

## A seasonal variation of calcitropic hormones, bone turnover and bone mineral density in early and mid-puberty girls – a cross-sectional study

Heli T. Viljakainen<sup>1\*</sup>, Anette Palssa<sup>1</sup>, Merja Kärkkäinen<sup>1</sup>, Jette Jakobsen<sup>2</sup>, Kevin D. Cashman<sup>3</sup>, Christian Mølgaard<sup>4</sup> and Christel Lamberg-Allardt<sup>1†</sup>

<sup>1</sup>Calcium Research Unit, Department of Applied Chemistry and Microbiology, University of Helsinki, Helsinki, Finland

<sup>2</sup>Danish Institute for Food and Veterinary Research, Søborg, Denmark

<sup>3</sup>Department of Food and Nutritional Sciences, University College, Cork, Ireland

<sup>4</sup>The Royal Veterinary and Agricultural University Research Department of Human Nutrition, Frederiksberg C, Denmark

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The importance of the seasonal variation of calcitropic hormones to growing skeleton has not been established. We studied whether there exists a seasonal variation in calcitropic hormones, bone mineral density (BMD) and bone remodelling markers in early puberty girls. One hundred and ninety-six girls, mean age 11.4 (SD 0.4) years, in Tanner stage 2 (early puberty) and 3 (mid-puberty) were studied during September to March. The BMD was measured from the lumbar vertebrae and the left femur by dual-energy X-ray absorptiometry. Their serum 25-hydroxyvitamin D (S-25-OHD), serum intact parathyroid hormone (S-iPTH), serum osteocalcin, urinary pyridinoline and urinary deoxypyridinoline were analysed from fasting samples. The concentration of S-25-OHD and serum osteocalcin differed among months ( $P < 0.01$ ), reflecting a seasonal variation. The parathyroid hormone correlated negatively with S-25-OHD ( $r = -0.325$ ,  $P < 0.001$ ). Moreover, the BMD in the femur ( $P = 0.047$ ) and to a lesser extent in vertebrae ( $P = 0.057$ ) differed between months in early puberty girls but this was not seen in mid-puberty. Seasonal variation in S-25-OHD and bone remodelling markers accompanied by negative correlation between S-25-OHD and S-iPTH was seen in this cross-sectional study of adolescent girls. In addition, the seasonal rhythm contributed 7.0–7.6% difference in the BMD of lumbar vertebrae and left femur in early puberty girls. This variation should be avoided since it could hamper peak bone mass attainment.

**Seasonal variation: 25-Hydroxyvitamin D: Bone mineral density: Bone remodelling markers: Adolescent girls: Cross-sectional study**

Seasonal variation in serum 25-hydroxyvitamin D (S-25-OHD) and other calcitropic hormones is believed to determine the seasonal variation of bone mass (Krall *et al.* 1989; Dawson-Hughes & Harris, 1993). In healthy adults, the seasonal variation in bone mass is shown to be around 2% (Bergstralh *et al.* 1990) whereas in the elderly it comprises 6–8% change (Rapuri *et al.* 2002; Bhattoa *et al.* 2004). Furthermore, a prospective community-based study demonstrated that the rate of bone fractures and falls follow this seasonal periodicity (Pasco *et al.* 2004). Similar findings have been reported before (e.g. Jakobsen *et al.* 1991) in retrospective studies. Hence, this problem faces many, but the seasonality of bone mineral density (BMD) in adolescence has not been established.

Bone growth accelerates at puberty. Any nutrient deficiency during this highly sensitive period could compromise the peak bone mass attainment and increase the risk of osteoporosis later in life (Matkovic *et al.* 1994). The importance of vitamin D during growth is evident (DeLuca, 1979). Furthermore, vitamin D is thought to limit bone mineral accretion (e.g. Lehtonen-Veromaa *et al.* 2002), as vitamin D insufficiency

is commonly seen among adolescents (Vieth *et al.* 2001; Gordon *et al.* 2004; Andersen *et al.* 2005).

Thus, the aim of the present study was to define the existence of seasonal variation in calcitropic hormones, site-specific BMD and biochemical markers of bone turnover in early puberty girls in a cross-sectional setting.

### Subjects and methods

#### Subjects and subject selection

A total of 196 adolescent girls, mean age 11.4 (SD 0.4) years were studied in the capital region of Helsinki (60°N), in southern Finland. Recruitment was conducted in primary schools. The subjects included were healthy, used no medicines known to affect calcium metabolism and were of Caucasian origin. Ethical approval was obtained from the Ethical Committee of Helsinki and Uusimaa Hospital District. The subjects and their parents gave informed written consent in agreement with the Helsinki Declaration before entering the study.

**Abbreviations:** BA, bone area; BMC, bone mineral content; BMD, bone mineral density; S-iPTH, serum intact parathyroid hormone; S-25-OHD, serum 25-hydroxyvitamin D; U-DPyr, urinary deoxypyridinoline; U-Pyr, urinary pyridinoline.

\* **Corresponding author:** Dr Heli T. Viljakainen, fax +358 9 19158269, email heli.viljakainen@helsinki.fi

† **Reprint requests:** Dr Christel Lamberg-Allardt, Department of Applied Chemistry and Microbiology, P.O. Box 66, FIN-00014, University of Helsinki, Finland, email christel.lamberg-allardt@helsinki.fi

### Laboratory measurements

The fasting serum samples were collected between 08.00 and 10.00 hours and stored at  $-20^{\circ}\text{C}$  before analysis. S-25-OHD concentration (S-25-OHD<sub>2</sub> and S-25-OHD<sub>3</sub>) in the samples was measured by HPLC analysis at the Danish Institute for Food and Veterinary Research, Søborg, Denmark (Andersen *et al.* 2005). The CV for the intra- and inter-assays were 4.3% and 6.3%, respectively.

Serum intact parathyroid hormone (S-iPTH) was measured with a commercial two-site immunoenzymometric assay (OCTEIA<sup>®</sup>; IDS, Boldon, UK) with intra- and inter-assay CV of 3.0% and 5.4%, respectively. Serum and urinary concentrations of calcium, phosphate and creatinine were analysed using an automated KoneLab spectrophotometer (Thermo Clinical LabSystems Ltd, Espoo, Finland) at the Department of Applied Chemistry and Microbiology, University of Helsinki, following routine methods. The intra- and inter-assay CV for these analyses were less than 7.5%, except for urinary calcium and creatinine, which were less than 15%. The urinary calcium and phosphate concentrations were expressed as mmol/mmol creatinine.

The bone remodelling markers and creatinine were analysed at the Department of Food and Nutritional Sciences and Biosciences Institute, University College, Cork, Ireland. Serum osteocalcin was measured in duplicate samples using an ELISA (N-MID<sup>™</sup>; Osteometer Biotech, Osteopark, Denmark). The intra-assay CV was 11%. Urinary pyridinoline (U-Pyr) and urinary deoxypyridinoline (U-DPyr) were analysed in duplicate, using an automated analysis system (Gilson ASPEC; Gilson S.A., Villiers-le-Bel, France) as described elsewhere (Cashman *et al.* 2001). The intra-assay CV for Pyr and DPyr were 5% and 3%, respectively. The mean U-Pyr and U-DPyr were expressed as nmol/mmol creatinine. Creatinine was determined in fasting second void urine samples using a diagnostic kit (Metra Creatinine Assay Kit, catalogue no. 8009; Quidel Corporation, San Diego, CA, USA). The intra-assay CV was 1.6%.

### Bone mineral density measurements

The BMD, bone mineral content (BMC) and bone area (BA) were measured with dual-energy X-ray absorptiometry (QDR 4500 A; Hologic, Waltham, MA, USA) from the lumbar vertebrae, L1–L4, and left femur. The left femur region includes the femoral neck, trochanter, Ward's triangle and inter-trochanter area which is analysed as a single area of interest. Calibration of the measurement was performed using a spine phantom; the inter-assay CV for the phantom was 0.31%. Intra-assay CV were determined with duplicate measurements of ten subjects. CV for BMD in the left femur and lumbar vertebrae were 0.67% and 1.39%.

### Tanner stage, nutrient intakes and other background data

The pubertal stage of the subject was assessed *ad modum* Tanner (Marshall & Tanner, 1969). A self-assessment protocol concerning the evaluation of breast development and timing of menarche was completed during the interview. Girls scored to Tanner stage 2 (early puberty) and Tanner stage 3 (mid-puberty) were selected for this cross-sectional study.

The subjects completed, together with their parents, a form containing questions on their medical history, use of vitamin D and calcium supplements, time spent out-of-doors, sunny holidays and physical activity. The dietary vitamin D and calcium intakes were evaluated using a food frequency questionnaire, which is a validated semi-quantitative questionnaire covering over seventy foods (Outila *et al.* 2001). The nutrient contents of the foods were calculated using the Finnish National Food Composition Database, Fineli<sup>®</sup>, version 2001, which is maintained by the National Public Health Institute of Finland, Nutrition Unit. The physical activity of the subjects consisted of school trips, guided activity in free time and free-time activity on their own (Outila *et al.* 2001). The activity was calculated as minutes per day. All forms were checked by the researchers and, if needed, additional information was requested.

### Statistics

Statistical analyses were performed by SPSS version 12.0 for Windows (SPSS Inc., Chicago, IL, USA). The normal distribution of variables was tested by Kolmogorov–Smirnov test. Comparison of two groups was performed with independent samples *t* test and more than two groups with ANOVA. *Post hoc* tests were performed with Tukey's HSD test. If variables were not normally distributed, non-parametric tests were applied. Results were considered statistically significant when  $P < 0.05$  while  $P$  values between 0.05 and 0.1 were considered as trends.

Girls in Tanner stages 2 and 3 were included in the statistical analyses. All analyses were performed on the whole group ( $n$  196), and separately on early puberty ( $n$  121) and mid-puberty girls ( $n$  75). Different  $P$  values are shown for each subgroup.

BMD, BMC and BA values were analysed with ANCOVA, in which age, height, weight, dietary intake of calcium and physical activity were used as covariant.

Data are presented as mean values and standard deviations.

## Results

### Baseline characteristics

The characteristics of the 196 girls are presented in Table 1.

### Serum 25-hydroxyvitamin D and parathyroid hormone concentration

The monthly mean value for S-25-OHD, the prevalence of deficiency, S-iPTH, elevated S-iPTH concentration ( $>4.1$  pmol/l) and dietary intakes of vitamin D and calcium are shown in Table 2. The mean S-25-OHD concentration differed among months (ANOVA;  $P < 0.001$ ), the highest were measured in September and October and the lowest in February. The opposite was observed for S-iPTH concentration, but the difference among months was not significant for the whole group ( $P = 0.627$ ), but among early puberty girls a tendency of variation was observed ( $P = 0.069$ ). The correlation between serum S-iPTH and S-25-OHD was  $r = -0.325$ ,  $P < 0.001$  (Fig. 1).

**Table 1.** Characteristics of early and mid-puberty girls (Mean values and standard deviations)

	Early ( <i>n</i> 121)		Mid ( <i>n</i> 75)		<i>P</i>
	Mean	SD	Mean	SD	
Age (years)	11.4	0.4	11.5	0.4	0.171
Height (cm)	147.2	7.2	151.5	6.4	<0.001
Weight (kg)	39.6	8.1	45.2	9.4	<0.001
Physical activity (min/d)	80.1	37.6	75.1	37.0	0.369
Calcium intake (mg/d)*	1250	700	1160	630	0.494
Vitamin D intake (µg/d)*	4.4	4.0	4.8	4.4	0.594
Users of vitamin D supplements	22	18.2	14	20.0	0.757

\* Includes intakes from supplements.

#### Seasonal variation in bone mineral density, bone mineral content and bone area

In the whole group, the femoral BMD (ANCOVA;  $P=0.046$ ), but not BMC ( $P=0.524$ ) nor BA ( $P=0.292$ ), reflected seasonal variation after adjusting for age, weight, height, physical activity and dietary intake of calcium. A similar tendency was observed in the lumbar vertebral BMD ( $P=0.093$ ), as well as in BMC ( $P=0.091$ ), but not in BA ( $P=0.348$ ).

After stratification to pubertal stage, the seasonality was seen only among early puberty girls in both sites. The femoral BMD differed among months (ANCOVA;  $P=0.047$ ) among early puberty girls. The BMD in October was 7.6% higher than in March ( $P<0.008$ , by contrast). Neither the femoral BMC nor BA followed the seasonal rhythm (ANCOVA;  $P=0.229$  and  $P=0.314$ , respectively; Fig. 2). In the lumbar vertebrae BMD, a trend for seasonal variation was seen ( $P=0.057$ ), although the difference between October and March was 7.0% significant (contrast;  $P=0.007$ ; Fig. 3). No seasonality was noted in the lumbar vertebrae BMC or BA (ANCOVA;  $P=0.168$  and  $P=0.619$ , respectively).

In the mid-puberty girls, the femur or lumbar vertebrae BMD did not differ among months (ANCOVA;  $P=0.903$  and  $P=0.762$ , respectively), neither did BMC nor BA in either site (data not shown). Although a decreasing pattern

in both BMD throughout the winter was observed, the number of subjects in mid-puberty varied among months and thus confused the interpretation.

#### Bone remodelling markers

In the whole group osteocalcin and U-DPyr concentrations varied between months after adjustment for age, puberty development, weight, height, physical activity and dietary intake of calcium (ANCOVA;  $P=0.062$  and  $P=0.035$ , respectively), whereas U-Pyr did not ( $P=0.635$ ).

Among early puberty girls the seasonal rhythm was seen only in osteocalcin concentration (ANCOVA;  $P=0.01$ ) which was 17.6% lower in March than in September (contrast;  $P<0.001$ ; Fig. 4). U-DPyr and U-Pyr did not vary among months ( $P=0.207$  and  $P=0.843$ , respectively; Fig. 5).

No seasonal variation was observed in bone remodelling markers (ANCOVA;  $P=0.200-0.572$ ) among mid-puberty girls.

#### Discussion

In this cross-sectional study, we found a seasonal variation in S-25-OHD and negative correlation between S-iPTH and S-25-OHD. In addition, the BMD of the left femur and lumbar vertebrae were 7.0% and 7.6% lower in March than in October in early puberty girls. Moreover, the bone formation marker, osteocalcin, supported the seasonal variation of BMD as it decreased during winter. To our knowledge, similar results in this age group have not been reported before.

#### Seasonal variation in serum 25-hydroxyvitamin D and parathyroid hormone concentrations

In the present study, the S-25-OHD concentration was highest in September, post-summer, and decreased until February. Typically the seasonality is seen in S-25-OHD and S-iPTH concentrations (e.g. Woitge *et al.* 2000; Rapuri *et al.* 2002), but rarely in 1,25-dihydroxyvitamin D, the concentration of which is tightly regulated in healthy subjects. From late October to April/May synthesis of vitamin D in the skin is deprived in

**Table 2.** Monthly mean values for serum 25-hydroxyvitamin D (S-25-OHD), serum intact parathyroid hormone (S-iPTH), and dietary intakes of vitamin D and calcium (for the prevalence of vitamin D deficiency (S-25-OHD < 25 nmol/l) and prevalence of elevated S-iPTH concentrations (> 4.1 pmol/l) the percentages for each month are provided)\*

(Mean values and standard deviations)

Month	<i>n</i>	S-25-OHD (nmol/l)		Vitamin D deficient (%)	S-iPTH (pmol/l)		Elevated S-iPTH (%)	Dietary vitamin D intake (µg/d)		Dietary calcium intake (mg/d)	
		Mean	SD		Mean	SD		Mean	SD	Mean	SD
September	35	59.5†	13.4	0‡	3.18§	1.16	11.4	4.8¶	4.8	1250**	550
October	57	54.7	13.4	0	3.30	0.91	21.1	4.4	3.0	1210	630
November	35	41.9	16.4	14.3	3.35	1.30	17.1	4.8	4.0	1410	910
February	29	37.3	15.5	17.2	3.41	0.85	13.8	4.4	4.0	1460	690
March	40	38.2	12.7	17.5	3.56	1.11	25.0	4.4	4.0	1370	710

\* For details of procedures, see p. 125.

†  $P<0.001$  for whole group,  $P<0.001$  for early puberty girls,  $P=0.027$  for mid-puberty girls.

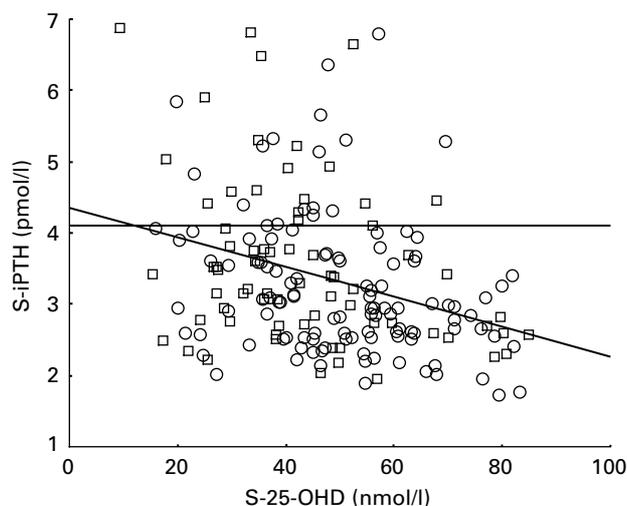
‡  $P=0.003$  for whole group,  $P=0.020$  for early puberty girls,  $P=0.122$  for mid-puberty girls.

§  $P=0.627$  for whole group,  $P=0.069$  for early puberty girls,  $P=0.643$  for mid-puberty girls.

||  $P=0.607$  for whole group,  $P=0.637$  for early puberty girls,  $P=0.576$  for mid-puberty girls.

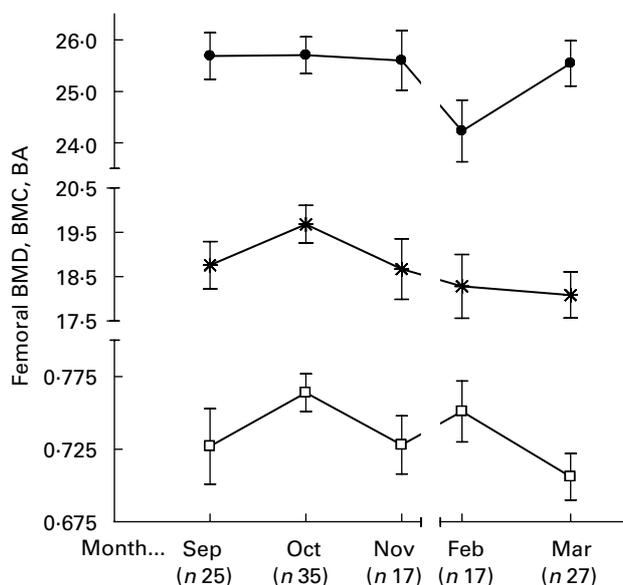
¶  $P=0.892$  for whole group,  $P=0.923$  for early puberty girls,  $P=0.220$  for mid-puberty girls.

\*\*  $P=0.789$  for whole group,  $P=0.696$  for early puberty girls,  $P=0.987$  for mid-puberty girls.

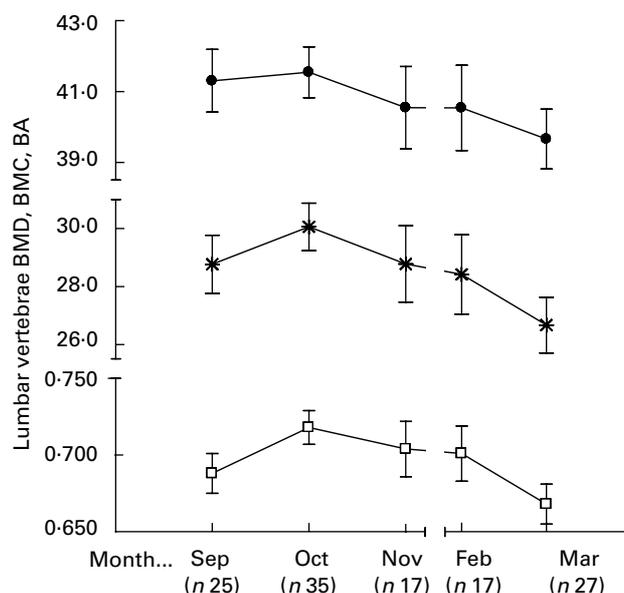


**Fig. 1.** Correlation between serum intact parathyroid hormone (S-iPTH) and serum 25-hydroxyvitamin D (S-25-OHD) concentration in early puberty girls (○) and mid-puberty girls (□) ( $n$  196). For details of procedures, see p. 125. Spearman's correlation:  $r = 0.325$ ,  $P < 0.001$ . The reference line for elevated S-iPTH concentration is shown at 4.1 pmol/l.

Finland due to limited solar exposure (Lamberg-Allardt, 1984) which contributes to mobilisation of body stores of vitamin D. The dietary intake of vitamin D could maintain the stores if it is adequate. However, the prevalence of vitamin D deficiency in the present study was 15.5% during the winter months. This suggests that the median dietary intake of vitamin D, 4.7  $\mu\text{g}/\text{d}$ , is not adequate, which has been recognised as the current recommendation has been increased from 5 to 7.5  $\mu\text{g}$  for this



**Fig. 2.** Variation in left femur bone mineral density (BMD), bone mineral content (BMC), and bone area (BA) across fall and winter months for early puberty girls. For details of procedures, see p. 125. The femoral BMD (□) differed between months after adjustment for age, weight, height, physical activity and dietary intake of calcium (ANCOVA;  $P = 0.047$ ). No variation between months was observed in BMC (○) or BA (●) ( $P = 0.229$  and  $P = 0.314$ , respectively). The number ( $n$ ) of studied subjects is indicated for each month. Values are means with their standard errors depicted by vertical bars.



**Fig. 3.** Variation in the lumbar vertebrae bone mineral density (BMD), bone mineral content (BMC) and bone area (BA) across fall and winter months for early puberty girls. For details of procedures, see p. 125. The lumbar vertebrae BMD (□) differed between months after adjustment for age, weight, height, physical activity and dietary intake of calcium (ANCOVA;  $P = 0.057$ ). No variation was observed in the BMC (○) or BA (●) ( $P = 0.168$  and  $P = 0.619$ , respectively). The number ( $n$ ) of studied subjects is indicated for each month. Values are means with their standard errors depicted by vertical bars.

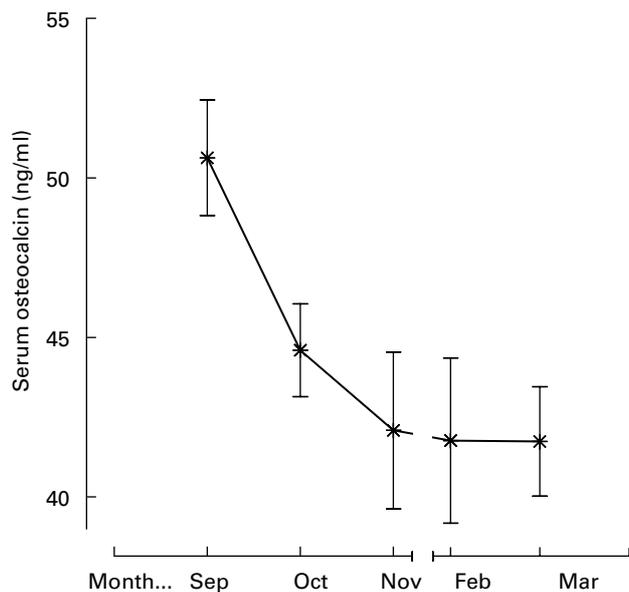
age group (Nordic Council of Ministers, 2004). Several other studies have suspected the same (Glerup *et al.* 2000; Vieth *et al.* 2001; Lehtonen-Veromaa *et al.* 2002).

Although the concentration of S-iPTH in the whole group did not vary between months, a negative correlation with S-25-OHD and S-iPTH was found, but not a plateau in the S-iPTH concentration, which agrees with earlier studies in adolescent girls (Outila *et al.* 2001; Cheng *et al.* 2003). However, the iPTH concentration has reached a plateau with increasing 25-OHD concentration in studies in adults and the elderly (Chapuy *et al.* 1997; Vieth *et al.* 2003), but not in all (Pasco *et al.* 2004). Hormones like oestrogens and growth hormone (Martin *et al.* 1997; Bass *et al.* 1999) and the dietary intake of calcium are known to suppress iPTH secretion independently of 25-OHD (Kärkkäinen *et al.* 1998), which cannot be ruled out here. For example, the mean dietary intake of calcium in these girls was 1240 mg, which is adequate when compared to the recommendation (Nordic Council of Ministers, 2004).

The lowest mean 25-OHD concentration was seen in February, but the highest S-iPTH followed in March, which could be predicted as causality (Rapuri *et al.* 2002).

#### Seasonal variation of bone mineral density, bone mineral content and bone size

Earlier studies have reported seasonal variation in bone mass in different populations (Bergstralh *et al.* 1990; Dawson-Hughes & Harris, 1993; Rosen *et al.* 1994; Rapuri *et al.* 2002) but others have not found such a variation (Vanderschueren *et al.* 1991; Tsai *et al.* 1997; Overgaard *et al.* 1998). We stated a 7.5–8.2% seasonal variation in site-specific BMD in early



**Fig. 4.** Bone formation marker, serum osteocalcin, in early puberty girls. For details of procedures, see p. 125. Serum osteocalcin differed between months (ANCOVA;  $P=0.01$ ) after adjustment for age, puberty development, weight, height, physical activity and dietary intake of calcium. Values are means with their standard errors depicted by vertical bars.

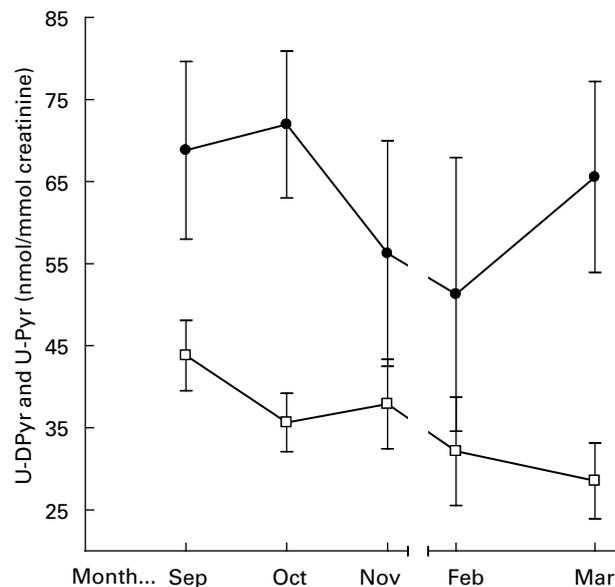
puberty girls, which seems to be somewhat higher than observed in healthy adults (Bergstralh *et al.* 1990; Dawson-Hughes & Harris, 1993).

BMC characterises bone mass and it is advised to use it in growth studies together with bone size to illustrate changes in bone strength (Heaney, 2005). There was some implication, at least in the lumbar vertebrae, that BMC is affected by season. The sub-groups did not indicate this, possibly due to smaller sample size. On the other hand, bone size did not show any variation at all. If less mineral is accumulated or even lost during winter, seasonal variation could complicate the bone mass attainment and increase fracture risk among adolescents (Bailey *et al.* 1989).

Recently Bonjour (2005) stated that during early puberty bone is most sensitive to nutrients and physical activity. Our present results support this as the developing vitamin D deficiency with elevated S-iPTH modulated BMD in early puberty but not in mid-puberty girls. In mid-puberty, high oestrogen concentration could damp out the interaction of low vitamin D status and elevated parathyroid hormone (Khosla *et al.* 1997). On the other hand, the pubertal stage-specific results in our present study could be due to the fewer number of subjects in mid-puberty than in early puberty.

#### *Mechanism of seasonal variation in bone mineral density*

Seasonal variation of calcitropic hormones are thought to influence bone mass by increasing resorption and decreasing bone formation during winter and *vice versa* during summer (e.g. Rosen *et al.* 1994). Our present results showed that bone formation reflected by osteocalcin was highest in September and decreased together with S-25-OHD concentration. Positive association between vitamin D status and



**Fig. 5.** Bone resorptive markers in early puberty girls. For details of procedures, see p. 125. Urinary deoxypyridinoline (U-DPyr,  $\square$ ) and urinary pyridinoline (U-Pyr,  $\bullet$ ) did not differ between months (ANCOVA;  $P=0.207$  and  $P=0.843$ , respectively). Values are means with their standard errors depicted by vertical bars.

formative markers has been described by others (Woitge *et al.* 2000; Lehtonen-Veromaa *et al.* 2002).

On the other hand, bone resorption markers were hard to interpret in this cross-sectional study. U-DPyr seemed to decrease during winter within the whole group, but not separately among early and mid-puberty girls. In separate groups, U-DPyr and U-Pyr did not follow the seasonal rhythm. In follow-up studies resorptive bone markers tend to increase during winter (Vanderschueren, 1991; Rosen *et al.* 1994; Woitge *et al.* 2000; Rapuri *et al.* 2002; Lehtonen-Veromaa *et al.* 2002), due to increased parathyroid hormone secretion. Although S-iPTH increased throughout the winter among early puberty girls, we did not observe an increase in resorptive markers: the use of one time specimen to measure bone remodelling markers in a cross-sectional study is not informative, because of many confounding factors or due to a small sample size (e.g. Woitge *et al.* 2000; Borderie *et al.* 2001; Fares *et al.* 2003).

#### *Limitation of the study*

A follow-up study could provide better evidence on seasonal variation of BMD. However, a cross-sectional study is ethically preferred in this kind of work because with repeated dual-energy X-ray absorptiometry measurement the radiation doses tend to increase.

The number of subjects differed among months but it is unlikely to affect the results since the monthly variances of BMD were equal throughout the study. In addition, longitudinal studies could provide more valuable evidence than a cross-sectional study, especially concerning the bone remodelling markers.

#### **Conclusions**

Our data suggested that the variation in calcitropic factors, mainly in S-25-OHD and S-iPTH, influences BMD. Decreased

bone formative markers accompanied with stable or decreased rates of resorptive markers could result in lowered BMD, as noted among early puberty girls in winter. In addition, in early puberty, the skeleton seems to respond more sensitively to modulating factors than in mid-puberty.

Any negative balance in bone metabolism during growth might disturb the natural bone mass acquisition, and lead to lower peak bone mass. Taking this into consideration, it is vital to avoid the seasonal variation of BMD by ensuring an adequate vitamin D intake during winter in adolescent girls. More studies are needed to define the adequate vitamin D intake for this target group.

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