta Hospital approved the experimental protocol.

Experimental protocol
Anthropometrical and maturity status measures
Weight, height, and sitting height were measured with the subjects barefooted and lightly clothed. Body mass index (BMI) was calculated as weight per height squared ($kg\,m^{-2}$). Participants were divided according to the World Health Organization (WHO) child growth standards for BMI in three groups; normal-weight group (BMI $\leq$ 85th percentile), overweight group (85th percentile $<$ BMI $<$ 97th percentile), and obese group (BMI $>$ 97th percentile) (20). Triceps and subscapular skinfolds thickness was measured with Harpenden's skinfold calipers (Baty International, West Sussex, England). Body fat percentage was calculated using Slaughter's prediction equation (21). Biologic maturity was assessed by incorporating anthropometric variables (weight, standing height, and sitting height) and was calculated using the equation of Mirwald (22); maturity offset $= -9.236 + (0.0002708*$leg length*sitting height$) + (-0.001663*$age*leg length$) + (0.007216*$age* sitting height$) + (0.02292*$weight by height ratio$)$. This assessment is a non-invasive and practical approved method of predicting age in years from the peak height velocity (PHV) as a measure of maturity offset. For the purpose of data analysis, children were divided into three groups: pre-PHV (-3 to -1 year from PHV), around PHV (-1 to $+1$ year from PHV), and post-PHV ($+1$ to $+3$ years from PHV).

Dietary intake
Nutritional inquiry was completed for 174 children. The daily vitamin D intake in children's diet was assessed using a 3-day food record (including 2 weekdays and 1 day over the weekend), combined with a food frequency questionnaire (FFQ) that the parents mostly completed. A 35-items FFQ to quantify the consumption of nutrients naturally rich in vitamin D, such as fatty fish, meat, eggs, milk, and other dairy products. The questionnaire was developed based on a validated FFQ (23). It was translated into Arabic language and pre-tested before use. The FFQ was modified to estimate vitamin D intake and consumption frequency across nine categories (never, 1 time/month, 1–2 times/week, 2–3 times/week, 3–4 times/week, 1 time/day, 2 times/day, 3 times/day, and $\geq$ 4 times/day). Ease of administration of this FFQ was enhanced by the use of a food photograph album of Tunisian food products that emphasized portion sizes. Four food groups were selected as follows: fish (50 g/serving), eggs (50 g/serving), red meat (50 g/serving), and milk (200 mL/serving). Consumption frequencies of milk and egg were standardized into servings per day and into servings per week for fish and red meat. The data about the mean daily intake of nutrients were processed using the professional Nutri Pro 7 program (Nutri Pro 7 software, CERDEN, Brussels, Belgium).

Biochemical analyses
Blood samples were collected following an overnight fast. Blood was centrifuged at $2000 \times g$ for 20 min and the plasma was frozen at $-40°C$ until analysis (within 3 months). Plasma glucose, calcium, phosphorus, and C-reactive protein (CRP) were assessed on Architect C8000 analyzer (Abbott Laboratories, Abbott Park, IL), using the respective reagents kits. Plasma 25-hydroxyvitamin D (25-OHD) and insulin concentrations were measured by chemiluminescence immunoassay methods using the Liaison analyzer (DiaSorin Inc., Stillwater, MN) and the respective reagents kit. Vitamin D status was evaluated according to the standards of the Institute of medicine (IOM). Vitamin D deficiency, insufficiency, and sufficiency were defined as plasma 25-OHD concentrations below 12 µg/L, 12 to 20 µg/L, and over 20 µg/L, respectively (24). Insulin sensitivity/resistance was assessed using two

indexes; the homeostasis model assessment of insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI), according to the following equations (25, 26): HOMA-IR $=$[(fasting insulin in $\mu U/mL) - $ (fasting glucose in mg/dL)/405]; QUICKI $= 1/$[log (fasting insulin in $\mu U/mL) + $ log(fasting glucose in mg/dL)].

Statistical analysis

Data were analyzed using SPSS for Windows (version 18.0; SPSS Inc., Chicago, IL). Continuous variables were tested for normality using Kolmogorov-Smirnov test. Values are expressed as mean (SD) or median (inter quartile range, IQR) for continuous variables and as a percent for categorical variables. Comparisons between groups were performed using analysis of variance or the Mann–Whitney test for continuous variables and Pearson chi-square test or Fisher's exact test for categorical variables as appropriate. The association between continuous variables was tested using a Pearson correlation test. Unadjusted and multi-adjusted odd-ratios with 95% confidence intervals were calculated as an estimate of the risk of vitamin D deficiency/insufficiency for several potential risk factors. A binary logistic regression model was used to identify predictors for vitamin D deficiency, while adjusting for possible confounding factors. Adjustment was performed on maturation status (pre-PHV/around and post-PHV); body mass (normal-weight/overweight and obese); skin color (fair/dark); and dichotomous variables for fat mass ($<20\%/ \geq 20\%$), HOMA-IR ($<1.5/ \geq 1.5$); and the daily intake of vitamin D ($<8.5/ \geq 8.5 \mu g$), milk ($<500/ \geq 500$ mL), red meat ($<100/ \geq 100$ g), fish ($<100/ \geq 100$ g), and eggs ($<50/ \geq 50$ g), defined as the respective continuous variables split at the median. The fit of logistic models was satisfactory. A two-tailed P-value less than 0.05 was considered statistically significant.

Results

The main characteristics of children according to body mass are shown in Table 1. Serum CRP and insulin concentrations and HOMA-IR and total energy intake were significantly higher and QUICKI lower in obese and overweight children compared to normal-weight children. Daily vitamin D intake was low and equivalent in the three groups. Plasma 25-OHD concentrations ranged between 3.80 and 31 $\mu g/L$, and did not differ according to body mass (Fig. 1). Vitamin D inadequacy was noted in about 85% of the children, with 40.9% having deficiency and 44% having insufficiency. Plasma 25-OHD was positively correlated with dietary intake of proteins ($r = 0.407$, $p < 0.001$), milk ($r = 0.542$, $p < 0.001$), red meat ($r = 0.282$, $p < 0.001$), fish ($r = 0.502$, $p < 0.001$), and eggs ($r = 0.512$, $p < 0.001$) (Fig. 2). However, no significant correlation was observed with PHV, BMI, fat mass, HOMA-IR, or QUICKI. Compared with vitamin-D-sufficient children, those with vitamin D deficiency or insufficiency showed lower intakes of proteins, milk, red meat, fish, and eggs (Fig. 3). In a multivariate analysis, vitamin D deficiency and insufficiency were associated with lower dietary intakes of vitamin D, proteins, milk, red meat, fish, and eggs. However, no association was observed with PHV, BMI, fat mass, HOMA-IR, or skin color (Table 2).

Discussion

This study showed that vitamin D inadequacy is common among active Tunisian children and is associated with a

Table 1. Clinical, nutritional, and biochemical characteristics of children according to body mass ($n = 225$)

	Normal-weight children ($n = 105$)	Overweight children ($n = 48$)	Obese children ($n = 72$)	P
Age, years	11.4 ± 2.81	11.1 ± 2.08	11.5 ± 2.04	0.203
Peak height velocity, years	-3.51 (1.90)	-2.80 (2.30)	-2.00 (2.30)	<0.001
Fat mass,%	17.5 (6.71)	28.3 (3.72)	35.3 (3.73)	<0.001
Total energy intake[a], cal/day	2550 ± 624	2652 ± 800	2843 ± 809	0.006
Vitamin D intake[a], $\mu g/day$	8.21 ± 3.60	8.53 ± 4.45	8.20 ± 4.55	0.224
Parathyroid hormone, $\mu g/L$	39.3 ± 22.0	37.4 ± 15.4	37.5 ± 27.3	0.832
Calcium, mg/L	95.1 ± 4.14	95.8 ± 3.72	96.2 ± 3.84	0.147
Phosphorus, mg/L	42.5 ± 3.91	42.8 ± 5.04	43.6 ± 4.42	0.702
Glucose, mg/dL	89.9 ± 6.77	89.6 ± 7.77	91.3 ± 7.01	0.337
Insulin, UI/L	5.35 (3.58)	8.55 (6.05)	11.7 (9.14)	<0.001
HOMA-IR	1.14 (0.79)	1.31 (1.44)	2.65 (2.10)	<0.001
QUICKI	0.375 ± 0.035	0.358 ± 0.029	0.335 ± 0.034	<0.001
C-reactive protein, mg/L	0.41 (0.60)	0.62 (2.71)	0.91 (2.32)	<0.001

Values are expressed as mean$\pm$SD or median (inter quartile range); HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index.
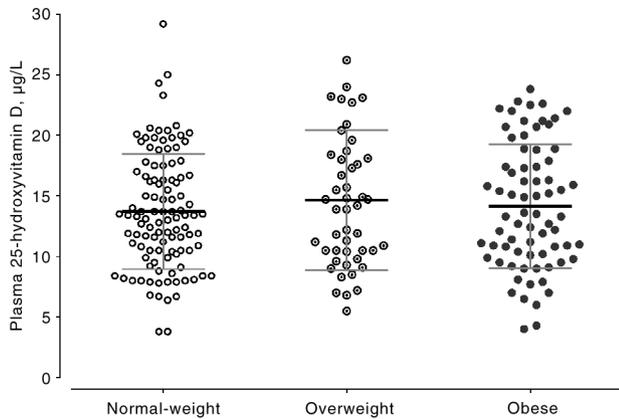[a]Collected in 174 children.

Fig. 1. Plasma 25-hydroxyvitamin D concentrations in Tunisian active children according to body mass ($n = 225$).

low intake of vitamin D food sources, but not with maturity status, adiposity, or insulin resistance. The high prevalence of vitamin D inadequacy in Tunisian children is consistent with similar findings in other parts of the world (4–10). This finding is somewhat surprising since these are active children who live in a sunny environment and engage in a reasonable level of outdoor activities. However, a number of factors may be behind this finding. Our study

had been conducted during the winter when plasma 25-OHD levels are usually at their nadir (3). Because of cool environmental temperatures, children were wearing clothes that covered the trunk and limbs, minimizing the skin area exposed to the sun's rays and preventing vitamin D synthesis. Also, the training sessions were held in the early mornings or late afternoons when the sun's rays are least efficient for vitamin D synthesis (7, 27). Although dark skin color is a risk factor for hypovitaminosis D, obviously, that distinction is of little importance when most of the skin areas are covered with the clothing. However, the most important factor behind the observed vitamin D inadequacy in most children is probably the inadequate intake of dietary vitamin D sources. Our data demonstrate that active children living in sunny environments may still have vitamin D inadequacy, which underlines the importance of monitoring plasma 25-OHD concentrations in children. The present study revealed the low dietary vitamin D intake in most children. In all the children participating in the present study, the daily oral vitamin D intake was well below the US Institute of Medicine Recommended Daily Allowance (RDA) of 15 mcg (24). There is no dietary recommendation for vitamin D for children and adolescents in Tunisia, as it is assumed
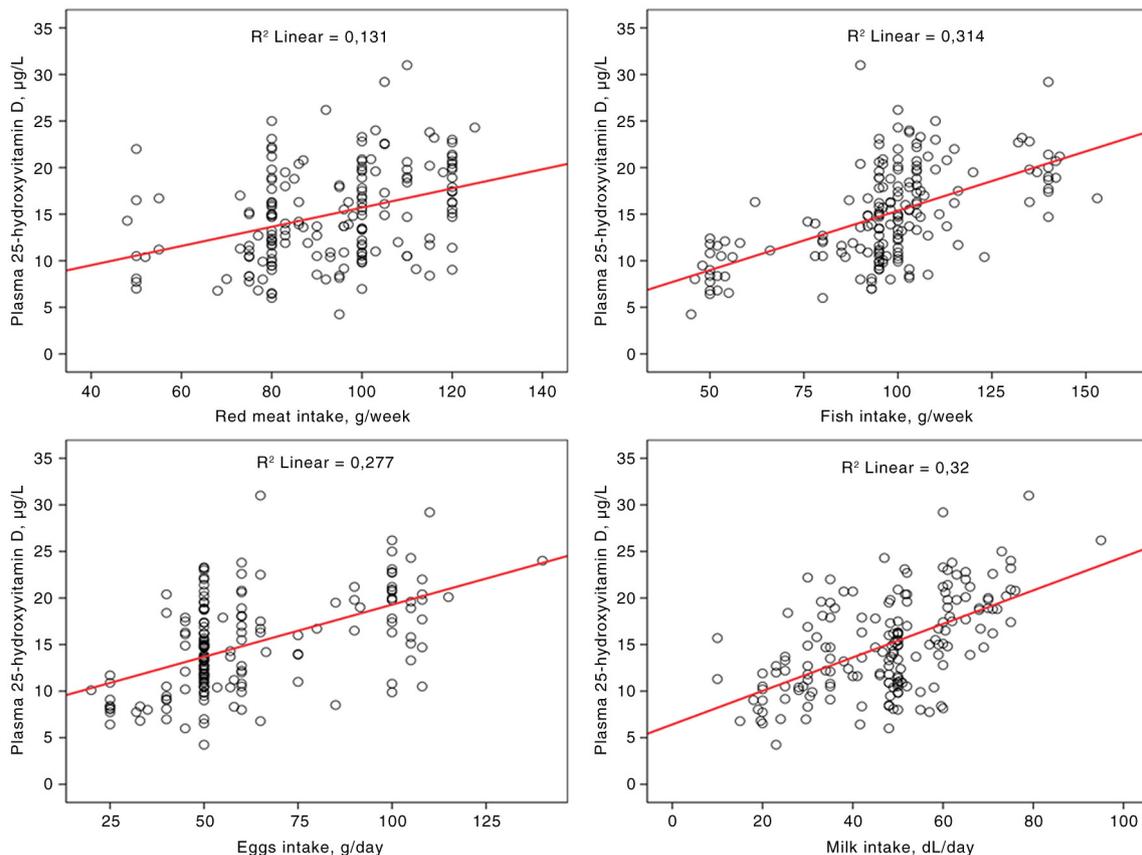

Fig. 2. Correlations of plasma 25-hydroxyvitamin D with daily intake of red meat, fish, eggs, and milk in Tunisian active children ($n = 174$).
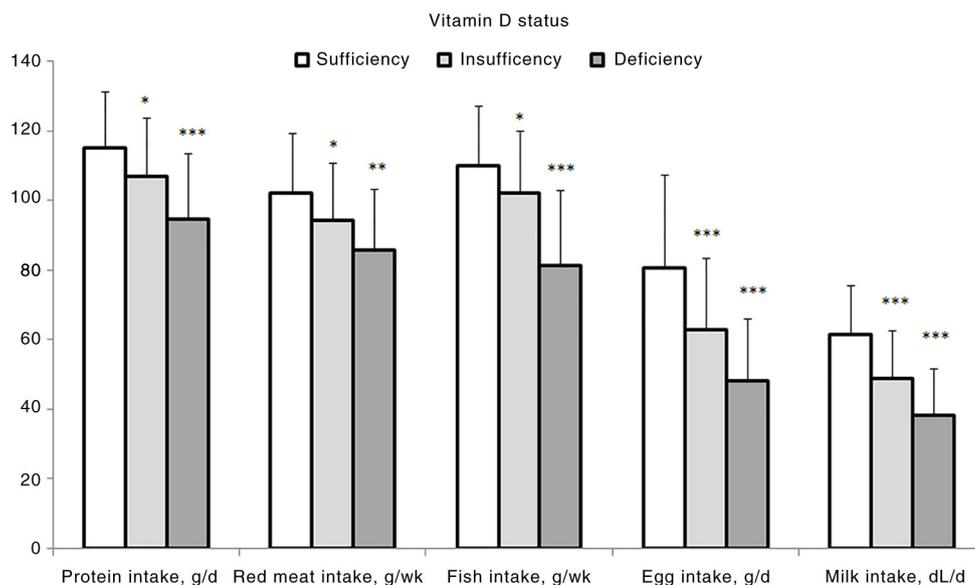
Fig. 3. Daily intake of proteins, red meat, fish, eggs, and milk according to the vitamin D status in Tunisian active children ($n = 174$).

that sun exposure will ensure an adequate vitamin D status. Our finding of low plasma 25-OHD levels in 85% of the participants highlights the need for evidence-based dietary

recommendations for Tunisian children. Our study also showed a clear relationship between vitamin D deficiency/insufficiency and low intake of vitamin D food sources

Table 2. Plasma 25-hydroxyvitamin D and multi-adjusted odd-ratios for vitamin D deficiency/insufficiency in children according to confounding variables

			Plasma 25-OHD, µg/L	Vitamin D deficiency[a]		Vitamin D insufficiency[b]	
		N	Mean (SD)	%	OR (IC à 95%)[c]	%	OR (IC à 95%)[c]
Maturity status	Pre-PHV	194	14.1 ± 5.25	40.7	–	83.0	–
	Around/post-PHV	31	13.5 ± 4.07	41.2	1.89 (0.54–6.67)	97.1	6.76 (0.89–51.2)
Body mass	Normal-weight	105	13.8 ± 4.79	40.0	–	89.5	–
	Overweight/obesity	120	14.3 ± 5.38	41.7	1.34 (0.50–3.64)	80.8	3.09 (0.81–11.8)
Adiposity	No	86	13.7 ± 4.52	39.3	–	91.7	–
	Yes	139	14.4 ± 5.39	41.0	0.81 (0.29–2.24)	80.6	4.41 (0.97–18.1)
Insulin resistance	No	111	14.2 ± 5.25	39.8	–	83.2	–
	Yes	114	13.9 ± 4.97	41.6	1.07 (0.47–2.45)	86.7	1.82 (0.60–5.66)
Skin color	Fair	59	15.9 ± 5.19	25.8	–	77.4	–
	Dark	166	13.3 ± 4.88***	46.4	1.40 (0.60–3.25)	88.0	1.89 (0.66–5.44)
Vitamin D intake	≥8.34 µg/day	85	15.3 ± 5.29	31.8	–	81.2	–
	<8.34 µg/day	89	14.7 ± 4.90	36.0	2.63 (1.06–6.51)*	82.0	1.45 (0.50–4.22)
Milk intake	≥500 ml/day	87	12.8 ± 4.25	19.5	–	69.0	–
	<500 ml/day	87	17.1 ± 4.97***	48.3	3.32 (1.32–7.90)**	94.3	5.83 (1.75–19.4)**
Red meat intake	≥100 g/week	86	13.3 ± 4.76	22.1	–	73.3	–
	<100 g/week	88	16.7 ± 4.87***	45.5	3.42 (1.46–8.01)**	89.8	3.63 (1.23–10.7)*
Fish intake	≥100 g/week	84	12.7 ± 4.53	13.1	–	70.2	–
	<100 g/week	90	17.4 ± 4.51***	53.3	6.23 (2.64–14.7)***	92.2	5.93 (1.87–18.7)**
Egg intake	≥50 g/day	73	13.3 ± 4.37	17.8	–	68.5	–
	<50 g/day	101	17.2 ± 5.12***	45.5	2.94 (1.25–6.92)*	91.1	3.74 (1.27–11.0)*

PHV, pick of high velocity; OR, odd-ratio; 95% CI, 95% confidence interval; 25-OHD, 25-hydroxyvitamin D; [a]25-OH D <12 µg/L; [b]25-OHD <20 µg/L; [c]adjusted for maturation status, BMI, skin color, fat mass, HOMA-IR, and intake of vitamin D, milk, red meat, fish, and eggs; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

(i.e. fish, meat, milk, and eggs). These findings are in line with the study of Areum et al. (10) showing a positive correlation of serum 25-OHD with the consumption of vitamin D food sources in Korean adolescents.

In sunny areas, although sun exposure is the major source of vitamin D in the body, vitamin D inadequacy may occur. This suggests that concomitant appropriate dietary intake is required. The assumption that, in sunny environment, sun exposure alone may provide adequate plasma 25-OHD levels is often false. When sun exposure is limited as a consequence of low-sunshine seasons, pollution, dark skin, or clothing, the dietary intake of vitamin D may be the more significant contributor to vitamin D status. In these conditions, lack of dietary intake may increase the risk of vitamin D inadequacy.

The present study showed no association between plasma 25-OHD and adiposity as assessed either by BMI percentile or fat mass. Some previous studies reported an inverse relationship between plasma 25-OHD and adiposity (7, 9, 11, 28), while others found no association (8, 13, 14, 29). In a sample of Quebec youth, BMI was negatively associated with 25-OHD levels in girls but not in boys (30). Hypothetic mechanisms of low plasma 25-OHD levels in obesity include sequestration of vitamin D in the fat depot, impaired mobilization from the fat depot, and reduced skin and dilution throughout the body. Other important factors in the obese include limited sun exposure due to few outdoor activities and reduced exposed skin area because of clothing (31, 32). The latter mechanism may be of great importance in explaining hypovitaminosis D in obesity. This may explain the lack of an association between vitamin D and adiposity in our series. Indeed, the overweight or obese and normal-weight children included in this study spend as much time outdoors and wear similar clothes, and thus receive the same dose of sunshine. The relationship between adiposity and the low vitamin D status described in some studies may be related to short exposure to the sun, rather than an excess of body fat *per se*. In line with the lack of an association with adiposity, our study showed no association between plasma 25-OHD and insulin resistance, a condition usually associated with obesity. In fact, the relationship between vitamin D and insulin resistance is still a subject of debate (17–20, 33).

Our study has focused on a broad sample of children and adolescents, and its findings arose from multivariate analyses adjusting on several potential confounders for vitamin D status. The study has controlled for the time spent outdoors and thus on the amount of sun exposure, which is an important predictor of vitamin D status. Although sun exposure was not measured with precision, all participants have comparable outdoor activities and equivalent sun exposure. This group of children is probably typical of urban and suburban children from the Mediterranean region. The study has also limitations. The trans-sectional design prevents the evaluation of vitamin D status year-round, rendering the findings only suitable for the winter season. The socioeconomic rank of children was not properly identified. However, based on parents' occupation, most participants have average to high socioeconomic status. Nutritional assessment was achieved in only 77% of participants and vitamin D intake was estimated using a non-Tunisian food database. Because the vitamin D content of foods is not given in the food composition database for Tunisia, the presumed concentrations of vitamin D were obtained from European food composition tables, which make estimations less precise. To overcome this issue, we considered the consumption of vitamin D food sources in Tunisian diet. The development and validation of a vitamin D database for Tunisian food is necessary to allow future estimations of vitamin D intake. Our study did not look for health problems that may be related to vitamin D deficiency, an aspect that is beyond the scope of the study. Further studies should address the health consequences of hypovitaminosis D in exposed populations.

In conclusion, Tunisian children and adolescents are exposed to a high risk of vitamin D inadequacy. They have inadequate sun exposure during winter despite living in a sunny climate. Vitamin D intake is also low in most children, due to little consumption of vitamin D food sources. Finally, circulating 25-OHD concentrations are related to vitamin D food sources intake, suggesting that dietary intake is a key contributor in vitamin D status when sun exposure is limited. Given the key role of vitamin D in growth and health in general, every measure should be undertaken to achieve sufficient vitamin D status in these children. These measures include education in order to ensure adequate and safe sun exposure and appropriate consumption of vitamin D-rich/fortified foods. Further research is needed to establish an optimal combination of sun exposure and food intake/supplementation ensuring year-round sufficient circulating 25-OHD in children and adolescents living in a sunny climate.

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