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ORIGINAL CONTRIBUTION

Prevalence of hypovitaminosis D and folate deficiency in healthy young female Austrian students in a health care profession

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Abstract
Purpose We performed a single-day cross-sectional study to assess the prevalence of vitamin D deficiency as well as folate status in healthy young female volunteers well educated with respect to health information.
Methods We assessed dietary intake of vitamin D and calcium, serum concentrations of 25-OH-vitamin D_3, folate, red blood cell folate and other dietary, laboratory, and lifestyle parameters in 215 young healthy women (age 18–30 years) on a single day at the end of the winter months. Primary aim was to investigate the prevalence of hypovitaminosis D. Folic acid status was a secondary study aim.
Results Mean daily ingestion of vitamin D was 2.25 μg/day with a daily calcium intake of 749 mg/day. 6.9% had hypovitaminosis D (25-OH-vitamin D_3 <30 nmol/L) and 89.3% were vitamin D insufficient (<75 nmol/L). Preplanned subpopulation comparison (lower vs. upper quartile) revealed a significant negative correlation ($P = 0.048$) between plasma PTH and 25-OH-vitamin D_3 levels. Fifteen individuals (6.9%) were folic acid deficient (<140 ng/mL RBC folate). Only 9.3% reached RBC folate concentrations regarded as optimal for the prevention of fetal neural tube defects (>400 ng/mL).
Conclusions The prevalence of hypovitaminosis D in healthy young women trained in health care professions is low but 89.3% can be classified as vitamin D insufficient in spring. Folate status can also be considered not sufficient. Considering the emerging role of higher vitamin D plasma levels for many health conditions, a timely correction of vitamin D status in the general Austrian population appears appropriate.

Keywords Vitamin D · Women · Diet · 25-(OH)-vitamin D · Hypovitaminosis D · Folic acid status · Calcium intake · Vitamin D intake

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Introduction

Vitamin D and folic acid deficiencies are common in healthy children and adults. Although vitamin D supplementation in infants has eradicated rickets as a major health problem, subclinical vitamin D deficiency and insufficiency are affecting a large fraction of men and women of all age groups, and in many geographic regions [1, 2]. Insufficient vitamin D status is mainly attributed to inadequate sunlight exposure and inadequate dietary supply. It is often associated with insufficient dietary calcium consumption [1, 2]. Vitamin D insufficiency also affects risk populations, such as pregnant women [3], infants, as well as postmenopausal women [1]. In pregnant women, this is associated with reduced bone mineral accrual in their offspring when examined after 9 years, suggesting that the fetal phase of growth has long-term effects on bone health and the risk of osteoporotic fractures later in life [3].

Recent health surveys also confirmed inadequate calcium, vitamin D, and folate intake in European countries, including Austria [2, 4, 5]. In Austria, a comprehensive study of calcium and vitamin D status in a large group of the apparently healthy adult population revealed vitamin D and calcium malnutrition in all age groups and more severe vitamin D deficiency (serum concentration <30 nmol/L) in 26% of the population during the winter months [2]. Levels of 25-OH-vitamin D_3 correlated negatively with increased PTH levels in both sexes, and this was most pronounced in individuals >60 years of age [2]. These data indicate that widespread recommendations for adequate diet (vitamin D, calcium supply) and/or sunlight exposure are not sufficient to achieve adequate status. Moreover, even adequate diet may not provide enough vitamin D to reach the serum concentrations currently considered sufficient for optimal health outcomes. This emphasizes the need for adequate sun exposure [6].

It is at present unclear to which extent educational factors affect adherence to recommendations for adequate vitamin D supply. We therefore investigated calcium and vitamin D nutritional status as well as the prevalence of hypovitaminosis D in a cross-sectional study of a well-educated population of young female students of health care professions (students of pharmacy, medicine, nursing, nutrition, and dietetics) in which awareness of health-related questions should be high. The study was carried out in all subjects on a single day at the end of the winter to test if the prevalence of hypovitaminosis D was lower in this well-defined cohort than in the general population of earlier reports [2].

Low folate levels are also a risk factor for young women at childbearing age because of a well-established correlation of low folic acid status in early pregnancy with an increased risk of neural tube defects (NTD) in offspring [7–9]. Red blood cell (RBC) folate concentrations below 140 ng/mL are considered as folate deficiency whereas NTD risk sharply decreases at concentrations above 400 ng/mL [7]. To prevent NTD, a sufficient folate status is important already during the periconceptual period [7], emphasizing the need for a continuous adequate folate supply in fertile women. Since food is not fortified with folic acid in Austria, we also investigated the folate status in the sample of young women recruited.

Subjects and methods

Study design

This cross-sectional mono-center study was designed to determine the prevalence of hypovitaminosis D in young female health care professional students. The study was initiated by student representatives as an interdisciplinary project with selected students involved in planning and conducting the trial under supervision of experienced investigators providing professional methodological support in all relevant disciplines. Blood sampling was conducted on a single day (April 2, 2008, before noon) to minimize the influence of vitamin D_3 production by sunlight exposure on data assessment. The study was approved by the Ethics Committee of the Medical University of Innsbruck.

Overall, female students studying pharmacy (University of Innsbruck), medicine (Innsbruck Medical University), dietetics and nutrition, or nursing (fhg, Innsbruck) were recruited as volunteers to participate in this investigation. The selection of this population was predefined to compare the results of health care–educated persons with the general Austrian population. All volunteers who responded to announcements of the study distributed on campus and who fulfilled the inclusion criteria were enrolled. With this process, we attempted to further select for participants with high awareness for their own health status. Inclusion criteria were female volunteers aged 18–30 years, signed informed consent, and participation in a health care profession educational program. On the day of the study, all participants signed a written informed consent after being informed about the study. After brief investigation by a medical doctor, a blood sample (forearm vein) was collected for the study. Sample collection was done by teams from the blood donor services of the University Hospital Innsbruck. Study participants had then the option to donate blood for a blood donation campaign. Questionnaires were filled in following blood sampling.

Dietary recall and lifestyle questionnaire

Since a major pre-specified objective of our study was to determine the average intake of calcium, vitamin D, and

phosphate, we assessed nutrition by combining a short-term and a long-term instrument using a single 24-h recall and a food frequency questionnaire (FFQ) for the last 4 weeks [10, 11]. Estimation of "true" calcium or vitamin D intake with reasonable precision for an individual was not aim of this study and would afford a different method [12]. The single 24-h recall was used to quantify the average intake of our group [10] rather than individual daily intake. The FFQ was used to estimate (selected) foods usually eaten and to rank individuals by food or nutrient intakes (semi-quantitative FFQ) in order to compare the characteristics of subgroups with high and low intakes [10, 13]. Since correlation coefficients for newly designed FFQs and those adapted from other questionnaires were found to be similar for calcium (0.54 vs. 0.55) [11], we slightly modified an existing FFQ [11] designed for assessing dietary calcium intake in the general population without age or gender discrimination. Since the additional assessment of vitamin D intake is limited to very few foodstuffs (such as eggs, liver, and fish), use of the same FFQ appeared justified.

For the 24-h recall, individual study participants underwent a structured interview by specifically trained personnel about the consumption of defined amounts of different foods within the last 24 h, including milk, yogurt, cheese, fluid intake, fast food, meal, vegetables, fruits, chocolate, eggs, potatoes, and fish. In addition, participants completed the FFQ about their food intake and nutritional behavior during the last 4 weeks under supervision of trained personnel. Estimates of vitamin D and calcium intake per day were calculated from nutrition databases using the software package soft and hard, Food-Control-Management-System FCMS/Diät 2000 (Version 7.0).

The questionnaire also assessed sports, smoking habits, and alcohol consumption. Participants were also asked about the location of sport activities (indoor, outdoor or both) in order to obtain an estimate of UV-radiation exposure even if the average UV-index is low in Austria during winter months and considered inadequate for sufficient vitamin D_3 production. Use of any medicinal products or food supplements was also documented for all study participants.

Laboratory parameters

Serum concentrations of calcium, phosphate, total alkaline phosphatase, and estradiol were measured by routine methods with a P 800 module (calcium, total alkaline phosphatase) and an E170 module (estradiol) from a modular analytics system (Roche Diagnostic GmbH, D-68298 Mannheim, Germany). Plasma concentrations of intact parathyroid hormone (PTH) and P1NP were determined with electro-chemoluminescence immunoassay (ECLIA) on the E170 system. Plasma 25-OH-vitamin D_3 concentrations were measured from EDTA-Plasma by ECLIA (E170 module). This vitamin D_3 assay employs a polyclonal antibody directed against 25-OH-vitamin D_3. Plasma folic acid concentrations and red blood cell (RBC) folic acid content were measured with a two-step competitive immunoassay using the IMMULITE 2000 folic acid assay (Siemens, Healthcare diagnostic Products, ltd. Lanberies, Gwynedd LL55 4EL, UK). The red blood cell folic acid content was determined in the hemolysate after incubation in ascorbic acid solution (0.5%). To avoid inter- and intra-laboratory variations, all samples were analyzed in the same laboratory within a few days after sample collection using a single lot of reagents for each analysis. All parameters were measured in single processing mode. In total, 215 samples of 225 recruited subjects could be analyzed for various parameters. In the remaining subjects, samples could either not be obtained or could not be analyzed ($n = 10$).

Statistical analysis

Statistical analysis was performed as predefined in the study protocol using SPSS software package. Continuous characteristics were expressed as mean ± SD, and categorical variables were displayed as frequencies. Associations between continuous variables were assessed using Pearson's correlation coefficient. For group comparisons, non-parametric Mann–Whitney test with effect size measures and confidence intervals was used. A P value <0.05 was considered statistically significant.

The primary study aim was to determine the prevalence of hypovitaminosis D prospectively defined as a plasma concentration of <30 nmol/L (12 ng/mL). This cutoff value was based on a previous study in the Austrian population [2]. Secondary criteria were the prevalence of folic acid deficiency (defined as ≤140 ng/mL; [7–9]), the prevalence of calcium and vitamin D malnutrition based on current recommendations of intake, the correlation of low 25-OH-vitamin D_3 plasma levels with the dietary uptake of calcium and vitamin D, plasma PTH, and plasma levels of the bone metabolism markers alkaline phosphatase and procollagen one N-terminal propeptide (P1NP). Recommended daily intake for the age of our study population is 700–1,000 mg for calcium and 5 μg (200 I.E.) of vitamin D (current recommendations of the Joint Austrian–German–Swiss Societies for Nutrition and the European Commission; [14]). All correlation analyses were preplanned as comparisons of the above parameters between the subpopulations of study participants within the lowest (Q1) and highest quartiles (Q4) of plasma 25-OH-vitamin D_3 levels of the overall population. This strategy ensured also the detection of differences not linearly dependent on 25-OH-vitamin D_3 concentrations. This is especially true

for plasma PTH concentrations, which were found to increase more steeply at 25-OH-vitamin D_3 levels below 30–50 nmol/L [15–17]. The cutoff points for the lowest and highest quartiles were calculated from the respective data post hoc as 39 and 59 nmol/L.

Sample size calculation was based on a significance level (α-error) of 0.05, a β-error of 0.20, and a power ($1-\beta$) of 0.80 to confirm a prevalence of hypovitaminosis D of less than 10% in study population. Considering a dropout rate of 5%, a total sample size of 225 was determined.

Results

Demographics

A total of 225 volunteers were recruited. For 10 volunteers, no blood samples could be analyzed for technical reasons, resulting in a dropout rate of 4% (expected 5%). In 215 volunteers, 25-OH-vitamin D_3 levels could be analyzed. This number is in accordance with the calculated sample size.

The average age of the study population was 22.1 years (SD 2.7), height 167 cm (SD 6), weight 59 kg (SD 7), and BMI was 21.1 kg/m^2 (SD 2.27). All healthy volunteers were female students of one of the health care professions in Innsbruck: 71 young female healthy volunteers were studying pharmacy (31.6%), 54 medicine (24%), 74 nursing (32.9%), 21 dietetics and nutrition (9.3%) and 5 others (2.2%). Forty-six healthy volunteers (20.4%) were current smokers. Sports activities were performed on average 2.6 (SD 1.76) days per week for an average of 1.27 (SD 0.71) hours per day. Alcohol was consumed on average 1.11 (SD 1.01) days per week in an amount of 2.09 (SD 1.51) drinks per week (Table 1).

Not only the predefined 25-OH-vitamin D_3 subgroups (Q1 vs. Q4) but also the other quartiles were comparable with respect to age and body mass index. The percentage of self-reported current smokers, reported alcohol consumption and sports activities (days/week) consistently decreased within the four quartiles and were significantly lower in the lowest 25-OH-vitamin D_3 quartile Q1 than in Q4 (for statistical comparisons within all quartiles see Table 1). No differences were found with regard to exercise outdoors activities.

About 50% of the students (108/225) reported use of hormonal contraceptives. Folic acid and vitamin D_3 were only supplemented in 8 subjects. Supplements containing vitamin D_2 were not taken.

Dietary intake

Overall average daily vitamin D intake was 2.25 μg/day (SD 4.92), reported via the 24-h—recall. Somewhat lower values were obtained with the 4-week FFQ 1.09 μg/day (SD 0.60). Therefore, 92% (24-h recall) and 100% (4-week FFQ) of the study population reported inadequate daily intake of vitamin D (i.e., below 5 μg/day).

Average daily calcium intake in the entire study population was calculated at 749 mg/day (SD 405) (24-h recall) and 1,194 mg/day (SD 548; (4-week FFQ). The average daily intake reported for phosphate was 1,026 mg/day (SD 395) (24-h recall) and 1,264 mg/day (SD 491) (4-week FFQ). Seventy-four percent and 46% reported inadequate daily intake of calcium (i.e., below 1,000 mg/day).

Daily vitamin D intake was essentially identical as reported for women below 40 years of age in the general population in Austria (2.3 μg/day).

Laboratory parameters

Mean 25-OH-vitamin D_3 plasma levels were 50.3 (SD 16.6) nmol/L and PTH concentrations were 45.9 (SD 14.6) ng/L. Mean serum concentrations for calcium and anorganic phosphate were 2.44 (SD 0.10) and 1.19 (SD 0.15) mmol/L, respectively.

The primary aim of the study was to determine the prevalence of hypovitaminosis D defined as plasma 25-OH-vitamin D_3 concentrations of less than 30 nmol/L (12 ng/mL). As shown in Fig. 1, 15 subjects (6.9%) were below this value. Eighty-nine percent of our study population can be considered vitamin D insufficient if plasma 25-OH-vitamin D_3 concentrations <75 nmol/L are taken as the currently most widely considered lower cutoff for insufficiency. Furthermore, 55.8% of individuals did not reach 50 nmol/L, a concentration considered to meet the needs of at least 97.5% of the population in the recently published report of the Institute of Medicine.

Another secondary study aim was to determine if 25-OH-vitamin D_3 plasma levels correlate with the dietary uptake of calcium, vitamin D, phosphate, plasma concentrations of PTH, and bone metabolism markers alkaline phosphatase and P1NP. This was achieved by the preplanned comparison of these parameters between the subpopulations of study participants within the lowest (Q1; post hoc <39 nmol/L) and highest quartiles (Q4, post hoc >59 nmol/L) of plasma 25-OH-vitamin D_3 levels of the overall study population. As illustrated in Fig. 2 and Table 1, PTH plasma concentrations were significantly higher in the Q1 subgroup as compared to Q4, although both groups remained within the normal range. This was supported by ordinary regression analyses revealing a borderline significant negative correlation between mean PTH and 25-OH-vitamin D_3 ($r = -0.13$, $P = 0.053$). Our findings demonstrate the correlation between higher plasma PTH and lower 25-OH-vitamin D_3 concentrations

Table 1 Demographic characteristics, nutrient intake, and laboratory parameters in the total study population as well as in all quartiles of 25-OH-vitamin D_3 (25-(OH)D_3) plasma concentrations used for correlation analysis

	All volunteers $N = 225$	Subpopulation Q1 25-(OH)-D3 <39 nmol $N = 57$	Subpopulation Q2 25-(OH)-D3 39–47 nmol $N = 54$	Subpopulation Q3 25-(OH)-D3 47–59 nmol $N = 49$	Subpopulation Q4 25-(OH)-D3 >59 nmol $N = 55$	Subpopulation 25-(OH)-D3 n.d. $N = 10$	P value
Age (years)	22.1 (2.7)	22.5 (2.5)*	22.4 (3.5)	21.4 (2.1)*	21.8 (2.6)	23.1 (2.6)	*$P = 0.036$ (Q1 vs. Q3)
Education (n, %)							
Pharmacy	71 (31.6)	31 (54.4)*$^{+\#}$	17 (31.5)$^{+}$	8 (16.3)$^{\#}$	13 (23.6)*	2 (22.2)	*$P = 0.0006$ (Q1 vs. Q4)
Medicine	54 (24)	11 (19.3)	14 (25.9)	16 (32.7)	10 (18.2)	3 (33.3)	$^{+}P = 0.024$ (Q1 vs. Q2)
Nursing	74 (32.9)	12 (21.1)	19 (35.2)	19 (38.8)	21 (38.2)	3 (33.3)	$^{\#}P = 0.001$ (Q1 vs. Q3)
Dietetics	21(9.3)	2 (3.5)	4 (7.4)	5 (10.2)	9 (16.4)	1 (11.1)	
other	5 (2.2)	1 (1.8)	–	1 (2.0)	2 (3.6)	1 (11.1)	
BMI (kg/m^2)	21.1 (2.27)	21.3 (2.3)	21.4 (2.6)	21.4 (2.5)	20.7 (1.7)	19.9 (1.5)	n.s.
Smoker (n, %)	46 (20.4)	6 (10.5)*	7 (13.0)$^{+}$	10 (20.4)$^{\#}$	23 (41.8)*$^{+\#}$	0 (0%)	*$P < 0.01$ (Q1 vs. Q4) $^{+}P = 0.001$ (Q2 vs. Q4) $^{\#}P = 0.020$ (Q3 vs. Q4)
Alcohol consumption[a]							
Days/week	1.11 (1.01)	0.9 (0.90)*	1.09 (0.90)	1.12 (0.93)	1.29 (0.98)*	1.60 (2.17)	*$P = 0.021$ (Q1 vs. Q4)
Drinks/week	2.09 (1.51)	1.9 (1.63)*	2.04 (1.28)$^{\#}$	2.03 (1.56)	2.4 (1.55)*	2.19 (1.71)	*$P = 0.035$ (Q1 vs. Q4)
Sport activities[a]							
Days/week	2.6 (1.76)	2.07 (1.77)*$^{+}$	2.28 (1.62)$^{\#}$	2.90 (1.69)$^{+}$	3.13 (1.76)*$^{\#}$	3.1 (1.91)	*$P < 0.01$ (Q1 vs Q4) $^{+}P = 0.004$ (Q1 vs. Q3) $^{\#}P = 0.008$ (Q2 vs. Q4)
Hours/day	1.27 (0.71)	1.24 (0.75)	1.36 (0.81)	1.17 (0.64)	1.28 (0.58)	1.56 (1.07)	n.s.
Exercise outdoors[a] (n, %)	109 (48)	27 (47)	25 (46)	23 (47)	28 (51)	6 (60)	–
Exercise outdoors[a] (hours/week)	3.50 (3.63)	3.26 (3.40)	3.10 (2.25)	3.22 (3.41)	3.54 (3.23)	7.08 (8.68)	–
Calcium intake (mg/day) 24 h recall	749 (405)	723 (437)	754 (409)	754 (396)	775 (375)	700 (496)	n.s.

Table 1 continued

	All volunteers $N = 225$	Subpopulation Q1 25-(OH)-D3 <39 nmol $N = 57$	Subpopulation Q2 25-(OH)-D3 39–47 nmol $N = 54$	Subpopulation Q3 25-(OH)-D3 47–59 nmol $N = 49$	Subpopulation Q4 25-(OH)-D3 >59 nmol $N = 55$	Subpopulation 25-(OH)-D3 n.d. $N = 10$	P value
Calcium intake (mg/day)	1,194 (548)	1,252 (607)	1,080 (463)	1,252 (516)	1,223 (604)	1,044 (400)	n.s.
4 week FFQ							
Vitamin D intake (μg/day)	2.25 (4.92)	1.58 (2.03)	2.30 (5.32)	2.19 (4.41)	2.65 (6.35)	4.09 (7.92)	n.s.
24 h recall							
Vitamin D intake (μg/day)	1.09 (0.60)	1.06 (0.16)	1.02 (0.47)	1.13 (0.50)	1.18 (0.86)	0.97 (0.32)	n.s.
4 week FFQ							
Phosphate (mg/day)	1,026 (395)	952 (407)	1,079 (359)	1,031 (403)	1,053 (407)	967 (439)	n.s.
24 h recall							
Phosphate intake (mg/day)	1,264 (491)	1,321 (537)	1,166 (404)	1,302 (466)	1,275 (604)	1,225 (438)	n.s.
4 week FFQ							
Plasma/serum laboratory parameters							
25(OH)D_3 (nmol/L)	50.3 (16.6)	32.4 (6.6)*	43.8 (2.5)*	52.7 (3.2)*	73.1 (10.7)*	–	*$P = < 0.01$ (Q1 vs. Q4) (Q1 vs. Q2) (Q1 vs. Q3) (Q2 vs. Q3) (Q2 vs. Q4) (Q3 vs. Q4)
Alk. phosphatase (U/L) (35–105)	57.8(14.7)	57.2 (13.1)	59.1 (14.5)	58.5 (13.1)	56.7 (17.9)	–	n.s.
PTH (ng/L) (15–65)	45.9 (14.6)	50.2 (16.5)*$^{+}$	43.5 (13.2)$^{+}$	45.7 (12.3)	44.0 (15.1)*	–	*$P = 0.048$ (Q1 vs. Q4) $^{+}P = 0.007$ (Q1 vs. Q2)
Estradiol (ng/L) (follicular phase 8–179; ovulation phase 47–518; luteal phase 40–284)	57.9 (69.2)	69.7 (77.4)*	67.1 (76.0)$^{+}$	63.4 (76.0)$^{\#}$	32.0 (32.2)*$^{+\#}$	–	*$P = < 0.01$ (Q1 vs. Q4) $^{+}P = 0.013$ (Q2 vs. Q4) $P = 0.032$ (Q3 vs. Q4)
P1NP (μg/L) (26–64)	52.4 (19.7)	53.8 (21.1)	57.7 (24.8)	54.7 (23.1)	52.1 (30.1)	–	n.s.
Plasma folate (ng/mL) (3–17)	8.24 (3.55)	8.00 (3.76)	8.09 (3.64)	8.72 (3.25)	8.23 (3.56)	–	n.s.
RBC folate (ng/mL) (calculated from plasma and whole blood folate)	251.7 (98.3)	243.9 (89.7)	248.4 (93.2)	255.2 (97.0)	259.7 (113.8)	–	n.s.
Calcium (2.09–2.54)	2.44 (0.10)	2.44 (0.09)	2.46 (0.10)	2.44 (0.11)	2.44 (0.11)	–	n.s.

Table 1 continued

	All volunteers $N = 225$	Subpopulation Q1 25-(OH)-D3 <39 nmol $N = 57$	Subpopulation Q2 25-(OH)-D3 39–47 nmol $N = 54$	Subpopulation Q3 25-(OH)-D3 47–59 nmol $N = 49$	Subpopulation Q4 25-(OH)-D3 >59 nmol $N = 55$	Subpopulation 25-(OH)-D3 n.d. $N = 10$	P value
Phosphate (0.87–1.45)	1.19 (0.15)	1.19 (0.18)	1.20 (0.14)	1.20 (0.15)	1.18 (0.14)	–	n.s.
Hormonal contraceptives (n, %)	108 (48.0)	24 (42.1)	21 (38.9)	27 (55.1)	35 (63.6)	1 (10.0)	–

Data are given for subpopulations of 25-OH-vitamin D_3 quartiles as described in the text. Data for individuals for which 25-OH-vitamin D_3 data could not be obtained (n.d., $n = 10$) are given as a separate subpopulation. Unless indicated otherwise, data are given as frequencies (%) or means ± SD

[a] Data are representative for previous 3 months. Reference intervals from our laboratory for women of the respective age group are given in parentheses. n.d

in a population of young healthy women as previously reported for adults and young children.

We also observed significantly higher estradiol plasma concentrations in the Q1 subgroup (as well as in Q2 and Q3) as compared to Q4 (Table 1), although all groups remained within the physiological range. This was supported by ordinary regression analyses revealing a significant negative correlation between mean estradiol and 25-OH-vitamin D_3 ($r = -0.257$, $P = 0.01$).

None of the other laboratory parameters was significantly different in the two groups, as was the case for the estimated daily calcium, vitamin D, and phosphate intakes (Table 1).

Interestingly, inspection of the distribution of 25-OH-vitamin D_3 concentrations (Fig. 1) indicated the presence of two populations with plasma concentrations ≤60 nmol/L ($n = 168$, 78%) and ≥62 nmol/L ($n = 47$, 22%; termed "high 25-OH-vitamin D_3 group"). We therefore analyzed whether these two populations differ with respect to the two major sources of vitamin D_3, that is, sun exposure and vitamin D dietary intake. No significant differences were found between these two groups. However, a significant difference in the hours spent exercising outdoors was found between the high 25-OH-vitamin D_3 group and the subpopulation with pronounced 25-OH-vitamin D3 deficiency (<30 nmol/L). In the latter, 80% of subjects exercised on average 2.63 (SD 3.77) hours/week whereas in the high 25-OH-vitamin D_3 group, 83% exercised on average 4.09 (SD 3.19) hours/week. This corresponds to a 1.6-fold higher exposure to sunlight in h/week ($P = 0.018$). Calcium and vitamin D dietary intake calculated by the 4-week FFQ were not different between these groups [vitamin D: 0.98 (SD 0.50) µg/day vs. 1.28 (SD 0.89) µg/day; $P = 0.308$; calcium: 1,323 (SD 755) mg/day vs. 1,283 (SD 622) mg/day, $P = 0.876$].

Our 25-OH-vitamin D assay preferentially detected 25-OH-vitamin D_3. However, this was unlikely to underestimate total 25-OH-vitamin D status because we could rule out significant sources of dietary vitamin D_2 by our food and lifestyle questionnaires (see below).

As a further secondary study aim, we also determined the prevalence of subjects with folic acid deficit defined as RBC levels below 140 ng/mL. For this purpose, RBC folic acid levels were determined as a measure of long-term folic acid status. The distribution of RBC folic acid concentrations is illustrated in Fig. 3. Mean RBC folate levels were 251.7 ± 98.3 ng/mL (Table 1), and plasma levels of folate were 8.24 ± 3.55 ng/mL. RBC levels correlated significantly with plasma folate concentrations ($r = 0.375$; $P = 0.01$).

Fifteen individuals (6.9%) presented with levels less than 140 ng/mL and were therefore considered deficient. None of these individuals showed signs of anemia or other laboratory abnormalities of red and white blood cell

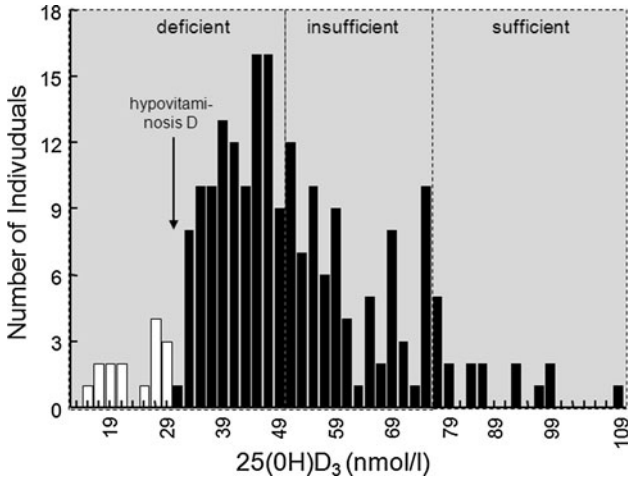

Fig. 1 Distribution of 25-OH-vitamin D_3 plasma concentrations. *Numbers* indicate bin centers (bin width = 2). Hypovitaminosis D was predefined as 30 nmol/mL (12 ng/mL) for comparability with a previous study in Austria. Other cutoffs are based on Holick [1]

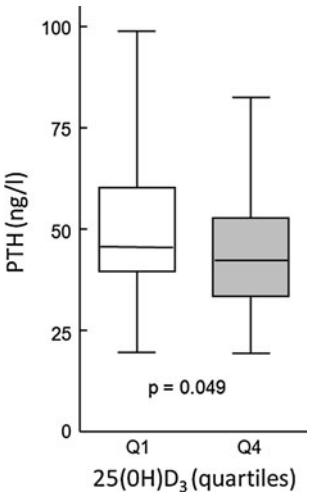

Fig. 2 Plasma PTH concentrations in the upper and lower quartiles of the study population. Significance was calculated using non-parametric Mann–Whitney test

parameters. Whereas RBC folate of <140 ng/mL is considered a risk factor for the development of anemia, periconceptual RBC folate concentrations of >400 ng/mL are currently considered optimal in young fertile women in order to minimize the risk for fetal neural tube defects (NTD). In our study population, only 20 individuals (9.3%) reached this concentration range (Fig. 3).

Discussion

We assessed vitamin D and folic acid status as well as dietary uptake of calcium and vitamin D in a country in which neither vitamin D (e.g., through supplementation of

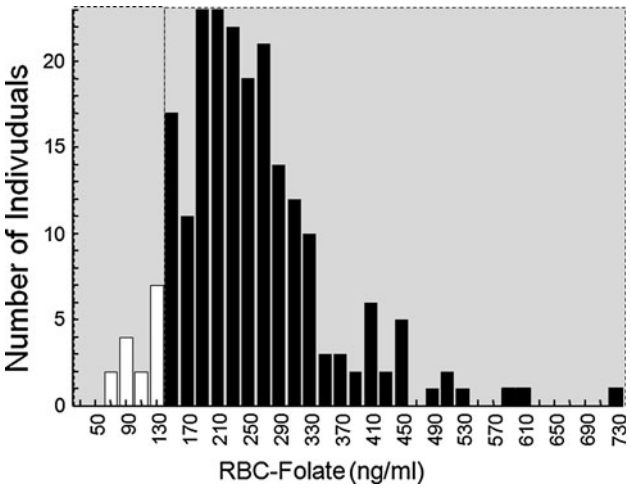

Fig. 3 Distribution of RBC folate concentration. *Numbers* indicate bin centers (bin width = 20). The *vertical line* indicates the cutoff for anemia (140 ng/mL)

milk) nor folic acid (e.g., through enrichment in cereal-grain products) fortification is enforced. In addition, exposure to UV-light during the winter months is estimated [18] to be too low to maintain efficient vitamin D_3 synthesis (mean noon UV-index <2 from November to February; UV research group, Medical University of Innsbruck, http://www.uv-index.at).

We selected a young female study population that would greatly benefit from an adequate vitamin D status especially with respect to long-term bone health and an adequate folic acid status with respect to minimizing the risk for fetal NTD. By selecting subjects currently undergoing training in a health care profession, aware of the role of nutrition for general health, and with efficient access to information regarding health-related topics, we could test whether the prevalence of hypovitaminosis D deficiency is lower in these subjects than previously reported for the general Austrian population. Furthermore, blood sampling and questionnaires were performed on a single day to minimize the influence of seasonal vitamin D production by sunlight on data assessment. The selected design thus excluded inter-day, inter-center, inter-age, inter-gender, and most inter-social status differences. The prevalence of smokers and weekly alcohol consumption were lower as reported for women of similar age groups in the Austrian population (smoking prevalence 29%, alcohol consumption 4.5 drinks/week ([19, 20]; cf. Table 1). This is in accordance with our effort to select a young population with a high awareness for its own health status.

We found that the prevalence of hypovitaminosis D (predefined as 25-OH-vitamin D_3 <30 nmol/L) was significantly lower (6.9%) than previously reported [2] in a large survey for the general population (26%, [2]), although the known inter-assay and inter-laboratory

differences must be considered when comparing 25-OH-vitamin D_3 data between studies [21]. The arithmetic mean (50.3, SD 16.6) in our population was very similar to that for women below age 40 in this previous study (54.7, SD 36.8) nmol/L. However, the lower standard deviation in our study is in accordance with the much higher homogeneity of our study population. Eighty-nine percent of our study population can be considered vitamin D insufficient if plasma 25-OH-vitamin D_3 concentrations <75 nmol/L are taken as the currently most widely considered lower cutoff for insufficiency [1, 22]. Our data also suggest that, due to very low dietary intake, vitamin D supply in our population must be mainly UV-derived. This is also in accordance with the observation that deficient individuals reported significantly fewer hours per week exercising outdoors. Compared to women below age 40 in the general Austrian population (560 mg/day), our study population reported higher daily calcium intake (Table 1) but the same vitamin D intake (2.25 μg/day vs. 2.3 μg/day in [2]). Therefore, a higher UV-exposure may contribute to the lower prevalence of hypovitaminosis D in our study.

Smoking has been reported to be associated with lower vitamin D levels [23–25]. However, smokers were less prevalent in our subpopulation with low 25-OH-vitamin D_3 levels (Table 1), and none of the 15 subjects with hypovitaminosis D reported current or previous smoking. Therefore, smoking is an unlikely explanation for the lower vitamin D status in our study population.

Despite our finding of a much lower prevalence of subjects with hypovitaminosis D, only 44.2 and 11% reached 25-OH-vitamin D_3 levels ≥50 and ≥75 nmol/L, respectively. These levels are currently considered to indicate sufficient vitamin D given that PTH levels begin to level off and intestinal calcium transport becomes maximal at these concentrations [1, 22]). In the absence of sufficient UV-exposure, regular supplementation of vitamin D_3 during the winter months appears as the only established strategy [1, 22] to maintain a sufficient vitamin D status as it is likely reached through sunlight exposure during the summer months in most individuals. Our questionnaire shows that vitamin D supplementation is uncommon in young women in Austria, as expected from the low recommended daily allowances according to current national or European recommendations (Joint Austrian–German–Swiss Societies for Nutrition and the European Commission; [14]). This is in contrast to children below 12 months of age and the elderly in which recommended daily uptake is not only higher but also enforced by supplementation. For the age group studied here, it is assumed that the currently (low) recommended daily supply for this age group (5 μg/day) can be reached by appropriate adherence to a vitamin D—rich diet and adequate sunlight exposure. Although possible in theory, our finding that dietary vitamin D supply is insufficient even in a well-educated population of young women with excellent access to health care information supports previous reports arguing against this assumption (Austrian Nutrition Report 2008; http://www.bmg.gv.at; [26, 27]), at least during the winter months. Our data also demonstrate that in young healthy women, plasma 25-OH-vitamin D_3 levels below <39 nmol/L (defining the cutoff for our lowest quartile) are associated with significantly higher plasma PTH concentrations than levels in the highest quartile (>59 nmol/L). Our cutoff point obtained for the lower quartile therefore suggests that plasma levels of at least 40 nmol/L 25-OH-vitamin D_3 protect from (periodic) PTH increase in this female population. Our finding that plasma PTH levels were significantly lower not only in Q4 but also in Q2 (Table 1) suggests a steep increase at the lowest 25-OH-vitamin D_3 levels. Such a non-linear relationship is in agreement with previous studies in postmenopausal women [28], medical inpatients [17] and young New Zealand children [16].

Taken together, despite a lower prevalence of hypovitaminosis D than in the general population, our selected female cohort would clearly benefit from an additional 500 IU/day (12.5 μg/day) of vitamin D_3 in addition to the 100 I.U. from daily dietary intake to reach the recommendation of 600 I.U./day recently published from the Institute of Medicine [29]. This should increase mean plasma 25-OH-vitamin D_3 levels by about 15 nmol/L [22, 30, 31], which may raise plasma levels above 30 nmol/L in our deficient subjects.

With the exception of estradiol, none of the other laboratory parameters determined showed a significant difference between these two 25-OH-vitamin D_3 subgroups. Estradiol levels were significantly lower in the highest quartile as compared to the lowest. Although our findings support previous data reporting higher plasma 25-OH-vitamin D_3 levels to be associated with significantly lower plasma estradiol (and progesterone) levels in a population of young healthy women [32], our data have to be interpreted with caution since our study design did not aim at controlling for hormonal contraceptive use and standardization of individual luteal phase of each volunteer.

Similar to vitamin D status, folic acid status was also insufficient (RBC folate <140 ng/mL) for 6.9% of our study population and only 9.3% reached RBC folate concentrations considered optimal for the prevention of fetal NTD (>400 ng/mL) [7, 9]. In the United States, the prevalence of folate deficiency in women in the age of 15–45 years decreased markedly from 37.6% before 1994 to 5.5% in 2003–2004 (NHANES survey, [8]). This is remarkably similar to our findings. Likewise, the reported median (248 ng/mL for age group 20–59 vs. 235 ng/mL in our study), 95% confidence interval (239–258 ng/mL vs. 239–265 ng/mL), and prevalence of optimal folate status

(about 12% estimated for total population from Fig. 1 in [8]) also closely resemble our data. Thus, the folate status in our study population, which has not been exposed to mandatory food fortification and in which folic acid supplements were only taken infrequently, is similar to the folate status reported for the US population in 2003–2004, a country with food fortification and more common supplement use. We did not attempt to quantify folate intake in our study population because the uptake of folate equivalents is difficult to assess.

Like in other countries, food fortification with folate is not recommended in Austria [33] because of safety concerns in population subgroups exposed to excess plasma levels, which may not benefit from fortification such as men at risk of prostate cancer [34, 35].

Taken together, our data indicate that hypovitaminosis D in Austria is infrequent in the young female population with full access to information about how to maintain adequate vitamin D status. However, dietary uptake is as low as in the general population and the prevalence of vitamin D insufficiency is high after the winter months. An additional daily 500 IU would be required from supplements to reach the 600 IU/day recommended by the Institute of Medicine [22, 29] and to minimize the prevalence of hypovitaminosis D in our study population.

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