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Dietary calcium intake relates to bone mineral density in premenopausal women

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Bone density and Ca intake were assessed in fifty-six healthy premenopausal women, aged 21–47 years. Bone density was measured at the spine (lumbar vertebrae 1–4, anterio-posterior view), the non-dominant femur (neck, Ward's triangle and trochanter) and radius (33% distal and ultradistal) using dual energy X-ray absorptiometry (Lunar DPX-L). The mean values (SD) for bone density (g/cm²) were 1·18 (0·10) at the lumbar spine, 0·81(0·10) at the trochanter and for Ca intake 783 (329) mg. Bone density values were close to published normal values for young women and the Ca intakes were close to the recommended levels for the UK. However, there was a wide range especially in dietary Ca intake, and 27% of the group were at or below 500 mg/d. Correlations between Ca intake and bone density were highly significant at all three femoral sites (neck *r* 0·41; Ward's triangle *r* 0·40, $P < 0·01$; trochanter *r* 0·47, $P < 0·001$), significant at the spine (*r* 0·27, $P < 0·05$) but not found at the radius. These correlations were independent of body mass. The low levels of Ca intake found in a substantial proportion of this selected group of young women and their association with low femoral bone density are cause for concern.

Bone mineral density: Calcium: Premenopausal women

Bone density in the 3rd and 4th decades of life in women is increasingly recognized as important for long-term avoidance of osteoporotic fracture (Stevenson *et al.* 1989). A high 'peak bone mass' can be seen as a safety margin to protect bone strength in the face of inevitable menopausal losses (Law *et al.* 1991). Some studies relating dietary history and fracture incidence show that a high Ca diet can reduce the long-term risk of fracture (Matkovic *et al.* 1979; Holbrook *et al.* 1988), although not all such studies find this (Cooper *et al.* 1988; Wickham *et al.* 1989). Some studies have also shown that low Ca intake is associated with low bone density in young women (Kanders *et al.* 1988; Picard *et al.* 1988) while others have found no association between the two (Mazess & Barden, 1991). There is debate about the amount of dietary Ca needed to maintain optimal bone density. The current UK recommended nutritional intake (RNI) is set at 700 mg/d (Department of Health, 1991). However, studies of Ca balance (Heaney *et al.* 1977) indicate a requirement in young women of between 1·2 and 1·5 g/d and the US National Institutes of Health (1984) recommend an intake of 1·0 g/d. In two recent studies a threshold was observed at 800 mg/d; Ca intakes below this amount correlated significantly with bone density but further increases in Ca were not matched by similar increases in bone density (Kanders *et al.* 1988; van Beresteijn *et al.* 1990). The present report describes dietary Ca intakes in young women and their relationship with bone density at the three sites which fracture most commonly and, therefore, are of concern clinically, namely the spine, femur and radius.

* For reprints.

METHODS

The subjects were premenopausal women recruited by postal invitation sent to every third name on the age/sex register of a large group practice. Of 160 initial volunteers (response rate 27%), fifty-six women aged 21–47 years satisfied the selection criteria and entered the study. Selection criteria included: Caucasian race, no recent injury, no prescribed medication other than oral contraceptives, not pregnant and at least 12 months postpartum. Of the volunteers, 86% were in paid employment. Those who were currently involved in vigorous physical activity either at work or in their leisure were excluded. Activities likely to influence bone density such as squash, jogging or weight training were cause for exclusion, but swimming or walking were allowed since they have been found to have no influence (Marcus *et al.* 1992). The district from which they came was predominantly social classes 3 and 4 (Office of Population Censuses and Surveys 1980). Ethical permission was granted by the Medical School Ethical Committee and subjects gave their informed consent. All measurements were made during March and April.

Bone density was assessed, using dual-energy X-ray absorptiometry (Lunar DPX-L; Lunar Radiation Corporation, Madison, WI, USA), in the lumbar spine (lumbar vertebrae 1-4, anterio-posterior view), non-dominant femur and distal radius. Subjects were measured on one occasion using standard procedures. The femoral neck, Ward's triangle and trochanteric regions of the proximal femur and the 33% distal site of the radius were selected for analysis according to Lunar software V.1.2. In addition an ultradistal site of the radius was selected, defined as a region extending 15 mm proximally from the point at which the projection of the distal ulna and radius overlap. This definition differs from the Lunar definition for an ultradistal region of interest (UD ROI). It was chosen to simplify the analysis procedure since there is no generally agreed definition for an UD ROI between manufacturers and techniques.

The densitometer was calibrated daily according to the manufacturer's protocol. An Al step wedge in 150 mm depth of water (a phantom) was used as an independent weekly check and observed over time. In a period of about 21 months (sixty-eight observations) the slope of the regression of bone mineral density *v.* time for the phantom was $-0{\cdot}0016$ g/cm^2 per year ($-0{\cdot}13$%/year) which was not statistically significantly different from zero (t $-1{\cdot}1$, $P = 0{\cdot}36$). *In vivo* repeatability within our laboratory was determined from repeat measurements made about 1 week apart in forty-three subjects. The coefficient of variation for the respective sites was derived from the standard error of the mean difference divided by the overall mean (Healy 1958), and found to be 1·4% (n 21), 1·9–4·0% (n 43) and 1·2–2·5% (n 43) in the lumbar spine, femur and radius respectively. The effective dose equivalent (EDE) is less than 1·0 μSv per scan for women of reproductive capacity. However, as a precaution, subjects were measured within 10 d of the start of their previous menstrual cycle.

Height was measured to the nearest 0·005 m using a free-standing stadiometer. Body mass was measured to the nearest 0·05 kg (Marsdens balance scales). Body mass index (BMI) was derived from the mass (kg) divided by the square of the height (m).

A structured food frequency questionnaire administered by one interviewer was used to assess the level of Ca nutrition. The questionnaire contained a list of twelve different foods known to have a high Ca content including dairy, fish, fruit, vegetable and cereal products. Taking each food, one at a time, subjects were asked whether they included it in their habitual diet. They were then asked how frequently they consumed this food (daily, once weekly etc.) and then how much of it. The interviewer explored appropriately for portion size, e.g. pints of milk, ounces of cheese (including distinctions between 'hard' and 'soft' cheese), or pots of yoghurt (125 g etc.). Ingredients included in cooking such as milk or cheese were

Table 1. *Physical characteristics, calcium intake and bone mineral density of premenopausal Caucasian women*

(Mean values and standard deviations for fifty-six subjects)

	Mean	SD	Range	Median
Age (years)	31·5	5·69	21–47	30·5
Height (m)	1·63	0·06	1·52–1·76	1·63
Wt (kg)	63·6	9·01	48·0–91·0	62·0
BMI (kg/m^2)	24·0	3·44	18·7–33·4	23·3
Ca intake (mg/d)	867·1	709·6	200–5500	810·0
	782·9*	328·9*	200–1550*	787·5*
Bone density (g/cm^2)				
Lumbar (L1–L4)	1·18	0·10	0·97–1·44	1·17
Femur: Neck	0·99	0·10	0·73–1·26	0·98
Ward's triangle	0·96	0·13	0·65–1·29	0·96
Trochanter	0·81	0·10	0·53–1·04	0·81
Radius: Ultradistal	0·46	0·05	0·32–0·58	0·47
33% Distal	0·83	0·05	0·72–0·95	0·83

BMI, body mass index (weight/height^2). L1–L4, lumbar vertebrae 1–4.
* Excluding one individual with high Ca intake.

also explored. The interviewer then used this information along with standard food tables (Holland *et al.* 1991) to arrive at a semi-quantitative estimate of average daily Ca intake for each woman.

In a separate study of twenty-nine women the food frequency questionnaire was administered twice with a 6-month interval. The two mean estimates of Ca intake (mg) were 1075 (SD 236) and 1102 (SD 290); they correlated well (r 0·94, $P < 0·001$) and the mean difference was 28 (SD106) mg giving a coefficient of variation of 6·9%. Comparison of the food frequency method with a 3 d weighed food intake in twenty-seven women showed that the two different methods were well correlated (r 0·71, $P < 0·01$). Mean Ca intakes were 1183 (SD 225) mg for the food frequency assessment and 1204 (SD 310) mg for the 3 d weighed intake. The mean difference was 22·0 (SD 217) mg.

Data are expressed as means (SD) unless otherwise stated. Pearson product moment correlations were used to explore simple relationships between the variables and two-tailed significance levels were obtained. The degrees of freedom were 54 (n 56) unless otherwise stated. Stepwise multiple-regression analyses (SPSSX3; SPSS Inc., 1988) were used to find which factors explained the most variance, using each bone density site as a separate dependent variable.

RESULTS

Descriptive results for all variables are given in Table 1. The women were on average similar in physique to a large representative British sample aged 25–34 years (height 1·64 m, body mass 64·5 kg, BMI 24·0; Fentem, 1992). One individual, who had an unusually high Ca intake (5500 mg/d) due to excessive consumption of cheese, was excluded from all analyses involving Ca. The mean daily Ca intake for the remaining group of fifty-five women was 783 mg, with an approximately normal distribution.

The relationships between bone density, body composition and Ca intake are given in Table 2. The bone densities were significantly correlated with each other except for the spine and the 33% distal site of the radius. Height and body mass were independent. Ca

Table 2. *Pearson product moment correlation coefficients (r) between bone density, anthropometry and calcium intake*

	Anthropometry				Bone densities					
						Femur			Radius	
	Wt	Ht	BMI	Ca†	L1–L4	Neck	Ward's triangle	Trochanter	Ultradistal	33 % Distal
Age (years)	NS	NS	NS	NS	NS	NS	−0·28	NS	NS	NS
Wt (kg)		NS	0·86	NS	NS	0·42	0·29	0·40	NS	NS
Height (m)			−0·27	NS	NS	NS	NS	NS	−0·27	NS
BMI (kg/m²)				NS	NS	0·37	NS	0·42	0·33	NS
Ca intake (mg/d)†					0·27	0·41	0·40	0·47	NS	NS
Lumbar (L1–L4)						0·45	0·52	0·42	0·45	NS
Femur: Neck							0·87	0·80	0·53	0·34
Ward's triangle								0·72	0·57	0·34
Trochanter									0·51	0·29
Radius: ultradistal										0·47

BMI, body mass index (weight/height²); NS, not significant.
Critical r: $P < 0{\cdot}05$ 0·26; $P < 0{\cdot}01$ 0·31; $P < 0{\cdot}001$ 0·42.
† (n 55).

intake was correlated with all bone densities except at the radial sites; the trochanter was the most highly correlated ($P < 0{\cdot}001$; see Fig. 1) followed by the femoral neck, Ward's triangle and the lumbar spine ($P < 0{\cdot}01$, $P < 0{\cdot}01$ and $P < 0{\cdot}05$ respectively). Femoral bone density was also correlated with body mass, the relationship being more significant for the neck and trochanter ($P < 0{\cdot}01$) than for Ward's triangle ($P < 0{\cdot}05$). Similarly, bone density for the neck and trochanter but not for Ward's triangle were correlated with BMI. For the radius, bone density for the ultra-distal site was negatively correlated with height ($P < 0{\cdot}05$) and positively correlated with BMI ($P < 0{\cdot}05$). No significant correlations were observed at the 33 % distal site except with other bone densities. Ca intake was not correlated with body mass nor BMI. An age-related decline was observed only at Ward's triangle ($P < 0{\cdot}05$).

All measured variables which were significant in simple regression were entered into stepwise multiple-regression analysis and the equations below provide the best descriptions found. Bone density is expressed as g/cm² and the explained variance is given as a percentage.

Lumbar spine: 0·086 Ca (g) + 1·108 — R 0·27, 7 %;

femoral neck: 0·115 Ca (g) + 0·004 mass (kg) + 0·625 — R 0·56, 31 %;

Ward's triangle: 0·145 Ca (g) − 0·006 age (year) + 0·004 mass (kg) + 0·810 — R 0·54, 29 %;

trochanter: 0·137 Ca (g) + 0·011 BMI + 0·436 — R 0·60, 36 %;

radius, ultradistal: 0·004 BMI + 0·363 — R 0·30, 9 %;

radius, 33 %: no variables were found.

Ca intake remained significant at all sites except the radius and its contribution to the total explained variance ranged from 7 % at the spine to 20 % at the trochanter. In addition either body mass or BMI were significant at the femur and radius. Age remained significant at Ward's triangle.

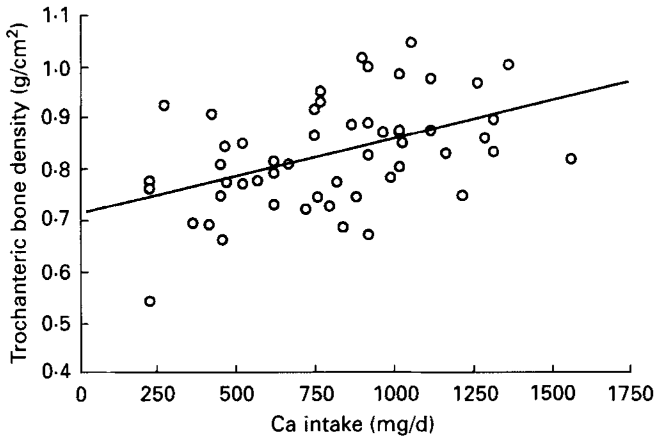

Fig. 1. Dietary calcium intake and femoral bone density in premenopausal women. Each point represents one value for one individual measured on one occasion; (——), the least-squares best-fit regression line (r 0·47, $P < 0·001$). For details of subjects and procedures, see pp. 78–79.

DISCUSSION

The repeatability of measurements of bone density using dual energy X-ray absorptiometry is now extremely good (Johnson & Dawson-Hughes, 1991), the scan times are short and the radiation dose low, so accurate information at all potential fracture sites can be obtained conveniently and safely. Mean bone densities for our group were not dissimilar to those reported in other studies of young healthy women which used the same methodology (Rockwell *et al.* 1990; Snowharter *et al.* 1990). The bone densities at the different sites were in general significantly correlated with each other, but some correlations were low. This confirms other reports (Seldin *et al.* 1988; Wahl *et al.* 1988) and shows that prediction of densities from one site to another for an individual would not be accurate for clinical diagnosis. The site of interest should be measured directly.

Assessment of dietary intake of any nutrient inevitably has a low repeatability compared with measurements of variables such as bone density. However, in general, food frequency methods compare quite well with weighed methods (Seldin *et al.* 1988; Wahl *et al.* 1988), and the repeatability and validity of the food frequency method adopted in the present study was good. The mean Ca intake of 783 (SD 329) mg was close to the RNI of 700 mg (Department of Health, 1991), but the range was wide with fifteen individuals (27% of the group) below 500 mg. Other reported studies of selected groups of young women give mean intakes of 576 (SD 383) mg (Picard *et al.* 1988), 871 (SD 318) mg (Kanders *et al.* 1988) and 909 (SD 351) mg (Mazess & Barden, 1991). Kanders *et al.* (1988) selected subjects for high levels of activity and they report a significant relationship between intakes of Ca and total energy. Variation in intakes was similar and rather large in all these studies; many individuals, therefore, have habitual dietary intake well below recommended amounts. Whether these intakes are adequate or not depends on efficiency of absorption (Heaney *et al.* 1977; Gallagher *et al.* 1979) as well as body size and individual needs.

Despite a wide range, age was not significant in the present study, except at Ward's triangle ($P < 0·05$). This may be because the mean age was only 31 years, so half the group may not yet have attained their peak bone mass (Rodin *et al.* 1990). Some studies show little or no age-related change in pre-menopausal women (Gallagher *et al.* 1987; Lindsay *et al.* 1992), and others show that bone density begins to decline slightly from about 30 years of age, well before the menopause (Riggs *et al.* 1981; Hansson & Roos, 1986; Smith, 1987;

Melton III *et al.* 1988, 1989). Our finding accords closely with Stevenson *et al.* (1989) who found a steep decline at Ward's triangle with only a slight change at the lumbar spine and other femoral areas.

Ca intake had a consistent, highly significant and numerically rather similar association with bone density at lumbar and all femoral sites. Although the variance explained by Ca intake was low, the outcome suggests that some intakes are inadequate. The slopes of the relationships suggest that, on average, increasing Ca intake by 200 mg could increase bone density by a few percent which might be useful for future protection from fracture. A number of studies in postmenopausal women (Recker *et al.* 1977; Ettinger *et al.* 1987; Riis *et al.* 1987; Smith *et al.* 1989; Nelson *et al.* 1991) and at least one in men (Kelly *et al.* 1990) have found associations between Ca intake and bone density at various sites, but only two other studies in premenopausal women have been found (Kanders *et al.* 1988). These both showed an association between radial bone density and Ca intake but findings for the spine were less convincing and the femur was not examined.

Femoral bone densities were also significantly and selectively associated with either body mass or BMI which is consistent with the view that these bone densities are sensitive to load-bearing and gravitational effects on carried body mass. Slight stature was associated with low bone density only in the radius. This is perhaps due to the heterogeneity of build within the group; lack of correlation between height and body mass is unusual. Those who were light or thin did appear to have lower bone densities at the hip and so they are likely to be more at risk in the long term from fractured femur.

Larger people may have greater energy intakes, and this in turn may be associated with higher Ca intakes. However, Ca intake was not related to body mass and multiple-regression analysis confirmed the independent effects of both body composition and dietary Ca. High levels of activity have also been thought to lead to increased Ca intake for similar reasons (Kanders *et al.* 1988; Kelly *et al.* 1990). However, it is necessary to distinguish between high levels of activity produced by prolonged moderate exercise which increases energy expenditure but is not thought to affect bone density (Marcus *et al.* 1992), and that produced by intermittent brief bouts of intense exercise which probably does affect bone density (Gleeson *et al.* 1990; Gutin & Kasper, 1992). The latter was an exclusion criterion for the present study; it seems unlikely, therefore, that the relations found between Ca intake and bone density could be attributed to variations in activity levels within the group.

The low levels of dietary Ca found in this selected group and their association with low bone density are cause for concern. If bone densities are below the optimum before the menopause then the likelihood of osteoporotic fracture in later life is increased.

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