

NUTRITION, LIFE-STYLE PRACTICE, SERUM VITAMIN D CONCENTRATION AND BONE DENSITY IN HUNGARIAN ADOLESCENTS

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This study investigates the nutritional status, life-style practices, serum vitamin D and bone density of adolescents. A total of 326 adolescents, aged between 11 and 14 years, were involved in the present examination: after winter 110 boys and 97 girls (March 2002 and February 2003), and after summer 66 boys and 53 girls (October 2002 and 2003) were investigated. Energy and nutrient intake and life-style practices were assessed. Body mass and height were measured, body mass index was calculated. Serum concentration of 25-hydroxyvitamin D [25(OH)D] was determined. Bone mineral density of calcaneus was measured. Protein intake was higher than the Hungarian RDA. All of the students had vitamin D intake far below the RDA, 33.3% of boys and 43.2% of girls had Ca intake below 70% of RDA, and sodium intake was more than twice higher than the RDA. After winter, 2% of boys and 8% of girls were considered vitamin D deficient and 11% and 19% vitamin D insufficient, respectively. After summer, 24% of boys and 21% of girls had 25(OH)D level over the reference range. The mineral content of bone was insufficient in 12.9% of boys and 10.4% of girls, respectively. The majority of the students had physical activity only in school, incorporated in the timetable.

Keywords: nutritional status, life-style, serum 25(OH)D, bone mineral density, adolescents

The regulation of bone growth and maintenance of its health involves genetic, cellular, hormonal and environmental factors including diet and physical activity (PRENTICE et al., 2003). The adult bone mass is acquired up to early adulthood and the peak bone mass is the most important determinant for the risk of osteoporosis associated with fractures in later life (MAHAN & ESCOTT-STUMP, 1996). The other words, it means that the growth period of adolescent is a critical time for bone mineral accretion. To develop and maintain the optimal bone density and to delay the process of osteoporosis, it is essential to build up a primary prevention program for youth as early as possible. In this point of view the most important factor is to reveal the possible deficiencies connected with environmental factors and to correct them. The purpose of this study was to assess nutritional factors, serum vitamin D level and bone density of adolescents.

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1. Materials and methods

1.1. Subjects

The study groups comprised altogether 326 healthy schoolchildren aged 11–14 years. The adolescents were recruited from 6 districts of Budapest, the capital. Teachers and parents were informed about the study protocol both personally and in written form.

The study protocol was approved by the Regional Medical-Ethics Committee of the Semmelweis University, Budapest and carried out in accordance with the Declaration of Helsinki. Informed written consent was obtained from parents of all volunteers.

1.2. Study protocol

A cross-sectional survey of schoolchildren, living in the capital was carried out. At the end of winter season 2002 (March) and 2003 (February) 110 boys and 97 girls were enrolled in the study, and after summer time 2002 and 2003 (October) 66 boys and 53 girls participated in the examinations.

For the determination of sexual maturity, the Tanner grading system was used (VAUGHAN & LITT, 1995). Tanner stage of each student was assessed by a paediatrician.

1.3. Dietary assessment

Dietary information was obtained from a food-intake diary kept for 2 non-consecutive work days and Sunday (RURIK et al., 2003). The diary was filled in by the help of mothers and a qualified dietician interrogated all participants and specified the data. Energy and nutrient intake was calculated by our computer program, NutriComp (BIRÓ et al., 1999). To check for underreporting, the ratio of reported energy intake (EI) and estimated basal metabolic rate (BMR) according to SCHOFIELD and co-workers (1985) was used as proposed by GOLDBERG and co-workers (1991). Record with an EI:BMR ratio <1.1 indicating a non-plausible individual was excluded from further analysis. Similarly, when the ratio was ≥ 2.7 the subject was excluded because his/her record was over-estimated.

1.4. Anthropometric data

Body mass and height were measured according to the WHO recommendations (REPORT OF WHO, 1995). Weight was measured to the nearest of 0.1 kg (SECA 707 scale) and height to the nearest of 0.1 cm (SECA 220 body height). Body mass index (BMI, kg m^{-2}) was calculated.

1.5. Life-style indicators

Parents with their children completed a questionnaire regarding hours spent reading and watching television, outdoor and physical activity per day.

1.6. Biochemical analyses

All blood samples were obtained from the students between 08:00 and 09:00 h after an overnight fast in the school doctor's office. The blood samples were centrifuged ($2100\times g$, 15 min) within 2 h of venipuncture and sera were stored frozen at -20°C . The serum samples for 25-hydroxyvitamin D [25(OH)D] were protected from light during processing and measured by enzyme immunoassay [IDS OCTEIA 25(OH) Vitamin D kit, GB]. Participants were categorised according to their 25(OH)D levels: vitamin D-deficiency was defined when 25(OH)D was $<25\text{ nmol l}^{-1}$, insufficiency between $25\text{--}40\text{ nmol l}^{-1}$, sufficiency from >40 to 120 nmol l^{-1} , excess $>120\text{ nmol l}^{-1}$ (VIETH, 1999; CHENG et al., 2003).

1.7. Densitometry

Bone mineral density (BMD, g cm^{-2}) was detected by SAHARA Clinical Bone Sonometer (Hologic Ultrasoundmeter). The bone ultrasound measures the attenuation of ultrasound across the heel. Decreased mineral content was diagnosed if Z-score of BMD was between -1 and -2 and osteoporosis if Z-score was under -2.0 .

1.8. Statistical analysis

Data were recorded on data sheets and saved with the Data Ease program in the form of personal records. Statistical evaluation was carried out using SPSS for Windows 9.0. Comparisons between groups were performed with ANOVA. Gender specific Z-scores of BMD for 11–14 y adolescents were also calculated. Data are presented as means and standard deviations.

2. Results and discussion

Body mass and height increased gradually with sexual maturation in both males and females, BMI increased only in girls in concert with the maturation (Table 1).

From the factors that are known to influence bone status we primarily focused on nutritional aspects. We attached major importance to intakes of protein, sodium, calcium, vitamins D and C.

Considering that there was no significant seasonal fluctuation in the energy and nutrient intake data were combined. After validation of nutritional reports, the intakes of 129 boys and 118 girls were accepted. Daily energy intake was matching for gender and age, however, students took more than 12% of their energy from protein (Table 2). Mean calcium intake was lower than the RDA and 33.3% of boys and 43.2% of girls had intakes below 70% of RDA. Sodium intake was more than 2.5 times higher than the RDA. In spite of the fact that mean vitamin C intake was higher than the RDA, more than 20% of students had an intake below 70% of RDA. Vitamin D intake was much below the Hungarian RDA (Table 3).

Table 1. Characteristics of adolescents

Tanner stage (T)	N (%)	Age (year)	Body mass (kg)	Body height (cm)	BMI (kg m ⁻²)
Boys (n=176)					
1	31 (17.6)	11.2±0.50	39.9±11.22	145.7±7.24	18.6±3.96
2	44 (25.0)	12.0±0.78	44.9±11.85	152.3±7.54	19.0±3.69
3	44 (25.0)	12.8±0.74	53.5±16.38	161.0±6.84	20.4±4.93
4-5	57 (32.4)	13.4±0.58	57.6±11.00	170.5±5.74	19.7±3.04
Girls (n=150)					
1	11 (7.3)	11.1±0.30	34.5±5.83	142.7±5.04	16.9±2.59
2	20 (13.3)	11.3±0.47	45.0±10.53	152.1±7.63	19.3±3.29
3	52 (34.7)	11.8±0.85	46.8±8.71	156.3±5.80	19.1±3.09
4-5	67 (44.7)	13.1±0.77	56.8±12.50	161.5±7.13	21.7±3.96

Table 2. Daily macronutrient intake of adolescents

	Boys (n=129)		Girls (n=118)	
	mean	±SD	mean	±SD
Energy (kJ)	11239	2188	9594	1733
(kcal)	2685	522.8	2292	414.1
Protein (g)	97.6	20.62	82.0	19.13
en%	14.6	1.66	14.3	1.94
Fat (g)	104.0	25.61	89.9	24.33
en%	35.2	4.95	34.9	5.28
Carbohydrate (g)	334.5	77.7	284.1	53.65
en%	49.9	5.15	50.4	5.53
Fibre (g)	23.2	6.22	19.9	5.65

Table 3. Daily intake of some minerals and vitamins of adolescents

	Boys (n=129)		Girls (n=118)		RDA ^a in Hungary	Boys ^b	Girls ^b
	mean	±SD	mean	±SD			
Vit B ₆ (mg)	1.76	4.516	1.52	4.314	1.8	8.5	3.4
Vit C (mg)	87.1	54.64	88.6	67.22	60	20.2	22.9
Vit D (µg)	2.6	1.16	2.3	1.11	10	100	99.2
Calcium (mg)	896	333.3	763	276.1	1000	33.3	43.2
Magnesium (mg)	400	107.3	336	84.3	350	4.7	11.0
Copper (µg)	1.1	0.35	0.9	0.25	1	8.5	20.3
Zinc (µg)	9.5	2.65	7.7	2.21	9	10.9	27.1
Phosphorus (mg)	1272	282.5	1094	239.2	1000	0	0
Sodium (g)	6.0	1.31	5.1	1.39	2.00	100 ^c	100 ^c

^a Source: Tápanyagtáblázat. (Food composition tables.) Medicina Könyvkiadó, Budapest, 1999

^b % of adolescents with intake below 70% of RDA

^c % of adolescents with intake higher than 250% of RDA

Protein is a crucial nutrient as it takes part in the collagen formation and seems to be involved in the regulation of Ca absorption (KERSTETTER et al., 1998, BARON, 1999). Excess protein and sodium intake increases urinary calcium loss: each gram of protein causes an increment in urine calcium loss of about 1 mg, and every 2300 mg of sodium excreted via kidney pulls approximately 40 mg of calcium out with it (NORDIN et al., 1993; HEANEY, 1997). According to our calculation, the protein intake was around 2.1 g protein/kg body weight for boys and 1.8 g kg⁻¹ for girls. Although the ideal protein intake is still a matter of investigation, the FAO/WHO/UNU experts suggested that the safe level of protein intake for 11–14 years old adolescents is between 0.94–1.00 g kg⁻¹ depending on the age and gender (REPORT OF JOINT FAO/WHO/UNU, 1985). Data for sodium intake of our studies were enormously high. Considering that both the protein and sodium intakes were high, it may indicate that the urinary calcium output was increased in our adolescents. Calcium is one of the primary bone-forming nutrients. Recently, the results of an open randomised intervention trial provided evidence that a higher calcium intake (1125 vs. 746 mg/day) consumed by milk was associated with a significantly higher bone mineral accumulation in adolescent girls (CADOGAN et al., 1997). In our study, approximately 33% of boys and 43% of girls had calcium intake lower than 700 mg/day. Average calcium intake may vary country by country. Daily intake of calcium in Spanish adolescents from Galicia was 916 mg for boys and 823 g for girls, respectively (AMORIM-CRUZ, 2000). On the other hand, daily calcium intake in Austrian adolescents was somewhat less than 750 mg (KOENIG & ELMADFA, 2000), a value that is very similar to ours. Since the optimal calcium intake is not yet unequivocally defined, therefore it may vary from 1000 to 1600 mg/day for adolescents (BALLABRIGA, 2000; ROLLAND-CACHERA et al., 2000). Ascorbic acid is essential for the synthesis of collagen, which is the main element of organic matrix, in which calcium salts are deposited to give bone great strength (SMITH, 2000). Despite the fact that the average intake of vitamin C was higher than the RDA, still approximately 20% of our adolescents had insufficient vitamin C intake.

Vitamin D mediates Ca homeostasis by stimulating absorption in the gut and resorption from bone, and by allowing Ca to circulate, increases bone formation. It is generally accepted that vitamin D intake by food is insufficient (KOENIG & ELMADFA, 2000; LEHTONEN-VEROMAA et al., 2002; CHENG et al., 2003). The results of our studies support this observation. Nevertheless, vitamin D can be obtained from three sources; the main dietary sources are fishes rich in fats, the consumption of which is very low in Hungary (around 2.7 kg/year for the population). It could also come from fortified milk and fortified dairy products, however, this is not applied considerably in the Hungarian practice. Margarine (the vitamin D content of it is not higher than that of butter) and supplements, which are consumed periodically and inconsistently, also represent sources of vitamin D. The main source of vitamin D is the biosynthesis of the molecule taking place in the skin. Due to the last possibility, we investigated vitamin D status after winter and summer. Out of 176 boys and 150 girls 166 and 144 came for blood sampling. In boys the mean levels of 25-(OH)D were 57.5±15.49 nmol l⁻¹ (n=100) after winter and 103.2±32.41 nmol l⁻¹ (n=66) after summertime, respectively, and the

difference was significant ($P < 0.001$). In the serum of girls the vitamin levels were as follows: $53.0 \pm 20.13 \text{ nmol l}^{-1}$ ($n=91$) after winter and $95.3 \pm 35.70 \text{ nmol l}^{-1}$ ($n=53$) after summer. The difference was also significant ($P < 0.001$).

There is now consensus that serum 25(OH)D concentration is a reliable indicator of vitamin D status, nevertheless, the optimal 25(OH)D concentration is not yet defined (PRENTICE et al., 2003). At present, most of the scientists agree on the cut-off points, which were also used by us (HOLICK, 1996; VIETH, 1999). After winter, vitamin D deficiency was recorded in 2% of boys and 8% of girls and insufficiency was detected in 11% of boys and 19% of girls, respectively. No vitamin D insufficiency was observed after summer, but 24% of boys and 21% of girls had serum 25(OH)D higher than the upper limit of the reference range (Fig. 1). Seasonal variation in vitamin D status was observed in young children as well as in adults (DAVIES et al., 1999; BROTH et al., 2001; VIETH et al., 2001). The higher level of 25(OH)D after summer should not be exclusively attributed to the increased exposure to sunlight as vitamin D supplementations has to be taken into consideration, as well. Although, the questionnaire contained inquiry on eventual use of supplements, answers were partly obscured or the vitamin intake was irregular. Likewise, we failed to receive informative answers for outdoor activities, nevertheless 22% of boys and 30% of girls were taking sun-bath.

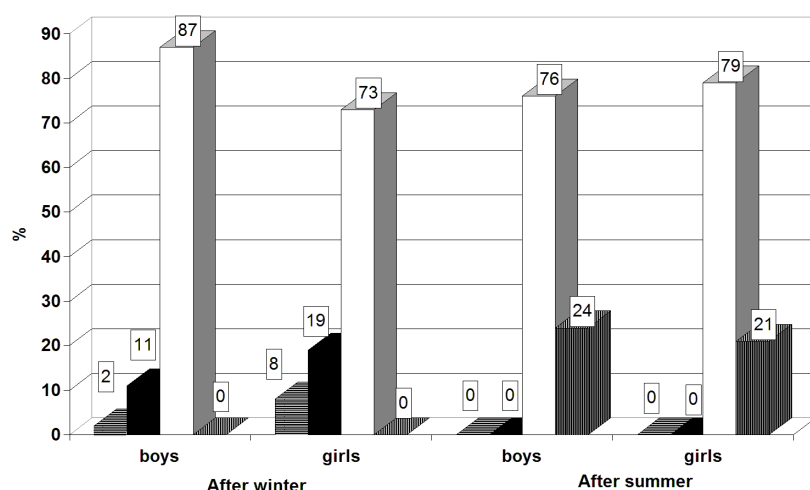


Fig. 1. Percent distribution of adolescents on the basis of their serum 25(OH)D content. $\square$: $<25 \text{ nM l}^{-1}$; $\blacksquare$: $25-40 \text{ nM l}^{-1}$; $\square$: $40-120 \text{ nM l}^{-1}$; $\blacksquare$: $>120 \text{ nM l}^{-1}$

Mean bone mineral density values measured by ultrasound showed only a mild fluctuation in association with maturation in both genders. On the basis of Z-score, one boy and one girl had osteoporosis ($Z\text{-score} < -2$), decreased bone mineral content

(Z-score was between -2 – -1) was seen in 12.9% of boys and 10.4% of girls. It is to be noted that the prevalence of decreased bone mineralization manifested earlier in boys than in girls, i.e. in boys decreased BMD reached the plateau from Tanner stage 2, whereas in girls it was attained from Tanner stage 3 (Table 4).

Table 4. Bone mineral density and maturation

	Tanner stage				
	1	2	3	4–5	Total
	BMD (g cm^{-3}) mean $\pm$ SD				
	Boys				
	0.51 $\pm$ 0.054	0.52 $\pm$ 0.095	0.51 $\pm$ 0.080	0.50 $\pm$ 0.078	0.51 $\pm$ 0.079
	Girls				
	0.49 $\pm$ 0.050	0.50 $\pm$ 0.064	0.54 $\pm$ 0.104	0.59 $\pm$ 0.138	0.56 $\pm$ 0.116
Z-score	n (%)				
	Boys				
< -2	–	–	1 (0.7)	–	–
> -2 – ≤ -1	2 (1.4)	6 (4.1)	5 (3.4)	6 (4.1)	19 (12.9)
-1 – > $+1$	26 (17.7)	31 (21.1)	31 (21.1)	39 (26.5)	127 (86.4)
Total	28 (19.0)	37 (25.2)	37 (25.2)	45 (30.6)	147 (100)
	Girls				
< -2	–	–	1 (0.7)	–	–
> -2 – ≤ -1	1 (0.7)	1 (0.7)	6 (4.4)	6 (4.4)	14 (10.4)
-1 – > $+1$	8 (5.9)	18 (13.3)	41 (30.4)	53 (39.3)	120 (88.9)
Total	9 (6.7)	19 (14.1)	48 (35.6)	59 (43.7)	135 (100)

BMD: bone mineral density

Lack of physical exercise and a sedentary life-style may have contributed to the inadequate accumulation of bone mass (MAHAN & ESCOTT-STUMP, 1996). In our study only 22% of boys and 14% of girls were involved in regular sporting activities in various sport clubs, the other students led a sedentary life-style.

3. Conclusion

Osteoporosis is a major public health problem not only for the aging population, but also for the younger generation with adverse changes in lifestyle and diet (BUNKER, 1994). In Hungary, the estimated prevalence of osteoporosis is 9%, i.e. around 600 000 women and 300 000 men are suffering from osteoporosis (LAKATOS, 2003). Results of our study appear to demonstrate that the risk factors for osteoporosis associated with nutrition and life-style are already present in adolescents. This fact may explain why around 0.9 million persons are suffering from osteoporosis in Hungary, a situation that calls for the implementation of an intervention program.

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