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Special Article - Vitamin D Deficiency

Vitamin D Intake and Status in Austria and Its Effects on Some Health Indicators

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Abstract

Considering the widespread insufficiency of vitamin D and its role in the regulation of various metabolic functions and the prevention of several health impairments, we evaluated vitamin D status and dietary intake in a nationally representative sample of 980 Austrians (6-80 years, women: 54.8%) and its associations with metabolic health using data from the Austrian Study on Nutritional Status 2012.

Dietary intake was assessed by 24h recalls in adults and elderly and a 3-day food record in children, plasma 25-OH-D concentrations were determined by HPLC with UV detection, anthropometric data of the participants as well as plasma lipid profile, fasting glucose, and HbA1c by standard procedures.

In adults, median (interquartile range) plasma 25-OH-D level was sufficient (≥50 nmol/l) (women: 57.4 (47.5) nmol/l, men: 55.9 (51.2) nmol/l) but not in children (girls: 44.9 (32.5) nmol/l, boys: 44.7 (36.0) nmol/l) and elderly (women: 42.3 (28.5) nmol/l, men: 41.8 (28.4) nmol/l). Insufficient 25-OH-Dlevels were found in 59% of the children and 64% of the elderly. No significant differences were observed between sexes. Season had a strong effect with better status during summer and early autumn, underlining the importance of endogenous synthesis. Median dietary vitamin D intake was below the recommended daily value but correlated only weakly with plasma status (r=0.100, p=0.007). In children and elderly, plasma 25-OH-D showed weak inverse correlations with body mass index (r=-0.165, p=0.005 and -0.306, p=0.001, respectively) and some metabolic health markers.

Our results show vitamin D as a potentially critical nutrient in children and the elderly.

Keywords: Vitamin D; Cardiovascular diseases; Metabolic health indicators

Introduction

The fat-soluble vitamin D is special among vitamins as it shows characteristics of a hormone [1]. For instance, it can be produced in the skin from 7-dehydrocholesterol under the influence of UV B-radiation and hydroxylation in the liver and kidneys. This is even the dominant source of 25-OH-cholecalciferol (25-OH-D_3), the major circulating form of vitamin D in the plasma [2]. Having long been thought to be mainly a regulator of calcium and phosphate absorption and thereby an essential factor for bone density and formation, vitamin D has in more recent times been associated with many more functions and health-related effects [1,3]. Inverse associations between the plasma concentration of 25-OH-D_3 and the risk for cardiovascular diseases [4] and certain cancer types (especially colorectal and bladder cancer) have been reported in epidemiological studies and meta-analyses [5]. In accordance, metabolic risk factors like hypertension, insulin resistance, dyslipidemia and vascular dysfunction were associated with lower 25-OH-D_3 plasma levels [3,6]. Vitamin D has also been found to act as a regulator of immune functions stimulating the response to infectious diseases while at the same time containing inflammatory events [7,8]. This is in line with its protective effect against chronic inflammation and atopic diseases observed in animal studies and human epidemiological surveys [9].

Vitamin D deficiency is commonly defined as a plasma 25-OH-D_3 concentration below 50 nmol/l (20 ng/ml) [10], although a lower threshold at 25 nmol/l (10 ng/ml) for marked deficiency has also been proposed [11]. However, in light of the recent epidemiological findings, a range of 75-100 nmol/l (30-40 ng/ml) has been suggested by some experts to further optimize health [10,12], but the need for such high levels has been questioned [13,14].

Against this background, the high prevalence of vitamin D deficiency worldwide gives rise to concern [15,16]. Indeed, low plasma concentrations of 25-OH-D_3 are not only encountered in populations living in higher latitudes with lower solar radiation intensity especially during winter but also in sunny countries where some studies found plasma 25-OH-D_3 levels to be even lower than in northern countries during winter. This can to some degree be ascribed to cultural and behavioral reasons like extensive clothing, more time spent indoors and the use of sunscreen in the absence of sufficient intake, especially in women from traditional environments [17-20]. Another at-risk group is elderly persons that show an age-related decline of endogenous synthesis and often have lower sun exposure particularly when suffering from frailty and disease [21-23].

Located between 47 and 49°N, Austria experiences low solar intensity during the winter months. Moreover, the lifestyle of a

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Table 1: Characteristics of the sample.

Population group	Schoolchildren (6-14 y)[#]		Adults (18-64 y)		Elderly (65-80 y)	
	Boys	Girls	Men	Women	Men	Women
N	169	163	148	232	72	89
Age (y)	10.0(9.7-10.3)[*]	10.2 (10.1-10.7)[*]	41.1(39.3-42.8)	41.2(39.4-43.0)	71.4(70.4-72.4)	71.6 (70.7-72.5)
Body mass index (kg/m^2)	19.2(18.7-19.8)	19.0 (18.4-19.5)	26.0 (25.5-6.5)[*]	23.9 (23.3-4.5)[*]	27.6 (26.7-28.5)[*]	29.3(28.4-30.3)[*]
Waist circumference (cm)	66.6 (65.4-67.9)[*]	64.0 (62.8-65.2)[*]	90.8 (89.1-2.5)[*]	78.1 (76.2-80.0)[*]	97.9 (95.6-100.3)[*]	92.3 (89.3-95.3)[*]
Body fat mass (%)	20.4 (19.3-21.4)[*]	23.0 (22.1-24.0)[*]	22.3 (21.2-3.3)[*]	29.6(28.3-30.8)[*]	24.1 (22.6-25.6)[*]	37.6 (36.1-39.1)[*]
Prevalence of overweight/ obesity (%)[a]	15.9 / 9.7	16.4 / 6.0	39.3 / 14.6[‡]	18.9 / 9.5[‡]	27.2[‡]	41.5[‡]
Smokers (current/former) (%)	1.3/5.0	5.7/5.1	21.3/22.2	25.2/18.5	10.4/43.7[‡]	6.1/21.0[‡]
Energy intake (MJ/d)[§]	8.1 (7.9-8.4)[*]	7.5(7.3-7.8)[*]	9.3(9.0-9.7)[*]	7.8(7.5-8.1)[*]	8.1 (7.7-8.6)[*]	7.2 (6.9-7.6)[*]
Protein intake (% of total energy)[§]	13.7 (13.3-14.1)	13.5(13.1-13.8)	15.3(14.7-15.9)[*]	14.5(14.1-15.0)[*]	14.4 (13.6-15.2)	15.0 (14.2-15.8)
Fat intake (% of total energy)[§]	34.3(33.5-35.1)	34.0(33.2-34.8)	36.3(35.4-37.3)	35.8(34.9-36.8)	34.9 (33.3-36.5)	36.9 (35.4-38.4)
Carbohydrate intake (% of total energy)[§]	50.9(49.9-51.8)	51.4(50.5-52.3)	43.8(42.6-44.9)[*]	47.0 (46.0-48.1)[*]	44.7(43.1-46.2)	44.5(42.9-46.0)
Supplement use (any kind) (%)	11.4	9.8	19.2[*]	33.5[*]	34.5	47.8
Calcium intake (mg/d)[§]	796.8 (744.8-848.9)[*]	717.0 (674.4-759.5)[*]	876.8 (816.6-936.9)	840.8 (792.7-888.9)	687.8 (626.9-748.7)	630.7 (568.2-693.2)
Total alkaline phosphatase in plasma (U/l)	227.6 ± 58.6[*]	211.0 ± 58.1[*]	72.8 ± 17.9	69.9 ± 20.2	74.7 ± 23.6[*]	84.0 ± 19.4[*]
Total cholesterol (mmol/l)	4.69 (4.58-4.79)	4.69 (4.57-4.80)	5.67 (5.49-5.86)	5.55 (5.42-5.68)	5.48 (5.23-5.74)	5.84 (5.60-6.08)
Triglycerides (mmol/l)	0.84 (0.77-.91)[*]	1.00 (0.92-.08)[*]	1.49 (1.35-.62)[*]	1.24 (1.05-1.42)[*]	1.46 (1.29-1.63)	1.38 (1.25-1.50)
LDL (mmol/l)	2.93 (2.83-3.02)	2.89 (2.78-3.00)	3.65 (3.50-.80)[*]	3.31 (3.19-3.43)[*]	3.33 (3.10-3.56)	3.71 (3.50-3.93)
HDL (mmol/l)	1.37 (1.33-1.42)	1.34 (1.29-1.39)	1.34 (1.30-.39)[*]	1.70 (1.64-1.76)[*]	1.51 (1.42-1.61)	1.50 (1.42-1.59)
Fasting plasma glucose (mmol/l)	4.49 (4.40-4.57)	4.41 (4.33-4.49)	5.01 (4.91-.12)[*]	4.80 (4.70-4.90)[*]	5.81 (5.50-6.11)[*]	6.04 (5.35-6.74)[*]
HbA1c (%)	4.34 (4.24-4.44)	4.39 (4.24-4.54)	4.41 (4.29-4.52)	4.34 (4.24-4.45)	5.24 (4.98-5.49)	5.04 (4.75-5.33)
Prevalence of the Metabolic syndrome[b] (%)	3.9	8.4	7.6	10.0	26.9	29.4

Data are means (95% CI). The nutritional data represent means of two 24h recalls.

[§]Misreporters (defined by an energy intake of ≤ 0.81 or ≥ 2.98 times the estimated basal metabolic rate (BMR) in children and ≤ 0.76 or ≥ 3.16 times the estimated BMR in adults and elderly calculated according to Goldberg, et al. 1991 [24]) were excluded from the analysis.

[#]Two boys were 15 years old.

[*]p<0.005 between sexes (Mann-Whitney-U-test).

[‡] p=0.009 between sexes (χ^2-test).

[a]Based on BMI categories by the WHO for adults < 65 y, on NRC, 1989 for adults >65 y and on reference percentile curves from Kromeyer-Hauschild, et al., 2001 [30].

[b]Defined in adults and elderly according to the IDF criteria [32], as the presence of waist circumference ≥94 cm in men and ≥80 cm in women and any two or more of the following: plasma triglycerides (TG) concentration ≥1.70 mmol/l; plasma HDL cholesterol <1.03 mmol/l in men and <1.29 mmol/l in women; fasting plasma glucose concentration ≥6.1 mmol/l; diagnosed diabetes mellitus; blood pressure ≥130/85 mmHg or treatment for hypertension. In children, metabolic syndrome was defined accordingly as having a waist circumference >90th percentile using waist circumference percentiles from Kromeyer-Hauschild, et al., 2008 [33] and any two of the following criteria: plasma TG ≥1.24 mmol/l, HDL ≤1.03 mmol/l and fasting plasma glucose concentration ≥6.1 mmol/l [38]. However, as in this study blood pressure was not measured, only data on self-indicated hypertension that was assessed in adults and elderly was evaluated.

modern Western industrial country and campaigns to reduce the risk of cutaneous melanoma may lead to reduced sun exposure in parts of the population [19,24]. In a study on sun exposure in Austrian adults a third of the participants (33.7%, n=1500) reported no outdoors sunbathing in the past year [25].

In a study involving a collective of 1048 Austrian adults (21-76 y) of both sexes, the mean intake of vitamin D (2.5 µg/d) did not reach the recommended level and 30.2% of the women and 22.8% of the men were found to be vitamin D deficient with plasma status of 25-OH-D3 levels below 30 nmol/l [26].

This underscores the importance of monitoring the vitamin D status of the population. Requisite data are available from the Austrian Study on Nutritional Status (ASNS) that is regularly conducted to evaluate the diet quality and energy and nutrient supply of the Austrian population. In 2010/2012, the assessment encompassed extensive biochemical analyses to gain a better insight into the nutritional status of different Austrian population groups [27]. The results on vitamin D status and dietary intake are presented in the following report also with regards to their association with metabolic health indicators.

Methods

Participants

Vitamin D status was assessed in the frame of the Austrian Study on Nutritional Status (ASNS) 2010/2012, a regularly conducted cross-sectional randomized survey to monitor the food consumption, nutritional status and potential other health determinants in the Austrian population. The quota sample was composed of 980 persons aged 6-80 years (54.8% female) from all Austrian regions (see Table 1 for sample characteristics). The sample was weighted for a better representation of the Austrian population with regards to age, sex

and regional distribution. The dietary data and blood samples were collected between May 2010 and February 2012 to account for seasonal variation [27].

The survey was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethical Committee of the City of Vienna (EK_10_037_0310). Written informed consent was obtained from all participants.

Anthropometric measurements and dietary assessment

The participants were weighed to the nearest 0.1 kg on a digital scale (Seca Bella 840, Seca Vogel &Halke, Hamburg, Germany) lightly dressed and wearing no shoes and their height was measured to 0.1 cm with a stadiometer (Seca 214, Seca Vogel & Halke, Hamburg, Germany). Each subject's weight in kg was divided by the squared height in m^2 to calculate the body mass index (BMI). This parameter was then used to categorize participants into normal weight, underweight, overweight and obese individuals according to the WHO criteria in adults (normal weight: BMI=18.5-24.99 kg/m^2; underweight: BMI<18.5 kg/m^2; overweight: BMI=25-29.99 kg/m^2; and obesity: BMI ≥30 kg/m^2) [28]. In elderly, the alternative criteria of the U.S. National Research Council's Committee on Diet and Health were applied (overweight: BMI ≥30 kg/m^2) [29], while overweight and obesity in children was defined by a BMI≥ the 90th and the 97th age and sex-specific percentile, respectively, according to Kromeyer-Hauschild, et al., 2001 [30].

Participants' waist circumferences were measured to 0.1 cm with an ergonomic measuring tape. In normal weight persons, waist circumference was taken at the narrowest part, in overweight persons at the top of the iliac crest, both after expiration [31]. In adults and elderly, a normal waist circumference was defined as <94 cm in men and <80 cm in women according to the International Diabetes Federation (IDF) [32]. In children, the 90th age- and sex-specific percentile according to Kromeyer-Hauschild, et al., 2008 [33] was used as the threshold.

In the adult and elderly participants, the dietary assessment consisted of two 24h recalls. The first recall was obtained in a direct interview by trained personnel, conducted following a modified version of the Automated Multiple-Pass Method (AMPM) developed by the US Department of Agriculture (USDA) [34]. The second recall was done via a telephone interview after two weeks. Food consumption of the children was assessed through a three-day estimated food record filled out on three consecutive days. A photo collection from the Second Bavarian Food Consumption Survey (BVS II) based on the EPIC-SOFT picture book was used to estimate the portion sizes [35,36]. The calculation of the energy and nutrient intake was done with the programme "nutritional. Software (nut.s) science" based on the German food composition database Bundeslebensmittelschlüssel 3.01 (Max RubnerInstitut, 2010, Karlsruhe, Germany, https://www.blsdb.de/bls) complemented by typical Austrian foods (datoDenkwerkzeuge, Vienna, Austria, http://www.nutritional-software.at/content/nuts-software/).

Additionally, participants were asked to complete a food frequency questionnaire on their habitual consumption of major food groups. In the case of the children, parents were also asked to fill out a questionnaire about their children's food consumption.

Biochemical analysis

For the biochemical analyses, blood samples were collected from the fasted subjects, stored on dry ice during transport to the study centre and immediately processed upon arrival. Plasma, serum and erythrocytes were stored at -80°C until analysis. Moreover, spot urine samples were obtained that were also cooled and stored at -80°C until analysis.

Vitamin D was determined as the sum of 25-OH-vitamin D_2 (ergocalciferol) and D_3 (cholecalciferol) by HPLC with UV detection using the ClinRep®DiagnosticalTestkit (Recipe, Munich, Germany, LOD: 2.8 µg/l for 25-OH-D_2 and 2.2 µg/l for 25-OH-D_3, LOQ: 4.6 µg/l for 25-OH-D_2 and 3.7 µg/l for 25-OH-D_3). Intraassay and interassay coefficients of variation (CV) were 4.1% and 6.2%, respectively.

Blood lipid profile, plasma glucose concentration and the activity of plasma total alkaline phosphatase (AP) were determined photometrically in a Vitros 250 autoanalyzer (Ortho-Clinical Diagnostics, Inc., Rochester, NY, HDL: intraassay CV: 2.23%, interassay CV: 4.44%; LDL intraassay CV: 2.10%, interassay CV: 7.72%; TG: intraassay CV: 1.33%, interassay CV: 2.35%; AP: intraassay CV: 0.89%, interassay CV: 2.32%). HbA1c was measured in whole blood with HPLC (ClinTest®-Standard test kit for HPLC Assay in Whole Blood, Recipe, Munich, Germany).

Statistical analysis

Misreporters of dietary intake were identified using the cut-off values from Goldberg, et al. 1991 [35,37] that are set at≤ 0.81 or ≥2.98 times the estimated basal metabolic rate (BMR) in children and ≤ 0.76 or ≥3.16 times the estimated BMR in adults and elderly for a mean physical activity level of 1.55 and a confidence interval of 99.7%.

Based on the data from the biochemical analysis and the questionnaires, the adult and elderly participants were categorized by their risk for the metabolic syndrome defined according to the criteria of the International Diabetes Federation (IDF) as the presence of a waist circumference ≥94 cm in men and ≥80 cm in women and any two or more of the following: plasma triglycerides concentration ≥1.70 mmol/l; plasma HDL cholesterol <1.03 mmol/l in men and <1.29 mmol/l in women; fasting plasma glucose concentration ≥5.6 mmol/l; diagnosed diabetes mellitus; blood pressure ≥130/85 mmHg or treatment for hypertension [32]. In children, metabolic syndrome was defined accordingly as having a waist circumference >90th age- and sex-specific percentile according to Kromeyer-Hauschild, et al., 2008 [33] and any two or more of the following: plasma triglycerides concentration ≥1.24 mmol/l; plasma HDL cholesterol <1.03 mmol/l; fasting plasma glucose concentration ≥5.6 mmol/l [38]. However, as in this study blood pressure was not measured, only data on self-indicated hypertension that was assessed in adults and elderly was evaluated. No data on hypertension and diagnosed diabetes mellitus were available in children.

Data were tested for normal distribution using the Kolmogorov-Smirnov test.

As the data on 25-OH-D concentrations and dietary vitamin D intake were not normally distributed, further analyses were done with non-parametric tests (Mann-Whitney-U test and Kruskal-Wallis test to study differences between subgroups). Between groups comparisons of categorical data were done with the χ^2-test.

Table 2: Status and intake of vitamin D by age and sex.

Population group	Age group (n)	Schoolchildren		Age group (n)	Adults		Age group (n)	Elderly	
		Boys	Girls		Men	Women		Men	Women
Vitamin D concentration in the plasma (nmol/l) (median (IQ range))	all (185/179)	44.7 (36.0)[‡]	44.9 (32.5)[‡]	all (163/240)	55.9 (51.2)	57.4 (47.5)	65-80 y (74/94)	41.8 (28.4)[a‡]	42.3 (28.5)[a‡]
	6-9 y (63/57)	45.8 (37.0)[a]	45.8 (27.2)[a]	18-24 y (18/38)	67.0 (56.5)[b]	54.4 (52.4)[bc]			
	10-12 y (97/87)	43.2 (41.1)[ab]	44.8 (34.8)[a]	25-50 y (95/148)	52.5 (50.9)[b]	53.9 (42.0)[b]			
	13-14 y (25/35)	51.6 (36.8)[ab]	40.2 (25.0)[a]	51-64 y (50/54)	61.4 (44.7)[b]	67.7 (48.6)[c]			
Dietary vitamin D intake (mg/d) (median (IQ range))[§]	all (169/163)	1.39 (0.93)	1.26 (1.00)	all (148/232)	2.17 (2.88)[‡]	1.83 (2.6)[‡]	65-80 y (72/89)	2.14 (4.64)[cd‡]	2.15 (4.26)[c‡]
	6-9 y (67/57)	1.58 (0.93)[a]	1.44 (1.09)[a]	18-24 y (17/37)	2.51 (2.28)[cd]	1.91 (2.18)[ac]			
	10-12 y (83/81)	1.22 (0.98)[b]	1.05 (1.02)[b]	25-50 y (87/143)	2.12 (2.74)[c]	1.79 (2.67)[ac]			
	13-14 y (19/25)	1.17 (0.92)[ab]	1.26 (1.11)[ab]	51-64 y (44/52)	2.42 (3.29)[d]	2.42 (3.50)[c]			

[§]Misreporters (defined by an energy intake of ≤ 0.81 or ≥ 2.98 times the estimated basal metabolic rate (BMR) in children and ≤ 0.76 or ≥ 3.16 times the estimated BMR in adults and elderly calculated according to Goldberg, et al. 1991 were excluded from the analysis.
[‡]denotes the lack of a statistically significant difference between the marked age groups of the same sex. Within each sex, statistically significant differences between the age subgroups are indicated by differing superscript letters.

Relationships between vitamin D plasma concentration, dietary vitamin D intake, anthropometric measures and metabolic parameters were evaluated in partial correlation analyses, controlling for the participants' age, sex, BMI, and their smoking status as well as total energy intake.

All analyses were performed with SPSS Statistics for Windows Version 24.0 (IBM Corp., Armonk, NY).

Results

Data on vitamin D intake and status were available for 964 and 935 participants, respectively. A total of 91 misreporters was identified according to the afore-described definition and excluded from the analyses on dietary vitamin D intake leaving 873 datasets for evaluation. However, as vitamin D concentrations in the blood are mainly determined by the endogenous synthesis, these samples were included in the analyses on biochemical status to get a good picture of the vitamin D status in Austria. An overview of some demographic, anthropometric, dietary and metabolic characteristics of the study population is given in Table 1.

Vitamin D status

In the adult subsample, the median concentration of 25-OH-vitamin D (written in the following as 25-OH-D) in the plasma of 56.3 nmol/l (IQ range: 51.2), 57.4 nmol/l (IQ range: 47.5) in the female participants and 55.9 nmol/l (IQ range: 41.3) in the male participants, was above the sufficiency threshold. Children and elderly both had significantly lower median plasma 25-OH-D levels than the adults, but did not differ significantly from each other (Table 2). No significant differences between sexes were observed within the age groups. Further subdividing the samples by age revealed that the 13-14 year-old girls had the lowest median 25-OH-D level (40.2 nmol/l, IQ range: 25.0) while the boys of this age class had a higher median level though not significantly so (51.6 nmol/l, IQ range: 36.8). Among the adult men, the median 25-OH-D level was highest in the subgroup aged 18-24 years (67.0 nmol/l, IQ range: 56.5), while among the women, the 51-64 year-olds had the best status (median 67.7 nmol/l, IQ range: 48.6). In this latter group, the percentage of individuals with vitamin D deficiency ([25-OH-D]<25 nmol/l) or insufficiency ([25-OH-D]25-<50 nmol/l) was also lowest (5.6% and 25.9%, respectively). Seventeen percent of the subjects had 25-OH-D levels between 75 and 100 nmol/l. About a fifth (22%) of the adult men also had plasma levels within this range and even 31% of those aged 18-24 years. However, 25-OH-D plasma levels below 50 nmol/l were also common in these subgroups (31.5% of the 51-64 year-old women, 41.3% of the 18-24 year-old men). In the children subgroup, 25-OH-D concentrations below 50 nmol/l were found in 62.3% and 56.2% of the girls and boys, respectively, and in 64.2% and 63.5% of the female and male elderly, respectively (Figure 1).

However, all the age groups had mean plasma total alkaline phosphatase activities within the respective reference ranges [39,40] (Table 1) and less than 10% of the participants had values above the reference range. Among those exceeding the upper reference threshold some were sufficient in vitamin D (25-OH-D ≥50 nmol/l) (data not shown). It also has to be noted that alkaline phosphatase activity is a rather unspecific marker being influenced by a wide range of physiological functions [41]. Nevertheless, this suggests that 25-OH-Dconcentration did not reach critically low levels that may entail clinical symptoms like osteomalacia.

Dietary vitamin D intake

Dietary intake of vitamin D was generally low across all age groups and did on average not meet the level recommended by nutrition societies (Table 2). The median in the whole sample was 1.67 µg/d (IQ range: 1.98), 1.52 µg/d (IQ range: 1.90) in women and 1.75 µg/d (IQ range: 2.05) in men. Children had particularly low intake levels (1.39 µg/d (0.93) in boys and 1.26 µg/d (1.00) in girls). Thus, only about 4% of the adult and elderly men and about 1% of the women had a vitamin D intake as recommended by the European Food Safety Authority EFSA (i.e., 15 µg/d) [42], while none of the children consumed this amount of vitamin D (Figure 1). In this context, it has to be noted that there is still some uncertainty about the adequate intake amounts of vitamin D as in healthy individuals this nutrient is mainly derived from endogenous synthesis. Accordingly, recommendations for dietary intake are generally made under the assumption of a minimal sun exposure resulting in an inadequate endogenous synthesis. The revised D-A-CH reference values of the

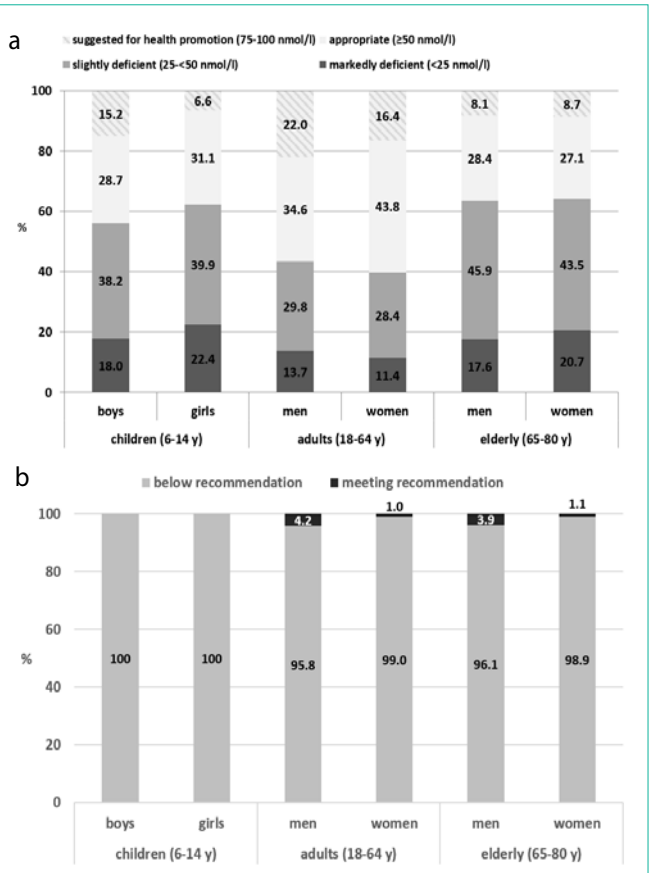

Figure 1: a) Status and b) intake of vitamin D by age group and sex. Prevalence given as % of the respective population.
Low vitamin D status defined through plasma 25-OH-vitamin D concentration according to the thresholds proposed by Cashman, et al., 2011 and Holick, et al., 2011: markedly deficient plasma vitamin D concentration <25 nmol/l; slightly deficient 25- <50 nmol/l, adequate >50 nmol/l, suggested for health promotion 75-175 nmol/l [10,11].
Adequacy of daily dietary intake of vitamin D was defined according to the recommendation of the European Food Safety Authority EFSA i.e., 15 µg/d for all persons aged ≥1 year (EFSA, 2016) [26].

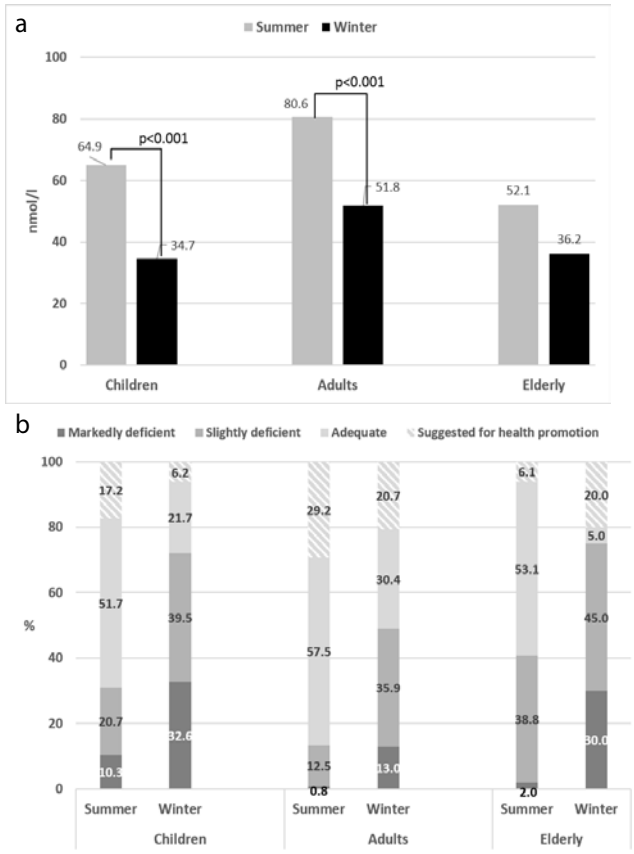

Figure 2: Seasonal effects on vitamin D status.
a) Comparison of the median plasma 25-OH-vitamin D concentrations measured during the summer (June to September) and winter (December to March) months by age and sex. Significant differences between sexes analyzed by Mann-Whitney-U test are indicated.
b) Distribution of vitamin D status during the summer (June to September) and winter (December to March) months by age and sex (% of the respective population groups), based on plasma 25-OH-vitamin D concentration according to the thresholds proposed by Cashman, et al., 2011 and Holick, et al., 2011: markedly deficient plasma vitamin D concentration <25 nmol/l; slightly deficient 25-<50 nmol/l, adequate >50 nmol/l, suggested for health promotion 75-175 nmol/l [10,11].

German, Austrian and Swiss Nutrition Societies recommend a daily intake of 20 µg/d [43] whereas the Institute of Medicine of the US National Academies has set a value of 15 µg/d for children from 1 year onwards, adolescents and adults up to 70 years, and 20 µg/d for persons >70 years of age [13]. An average intake level of 20 µg/d or more was also reached by 4% of the adults of both sexes and the elderly men meeting the EFSA recommendation but not by the elderly women. Moreover, the elderly men with sufficient vitamin D intake were all aged <70 years so that the older persons that represent a special at-risk group for nutrient deficiencies were not adequately supplied.

Vitamin D intake was only very weakly correlated to plasma 25-OH-D level (r=0.100, p=0.007, adjusted for age, sex, total energy intake and smoking status) and upon dividing the sample by age groups and sex was only retained in the adult subsample (r=0.147, p=0.005, adjusted for age, sex, total energy intake and smoking status) and the men (r=0.117, p=0.028 in all men, r=0.181, p=0.015 in adult men only, both adjusted for age, sex, total energy intake and smoking status).

Nevertheless, the participants reaching dietary vitamin D intake levels ≥20 µg/d had a significantly higher median plasma 25-OH-D level than those consuming less vitamin D (101.5 nmol/l, IQ range: 35.8 *vs.* 60.6 nmol/l, IQ range: 50.7for intakes of 5 µg/d (10 in the elderly) to <20 µg/d, p=0.003, and 47.5 nmol/l, IQ range: 37.3 for intakes <5 µg/d (10 in the elderly), p<0.001). However, the number of persons with such a high consumption level was very small (n=12 and n=58 with intakes 5(10) µg/d to <20 µg/d) and when comparing the median plasma 25-OH-D level of subjects within the different age- and sex-specific quartiles of vitamin D intake, no clear relationship was visible.

Fish consumption was a major contributor to vitamin D intake. Indeed, 4% of the participants who indicated a regular fish consumption (at least twice weekly) met the recommended vitamin D intake level of 15 µg/d whereas none of those who stated that

Table 3: Correlations between plasma 25-OH-D level and indicators of body weight and composition and of metabolic health.

	Unadjusted	adjusted for age, sex and smoking	adjusted for for age, sex, smoking and BMI	adjusted for for age, sex, smoking and waist circumference	adjusted for for age, sex, smoking,calcium and total energy intake*
BMI	children: r=-0.120 (p=0.022) elderly: r=-0.299 (p<0.001)	children: r=-0.165 (p=0.005) elderly: n.s.	-	children: n.s. adults: -0.228 (p<0.001) elderly: r=-0.333 (p<0.001)	children: r=-0.164 (p=0.010) elderly: r=-0.318 (p=0.001)
Waist circumference	children: r=-0.144 (p=0.006)	children: r=-0.205 (p<0.001)	children: r=-0.126 (p=0.032) adults: r=0.253 (p<0.001) elderly: r=0.205 (p=0.032)	-	children: r=-0.207 (p=0.001)
Body fat %	n.s	n.s.	adults: r=-0.412 (p<0.001) elderly: -0.133 (p=0.025)	n.s.	n.s.
Plasma triglycerides	children: r=-0.114 (p=0.030)	children: r=-0.114 (p=0.030)	n.s.	n.s.	children: -0.131 (p=0.039)
Total plasma cholesterol	elderly: r=0.220 (p=0.005)	elderly: r=0.221 (p=0.020)	n.s.	n.s.	elderly: r=0.269 (p=0.007)
HDL cholesterol	elderly: r=0.261 (p=0.001)	elderly: r=0.266 (p=0.005)	n.s.	n.s.	elderly: r=0.308 (p=0.002)
LDL cholesterol	elderly: r=0.214 (p=0.006)	elderly: r=0.224 (p=0.018)	n.s.		elderly: r=0.285 (p=0.004)
Fasting plasma glucose	children: -0.111 (p=0.035) elderly: r=-0.199 (p=0.031)	n.s.	n.s.	n.s.	n.s.

*Misreporters were excluded for this analysis.

No significant associations were observed between plasma 25-OH-D and HbA1c in any of the age groups.

they (almost) never consumed fish achieved this target. While the percentage of individuals with plasma 25-OH-D ≥50 nmol/l did not change with frequency of fish consumption, those with regular fish intake showed a lower prevalence of markedly low plasma levels (<25 nmol/l) than the non-consumers (11.4% *vs.* 19.6%, n.s.). This group also had a higher median 25-OH-D plasma level than the non-consumers (53.1 nmol/l, IQ range: 34.2 *vs.* 51.0 nmol/l, IQ range: 38.2, n.s.). Although these differences were not statistically significant they indicate a positive effect of fish consumption. However, of the participants for whom data on consumption was available only 8.6% consumed fish at least twice per week.

In turn, micronutrient supplement use that was assessed through a questionnaire did not significantly contribute to vitamin D status. Elderly persons who indicated that they consumed supplements even had a significantly lower median 25-OH-D concentration (43.9 nmol/l, IQ range: 33.0 *vs.* 53.8 nmol/l, IQ range: 33.8 in non-users, p=0.001). However, information about the type of supplement consumed was not always available and many vitamin supplements do not contain vitamin D. Among adults and elderly, supplement use was more frequent in women than in men (adults: 33.5% *vs.* 19.2%, p=0.001, and elderly: 47.8% *vs.* 33.5%, n.s.).

Seasonal variation of vitamin D status

In light of the seasonal variation of sun intensity in Austria, the vitamin D status of the participants was studied in relation to the date the sample was obtained. In the children and adults, the median plasma concentration of 25-OH-D was significantly higher during the summer and early autumn months (summed up in the following as summer corresponding to June to September than in winter and early spring (i.e. December to March) (Figure 2A). During the summer season, there were also a markedly lower percentage of subjects with deficient plasma 25-OH-D levels (Figure 2B). In the elderly, there was only a small seasonal difference in median plasma 25-OH-D concentration that was not statistically significant (Figure 2A). Moreover, while the percentage of markedly deficient persons was only 2% in the summer compared to 30% in the winter, 38.8% were still below the desirable plasma concentration in the winter compared to 45% in the summer (Figure 2B).

Overweight and obese children and elderly sampled during the summer months tended to have a lower median plasma 25-OH-D concentration than their normal weight counterparts (children: 40.1 nmol/l, IQ range: 36.3 *vs.* 47.6 nmol/l, IQ range: 28.1; elderly: 52.1 nmol/l, IQ range: 21.8 *vs.* 54.2 nmol/l, IQ range: 38.0) and in children, there was also a higher prevalence of insufficient vitamin D status (70% *vs.* 53.1%) but these differences were not statistically significant.

Users of any micronutrient supplement had a higher median plasma 25-OH-D level than non-users during winter time (41.2, IQ range: 32.9 *vs.* 52.5 (IQ range: 58.6) but this difference was not significant (p=0.052).

Association of body weight and composition with vitamin D status

In children and elderly, overweight or obese individuals had a lower median plasma 25-OH-D concentration than normal weight or underweight persons (37.9 nmol/l, IQ range: 36.6 *vs.* 46.8 nmol/l IQ range: 32.9, p=0.006 in children, 35.0, IQ range: 23.3 *vs.* 43.9, IQ range: 36.9, p=0.013 in elderly). Additionally, children with a waist circumference exceeding the 90th age- and sex-specific percentile also had a lower median plasma 25-OH-D concentration (40.2 nmol/l, IQ range: 36.5 *vs.* 47.1 nmol/l, IQ range: 32.1 in those with normal WC, p=0.001) while no significant difference was observed in the other age groups. A higher percentage of overweight and obese children

and elderly had plasma 25-OH-D levels <50 nmol/l compared to their normal or underweight counterparts (64.4% *vs*. 57.5%, n.s., in children and 75.4% *vs*. 57.8%, p=0.025, in elderly) although it was significant only in the elderly. Deficient plasma 25-OH-D levels were also more frequently observed in children (65.8% *vs*. 56.3% in children with a normal WC, n.s.) and elderly (53.4% *vs*. 48.3% in elderly with a normal WC, n.s.) with increased waist circumference (WC) although not significantly so. Overweight children had a RR of 1.12 of having low plasma 25-OH-D, overweight elderly of 1.3. However, no differences between weight categories were found in adults.

Relationship between vitamin D and metabolic health markers

In the children group, plasma 25-OH-vitamin D showed weak but significant inverse correlations to plasma triglyceride levels (r=-0.114, p=0.030), fasting plasma glucose (FPG) (r=-0.111, p=0.035), body mass index (r=-0.120, p=0.022) and waist circumference (r=-0.144, p=0.006). Apart from the association with FPG, these correlations in children were maintained after adjusting the data for sex, age and smoking status (plasma triglyceride levels: r=-0.114, p=0.030; body mass index: r=-0.165, p=0.005; and waist circumference: r=-0.205, p<0.001). In the elderly, plasma 25-OH-D was weakly correlated to plasma total cholesterol (r=0.220, p=0.005), HDL cholesterol (r=0.261, p=0.001) and LDL cholesterol (r=0.214, p=0.006) levels, as well as inversely to FPG (r=-0.199, p=0.031) and BMI (r=-0.299, p<0.001). Adjusting for sex, age and smoking status left only the associations with plasma total cholesterol (r=0.221, p=0.020), HDL cholesterol (r=0.266, p=0.005) and LDL cholesterol (r=0.224, p=0.018) levels in elderly (Table 3). No significant relationships between plasma 25-OH-D levels and metabolic markers were observed among adults.

In a further step, the data were also adjusted for calcium intake as this mineral also plays a role in the prevention of many nutrition-related chronic diseases [44]. However, this measure (accompanied by correction for total energy intake) did not result in significant changes of the observed relationships described above (Table 3).

In turn, after adjusting data for BMI in addition to sex, age and smoking status, the association of 25-OH-D with triglycerides in children was no longer significant and the one with waist circumference was weakened (r=-0.126, p=0.032). Likewise, correlations of 25-OH-D with HDL and LDL cholesterol in elderly were lost after adjusting for BMI. In turn, in adults and elderly plasma 25-OH-D was weakly positively correlated with waist circumference (r=0.253, p<0.001 and r=0.205, p=0.032, respectively) and weakly and moderately with relative body fat mass (r=0.133, p=0.025 and r=0.412, p<0.001, respectively) after adjusting for BMI (Table 3).

Adjusting for age, sex, smoking and waist circumference resulted in losses of all the correlations between plasma 25-OH-D and metabolic markers as well as with BMI in children, while in the elderly the relationships between 25-OH-D and HDL cholesterol (r=0.231, p=0.015) as well as BMI (r=-0.333, p<0.001) were maintained. In adults, there was also a significant inverse correlation between plasma 25-OH-D and BMI (r=-0.228, p<0.001) under these circumstances (Table 3).

In elderly, median 25-OH-D concentration in the plasma was lower in those with self-indicated hypertension than in those that stated not suffering from it (37.2 nmol/l, IQ range: 32.4 *vs*. 56.0 nmol/l, IQ range: 30.4, p=0.001) and in those with self-indicated cardiovascular disease (43.1 nmol/l, IQ range: 38.7 *vs*. 49.8 nmol/l, IQ range: 39.4, p=0.011) while no significant differences were observed for diabetes mellitus or atopic diseases.

According to the definition of the International Diabetes Federation (IDF), 110 participants could be classified as having the metabolic syndrome [32]. Of these, 27.9% were obese, 63.1% had a HDL below the reference, 55.4% had elevated triglyceride levels and 58.4% elevated fasting plasma glucose levels. Self-reported hypertension was present in 46.8% of the adults and elderly. The 95 persons with available 25-OH-D plasma levels had a lower median plasma 25-OH-D concentration than those without metabolic syndrome (46.3 nmol/l, IQ range: 35.9 *vs*. 48.6 nmol/l, IQ range: 38.4) but this difference was not statistically significant and neither was the slightly higher prevalence of 25-OH-D levels <50 nmol/l (53.7 *vs*. 52.1%).

Discussion

The findings of the here-presented study in a representative Austrian population support the position that at least certain at-risk groups like children and elderly persons, for instance, may have an increased risk of vitamin D deficiency. They are in line with other studies in populations living in comparable geographic latitudes [15,45,46]. Thus, in the German National Health Interview and Examination Survey for Children and Adolescents (KiGGS), about two thirds of the participants aged between 3 and 17 years had 25-OH-D plasma levels <50 nmol/l with an even higher prevalence in those with a migration background (75.9% in boys and 76.6% in girls) [45]. In a multicenter survey on vitamin D status of adolescents from nine different European countries, the overall prevalence of 25-OH-D plasma levels <50 nmol/l was 42.4%. Blood sampling took place between June and October so that a major proportion of the data was obtained during winter or end of winter possibly contributing to a higher prevalence of low vitamin D concentrations. However, in this survey, the Austrian (Viennese) subsample and particularly the female population showed a somewhat better status compared to those from other locations, with the third-highest mean 25-OH-D plasma level of 63.7 nmol/l for the total sample and the second-highest of 67.2 nmol/l for the female group [47].

Vitamin D deficiency defined by low 25-OH-D plasma levels have repeatedly been reported in elderly populations as well (for an overview see [22]). In an Austrian population of 1089 individuals aged 21 to 76 years, mean plasma 25-OH-D levels were lower in the women >60 years of age than in their younger counterparts (40.3±27 nmol/l *vs*. 54.3±36.8 nmol/l and 49.5±30.8 nmol/l in those aged <40 y and 40-60 y, respectively) and showed a weak but significant inverse correlation with age (r=-0.11, p<0.005) but this was not observed in the men (25-OH-D= 50.9±31.0 nmol/l in the <40 y-old to 56.5±30.8 nmol/l in the > 60 y-old). Moreover, 22.8% of the men and 30.2% of the women had values below 30 nmol/l. Intake of vitamin D did on average not reach the recommended level (2.78±1.13 µg/d in men and 2.23±1.10 µg/d in women) despite a slight but significant increase with age in the female group [26].

The high prevalence of 25-OH-D levels considered deficient that has been reported from various regions of the world positions vitamin D in a category with other critical micronutrients like iron, iodine, vitamin A or zinc and has raised concerns about its potential negative consequences for public health [48,49]. Such a pandemic as it is often termed raises the question about the validity of the cutoff values used to define deficiency. Indeed, there is still some controversy about the desirable level of 25-OH-D in the plasma or serum. So far, there is no definitive proof for benefits conferred by plasma 25-OH-D levels higher than 50 nmol/l at population level [13,44]. Part of the inconsistencies arises from the great variety of physiological functions accorded to vitamin D. Indeed, the definition of reference values has been mainly focused on bone health and maintenance while the evidence on the non-skeletal functions of vitamin D is still insufficient for this purpose [13,42].

However, under sun exposure plasma 25-OH-D concentrations in the range of 75 to 100 nmol/l are readily reached in healthy individuals, especially in those with light pigmentation [50,51]. This is also supported by the present study, where a marked seasonal variability was found with a significantly lower prevalence of vitamin D deficiency during the summer in all age groups. This is in accordance with reports from other regions in higher geographic latitudes and underscores the role of endogenous synthesis as the main source of vitamin D [52-56]. Notably, a study in Danish female adolescents and elderly women reported an influence of 25-OH-D concentration during the summer on the levels in the following winter. It was estimated that a 25-OH-D concentration of about 100 nmol/l was necessary during the summer to achieve the threshold level of 50 nmol/l in the following winter [56].

In the German GISELA Study 19.1% of the 162 participants between 66 and 96 years had plasma 25-OH-D levels <50 nmol/l (21.2% of the women and 14.3% of the men, n.s.) while values <25 nmol/l were not found. This notably lower prevalence of deficient vitamin D status may be related to the fact that all blood samples were obtained from July to September and that participants belonged to a highly-educated, health-conscious collective with a relatively high physical activity level and relatively high amount of time spent outdoors [57].

Therefore, the lower sun exposure associated with modern lifestyle or other reasons for sun avoidance in many individuals may lead to a high prevalence of vitamin D deficiency. The importance of endogenous synthesis is further exemplified by the overall low dietary intake of vitamin D also observed in our sample and its weak or even lacking association with plasma vitamin D levels. Indeed, across all age groups, less than 5% of the participants achieved the recommended intake amount of 15 µg/d, emphasizing the difficulty of covering the requirements for vitamin D through the diet. This suggests that the assessment of dietary vitamin D intake is not an adequate marker for the evaluation of vitamin D status and that plasma 25-OH-D and ideally also PTH concentrations have to be measured.

Against this background, the decrease of the capacity for endogenous synthesis with aging becomes relevant as a major cause of insufficiency in elderly. This effect of aging was described by MacLaughlinand Holick as early as 1985 who found that previtamin D_3 synthesis after exposure to UV radiation in 82 year-old persons had declined to 40% of the amount produced at the age of 8 years. Moreover, the concentration of 7-dehydrocholesterol, the precursor of vitamin D in the skin, also decreased with age as did skin thickness [21].The decline in skin thickness with age and its association with vitamin D synthesis was also observed by Need, et al. [58]. The lower cutaneous production of 25-OH-D_3 is also reflected by the lesser effect of season in the elderly subgroup observed in the present study. Nevertheless, it was also observed that elderly subjects could achieve a sufficient vitamin D status by adequate exposure to sunlight [59].

A small negative effect of overweight and obesity was found in children and elderly. Lower concentrations of 25-OH-D in overweight and obese individuals have previously been reported and are ascribed to various causes. For instance, it has been assumed that storage of the fat-soluble 25-OH-D in the adipose tissue reduces its availability in persons with a high fat mass [60]. This is in line with the relationship between plasma 25-OH-D and waist circumference that we found in the children subgroup. Another possible explanation suggested is that overweight and obese persons could spend less time on outdoor activities thus receiving less sun exposure. Alternatively or additionally, a causal role of vitamin D deficiency in the development of overweight an obesity has also been proposed based on the observation that the active form 1,25-$(OH)_2$-D acts as a regulator of adipocyte growth and differentiation through the vitamin D receptor that is expressed by these cells in their immature state [61].

Recently, genetic factors have also been shown to influence plasma 25-OH-D levels. In Caucasian study populations, this was particularly been reported of the gene GC that codes for the vitamin D transport protein in the blood and to a lesser degree of DHCR7 and CYP2R1, encoding the enzymes 7-dehydrocholesterol reductase and microsomal vitamin D 25-hydroxylase, respectively, that are both involved in the synthesis of active vitamin D. Carrying the minor polymorphisms was associated with lower plasma 25-OH-D concentrations and a higher risk for vitamin D insufficiency (up to two-fold) [55,62,63].

Regardless of the underlying causes for vitamin D insufficiency, the significance of a sufficient status of this nutrient is supported by a number of studies identifying vitamin D deficiency as a risk factor for some common non-communicable diseases such as cardiovascular diseases, hypertension, disturbances of glucose metabolism or diabetes, among others [4,14,38]. Therefore, we also looked at potential associations between vitamin D status and some metabolic health indicators that were also measured in the present sample. However, overall, the relationship between vitamin D plasma levels and cardiovascular as well as glycemic risk factors was small and were partly lost after adjustment for BMI or waist circumference, suggesting that these factors act as the causal effectors for both, vitamin D deficiency and increased cardiometabolic risk as discussed previously [14,64]. Indeed, an involvement of inflammatory markers and adipocytokines in the negative effects of obesity on metabolic health and insulin sensitivity in particular is now widely acknowledged and vitamin D has been shown to counteract these effects through its anti-inflammatory action [61,65]. In turn, blood lipids may rather play a role in the transport of vitamin D. In this regard, the weak positive association of plasma 25-OH-D with LDL cholesterol observed in the elderly subgroup is in line with the observations of Speeckhaert, et al.

who found an association of vitamin D-binding protein with blood lipoproteins [66].

Limitations of our study include its cross-sectional nature and the fact that the survey was not specifically designed to study vitamin D, particularly regarding its associations with disease. There was also no information available on skin type and sunlight exposure, both of which are important determinants of vitamin D status [10]. However, among its strength are the large sample size and its representativeness of the Austrian general population as well as the availability of both, data on biochemical status and dietary vitamin d intake assessed through a quantitative approach.

Nevertheless, the findings of this study suggest a risk for vitamin D deficiency at least in some parts of the Austrian population, namely children and older persons and especially during the winter months. In turn, the markedly better status during the summer months suggests that endogenous synthesis can be sufficient and underscores the importance of a regular sun exposure. A potential concern is the association of vitamin D insufficiency with overweight and obesity that has been described previously and was also found in the present study. This aspect might further add to the impact of the globally rising prevalence of excessive body weight on public health. Moreover, although good evidence for an association between vitamin D and metabolic health is still lacking, non-communicable diseases are the primary cause of death worldwide. Even if vitamin D is only one among many influencing factors, optimization of the status might contribute to improved health. It is, however, debatable whether the high plasma 25-OH-D levels recently proposed by some entities and a wide-spread supplementation with vitamin D are needed for health promotion. At least in healthy individuals, an adequate vitamin D status can likely be achieved by sufficient sun exposure preferably combined to physical activity, a proven health promoter on its own.

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