Status of Calcium and Vitamin D of Different Population Groups in Austria

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Abstract: Vitamin D and calcium are essential for bone metabolism and the status of both nutrients is discussed in relation to osteoporosis and osteoporosis-related disorders. Thus, it is important to monitor the status of these nutrients in the population to early recognise insufficiences and to establish preventive measures. Intakes of calcium and vitamin D have been evaluated in different Austrian population groups (pre-school children, school children, adolescents, adults, elderly, pregnant and lactating women) by dietary records (3 d and 7 d weighed records, 24 h recalls). From pre-school children, school children, elderly, pregnant and lactating women fasting blood and spontaneous urine samples were collected and analysed on concentrations of calcium in serum and urine (adjusted to creatinine) and of 25-OH-cholecalciferol in serum.

Average calcium intake $(834 \pm 422 \text{ mg/d})$ below the specific recommended allowances of the according age group were found for 58% of the total population with the highest incidence of low supply in adolescents at 15–19 years of age and adults at 46–65 years of age. Vitamin D intakes $(2.1 \pm 1.4 \,\mu\text{g/d})$ were even lower with 68% of the total population being not able to meet the recommendations. Again, adolescents at 15–19 years showed the highest incidence of insufficient vitamin D intakes. Calcium concentrations in serum were satisfying for children and adolescents. Vitamin D status was found below the reference range (15 nmol/l) for 50% of all samples with the highest frequency in elderly people. Basing on the applied method for the determination of 25-OH-cholecalciferol concentrations a reference range for the Austrian population is suggested.

The results show that low intakes of calcium and vitamin D lead to decreased status of these nutrients especially for adolescents, elderly, pregnant and lactating women, indicating the highest risk for the development of disorders of bone metabolism in these groups.

Key words: Vitamin D, 25-OH-cholecalciferol, calcium, intake, status, pre-school children, school children, adolescents, adults, elderly, pregnancy, lactation, reference range

Introduction

Generally, the supply of the Austrian population with energy, major and minor nutrients is categorised as satisfying [1]. For selected groups, however, an insufficient supply and thus a critical status of single minor nutrients is to be expected (e.g. vitamin B₆, folic acid, zinc, iodine).

Of special importance in this background is the populations supply with calcium and vitamin D. Due to their biochemical interactions and their role in bone metabolism, both nutrients are of particular influence for maintenance of health and physical performance in almost all periods of human life. In all developed countries with increasing proportion of elderly people there is also an increased incidence of degenerative bone disorders

including related economical and social consequences [2].

Preventive measures which are able to avert or delay the occurrence of osteoporosis represent the best way to achieve and maintain a high quality of living. A recent press release from the World Health Organization stated that worldwide the majority of hip fractures occur in Europe and in North America. According to the International Osteoporosis Foundation, every 30 seconds someone in the European Union has a fracture as a result of osteoporosis and annual direct medical cost to treat 2.3 million osteoporosis fractures in Europe and the United States of America come up to USD 27 000 million [3].

Double emittor x-ray analysis (DEXA) is considered as the most important screening parameter for a satisfying bone mineralisation [4]. Studies of different subject groups in Austria showed a high incidence of an insufficient bone mineralisation indicating measures to minimise the risk factors for osteoporosis development to be one of the main targets for health policy. According to the health report of elderly people in Vienna from 1997 the age standardised incidence of osteoporosis adjusted to the elderly population (60 years and above) shows a twofold higher incidence for women than for men. For subjects at an age of 85 and above the yearly risk of a fracture of the femoral bone was found to be 2% for men and 4% for women. Mortal complications following fractures of the femoral neck can be found in 10% of the male and 6% of the female elderly people being delivered into the hospital for this reason at an age of 60 years and above [5]. Although dietary related factors do not represent the only factors of influence for osteoporosis prevention, programmes should be developed integrating these factors into according prevention measures.

It has been recognised that the predisposition for the development of osteoporosis-related degenerations is determined in the early periods of life [6]. Consequently, the regular screening of large population groups is of central importance for the early recognition of possible deficiencies and for the early establishing of correcting measures. The status of calcium and vitamin D was evaluated within the Austrian Study on Nutritional Status (ASNS), which comprises several population groups and applied a broad spectrum of biochemical markers.

Subjects and Methods

Involved population groups

Within several sub-studies of the ASNS combining different methodological aspects the involved subjects were also evaluated on their nutritional status, whenever possible by both measurement of nutrient intake and assessment of concentration of calcium and 25-OH-vitamin D₃ in serum. Starting with the study on Austrian school-children in 1991, in the mean-time results from laboratory assessment are available for Austrian infants (4-6 years, n = 35), young school-children and adolescents (7–9 years, n = 119, 10-12 years, n = 355, 13-14 years, n = 269, 15-19years, n = 365), pregnant women (25–35 years, n = 231) and elderly people (55–85 years, n = 78). Nutrient intake was calculated from weighed 7d-records (4–6 years, n = 89, 7-9 years, n = 429, 10-12 years, n = 760, 13-14 years,n = 517, 15-19 years, n = 378, elderly, n = 78) from weighed 3d-records (pregnant women, n = 231, lactating women, n = 43) and from 24-h-recalls (20–25 years, n =573, 26-35 years, n = 780, 36-45 years, n = 569, 46-56years, n = 342, 56–65 years, n = 183). From adults no serum and urine samples, and from pregnant and lactating women no urine samples were available. Dietary records and/or blood and urine samples from school-children and adults could be collected from all over Austria, for all other population groups samples were drawn mainly from Vienna and regions of Lower Austria.

Methods

Fasting blood samples were drawn from all participating individuals by puncture of the arm vein. For further determinations blood serum was separated by centrifugation and the samples were stored at -80° C until final laoratory assessment of the selected biomarkers. As far as the participants were willing to co-operate spontaneous urine samples were also collected and after allocating to separate vessels and acidifying with acetic acid the samples were also stored at -80° C until final determination.

For the determination of calcium concentrations in serum and urine a semi-automated analyser was used based on ion-selective electrodes (Nova Biomedical). Intra-assay variation for calcium determination measured by repeated analysis of certified standard serum (Boehringer Mannheim) was 2.9% and the inter-assay variation was 4.8% measured by analysis of pooled serum over several days. Since urine samples were collected spontaneously, creatinine concentrations for the adjustment of calcium excretion were determined according to the Jaffe-method.

Vitamin D-status in serum was measured by the radio-immunological assessment of the concentrations of 25-OH-Vitamin D_3 using a competitive protein bound radio-immuno assay containing [3 H]-25-OH-Vitamin D_3 (Bühlmann Laboratories, Basel). The intra-assay variation for this method was found within a range of 2–10% using standard solutions and 7% for serum samples; inter-assay variation for serum samples was 11% [7].

Results

Figure 1 shows a box-plot of urinary calcium excretion (mmol Ca/mmol creatinin) and serum concentrations of 25-OH-cholecalciferol (nmol/l serum) for the studied subject groups in Austria. The medians of urinary calcium excretion are steadily decreasing from the youngest age group (0.21 mmol/mmol creatinine) down to the group at 13–14 years of age (0.08 mmol/mmol creatinine) followed by an only small increase for the adolescents (0.11 mmol/mmol creatinine). Elderly people showed median calcium excretion which were on a relatively high level of 0.27 mmol/mmol creatinine but also revealed a high variation of these values. Due to the high standard deviations, no significant differences were observed on the 5%-level for the different groups studied.

Median serum concentrations of 25-OH-cholecalciferol are steadily decreasing from the age group of children at 4–6 years down to the group of 13–14 years followed by a small increase in the group of adolescents at 15– 18 years. The lowest serum concentrations were found for elderly and pregnant women. Again, no significant differences were observed on the 5%-level for the different groups studied. Table I shows the averages and standard deviation of the studied population groups for both intake and status of calcium and vitamin D. Calcium intakes of the total

Table I: Intake and status of calcium and vitamin D (25-OH-cholecalciferol) of different Austrian subject groups (mean \pm standard deviation)

age (years)	calcium intake* (mg/d)	status* (mmol/l serum)	vitamin D intake* (µg/d)	status* (nmol/l serum)
4–6	1095 ± 468	2.35 ± 0.16	2.3 ± 1.5	15.6 ± 10.9
7–9	770 ± 272	2.26 ± 0.20	1.5 ± 0.8	12.1 ± 6.9
10-12	747 ± 313	2.38 ± 0.34	1.7 ± 1.3	9.9 ± 6.1
13-14	726 ± 294	2.39 ± 0.21	1.7 ± 1.1	9.2 ± 6.9
15-19	743 ± 313	2.36 ± 0.29	1.6 ± 1.0	11.2 ± 8.1
20-25	870 ± 633	n.a.	3.1 ± 5.2	n.a.
26-35	867 ± 595	n.a.	4.1 ± 14.4	n.a.
36-45	840 ± 536	n.a.	4.2 ± 7.2	n.a.
46-55	804 ± 523	n.a.	4.8 ± 16.8	n.a.
56-65	774 ± 431	n.a.	4.4 ± 12.2	n.a.
> 65	734 ± 213	2.29 ± 0.09	2.5 ± 1.8	9.5 ± 10.7
pregnan	it 1009 ± 300	n.a.	2.9 ± 1.8	17.0 ± 33.1
lactating	g 1097 ± 399	n.a.	3.0 ± 2.5	n.a.
total	834 ± 422	2.27 ± 0.31	2.1 ± 1.4	11.0 ± 7.5

n.a.: not analysed.

^{*} no statistical significant differences were observed on the 5% level.

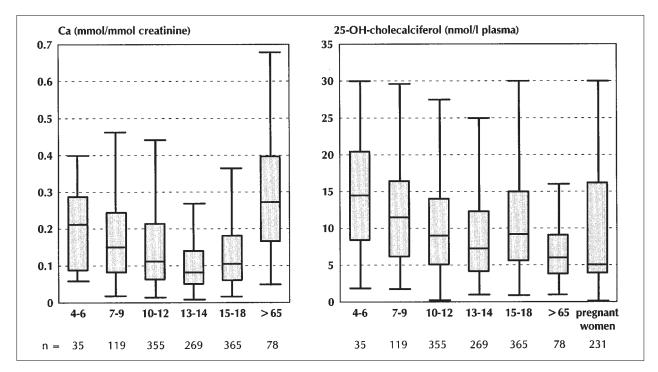


Figure 1: Box-Plot* of urinary calcium excretion** (mmol Ca/mmol creatinin) and serum concentrations of 25-OH-cholecalciferol (nmol/l serum) for the studied subject groups in Austria (no statistical significant differences were observed between the different groups on the 5% level)

^{*} whiskers represent minimum and maximum values, the box represents the interquartile range and contains the median

^{**} for adults no serum and urine samples were available, for pregnant women no urine samples could be collected, hence, results for these groups can not be shown.

population are on a rather low level with an average intake of 834 ± 422 mg/d. Children at an age of 4–6 years and pregnant and lactating women show the highest intakes of calcium reaching an amount of 1095 ± 468 mg/d, 1009 ± 300 mg/d, and 1097 ± 399 mg/d, respectively. Calcium status based on the determination of serum concentrations of children, adolescents and elderly were within a relatively narrow range of 2.26–2.39 mmol/l serum.

Vitamin D intakes of children at an age of 4–6 years reached a level of $2.3\pm1.5~\mu g/d$. All other age groups during childhood and adolescence showed lower intakes. The intake of vitamin D again increases during adulthood up to $4.8~\mu g/d$ accompanied by large variations within the age groups. For elderly as well as for pregnant and lactating women relatively low intakes of vitamin D (from $2.5-3.0~\mu g/d$) had to be noted. The average serum concentration of 25-OH-cholecalciferol for the total population in this study was $11.0\pm7.5~nmol/l$ with a clear deviation from this average observed for children at an age of 4–6 years ($15.6~\pm~10.9~nmol/l$) and pregnant women ($17.0\pm33.1~nmol/l$). No serum samples were available for adults and insufficient samples volumes for vitamin D determination could be collected for lactating women.

Discussion

The evaluation of nutrient intakes as well as serum concentrations of nutrients or their related biomarkers solely on the bases of their deviation from the recommended daily intake or the according reference values may lead to overinterpretation of low values especially when a high amount of data close to the according borderlines is found. Consequently, not only the estimation of the frequency of values below these reference ranges but also the estimation of the average deviation from the reference ranges for low values should be included in the classification of low intakes and concentrations of biomarker. Table II thus shows the frequency of values below the recommended dietary allowances and below the reference range and the average deviation of these values from the according borderline.

Although relatively high average intakes of calcium were found for preschool children at an age of 4-6 years still 13.5% of this population group show calcium intakes below the RDA of 700 mg/d based on the high variation of intakes. The percentage of insufficient calcium intakes compared to the according RDA is increasing through childhood and adolescence reaching a maximum at an age of 15-19 years, where RDA are highest, with 89.7% of observed calcium intakes below the RDA and an absolute intake of 743 ± 313 mg/d. During adulthood the amount of values below the RDA is quite constant between 68 and 74% of the age groups and maintains on this level for elderly people with an absolute calcium intake of 734 ± 213 mg/d. Pregnant and lactating women showed calcium intakes at a level of $1009 \pm 300 \text{ mg/d}$ and $1097 \pm 399 \text{ mg/d}$, respectively, leading to a frequency of low intakes between 41 and 53%. The evaluation of the deviation from the according RDA, however, showed that those values of the

Table II: Frequency of values below and average deviation from the recommended dietary allowances [8] and below the reference range [9]

age (years)		dietary	supply					statu	S		
	Ca- intake < RDA*	average deviation (%)	RDA (mg/d)	vit. D- intake < RDA*	average deviation (%)	RDA (µg/d)	Ca*	average deviation (%)	ref. (mmol/l	vit. D*	average deviation (%)	ref. (nmol/l)
4–6	13.5	-17.1	700	34.8	-19.7	5	2.8	- 1.0	2.1	28.6	-63.2	15
7–9	19.6	-29.9	900	27.3	<-30.8	5	5.9	-17.0	2.1	63.0	-68.5	15
10-12	41.3	-39.2	1100	45.5	-32.2	5	3.7	- 4.8	2.1	53.5	-73.6	15
13-14	48.7	-43.3	1200	50.9	-33.9	5	1.5	- 9.3	2.1	50.9	-75.5	15
15-19	89.7	-16.9	1200	94.4	-37.1	5	3.3	- 9.2	2.1	52.1	-71.3	15
20-25	68.2	-42.8	1200	85.5	-68.1	5	n.a.			n.a.		
26-35	67.8	-44.8	1000	80.8	-64.1	5	n.a.			n.a.		
36-45	67.5	-45.5	1000	77.0	-67.5	5	n.a.			n.a.		
46-55	72.5	-44.0	1000	74.6	-66.1	5	n.a.			n.a.		
56-65	73.8	-43.1	1000	81.4	-64.9	5	n.a.			n.a.		
> 65	71.8	-31.4	1000	78.2	-77.1	10	0.0	_	2.1	73.2	-78.1	15
pregnant	52.8	-21.2	1000	89.6	-51.4	5	n.a.	_	2.1	31.6	-79.1	15
lactating	40.5	-27.0	1000	85.8	-56.4	5	n.a.	_	2.1	n.a.		15
total	58.0	-38.2		68.8	-50		3.9	- 7.6		50.4	-71.7	

n.a.: not analysed.

^{*} percentage of values below the according recommendation (dietary supply) and below the according reference range (status), respectively.

young children (4–6 years) and adolescents (15–19 years) which were below the RDA had an average difference of –17% from this recommendation. This difference is considered as relatively small and can be easily improved by a small increase of the consumption of calcium rich food. Children between 7 and 14 years of age and during adult-hood both show high frequencies of calcium intakes below the according RDA and a high average deviation of these intakes of approximately –40% below the recommendation was found. These results require a clear emphasis on increasing the consumption of calcium rich food within nutrition programmes for children and adults. This has also to be recommended for elderly, pregnant and lactating women, although to a lesser extent but still with a high frequency of low intakes.

Similar tendencies were observed for the intakes of vitamin D. Again, adolescents at an age of 15-19 years show the highest incidence with 94% of vitamin D intakes below the recommended amounts, while the frequency of insufficient supplies in the younger age groups is clearly lower between approximately 27 and 51% (cf. Table II). During adulthood vitamin D intakes are on a higher level when measured in absolute amounts (between 3.1 and 4.8 μg/d) but the frequency of insufficient intakes is still on a high level of 75–86%. As can be seen from the standard deviations in Table II, adults show a very large variation in vitamin D-intake leading to the high frequencies of observed insufficient intakes. Elderly people at an age of 65 and older again show very low absolute intakes of $2.5 \pm 1.8 \,\mu \text{g/d}$ leading to a frequency of insufficiently supplied subjects of 78%. The recommendations of the German Society of Nutrition for vitamin D intakes of pregnant and lactating women have been reduced from 10 to 5 μ g/d in the current issue of the RDA [8]. However, still high frequencies of intakes below the recommendation together with an average deviation of -51--56% from the RDA were observed. Compared to the evaluation of calcium intakes the deviation of low vitamin D intakes reveals an even more unsatisfying situation for this nutrient in all age groups with the exception of the preschool children. Thus, the improvement of vitamin D intake by promotion of food consumption of good vitamin D sources, such as marine fish, is required.

For 25-OH-Cholecalciferol-concentrations in serum incidences of values below a normal range of 15 nmol/l were observed between approximately 29 and 63% for the children and adolescents, where the youngest age group showed the smallest amount of low serum concentrations. The highest amount of low values with 73% of all analysed blood samples was found for elderly people. Although pregnant women were not able to reach vitamin D intakes according to the RDA a relatively small frequency of

31.6% of low serum values was found for this population group. Again, the deviation of these low values from the reference range emphasises the need for the improvement of vitamin D intake.

Principally, the comparison of data from different subject groups and different methodology is rather critical. Therefore, a characteristic normal range has to be set for the study area and the applied method. Since only single values are available for Austria, international reference values were used for the characterisation of the data from the present study. However, a distinct classification of the available data as marginally or excessively supplied is not possible. Consequently, the values are classified as suboptimal and normal. The used reference ranges are depicted in the according figures or are given together with the selected parameters.

The calcium serum concentration underlies an extremely rapid and dynamic equilibrium with the calcium pool of the extracellular fluid with an efficient fine regulation by the exchange of calcium with the calcium pool of the bones [10]. An additional regulation of serum calcium levels occurs by alterations of gastrointestinal calcium absorption and renal clearance, while these modes of regulation play a less important role compared to the regulatory function of the bones [11]. Thus, the adaptability of the organism to changes of calcium metabolism is predominant for the physiological requirement of a finely regulated serum calcium concentration. The estimation of calcium status of the body on the basis of serum concentrations is rather limited. However, the evaluation of the variation of individual serum levels offers the possibility to determine the adaptability of the studied population groups, as it is likely that subjects with evidently decreased serum levels compared to the entire sample are less able to adapt easily and rapidly to unexpected deviations from calcium homeostasis.

The results of serum calcium determination can partly be seen as an indicator that primarily children and adolescents are in danger of an insufficiently rapid adaptation of calcium metabolism based on the evaluated high variations of individual serum levels. This is a critical situation especially for this population group as the skeleton is still growing and a rapid utilisation of serum calcium is required for bone formation [12]. The maximum peak bone mass is determined beside other factors by calcium bioavailability during bone growth, underlining the importance of a sufficient calcium supply of the bone particularly in young age in respect to the prevention of osteoporosis and osteoporosis-related disorders. The present data, however, do support this statement only in a limited way since other parameters which regulate calcium metabolism are not available and therefore should be complemented in further studies.

In contrast to homeostatically regulated calcium serum concentrations, the serum levels of 25-OH-cholecalciferol depend on dietary supply of vitamin D together with vitamin D production in the skin [13]. Thus, serum concentrations of this vitamin D-metabolite is considered as highly sensitive biomarker for human vitamin D-status [14]. As it is shown in Figure 1, the vitamin D supply of Austrian population groups with the exception of lactating women is below the normal range for healthy subjects of 15 nmol/l serum (6 μ g/l) for numerous individuals.

Unfortunately, there is no standardised method for the determination of serum levels of 25-OH-cholecalciferol, thus the comparison of different studies to vitamin D-status is only possible with some limitations. A meta study on the dependence of vitamin D-status from geographical latitude in different countries showed mean vitamin Dconcentrations on the basis of the determination of 25-OH-cholecalciferol in a range of 4.8 nmol/l (United Kingdom) to 68 nmol (USA) [15]. The high values in the USA are the result of the relative common food fortification of milk, butter and margarine with vitamin D. Austria with a mean concentration of 11 nmol/l is among the industrialised countries with the lowest vitamin D-serum levels. Main dietary sources of vitamin D in Austrian nutrition are marine fish and fish products and eggs. Since only very low amounts of marine fish are consumed in Austria compared to other European countries (6 kg/capita and year vs. an average consumption of 25 kg/capita and year in Europe [1, 16]) these low values have to be explained at least partly by this low fish consumption.

The vitamin D-supply of the Austrian subject groups has to be classified as suboptimal compared to other countries. However, the incidence of typical vitamin D-related deficiency disorders does not differ substantially from other European countries. As already mentioned, no standard methods for vitamin D-status assessment exist and, thus, no uniform reference ranges. Sauberlich suggested a guideline for the evaluation of vitamin D status with an acceptable/desired serum concentration of 25-OH-cholecalciferol of > 30 nmol/l and a low concentration between 12 and 25 nmol/l [17]. This again has to be seen as a reference range for populations with a high consumption of diets rich in vitamin D and no specific method for the determination of vitamin D serum concentrations is given. Consequently, a specific reference range for Austria for the classification of vitamin D status should be established due to the high variability of the different methods used. On the basis of the present results for serum concentrations of 25-OH-cholecalciferol Table III shows the suggested reference ranges for the classification of vitamin D-status using a competitive protein binding (CPB) radioimmunoassay with [3H]-25-OH-Vitamin D₃ for those Austrian subject groups with a sufficient amount of available

Table III: Suggested reference range for the classification of vitamin D-status using a competitive protein bound (CPB) radio-immunoassay with [³H]-25-OH-vitamin D₃ for Austrian subject groups (nmol/l serum following ethanol-extraction)

age (years)	25.–75. perc.	suggested r	eference ran	ige (nmol/l normal
4–6	7.5–16.0	11.3–21.5	10-<16	16–20
7–9	6.0 - 16.0	11.0-17.8	10 - < 16	16–20
10-12	5.3-13.8	9.0 - 18.5	10 - < 15	15-20
13-14	4.3 - 12.0	7.0 - 17.0	10 - < 15	15-20
15-19	5.5-13.8	8.8-20.1	10 - < 15	15-20
> 65	3.8-9.0	6.0 - 24.0	6–<9	10-25
pregnant	3.8-8.8	8.8-22.3	9-< 10	10-20

values. This reference range has been calculated on the basis of the 75th and 90th percentiles, which is a relatively conservative approach and has been chosen since dietary vitamin D supply of the studied subject groups in Austria has been classified as unsatisfying (cf. Table II). The calculated reference range for the group of children and adolescents is in accordance with the recommendations of the US Food and Nutrition Board for a sufficient serum concentration of 25-OH-cholecalciferol (11 nmol/l), which has also been used for the development of the new recommended dietary allowances [18]. Vitamin D requirements are highest during this age period for the development of the maximum peak bone mass, thus, a lower reference range can be established for the subject groups of higher age.

Interestingly, no additional recommendation has been formulated for pregnant women by the US committee since no low serum concentrations of vitamin D could be found even for intakes as low as 3.8 µg vitamin D/d when the subjects were sufficiently exposed to sunlight [19]. Thus, a lower reference range is suggested for pregnant women as well as for elderly people. No data are available for adults, further studies have to show whether the recommended reference range will be valid for this group as well. It is, however, likely that this recommendation can also be used for adults.

Conclusions

The present study on nutrition related factors influencing bone stability of Austrian subject groups shows that an optimisation mainly of vitamin D-status, but also calcium status is required as a measure for osteoporosis prevention. The development of an early health conscience is important since research has demonstrated that the prevention of osteoporosis and osteoporosis-related fractures may best be achieved by initiating sound health behaviour

early in life and continuing throughout life. The according optimisation of nutrition through a stronger focus on a higher intake of milk and low-fat milk products and mainly marine fish and fish products of all subject and age groups will be an important contribution. However, the promotion of a higher consumption of food rich in vitamin D has been rather ineffective during the past years. Therefore, the fortification of appropriate food with vitamin D (mainly margarine and other fat spreads) and with calcium (dairy products, fruit juices) seems to be a promising measure to be considered when discussing means of possibilities to improve vitamin D intake.

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