

## Parathyroid hormone is elevated but bone markers and density are normal in young female subjects who consume inadequate dietary calcium

D. Bonofiglio<sup>1</sup>†, M. Maggiolini<sup>1</sup>†, S. Catalano<sup>2</sup>, S. Marsico<sup>2</sup>, S. Aquila<sup>2</sup>, A. Giorno<sup>2</sup> and S. Andò<sup>3</sup>\*

<sup>1</sup>Department of Pharmaco-Biology, <sup>2</sup>Health Centre and <sup>3</sup>Department of Cellular Biology, University of Calabria, 87036 Rende (CS), Italy

(Received 3 March 1999 – Revised 18 October 1999 – Accepted 16 November 1999)

Dietary Ca and osteocalcin (OC), parathyroid hormone (PTH), 25-hydroxyvitamin D (25-OH-D), insulin-like growth factor (IGF)-I and sex hormone binding globulin (SHBG) were assessed simultaneously to bone mineral density (BMD) in 200 adolescent girls (aged 11–15 years) and 100 young women (aged 20–23 years), selected from the lowest and highest end of the Ca intake distribution of a larger population sample. Ca intake was evaluated by food frequency questionnaires, BMD was measured by dual energy x-ray absorptiometry at ultradistal and proximal radius of non-dominant arm, bone age was estimated from x-rays of left hand and wrist according to Tanner *et al.* (1983). Surprisingly, mean Ca intakes were below the dietary reference intakes in the subgroups of girls and women with the highest measured Ca consumption. Postmenarcheal, but not premenarcheal girls showed radial densities as high as the women and in no group was BMD associated with Ca intake. In all adolescents serum PTH was negatively related to dietary Ca. In girls before menarche IGF-I was positively associated with bone age, while in the same subjects the negative relationship between SHBG and BMD pointed to the crucial role of bioavailable sex steroids on bone mass apposition in early puberty. OC levels decreased progressively with age, while serum 25-OH-D significantly increased after menarche. In conclusion, although in adolescents low Ca intake has not been shown to induce any immediate deleterious effect on radial density, the compensatory hypersecretion of PTH supports the need for an adequate Ca intake to achieve peak bone mass.

### Calcium: Bone mineral density: Parathyroid hormone

Genetic and environmental factors such as nutrition and exercise are the main determinants of skeletal mineralization (Matkovic *et al.* 1990; Slemenda *et al.* 1991; Anderson *et al.* 1993). Amongst other nutrients, an adequate Ca intake during the first two decades of life contributes to the attainment of the genetically-determined maximal peak bone mass (PBM) (Sentipal *et al.* 1991; Johnston *et al.* 1992; Lloyd *et al.* 1993; Kanis, 1994; Chan *et al.* 1995; Slemenda *et al.* 1997). Bone mineralization, which is gradual in childhood, strongly accelerates during pubertal development, when approximately 37% of the total skeletal mass of the adult is accumulated (Gilsanz *et al.* 1988; Glastre *et al.* 1990; Katzman *et al.* 1991; Matkovic, 1992). During the period of rapid bone growth osteocalcin (OC) increases dramatically reflecting the rate of bone formation (Johansen *et al.* 1988; Calvo *et al.* 1996). Similarly, measurement of indirect variables allows the evaluation of bone metabolism, e.g. parathyroid hormone

(PTH), a regulator of mineral homeostasis (Habener *et al.* 1984; Roodman, 1996); 25-hydroxyvitamin D (25-OH-D), a reliable indicator of vitamin D status (Sherman *et al.* 1990; Ooms *et al.* 1995); insulin-like growth factor (IGF)-I, an indirect index of growth and local modulator of osteoblastic activity (Jones & Clemmons, 1995); sex hormone binding globulin (SHBG), the major inverse determinant of bioavailable sex steroids (van Hemert *et al.* 1989; Selby, 1990). To measure bone mineral density (BMD) in a young population sample, dual energy x-ray absorptiometry is the method of choice due to its great precision and accuracy with low dose of radiation (Mazess *et al.* 1990; Johnston *et al.* 1991; Boot *et al.* 1997).

In the present investigation a subset of data from a multicentre study carried out in six European countries (Kardinaal *et al.* 1999) was used to assess the influence of Ca intake on radial density, evaluating simultaneously OC, PTH, 25-OH-D, IGF-I and SHBG.

**Abbreviations:** BMD, bone mineral density; IGF, insulin-like growth factor; OC, osteocalcin; 25-OH-D, 25-hydroxyvitamin D; PBM, peak bone mass; PTH, parathyroid hormone; SHBG, sex hormone binding globulin.

\* **Corresponding author:** Professor Sebastiano Andò, fax +39 984 401275, e-mail sebando@tin.it

† The first two authors contributed equally to the work.

## Methods

### Subjects

Girls ( $n$  1079, range 11–15 years) and women ( $n$  350, range 20–24 years) all from secondary schools in the town of Rende and from the University of Calabria (Cosenza, Italy) respectively, were randomly invited to participate in the present study. Subjects were excluded if they were not of Caucasian origin, had chronic systemic diseases related or not to bone tissue, used corticosteroids, had irregular menstruation and previous pregnancy (for the women only), had lifestyle features such as sporting activity for more than 7 h/week, were vegetarian or followed any prescribed diet (except an energy-restricted diet).

Subsequently, 722 girls and 258 women filled a food frequency questionnaire in order to select our population sample from the lowest end (100 girls and fifty women) and the highest end (100 girls and fifty women) of the Ca intake distribution. Local medical-ethical committees approved our study protocol and all subjects (or parents if required) gave their written consent.

### Design

The evaluation of Ca consumption in the month before the interview was made by a twenty-item food frequency questionnaire adapted for both girls and women of an Italian population. Consumption frequency was reported in ten categories: rarely/never, on 1 day per month, on 2 days per month, on 1 day per week and subsequently on 2, 3, 4, 5, 6, 7 days per week. Portion sizes were quantified by subjects in terms of household measures (slices, spoons, cups, glasses) according to which standard weights were assigned.

To confirm in a comparable way the level of Ca intake, all subjects performed a 3 d food diary recording everything they consumed during a consecutive Wednesday, Thursday and Friday, the week before their visit to our Institute (Hartman *et al.* 1990; Larkin *et al.* 1991). Food item and quantity, and recipes of composite dishes were recorded and if necessary the parent responsible for meal preparation was invited to assist in completing the food diaries. Mean daily consumption of food products was then used to calculate Ca intake (mg/d) using the Italian food composition tables (Carnovale & Miuccio, 1989). The same investigator measured height and weight (by which BMI was calculated) and pubertal stages were assigned to girls according to the method of Tanner (1978).

### Experimental techniques

Bone mineral content and bone area were measured by dual energy x-ray absorptiometry (DXA Osteoscan, Niderburg BV, Bunschoten, The Netherlands) in the non-dominant arm at the ultradistal and proximal radius (one-third distal point between the styloid process and the tip of olecranon of the elbow) representing the trabecular and cortical bone components respectively (Leboff *et al.* 1992). Bone mineral content (g) divided by the projected area of the bone was used to calculate BMD ( $\text{g}/\text{cm}^2$ ) as a means of normalizing result for bone size. The same two technical operators collected all densitometric data calibrating the osteoscan every day against a reference phantom. The CV for ten

measurements of the same subject (with repositioning) was 2.15 % for ultradistal-BMD and 1.75 % for proximal-BMD. Left hand and wrist x-ray was performed in girls to establish bone age according to the method of Tanner *et al.* (1983).

Morning (08.00 hours) blood samples during early spring season were drawn from each subject after an overnight fast to measure serum levels of OC, PTH, IGF-I, SHBG and 25-OH-D. After collection blood samples were immediately centrifuged and portions of serum stored at  $-70^\circ$  to perform the assays in duplicate.

Serum intact OC concentrations were measured by immunoradiometric assay (Diagnostic System Laboratories, Inc., Webster, TX, USA). The intra- and inter-assay CV were 2.9 % and 4.7 % respectively. Serum intact PTH was determined by immunoradiometric assay (ICN Pharmaceuticals, Inc., Costa Mesa, CA, USA). The intra- and inter-assay CV were 3 % and 5.2 % respectively. IGF-I concentrations were quantified by immunoradiometric assay (Diagnostic System Laboratories, Inc.) after HCl-ethanol extraction in order to remove binding protein interference. Serum samples were diluted 250-fold before assay. The intra- and inter-assay CV were 3.2 % and 4.7 % respectively. Serum SHBG levels were measured by immunoradiometric assay (Diagnostic System Laboratories). The intra- and inter-assay CV were 2.8 % and 8.8 % respectively. Serum levels of 25-OH-D were determined after extraction with acetonitrile by radioimmunoassay kit (Incstar Co., Stillwater, MN, USA). The intra- and inter-assay CV were 7.8 % and 8.6 % respectively.

### Statistical analysis

Statistical analysis was performed separately for girls and women selected according to low and high Ca intake calculated from the food frequency questionnaires. Data for all variables were presented as means with their standard error. Comparisons of means were made using unpaired Student's *t* test and relationships between variables were identified by linear regression analysis. In this present investigation *P* values  $<0.05$  were considered significant.

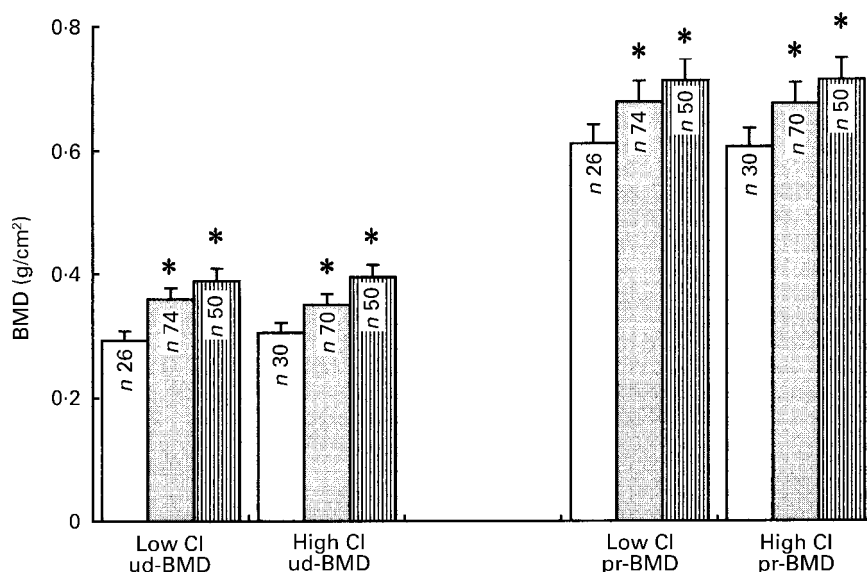
## Results

Table 1 shows anthropometric characteristics and dietary Ca of the two subgroups of girls and women selected following the criteria indicated in methods section (see Subjects section). Surprisingly, we found Ca intake values below the dietary reference intakes (Vedral, 1997) in both subgroups of girls and women with high measured Ca consumption (Table 1).

Postmenarcheal, but not premenarcheal girls showed radial densities as high as the women, and in no group was BMD influenced by Ca intake (Fig. 1). PTH was the only variable related to dietary Ca in all girls, being significantly elevated in adolescents with low Ca intake (Table 2). Serum OC progressively decreased after menarche occurrence and furthermore in young women (Table 2). In pre- and postmenarcheal girls OC was inversely related to BMD in the ultradistal and proximal radius ( $r$   $-0.57$ ,  $P < 0.001$  and  $r$   $-0.35$ ,  $P = 0.006$  respectively), while only in the postmenarcheal group was it negatively related to bone age ( $r$   $-0.66$ ,  $P < 0.001$ ). In

**Table 1.** Anthropometric characteristics and dietary calcium from 3 d records in the two subgroups of selected subjects with low and high calcium intakes (CI)  
(Mean values with their standard errors)

	Girls				Women			
	Low CI (n 100)		High CI (n 100)		Low CI (n 50)		High CI (n 50)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Age (years)	14.2	0.1	13.7	0.1	21.9	0.2	22	0.1
Height (cm)	155.9	0.7	156.8	0.7	160.1	0.7	160.9	0.8
Weight (kg)	54.9	1.1	53.3	1.4	58.7	1	56.6	1.1
BMI (kg/m <sup>2</sup> )	22.5	0.4	21.1	0.3	22.9	0.4	21.8	0.41
Age at menarche (years)	11.6	0.1	11.7	0.1	11.9	0.1	11.8	0.12
Dietary calcium (mg/d)	387.4	12.4	835.4	20.9	442.9	19.9	913	33.3



**Fig. 1.** Bone mineral density (BMD) at ultradistal (ud) and proximal (pr) radial sites in girls before (□) and after menarche (▨) and in women (▩) at low and high calcium intakes (CI). Mean values were significantly different in girls before and after menarche and in women compared with girls after menarche; \*  $P < 0.001$ .

**Table 2.** Serum osteocalcin (OC), 25-hydroxyvitamin D (25-OH-D) and parathyroid hormone (PTH) levels in pre- and postmenarcheal girls and in women at low and high calcium intakes (CI)  
(Mean values with their standard errors)

	Girls								Women			
	Premenarcheal				Postmenarcheal				Low CI		High CI	
	Low CI (n 18)	High CI (n 27)	Low CI (n 72)	High CI (n 56)	Low CI (n 45)	High CI (n 45)	Mean	SE	Mean	SE		
PTH (pg/ml)	36.2	19.7**	32	12.3***	26.6	24.7	5.9	5				
OC (ng/ml)	8.5	8.9	4.9	5.3	2.2†	2.3†	0.3	0.2				
25-OH-D (ng/ml)	19.3	19.9	24.3*	22.8*	25.9	25.3	1.2	1.1				

Mean values were significantly different from premenarcheal girls: \*  $P < 0.05$  (Student's *t* test).

Mean values were significantly different from postmenarcheal girls: †  $P < 0.01$  (Student's *t* test).

Mean value was significantly different from that of premenarcheal girls with low CI: \*\*  $P < 0.01$  (Student's *t* test).

Mean value was significantly different from that of postmenarcheal girls with low CI: \*\*\*  $P < 0.001$  (Student's *t* test).

**Table 3.** Insulin like growth factor-I (IGF-I) and sex hormone binding globulin (SHBG) in pre- and postmenarcheal girls and in women (Mean values with their standard errors)

	Girls					
	Pre-menarcheal (n 45)		Post-menarcheal (n 128)		Women (n 90)	
	Mean	SE	Mean	SE	Mean	SE
IGF-I (ng/ml)	458.6	19	433.6	24.7	301.6***	19.8
SHBG (nmol/l)	149.7	11.1	135.2	17.4	112.5	12.9

Mean value was significantly different from that of pre- and postmenarcheal girls: \*\*\*  $P < 0.001$  (Student's *t* test).

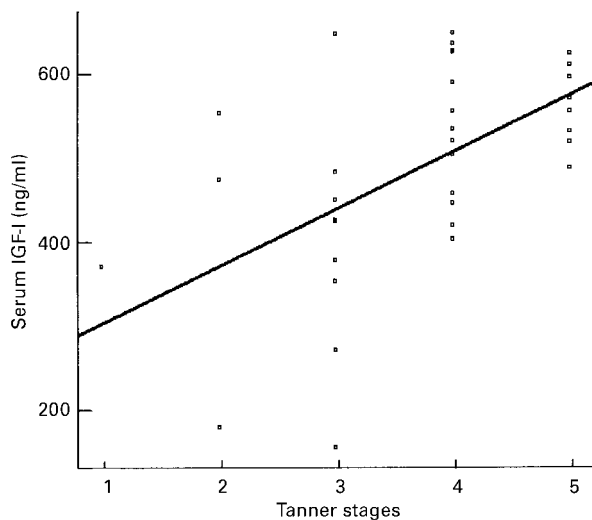
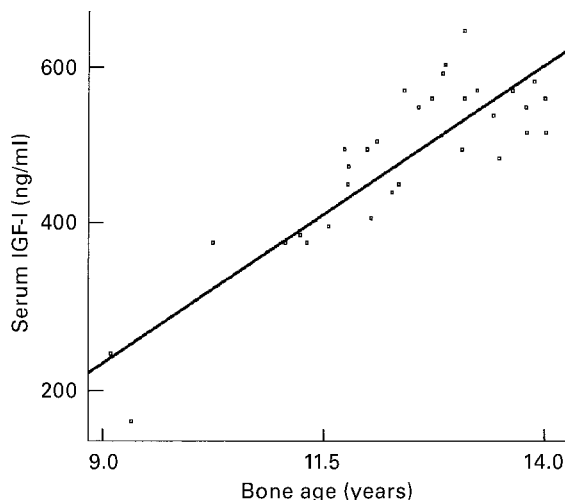
adolescents after menarche 25-OH-D increased to levels observed in young women (Table 2).

IGF-I and SHBG values showed no difference in girls according to menarche event, but both variables decreased in young women although in the latter group not significantly so (Table 3). Moreover, in girls before menarche IGF-I was positively associated to pubertal stages (Fig. 2) as well as to bone age (Fig. 3).

It is worthwhile noting that SHBG, an inverse determinant of free sex steroid fractions, was negatively related to both radial densities in premenarcheal girls (ultradistal BMD:  $r -0.33$ ,  $P < 0.05$  and proximal-BMD:  $r -0.35$ ,  $P < 0.05$  respectively) as well as to bone age in postmenarcheal girls ( $r -0.42$ ,  $P < 0.05$ ).

## Discussion

In this present investigation elevated levels of PTH and normal radial density were found in adolescent girls who consume low dietary Ca. Our data on BMD are comparable with those reported in a recent cross-sectional multicentre study (Kardinaal *et al.* 1999) suggesting no evidence of the role of dietary Ca in the attainment of PBM, as generally

**Fig. 2.** Relationship between serum insulin-like growth factor (IGF)-I and Tanner stages (Tanner, 1978) in girls before menarche;  $P < 0.01$  (linear regression analysis).**Fig. 3.** Relationship between serum insulin-like growth factor (IGF)-I and bone age in girls before menarche  $P < 0.001$ ; (linear regression analysis).

observed (Glastre *et al.* 1990; Katzman *et al.* 1991; Southard *et al.* 1991; Kröger *et al.* 1993; Young *et al.* 1995). Nevertheless, in other studies Ca consumption at more than adequate levels from foods or supplements has been attributed to the achievement of maximum PBM (Matkovic *et al.* 1990; Lloyd *et al.* 1993) and subjects consuming greater quantities of Ca early in life had greater bone mass later on (Halioua & Anderson, 1989; Murphy *et al.* 1994).

To date, in this study Ca intake below the dietary reference intake (Vedral, 1997) in low as well as high Ca consumers could be responsible for the lack of difference in BMD between these subgroups. However, adolescents of the low subgroup had elevated serum PTH levels as hormonal compensatory mechanism and apparently had no deleterious effects on BMD. These changes would last as long as there was an increased requirement by the bone for Ca, since the integrated actions of PTH allow the regulation of mineral homeostasis increasing intestinal absorption as well as the kidney transport of Ca and P (Habener *et al.* 1984).

With respect to OC, the main marker of bone formation, we found maximal values in premenarcheal girls and a progressive decrease in postmenarcheal girls as well as in women. It has been previously reported that during the process of skeletal accretion OC levels reflect bone growth rates, exhibiting peak values in early puberty (Rubin *et al.* 1993; Blumsohn *et al.* 1994). Indeed, *in vitro* studies confirmed the pattern of OC increase which was associated to the progressive osteoblastic differentiation and mineralization (Harris *et al.* 1995; Calvo *et al.* 1996).

In girls both before and after menarche IGF-I levels remained significantly higher compared with those of women, which is in agreement with other observations showing high levels of IGF-I during whole pubertal development (Cara *et al.* 1987; Blumsohn *et al.* 1994; Juul *et al.* 1994). Indeed, a positive association between serum free IGF-I and growth rate acceleration has been recently reported (Kawai *et al.* 1999) as well as a strong

correlation between IGF-I and some markers of bone turnover (Collins *et al.* 1998).

Interestingly, BMD was negatively related to SHBG (an indirect index of bioavailable oestrogen levels) in premenarcheal girls emphasizing the influence of sex steroids on bone maturation even before menarche onset (Turner *et al.* 1994; Saggese *et al.* 1997).

Evaluating serum 25-OH-D, the major form of vitamin D in humans reflecting sunlight exposure and dietary intake (Lawson *et al.* 1979; Chesney *et al.* 1981; Sherman *et al.* 1990; Ooms *et al.* 1995), we failed to find correlations with the other variables determined. However, enhanced levels of 25-OH-D were present in post- v. premenarcheal girls, probably due to the up regulation of vitamin D binding protein by increased oestrogen secretions (Cooke & Haddad, 1989).

The present study suggests that hormonal changes related to menarche occurrence strongly contribute to bone mass apposition, which is apparently irrespective of dietary Ca. The elevated PTH levels in adolescent low Ca consumers could have a potentially adverse effect on bone metabolism. Since we cannot predict whether this mechanism would become exhausted later on, an adequate Ca intake should be provided to our subjects to normalize serum PTH and to ensure the achievement of maximal PBM. On the basis of PTH enhancement supporting the actual need for Ca, we suggest consumption of the amount of this mineral as recommended by the dietary reference intake (Vedraal, 1997) instead of that indicated in the main recent multicentre study (Kardinaal *et al.* 1999).

### Acknowledgements

The authors very much thank the operators Liesbeth Abbink and Geertje Huijbers for collecting densitometric data.

### References

- Anderson JJB, Tylavsky FA, Halioua L & Metz JA (1993) Determinants of peak bone mass in young adult women: A review. *Osteoporosis International* **1**, Suppl., S32–S36.
- Blumsohn A, Hannon RA, Wrate R, Barton J, Al-Dehaimi AW, Colwell A & Eastell R (1994) Biochemical markers of bone turnover in girls during puberty. *Clinical Endocrinology* **40**, 663–670.
- Boot AM, De Ridder MAJ, Pols HAP, Krenning EP & De Muinck Keizer-Schrama SMPF (1997) Bone mineral density in children and adolescents: relation to puberty, calcium intake, and physical activity. *Journal of Clinical Endocrinology and Metabolism* **82**, 57–62.
- Calvo MS, Eyre DR & Gundberg CM (1996) Molecular basis and clinical application of biological markers of bone turnover. *Endocrine Reviews* **17**, 333–368.
- Cara JF, Rosenfield RL & Furlanetto RW (1987) A longitudinal study of the relationship of plasma somatomedin-C concentration to pubertal growth spurt. *American Journal of Diseases of Children* **141**, 562–564.
- Carnovale E & Miuccio F (1989) *Tabella di composizione degli alimenti (Food composition tables)*. Rome: Istituto Nazionale della Nutrizione.
- Chan GM, Hoffmann K & McMurray M (1995) Effects of dairy products on bone and body composition in pubertal girls. *Journal of Pediatrics* **126**, 551–556.
- Chesney RW, Rosen JF, Hamstra AJ, Smith C, Mahaffey K & DeLuca HF (1981) Absence of seasonal variation in serum concentrations of 1,25-dihydroxyvitamin D despite a rise in 25-hydroxyvitamin D in summer. *Journal of Clinical Endocrinology and Metabolism* **53**, 139–142.
- Collins D, Woods A, Herd R, Blake G, Fogelman I, Wheeler M & Swaminathan R (1998) Insulin-like growth factor-I and bone mineral density. *Bone* **23**, 13–16.
- Cooke NE & Haddad JG (1989) Vitamin D binding protein. *Endocrine Reviews* **10**, 294–307.
- Gilsanz V, Gibbens DT, Roe TF, Carlson M, Senac MO, Boechat MI, Huang HK, Schulz EE, Libanati CR & Cann CC (1988) Vertebral bone density in children: effect of puberty. *Radiology* **166**, 847–850.
- Glastre C, Braillon P, David L, Cochat P, Meunier PJ & Delmas PD (1990) Measurement of bone mineral content of the lumbar spine by dual energy x-ray absorptiometry in normal children: correlations with growth parameters. *Journal of Clinical Endocrinology and Metabolism* **70**, 1330–1333.
- Habener JF, Rosenblatt M & Potts JT Jr (1984) Parathyroid hormone: chemistry, biosynthesis, secretion, action, and metabolism. *Physiology Reviews* **64**, 985–1053.
- Halioua L & Anderson JJ (1989) Lifetime calcium intake and physical activity habits: independent and combined effects on the radial bone of healthy premenopausal Caucasian women. *American Journal of Clinical Nutrition* **49**, 534–541.
- Harris S, Enger R, Riggs B & Spelsberg T (1995) Development and characterization of a conditionally immortalized human fetal osteoblastic cell line. *Journal of Bone and Mineral Research* **10**, 178–186.
- Hartman AM, Brown CC, Palmgren J, Pietinen P, Verkasalo M, Myer D & Virtamo J (1990) Variability in nutrient and food intakes among older middle-aged men. Implications for design of epidemiologic and validation studies using food recording. *American Journal of Epidemiology* **132**, 999–1012.
- Johansen JS, Giwercman A, Hartwell D, Nielsen CT, Price PA, Christiansen C & Skakkebaek NE (1988) Serum bone gla protein as a marker of bone growth in children and adolescents: correlation with age, height, serum insulin-like growth factor I, and serum testosterone. *Journal of Clinical Endocrinology and Metabolism* **67**, 273–278.
- Johnston CC, Miller JZ, Slemenda CW, Reister TK, Hui S, Christian JC & Peacock M (1992) Calcium supplementation and increases in bone mineral density in children. *New England Journal of Medicine* **327**, 82–87.
- Johnston CC, Slemenda CW & Melton LJ (1991) Clinical use of bone density. *New England Journal of Medicine* **324**, 1105–1109.
- Jones JI & Clemmons DR (1995) Insulin-like growth factors and their binding proteins: biological actions. *Endocrine Reviews* **16**, 3–34.
- Juul A, Bang P, Hertel NT, Main K, Dalgaard P, Jorgensen K, Muller J, Hall K & Skakkebaek NE (1994) Serum insulin-like growth factor-I in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. *Journal of Clinical Endocrinology and Metabolism* **78**, 744–752.
- Kanis JA (1994) Calcium nutrition and its implications for osteoporosis. Part I. Children and healthy adults. *European Journal of Clinical Nutrition* **48**, 757–767.
- Kardinaal AFM, Andò S, Charles P, Charzewska J, Rotily M, Väänänen K, Van Erp-Baart AMJ, Heikkinen J, Thomsen J, Maggiolini M, Deloraine A, Chabros E, Juvin R & Schaafsma G (1999) Dietary calcium and bone density in adolescent girls and young women in Europe. *Journal Bone and Mineral Research* **14**, 583–592.
- Katzman DK, Bachrach LK, Carter DR & Marcus R (1991)

- Clinical and anthropometric correlates of bone mineral acquisition in healthy adolescent girls. *Journal of Clinical Endocrinology and Metabolism* **73**, 1332–1339.
- Kawai N, Kanzaki S, Takano-Watou S, Tada C, Yamanaka Y, Miyata T, Oka M & Seino Y (1999) Serum free insulin-like growth factor I (IGF-I), total IGF-I, and IGF-binding protein-3 concentrations in normal children and children with growth hormone deficiency. *Journal of Clinical Endocrinology and Metabolism* **84**, 82–89.
- Kröger H, Kotaniemi A, Kröger L & Alhava E (1993) Development of bone mass and bone density of the spine and femoral neck – a prospective study of 65 children and adolescents. *Bone Mineral* **23**, 171–182.
- Larkin FA, Metzner HL & Guire KE (1991) Comparison of three consecutive-day and three random-day records of dietary intake. *Journal of the American Dietetic Association* **91**, 1583–1542.
- Lawson DEM, Paul AA, Black AE, Cole TJ, Mandal AR & Davie M (1979) Relative contributions of diet and sunlight to vitamin D state in the elderly. *British Medical Journal* **2** (6185), 303–305.
- Leboff MS, Fuleihan GE, Angell JE, Chung S & Curtis K (1992) Dual-energy x-ray absorptiometry of the forearm: reproducibility and correlation with single-photon absorptiometry. *Journal of Bone and Mineral Research* **7**, 841–846.
- Lloyd T, Andon MB, Rollings N, Martel JK, Landis JR, Demers LM, Egli DF, Kieselhorst K & Kulin HE (1993) Calcium supplementation and bone mineral density in adolescent girls. *Journal of the American Medical Association* **270**, 841–844.
- Matkovic V (1992) Calcium intake and peak bone mass. *New England Journal of Medicine* **327**, 119–120.
- Matkovic V, Fontana D, Tominac C, Goel P & Chesnut CH III (1990) Factors that influence peak bone mass formation: a study of calcium balance and the inheritance of bone mass in adolescent females. *American Journal of Clinical Nutrition* **52**, 878–888.
- Mazess RB, Barden HS, Bisek JP & Hanson J (1990) Dual energy x-ray absorptiometry for total body and regional bone mineral density and soft tissue composition. *American Journal of Clinical Nutrition* **51**, 1106–1112.
- Murphy S, Khaw KT, May H & Compston JE (1994) Milk consumption and bone mineral density in middle aged and elderly women. *British Medical Journal* **308**, 939–941.
- Ooms ME, Lips P, Roos JC, Van der Vijgh WJF, Popp-Snijders C, Bezemer PD & Bouter LM (1995) Vitamin D status and sex hormone binding globulin: determinants of bone turnover and bone mineral density in elderly women. *Journal of Bone and Mineral Research* **10**, 1177–1184.
- Roodman DG (1996) Advances in bone biology: the osteoclast. *Endocrine Reviews* **17**, 308–332.
- Rubin K, Schirduan V, Gendreau P, Sarfarazi M, Mendola R & Dalsky G (1993) Predictors of axial and peripheral bone mineral density in healthy children and adolescents, with special attention to the role of puberty. *Journal of Pediatrics* **123**, 863–870.
- Saggese G, Bertelloni S & Baroncelli GI (1997) Sex steroids and the acquisition of bone mass. *Hormone Research* **48**, 65–71.
- Selby C (1990) Sex hormone binding globulin: origin, function and clinical significance. *Annals of Clinical Biochemistry* **27**, 532–541.
- Sentipal JM, Wardlaw G M, Mahan J & Matkovic V (1991) Influence of calcium intake and growth indexes on vertebral bone mineral density in young females. *American Journal of Clinical Nutrition* **54**, 425–428.
- Sherman SS, Hollis BW & Tobin JD (1990) Vitamin D status and related parameters in a healthy population: the effects of age, sex and season. *Journal of Clinical Endocrinology and Metabolism* **71**, 405–413.
- Slemenda CW, Christian JC, Williams CJ, Norton JA & Johnston CC (1991) Genetic determinants of bone mass in adult women: a reevaluation of the twin model and the potential importance of gene interaction on heritability estimates. *Journal of Bone and Mineral Research* **6**, 561–567.
- Slemenda CW, Peacock M, Hui S, Zhou L & Johnston CC (1997) Reduced rates of skeletal remodeling are associated with increased peak bone mineral density during the development of peak skeletal mass. *Journal of Bone and Mineral Research* **12**, 676–682.
- Southard RN, Morris JD & Mahan JD (1991) Bone mass in healthy children: measurement with quantitative DXA. *Radiology* **179**, 735–738.
- Tanner JM (1978) Physical growth and development. In *Textbook of Paediatrics*, pp. 249–303 [JO Forfar and CC Arnell, editors]. Edinburgh: Churchill Livingstone.
- Tanner JM, Whitehouse RH, Cameron N, Marshall WA, Healy MJR & Goldstein H (1983) *Assessment of Skeletal Maturity and Prediction of Adult Height*. London: Academic Press.
- Turner RT, Riggs LB & Spelsberg TC (1994) Skeletal effects of estrogen. *Endocrine Reviews* **15**, 275–300.
- van Hemert AM, Birkenhäger JC, de Jong FH, Vandenbroucke JP & Valkenburg HA (1989) Sex hormone binding globulin in postmenopausal women: a predictor of osteoporosis superior to endogenous oestrogens. *Clinical Endocrinology* **31**, 499–509.
- Vedra J (1997) *Dietary Reference Intakes: Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride*. (1997) Washington, DC: National Academy Press.
- Young D, Hopper JL, Nowson CA, Green RM, Sherwin AJ, Kaymakci B, Smid M, Guest CS, Larkins RG & Wark JD (1995) Determinants of bone mass in 10- to 26-year-old females: A twin study. *Journal of Bone and Mineral Research* **10**, 558–567.