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Vitamin D intake is low and hypovitaminosis D common in healthy 9- to 15-year-old Finnish girls

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Objectives: To study the prevalence of hypovitaminosis D, the effect of vitamin D supplementation on serum 25-hydroxyvitamin D [S-25(OH)D], and the intakes of vitamin D and calcium in Finnish 9- to 15-year-old athletic and nonathletic girls.
Design: 1-year follow-up study (February 1997-March 1998) with three months of vitamin D supplementation (10 μg/d) from October to January.
Setting: Turku University Central Hospital, Finland.
Subjects: 191 female volunteers aged 9–15 y (131 athletes and 60 controls).
Methods: Vitamin D and calcium intakes were estimated by a four-day food recording and a semi-quantitative food frequency questionnaire (FFQ). S-25(OH)D was followed by radioimmunoassay (RIA).
Results: At baseline the mean S-25(OH)D concentration was 33.9 nmol/l among all girls. In winter severe hypovitaminosis D (S-25(OH)D < 20 nmol/l) occurred in 13.4% of the participants and in 67.7% S-25(OH)D was below 37.5 nmol/l. By the next summer the mean S-25(OH)D concentration was 62.9 nmol/l and in 1.6% of the subjects it was below 37.5 nmol/l. The prevalence of severe hypovitaminosis D was not significantly reduced by three months of vitamin D (10 μg/d) supplementation. At baseline, the mean intake of vitamin D was 2.9 μg/d by food recording and 4.3 μg/d by FFQ. The mean calcium intake was 1256 mg/d and 1580 mg/d, respectively. The intakes of vitamin D and calcium remained unchanged during the follow-up period. The athletes consumed more calcium than nonathletic controls, whereas the intake of vitamin D was quite similar among both groups. The vitamin D intake by FFQ correlated with the S-25(OH)D concentration in wintertime ($r = 0.28$, $P < 0.01$).
Conclusion: Hypovitaminosis D is fairly common in growing Finnish girls in the wintertime, and three months of vitamin D supplementation with 10 μg/d was insufficient in preventing hypovitaminosis D. The daily dietary vitamin D intake was insufficient (< 5 μg/d) in the majority of participants, while the calcium intake was usually sufficient.
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Descriptors: calcium intake; hypovitaminosis D; serum 25-hydroxyvitamin D; vitamin D intake

Introduction

The sources of vitamin D are the diet and cutaneous synthesis under the influence of sunlight. At high latitudes, where there is only little sunlight in the winter there is reduced production of vitamin D for 4–6 months of the year (Webb *et al,* 1988). The recommended dietary allowance of vitamin D is 5 μg/d among 4- to 60-year-old subjects in Finland. Since the natural dietary sources of vitamin D are limited, rickets was previously fairly common in Finland, as in the other Scandinavian countries, until supplementation of the diet with vitamin D was started in the 1940s (Hallman *et al,* 1964). Currently vitamin D deficiency occurs in elderly people in Europe (Heikinheimo *et al,* 1996; Lips *et al,* 1996) and it may be an important risk factor for osteoporotic hip fractures (Lips *et al,* 1987), as implied by the fact that supplementation with vitamin D and calcium reduces the risk of hip fractures and other nonvertebral fractures among elderly women (Chapuy *et al,* 1992; Dawson-Hughes *et al,* 1997). Many studies have shown a significant correlation between the dietary vitamin D intake and the serum 25-hydroxyvitamin D [S-25(OH)D] concentration in the elderly (Lips *et al,* 1987; Krall *et al,* 1989; Salamone *et al,* 1993; Thomas *et al,* 1998). Vitamin D from the diet is clearly important in the northern countries like Finland (Lamberg-Allardt, 1984).

The most important source of calcium is milk products. Adolescents absorb a greater percentage of the calcium in their diet than adults. The benefits of a high calcium intake with respect to the development of the skeleton is most

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apparent in puberty (Abrams *et al,* 1997). Several studies have shown that the intake of calcium is important for the development of the bone mineral density (Johnston *et al,* 1992; Chan *et al,* 1995). The daily recommended intake of calcium among peripubertal girls is from 700 to 900 mg/d in Finland.

The purpose of this study was to investigate the daily dietary intake of vitamin D and calcium and to compare dietary intake of vitamin D with the S-25(OH)D concentration in the athletic and nonathletic 9- to 15-year-old girls during one year follow-up. We hypothesised that athletes may consume more vitamin D and calcium than controls owing to higher energy expenditure. The aim was also to evaluate the effect of vitamin D supplementation on the S-25(OH)D concentration in wintertime.

Subjects and methods

Subjects
The study group comprised 191 healthy Caucasian girls aged 9–15 y (66 competing gymnasts, 65 competing runners, and 60 nonathletic controls). The participants were enrolled as volunteers who were recruited from October 1996 to January 1997 from local sports clubs and schools in the city of Turku and its vicinity. The participant was considered as pursuing competitive athletics if she had participated regularly in competitive sports at a local, provincial or national level for at least one year. The group of runners consisted of long-distance runners and orienteers. A subject was referred to the control group if she did not participate in any kind of regular or organized sports. All participants were healthy and had no chronic diseases that could affect growth or the metabolism of calcium or vitamin D.

The study protocol was approved by the joint ethics committee of the Turku University and the Turku University Central Hospital. The study was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all volunteers and their parents.

Study design
All subjects were studied at baseline over an eight-week period in February–March 1997. Weight and height were recorded. Height was measured with a fixed stadiometer (Harpenden Stadiometer; Holtain, Crymych, UK) and weight with a regularly calibrated electronic scale (EKS exlusive, EKS International, Sweden). The body mass index was calculated and recorded (kg/m^2). All measurements were made between 0800–1000 h by the same observer (M L-V). The measurements were repeated six and twelve months later. At the six-month visit multivitamin supplementation (Optivit® (Leiras, Turku, Finland) containing vitamin D10 μg) was given to all participants. The participants were asked to take one tablet per day from the beginning of October for at least three months. They stopped the supplementation, which continued for three months, one month before the end of the follow-up period. Calcium supplementation (Puru-Calsor® (Orion, Espoo, Finland) containing 500 mg calcium) were given to those who consumed calcium less than 1000 mg/d.

Questionnaires
Questionnaires were administered to all subjects to determine vitamin D intake, calcium intake, physical activity, and medical history. All travels to lower latitudes and the duration of any such visits were also documented on the questionnaire. If the participant was a young child all questionnaires were answered by one of the parents together with the child, otherwise by the participant herself.

Assesessment of nutrient intake
Interviewer administered semi-quantitative food frequency questionnaires (FFQ) included questions on supplement use and pictures of portion sizes and were used to estimate the intakes of vitamin D and calcium. Two FFQs were administered: one covering the preceeding three months (vitamin D) and another covering the preceeding 1 month (calcium). The questionnaires have been used previously in a study by Välimäki *et al* (1994). The subjects kept a 4-d food recording for three weekdays and one weekend day. Daily intakes of energy, total fat, carbohydrate, vitamin D and calcium were calculated by a trained dietitian who used a validated Finnish nutrient data base (Rastas *et al,* 1993).

Laboratory studies
Morning blood samples were taken of the participants after an overnight fast. Local anesthetic patches (Emla® Astra, Södertälje, Sweden) were used to reduce the discomfort of venipuncture. Blood samples for determination of S-25(OH)D were centrifuged within two hours of venipuncture (2110 g) and frozen at -20°C. The serum samples for 25(OH)D were protected from light during processing. Samples obtained at each study visit were assayed in one run by RIA (Incstar Corporation, Stillwater, Minnesota, USA). The samples were run in duplicate; the interassay coefficient of variation was 8.3% at the level 35.3 nmol/l ($n = 50$) for 25-hydroxyvitamin D. Routine blood chemistry included serum calcium and alkaline phosphatase and was analysed by Hitachi 717 and 917 analysers (Hitachi Ltd, Tokyo, Japan).

Hypovitaminosis D
We defined severe hypovitaminosis D as S-25(OH)D below 20 nmol/l and moderate hypovitaminosis D as S-25(OH)D between 20 and 37.5 nmol/l (Thomas *et al,* 1998). The definition of hypovitaminosis D was determined from published data demonstrating that serum parathyroid hormone concentration is inducing an increase in patients who have serum 25(OH)D concentrations ≤ 37.5 nmol/l (Thomas *et al,* 1998).

Statistical analysis
The SAS 6.12 statistical software (SAS Institute Inc., Cary, NC, USA) for Windows were used to run the statistical analyses. The results were expressed as means ± standard deviation (s.d.). Comparisons of means between groups were done with two-sample *t*-tests. Fisher's exact tests were performed to compare proportions between athletes and controls. Paired sample test t-tests were used in comparing means between questionnaire and food record and Mc Nemar's tests in comparing corresponding paired sample proportions. If the assumptions of *t*-tests were questionable, corresponding non-parametric tests were also performed (Mann-Whitney test with unpaired data and Wilcoxon signed-rank test with paired data). There were no practical differences in results. Spearman's rank correlations were used in correlation analyses. The significance level was set at $P < 0.05$.

Results

One hundred eighty-six girls completed the one year follow-up; five participants discontinued after baseline evalution for reasons unrelated to the trial design. The baseline data of the study subjects are presented in Table 1. There were no significant differences between the groups concerning height or weight. The athletes consumed significantly more energy, carbohydrate, total fat and protein than the controls.

Intakes of vitamin D and calcium

The mean daily intake of vitamin D was quite similar among the athletes and the controls as assessed either by food recording or FFQ throughout the three evaluation periods (Figure 1). The athletes consumed significantly more calcium than controls during the whole study period, when the intake of calcium was estimated by both methods (Figure 1). The figures describing vitamin D intake at baseline were significantly lower by food recording than by FFQ (2.9 μg/d (s.d. 1.5) *vs* 4.3 μg/d (s.d. 2.1), $P < 0.001$). The corresponding figures for calcium intake were 1256 mg/d (s.d. 426) *vs* 1580 mg/d (s.d. 626), $P < 0.001$, respectively. The Spearman correlation coefficient for the intakes of calcium ranged from 0.54 to 0.64 ($P < 0.001$) and for the intakes of vitamin D from 0.27 to 0.44 ($P < 0.05$) between these two methods during the study.

Twenty-two percent of the participants took vitamin D supplementation at least four times a week at baseline and no less than 93.4% at the 12-month visit. The corresponding figures concerning calcium supplementation were 4.2% and 19.8%. There were no significant differences in the use of supplementation among the athletes and controls.

Serum 25-hydroxyvitamin D concentration and intake of vitamin D

At baseline, which was in midwinter (February–March) 1997, the mean S-25(OH)D concentration in all participants was 33.9 nmol/l. The participants who took vitamin D supplementation at the beginning of the study had a significantly higher S-25(OH)D concentration than the participants who did not (43.3 nmol/l *vs* 31.2 nmol/l, $P < 0.001$) (Table 2). The S-25(OH)D concentration at the six-month visit, which was in summertime (August–September) 1997, was significantly higher than the baseline and the 12-month values. Although 93.4% of the participants took vitamin D supplementation 10 μg/d (at least four times a week) during three months between October 1997 and January 1998, the mean S-25(OH)D concentration was only 33.7 nmol/l at the end of the study (Table 2).

Table 1 Baseline characteristics of study subjects

Variable	*Athletes*	*Controls*	*P*
Number	131	60	
Age (y)	12.9 (1 8)	13.1 (1.7)	NS
Height (cm)	155.0 (10 7)	156.4 (9.7)	NS
Weight (kg)	43.9 (10 3)	46.5 (10.4)	NS
BMI (kg/m^2)	18.0 (2 3)	18.8 (2.7)	< 0.05
Energy intake (KJ/kg/d)	193.2 (50 0)	164.8 (45.4)	< 0.001
Total fat intake (g/d)	72.1 (20 8)	64.9 (18.2)	< 0.05
Protein intake (g/d)	77.4 (17 9)	68.0 (16.9)	< 0.001
Carbohydrate intake (g/d)	241.3 (62 5)	218.6 (50.9)	< 0.05

Results are mean (s.d.); NS, not significant.

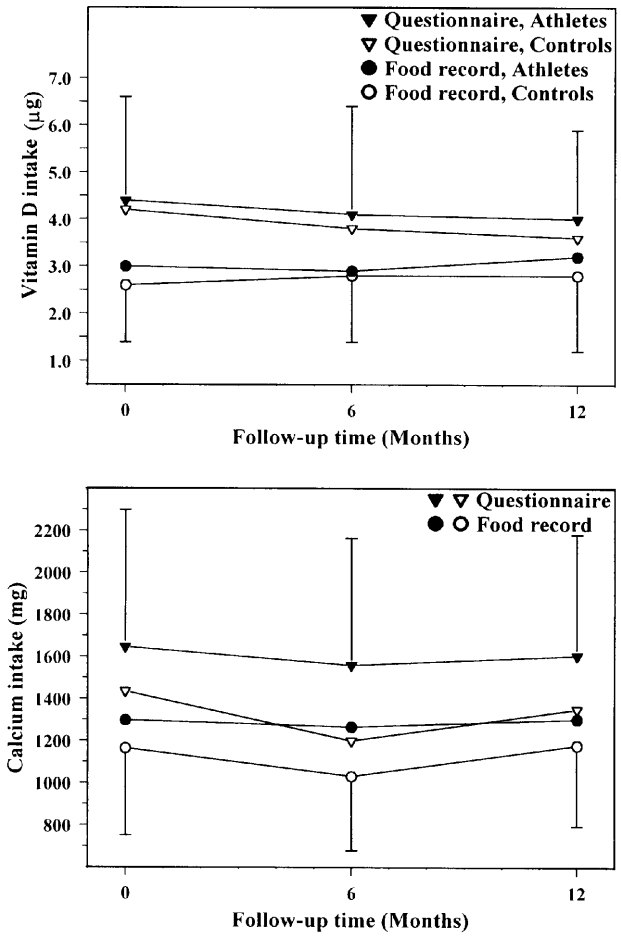

Figure 1 Daily dietary vitamin D (upper) and calcium (lower) intakes (mean ± s.d.) by 4-d food recording and food frequency questionnaire (FFQ) among the athletes and controls.

Vitamin D intakes assessed by the two different methods were compared to the S-25(OH)D concentrations in the subgroup of participants, who had not used vitamin D supplementation and who had not been on sunny holidays during the winter. The FFQ correlated weakly with S-25(OH)D concentration ($r = 0.28$, $P < 0.01$) while food recording did not ($r = 0.18$, NS). The S-25(OH)D concentration among those subjects in this subgroup who ingested more than 5 μg/d vitamin D as estimated by FFQ was higher than among those who ingested less than 5 μg/d (34.4 nmol/l *vs* 27.4 nmol/l, $P < 0.01$) (Figure 2).

The prevalence of severe hypovitaminosis D among 9- to 15-year-old girls was 13.4%, and of moderate hypovitaminosis D 67.7% at baseline in midwinter (February–March). At the same time 2.2% of the participants had the S-25(OH)D concentration below 10 nmol/l (Table 3). After the very sunny summer of 1997 in Finland only three participants (1.6%) in the study group had the S-25(OH)D concentration below 37.5 nmol/l. At the 12-month visit after three months of vitamin D supplementation 63.4% of the participants had still hypovitaminosis D, and 9.1% had severe hypovitaminosis D. The prevalence of hypovitaminosis D between the baseline visit and the 12-month visit did not differ significantly in the group of participants who took 10 μg/d of vitamin D supplementation for three months (Table 3).

Table 2 Mean (s.d.) S-25(OH)D (nmol/l) concentration during 1-year of follow-up

	Whole group		*Participants not taking supplementation at baseline*		*Participants taking supplementation at least 4 times a week at baseline*	
Follow-up visit	*n*	*S-25(OH)D nmol/l*	*n*	*S-25(OH)D nmol/l*	*n*	*S-25(OH)D nmol/l*
At baseline (February-March)	186	33.9 (13.9)	144	31.2 (12.2)	42	43.3 (15.2)[a ***]
At 6 months (August-September)	187	62.9 (15.0)[b,c ***]	145	62.7 (14.8)[b,c ***]	42	63.8 (15.6)[b,c ***]
At 12 months (February-March)	186	33.7 (11.4)	145	32.9 (11.2)	41	36.6 (11.7)[d *]

[a]S-25-OH-D at baseline in the supplementation group differ from without supplementation group.
[b]S-25-OH-D at the 6 months differ from baseline.
[c]S-25-OH-D at the 6 months differ from 12 months.
[d]S-25-OH-D at the 12 months differ from at baseline.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

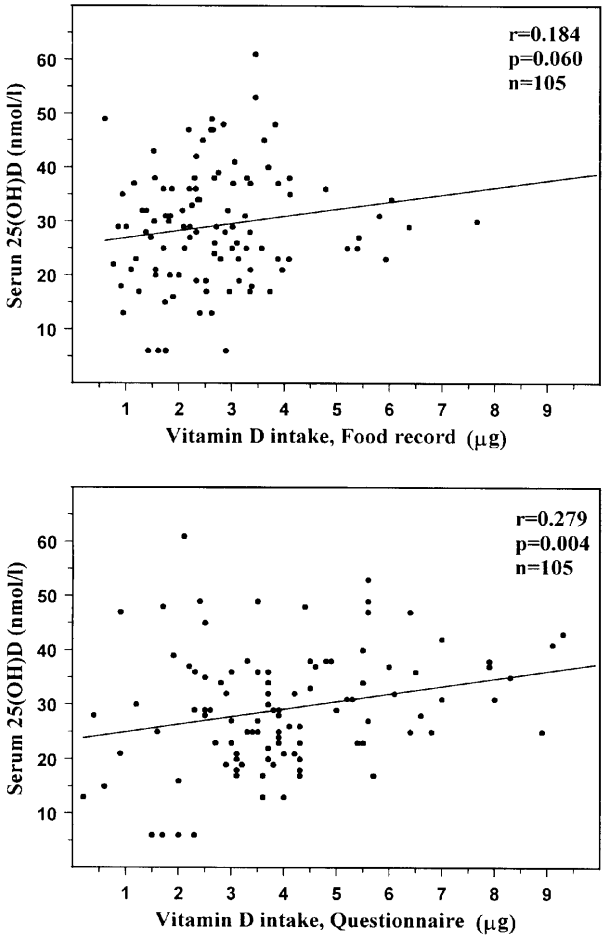

Figure 2 Daily vitamin D intakes assessed by food recording (upper) and food frequency questionnaire (FFQ) (lower) at baseline compared with S-25(OH)D concentration in the peripubertal girls, who had neither taken vitamin D supplementation nor been on sunny holidays during the winter.

The concentration of serum calcium and activity of alkaline phosphatase of all participants were normal during the follow-up period.

Discussion

The serum concentration of 25(OH)D is the most sensitive clinical marker of subject's vitamin D status (Hollis, 1996).

Table 3 Prevalence (%) of hypovitaminosis D in wintertime and summertime and in the next winter after 3 months of vitamin D supplementation

S-25-OH-D. nmol/l	*At baseline February–March*	*At 6 months August–September*	*At 12 months February–March*
≤ 37.5 nmol/l	126/186 (67.7%)	3/187 (1.6%)[a,b***]	118/186 (63.4%)
< 30 nmol/l	79/186 (42.5%)	0/187 (0%)[a,b***]	68/186 (36.6%)
< 20 nmol/l	25/186 (13.4%)	0/187 (0%)[a,b***]	17/186 (9.1%)
< 10 nmol/l	4/186 (2.2%)	0/187 (0%)	2/186 (1.1%)

[a]Frequency of participants at the six-month visit differ from that at baseline.
[b]Frequency of participants at the six-month visit differ from that at twelve-month visit.
***$P < 0.001$.

The present 1-year follow-up study of 186 girls demonstrated a frequency of severe hypovitaminosis D of 13.4% in midwinter (February–March), which is a fairly high figure. The next summer 1997, which was unusually sunny in our country, none of the participants had severe hypovitaminosis D. Although 93.4% of the participants used vitamin D supplementation 10 µg/d (400IU) at least four times a week during at least three months from October 1997 to January 1998, we found that 9.1% of the participants had severe hypovitaminosis D the next winter (in February–March). Three months of vitamin D supplementation did not significantly reduce the prevalence of hypovitaminosis D. The dose of vitamin D supplementation was apparently too low in Finland, located at 61°30' north. The vitamin D supplementation was discontinued one month before the sample collection which explains partly the insignificant change in S-25(OH)D concentration. The half-life of 25(OH)D in serum is approximately three weeks (Holick, 1990). We do not believe that poor compliance explains the insignificant change in S-25(OH)D, while girls reported the frequency of vitamin D supplementation by a self-administered questionnaire, and we included this information to the analysis.

The average dietary vitamin D intake of girls aged 12 or 15 y in Finland has previously been reported to be 2.1 to 3.0 µg/d; the total intake of girls of this age including supplements ranged from 2.5 to 3.4 µg/d (Lamberg-Allardt, 1984). These figures agree with the present study (Figure 1). Although the daily intake of vitamin D in our study was below the recommended daily allowance of vitamin D in Finland, only 22.0% of the participants took vitamin D

Table 4 S-25(OH)D concentration among the healthy subjects in the former studies

				S-25(OH)D, nmol/l		
Study	*Country*	*Age (y)*	*Number of the subjects*	*In the summer*	*In the winter*	*Unknown season*
Savolainen *et al,* 1980	Finland	3–81	41	82.5	36.3	
Vik *et al,* 1980	Norway	22–46	17	84.2	51.5	
Aksnes *et al,* 1982	Norway	8–18	34	142	55	
König *et al,* 1993	USA	3–18	61			53.8
Hillman *et al,* 1994	USA	mean 11.8	37			53.2
Moulas *et al,* 1997	Greece	5–10	15	64	48	
		11–23	19	68	37.5	
Kristinsson *et al,* 1998	Iceland	16	71		41.4	
		20	118		45.8	

supplementation at least four times a week at the beginning of the study. McKenna (1992) reported that the mean vitamin D intake is significantly lower in Central Europe (2.5 μg) than in North America (6.2 μg) or Scandinavia (5.2 μg). Generally young girls consume only a little vitamin D (Andersen *et al,* 1995b; Samuelsson *et al,* 1996), although in Iceland the situation is much better (Kristiansson *et al,* 1998).

In our study the mean S-25(OH)D concentration was lower (33.9 nmol/l) than those observed in earlier studies in wintertime (Savolainen *et al*, 1980; König *et al*, 1993; Hillman *et al*, 1994; Moulas *et al*, 1997) (Table 4). However, in the studies of the 1980s S-25(OH)D was assayed by a different technique, and it is thus difficult to compare those results with ours. Vik *et al* (1980) concluded that the dietary intake of vitamin D is sufficient to maintain a fairly high and nearly constant S-25(OH)D concentration despite the complete absence of sunshine for 2 months annually in Tromsö. This difference might due to eating habits between Finland and other Scandinavian countries. Probably the consumption of fish is more common in Iceland than in Finland, while the daily vitamin D consumption is at least 7.5 μg/d (Kristiansson *et al,* 1998).

In our study 65% of the participants did not use vitamin D supplementation at baseline, and in this group the mean S-25(OH)D concentration was very low (31.2 nmol/l). Those who reported taking multivitamins at the beginning of the study had a significantly higher (43.3 nmol/l) S-25(OH)D concentration. There is a lack of studies concerning the effect of vitamin D supplementation in young people. However, Zeghoud *et al* (1995) reported the use of vitamin D injections of 100 000 IU to keep the S-25(OH)D concentrations in the normal range 1–2 months. A Norwegian study on participants aged 8–18 y found that supplementation of the diet with 10 μg/d (400 IU/d) vitamin D raised the S-25(OH)D concentration from 55 nmol/l to 74 nmol/l. In the same study, the seasonal variation raised the S-25(OH)D to 142 nmol/l (Aksnes *et al,* 1982). The effect of vitamin D supplementation on the S-25(OH)D concentration has been demonstrated in several studies on elderly people worldwide (Chapuy *et al,* 1992; Heikinheimo *et al,* 1996; Lips *et al,* 1996). Barger-Lux *et al* (1998) reported that an 8-week course of vitamin D3 of 10 μg/d raised S-25(OH)D by 11 nmol/l in healthy adult males. In our study the mean S-25(OH)D concentration was about 25 nmol/l among the subjects who had the lowest dietary vitamin D intake (1 μg/d) and about 35 nmol/l among those with the highest intake (9 μg/d); these figures agree with the above-mentioned results. Significantly the mean increase in S-25(OH)D caused by sunny weather was about 30 nmol/l which corresponds to more than 20 μg/d of a daily dietary vitamin D intake. We agree with the opinion of Utiger (1998) who suggests that vitamin D supplementation should be used more widely, and forward the suggestion that also peripubertal children should consume daily vitamin D supplementation.

Several studies have demonstrated a positive correlation between S-25(OH)D concentration and bone mineral density in elderly persons (Chapuy *et al,* 1992; Collins *et al,* 1998). Also treatment with vitamin D and calcium reduces the risk of fractures (Chapuy *et al,* 1992; Dawson-Hughes *et al,* 1997), however a recent cross-sectional study did not show any significant association between S-25(OH)D and bone mineral density in adolescent girls (Kristinsson *et al,* 1998).

The participants in the present study took generally speaking the recommended Finnish dietary allowance of calcium of 700–900 mg/d. There was substantial individual variation. In general, the daily intake of calcium was sufficient because the girls used dairy products. Although the calcium intake of young girls in the USA is low (Johnston *et al,* 1992), the dietary calcium intake among young girls in the northern part of Europe is generally adequate (Andersen *et al,* 1995b; Samuelsson *et al,* 1996; Boot *et al,* 1997; Kristinsson *et al,* 1998).

In the present study the correlation between the two methods estimating dietary intake of vitamin D was statistically significant but weak, which may be due to the fact that almost all participants consumed only small amounts of vitamin D. The Spearman rank correlation coefficient between the methods ranged from 0.27 to 0.44. In a Norwegian study the Spearman correlation coefficients between FFQ and 7-day food recording for vitamin D was even lower 0.14 (Andersen *et al,* 1995a). The correlation between S-25(OH)D and dietary intake of vitamin D has been 0.13 to 0.80 in previous studies (Lips *et al,* 1987; Krall *et al,* 1989; Salamone *et al,* 1993). In the present study at baseline, there was a statistically significant but weak correlation between S-25(OH)D and intake of vitamin D as assessed by FFQ, but not by food recording. This reflects the fact that even at this northern latitude the S-25(OH)D concentration is only partly determined by the dietary intake.

Conclusion

Almost all Finnish 9- to 15-year-old girls in our study had a lack of vitamin D in their diet, while the daily intake of calcium was generally sufficient. The prevalence of hypovitaminosis D was remarkably high in this group of young

fast growing girls. The supplementation with vitamin D (10 μg/d) did not significantly succeed in reducing the prevalence of hypovitaminosis D. Although none of the subjects had rickets, the results from this study suggest that the intake of vitamin D is inadequate in this age group of females. This might be an important public health problem and in addition to its effect on the growing skeleton, hypovitaminosis D may affect other organ systems adversely.

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