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Dietary calcium, sex hormones, and bone mineral density in men

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Abstract

Objective—To evaluate the factors that determine bone mineral density at axial and appendicular sites in normal men.

Design—Measurement of bone mineral density of the radius by single photon absorptiometry and of the lumbar spine and hip by dual photon absorptiometry to assess their relation with various determinants of bone mineral density. Dietary calcium was assessed from a questionnaire validated against a four day dietary record.

Setting—Local community, Sydney, Australia.

Patients—48 Men (aged 21-79, median 44) recruited from the local community including 35 male cotwins of twin pairs of differing sex recruited from the Australian National Health and Medical Research Council twin registry for epidemiological studies on determinants of bone mineral density.

Main outcome measures—Bone mineral density of the axial and appendicular skeleton and its relation to age, anthropometric features, dietary calcium intake, and serum sex hormone concentrations.

Results—Dietary calcium intake (g/day) was a significant predictor of bone mineral density of axial bones, explaining 24% and 42% of the variance at the lumbar spine and femoral neck respectively. This effect was independent of weight. In contrast with the axial skeleton, bone mineral density at each forearm site was predicted by weight and an index of free testosterone but not by dietary calcium intake.

Conclusions—Dietary calcium intake has a role in the determination or maintenance, or both, of the axial but not the appendicular skeleton in adult men.

Introduction

Although osteoporosis is more common in elderly women, men also have an increased risk of sustaining fractures of the hip, spine, and forearm with aging.¹ Moreover, a survey done in Sweden has suggested that, though the age adjusted incidence of fractures of the hip may be declining in women, the incidence of such fractures in elderly men is increasing.² In contrast with women, however, few data have been published on the factors that determine bone density in normal men.

With cotwins of differing sex we found that though men had substantially greater bone mineral density of their forearms and were appreciably heavier than their female cotwins, the women tended to have greater bone mineral density of their lumbar spines and there was no difference in femoral neck density.³ Those contrasting differences in bone mineral density with sex at various skeletal sites led us to assess the determinants of mineral density of axial and appendicular bones in normal men. We studied the relation between dietary calcium, anthropometric measures, sex hormones, and biochemical indices of bone turn-

over and bone mineral density of the lumbar spine, proximal femur, and forearm in a group of normal men.

Methods

Forty eight men aged 21-79 (median 44) who had been recruited for epidemiological studies on bone density were included in the study and 35 of these were cotwins of twin pairs of differing sex. The study group also included four pairs of monozygotic twins recruited for studies of the genetic effect on bone density. All twins were recruited from the Australian National Health and Medical Research Council twin registry. All subjects were eugonadal as determined by serum testosterone concentrations, and none had a history of fracture or of bone disease or were taking medication known to affect bone.

The bone mineral density (g/cm²) at both axial skeletal sites was estimated as the bone mineral content corrected for the area (cm²) of skeletal tissue scanned. Single photon absorptiometry was used to determine the bone mineral density of the radius. This method measures the bone mineral content of a given length of the distal radius and then estimates the bone mineral density (g/cm²) by correcting the bone mineral content for the width of the bone. Bone mineral density was measured at the L2-L4 part of the lumbar spine and at three regions of the proximal femur (femoral neck, Ward's triangle, and trochanteric region) with a Lunar DP3 dual photon absorptiometer (Lunar Radiation, Madison, Wisconsin, United States).⁴ The coefficient of variation was 1.4% with cadaveric vertebrae and 2.6% at the femoral neck in normal volunteers.⁴ Radiographs of the lumbar spine were obtained in all subjects aged over 40 to exclude the presence of fractures, severe osteoarthritis, or calcification of the aorta, because these may spuriously increase the calculated bone mineral density of the lumbar spine. Bone mineral density was also measured at the distal and ultradistal radius of the non-dominant forearm in 30 subjects by single photon absorptiometry with a Lunar SP-2 densitometer (Lunar Radiation, Madison, Wisconsin, United States). The precision of this method was 1.6% in normal volunteers (unpublished data). Lean body mass and fat mass were calculated from height, weight, and age.⁵ Dietary calcium (g/day) was estimated from a questionnaire validated against a four day weighed diet record.⁶ Ten of the subjects, who had taken part in the validation of the dietary calcium questionnaire, had data available on their total daily energy intake as assessed by a four day dietary weight record.

Blood samples were taken in the early morning after an overnight fast. The serum osteocalcin concentration was measured in our laboratories by a radioimmunoassay with rabbit antiserum osteocalcin antiserum, a method that has intra-assay and interassay coefficients of variation of 8% and 10% respectively. The hydroxy-

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Br Med J 1990;300:1361-4

proline:creatinine ratio was calculated by measuring the concentrations of creatinine and hydroxyproline in samples of urine collected over two hours after an overnight fast.⁷ Serum concentrations of oestradiol and testosterone were measured in our laboratories by radioimmunoassay of benzene extracted serum with charcoal separation. The intra-assay and interassay coefficients of variation were 9.4% and 10.1% for serum oestradiol concentration and 6.0% and 8.3% for serum testosterone concentration. The serum dehydroepiandrosterone sulphate concentration was measured by specific radioimmunoassay with a tritiated tracer and a charcoal separation step, the intra-assay and interassay coefficients of variation being 7.3% and 12.5% respectively. Serum sex hormone binding globulin concentration was measured in 32 subjects by solid phase second antibody immunoradiometric assay (Farmos Diagnostica, Finland), the intra-assay and interassay coefficients of variation being 6.4% and 9.0% respectively. An index of free testosterone concentration was estimated as the ratio of testosterone to sex hormone binding globulin.⁸ The concentrations of follicle stimulating hormone and luteinising hormone were measured by radioimmunoassay (Micromedic), with intra-assay and interassay coefficients of variation of 6.0% and 10.0% for follicle stimulating hormone and 7.0% and 9.0% for luteinising hormone.

The predictors of bone mineral density at each site were determined by stepwise linear regression analysis using the Statview 512+ statistical package (Abacus Concepts, Berkeley, California). The serum osteocalcin, oestradiol, testosterone, and sex hormone binding globulin concentrations and the free testosterone index and urinary hydroxyproline:creatinine ratio were not normally distributed and were therefore logarithmically transformed before analysis.

Results

The mean (95% confidence intervals) of the variables measured in the subjects were 175.7 cm (173.5 to 177.5) for height, 77.2 kg (72.5 to 79.6) for weight, 25.0 kg/m² (23.0 to 25.8) for body mass index, 62.8 kg (60.3 to 64.3) for lean body mass, and 14.9 kg (12.5 to 16.0) for fat mass. The median serum testosterone concentration was 17.8 nmol/l (range 11.6 to 37.0) and median free testosterone index was 0.69 (range 0.47 to 1.15).

There was a negative relation between age and lean body mass ($r = -0.40$, $p < 0.01$) but no relation between age and body mass index ($r = 0.04$) or fat mass ($r = 0.12$, $p = 0.42$). Bone mineral density declined with age at the femoral neck ($r = -0.40$, $p < 0.01$) and Ward's triangle ($r = -0.51$, $p = 0.0006$) but not at the trochanteric region ($r = -0.06$). There was no relation between age and bone mineral density at the lumbar spine or either forearm site (table).

There was no significant relation between age and serum testosterone concentration ($r = 0.06$), age and serum oestradiol concentration ($r = 0.14$, $p = 0.36$), or age and serum sex hormone binding globulin concentration ($r = 0.22$, $n = 32$, $p = 0.24$). The log free testos-

terone index did, however, fall significantly with age ($r = -0.55$, $p = 0.002$, $n = 32$), as did the serum dehydroepiandrosterone sulphate concentration ($r = -0.55$, $p = 0.0001$, $n = 48$). These relations remained significant at the < 0.0025 level even when the significance level was adjusted to account for the number of comparisons made (Bonferroni correction). There was no significant relation between age and log serum osteocalcin concentration ($r = -0.04$), although there was a tendency for the log urinary hydroxyproline:creatinine ratio to decline with age ($r = -0.27$, $p = 0.07$). There was no significant relation between indices of bone turnover and serum sex hormone or dehydroepiandrosterone sulphate concentrations or between indices of bone turnover and bone mineral density at any site.

Figure 1 shows the positive relation that was found between dietary calcium intake and bone mineral density of the lumbar spine ($r = 0.49$, $p < 0.001$) and femoral neck ($r = 0.65$, $p = 0.0001$). This positive

FIG 1—Relation between dietary calcium and bone mineral density at lumbar spine and femoral neck in normal men

relation was also found at Ward's triangle ($r = 0.46$, $p = 0.0012$) and the trochanteric region ($r = 0.42$, $p < 0.004$). Though a higher dietary calcium intake was associated with greater bone mineral density at the distal radius, this was not significant at the ultradistal radius (table). When the bone mineral density values corresponding to the three subjects whose dietary calcium intake exceeded 1.30 g/day were removed from the analysis the relation remained significant at both the femoral neck ($r = 0.44$, $p < 0.006$) and lumbar spine ($r = 0.29$, $p = 0.05$) but there was no longer a significant relation at the trochanteric region ($r = 0.24$, $p = 0.12$) or at Ward's triangle ($r = 0.20$, $p = 0.20$). Alternatively when the data were analysed non-parametrically (Spearman's rank correlation) a significant positive relation remained at all the axial sites ($z = 3.05$, $p < 0.01$ for the lumbar spine; $z = 4.03$, $p < 0.001$ for the femoral neck; $z = 2.55$, $p < 0.01$ for Ward's triangle; and $z = 2.41$, $p < 0.02$ for the trochanteric region). The relation at the distal radius was no longer significant ($z = 1.64$, $p = 0.10$).

In the 10 subjects who had data on both dietary

Correlations between dietary calcium (g/day) and bone mineral density at axial and appendicular sites and anthropometric measures of height, weight, body mass index, lean body mass, and fat mass

	Dietary calcium	Lumbar spine	Femoral neck	Distal radius	Ultradistal radius
Dietary calcium		0.49***	0.65***	0.34*	0.25
Age	-0.24	-0.06	-0.40**	-0.12	0.0002
Weight	0.23	0.45**	0.49**	0.55***	0.55***
Height	0.27	0.20	0.17	0.12	0.21
Body mass index	0.05	0.34**	0.42**	0.37**	0.43**
Lean body mass	0.27**	0.36**	0.51**	0.45**	0.48**
Fat mass	0.06	0.34**	0.39**	0.42**	0.51**

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

calcium and energy intake we analysed the relation between these variables and bone mineral density. There was no significant relation between energy intake and bone density at either the lumbar spine or femoral neck, but there remained significant positive relations between dietary calcium (mmol/4187 J) and bone mineral density at the lumbar spine ($r=0.69$, $p=0.03$) and femoral neck ($r=0.80$, $p<0.01$).

The simple relations between bone mineral density at the axial and appendicular sites and anthropometric measures are given in the table. The relative importance of these predictors of bone mineral density at each site were determined by stepwise regression analysis including age (years), height (cm), weight (kg), and estimated daily dietary calcium intake (g/day). Dietary calcium remained an important independent predictor of bone mineral density at all axial sites.

REGRESSION EQUATIONS

The regression equations obtained from this analysis were as follows.

$$\text{Lumbar spine bone mineral density} = 0.73 + 0.20 \text{ dietary calcium} + 0.004 \text{ weight}$$

$R^2=0.32$, $p=0.00002$, partial regression coefficients significant at the <0.01 and <0.05 level respectively (fig 1).

$$\text{Femoral neck bone mineral density} = 0.45 + 0.20 \text{ dietary calcium} + 0.004 \text{ weight}$$

$R^2=0.51$, $p=0.0001$, partial regression coefficients significant at the <0.0001 and 0.002 level respectively (fig 1). Similar results were found for Ward's triangle and the trochanteric region. Age, however, was an independent predictor of the bone mineral density of Ward's triangle.

Anthropometric measures predicted bone mineral density at the forearm sites but dietary calcium and age did not. The final regression equations for the forearm sites were:

$$\text{Distal radius bone mineral density} = 0.25 + 0.003 \text{ weight} \quad (r=0.55, p<0.001).$$

$$\text{Ultradistal radius bone mineral density} = 0.21 + 0.003 \text{ weight} \quad (r=0.55, p<0.005).$$

The relations between serum sex hormone and dehydroepiandrosterone sulphate concentrations and bone density at each site were assessed by multiple linear regression analysis in the subgroup of 30 subjects in whom serum sex hormone binding globulin concentrations were available. Forearm densitometry data were available for 23 of these 30 subjects. The predictors incorporated in the analysis were age, serum dehydroepiandrosterone sulphate concentration, log serum testosterone concentration, log serum oestradiol concentration, and log free testosterone index. Age remained the only significant predictor of bone mineral density at the femoral neck, and none of these variables predicted bone mineral density at the other proximal femur regions or the lumbar spine. The log free testosterone index was, however, the best predictor of bone mineral density at both forearm sites (fig 2):

$$\text{Distal radius bone mineral density} = 0.50 + 0.28 \text{ log free testosterone index} \quad (r=0.46, p<0.05).$$

$$\text{Ultradistal radius bone mineral density} = 0.46 + 0.29 \text{ log free testosterone index} \quad (r=0.51, p<0.05).$$

When weight was included in these regression equations the log free testosterone index was no longer a significant predictor of bone mineral density at the distal radius. Weight and log free testosterone index were, however, significant independent predictors of bone mineral density at the ultradistal radius ($R^2=0.44$).

FIG 2—Relation between free testosterone index (ratio of testosterone to sex hormone binding globulin) and bone mineral density at distal and ultradistal radius in normal men

Discussion

We have studied potential predictors of bone mineral density at the lumbar spine, proximal femur, and forearm in a group of normal men. Dietary calcium intake was an important predictor of bone mineral density at the hip and lumbar spine, accounting for 24% and 42% of the variance in bone mineral density at each site respectively. Such a relation between dietary calcium intake and bone mineral density has not been reported in men or women.

There have been numerous studies suggesting that bone loss with aging and the incidence of fractures are not related to dietary calcium intake.⁹⁻¹¹ Most studies have, however, concentrated on bone mineral density in women. Interestingly, in a recent case-control study, though no association was found between dietary calcium intake and the incidence of fractures of the hip in women, men who had a dietary calcium intake greater than 1 g/day had a lower risk of sustaining a fracture.¹² A Californian study found that fractures of the hip were commoner in men and women who had lower dietary calcium intakes.¹³ Though there is no evidence that the rate of loss of bone mineral density, at least in women, is related to current dietary calcium intake, there are data to suggest that peak adult bone density might be influenced by dietary calcium.¹⁴ One group has found that the estimated calcium intake in young adulthood might be used to predict lumbar bone mineral content in premenopausal women aged 40-50.¹⁵ Block *et al* found a positive correlation between bone mineral density at the femoral neck and dietary calcium intake in a group of weightlifters, though no such relation was found among sedentary men.¹⁶ Others have suggested an interaction between physical activity and dietary calcium intake.¹⁷

It may be argued that those individuals who had the greater calcium intake also had a greater total energy intake and were more physically active. In the group of 10 subjects in whom data about energy intake were available, however, the strong positive relation

between calcium intake (corrected for total energy intake) and bone mineral density at both the femoral neck and lumbar spine remained. Interestingly, Holbrook *et al* found a higher incidence of fractures of the hip in subjects with a lower dietary calcium intake corrected for energy intake.¹³ Though we know of no studies that have directly assessed the relation between physical activity and dietary calcium, we found that dietary calcium intake in a group of 141 women did not correlate with their predicted maximal oxygen uptakes, which were used as an index of physical activity ($r=0.05$ unpublished data). Furthermore, Kanders *et al* found that subjects expending more than 4061 kJ energy a day did not differ in their dietary calcium intake from those expending less than 4061 kJ energy a day. In the same study subjects with a dietary calcium intake of less than 0.8 g/day did not differ in their total energy expenditure but had a lower bone density at the lumbar spine and femoral neck than those whose dietary calcium intake was greater than 0.8 g/day. These observations suggest that the relation between dietary calcium and bone mineral density is not due to the effect of physical activity on bone density.

It is perhaps surprising that the current dietary calcium intake should have a predictive relation to the current bone mineral density at any site. Logically dietary calcium might be expected to exert its effect over many years. Hence one possible explanation is that dietary intake in general, including calcium, might be more stable in men than in women, being less affected by media information and dietary fads, which tend to be targeted at women. Such variations might explain why a similar effect of dietary calcium on the mineral density of axial bones in women has not been shown, although certain studies have suggested that long term calcium intake might influence the attainment and maintenance of peak bone mass.^{14,15} Thus the negative studies might have been confounded by the greater variability of diet between the time when dietary intake was assessed and the extended periods of time over which dietary calcium would be expected to have influenced the attainment—that is, young adulthood—or maintenance, or both, of peak bone mass.

We have found that though the bone mineral density of the forearm is up to 27% higher in men than in their female cotwins, there is little sex difference in bone mineral density at the axial sites.³ Interestingly, in our present study there was no relation between serum sex hormone concentrations and bone mineral density of either the hip or the lumbar spine, but a free testosterone index predicted bone mineral density at both forearm sites. No previous studies have shown a positive relation between circulating serum gonadal steroid hormone concentrations and bone mineral density of the forearm in normal men. Foresta *et al*, using metacarpal radiography, have, however, shown a positive correlation between cortical bone mass at this site and plasma testosterone concentrations in elderly men.¹⁸ In contrast, McElduff *et al* found an appreciable but small negative relation between total serum testosterone concentration and bone mineral content of the non-dominant forearm whereas neither total nor free testosterone concentrations predicted the bone mineral content of the dominant forearm. It has been well established that vertebral and forearm osteopenia occurs in hypogonadal men, but sex hormone concentrations do not predict the bone density in these subjects.^{20,21} Greenspan *et al* assessed forearm and spine density in men with hyperprolactinaemic hypogonadism.²² Those subjects who had successful treatment and restoration of gonadal function had appreciable increases in radial shaft density, but vertebral trabecular density increased only slightly. Others

have found that in men with hypogonadotrophic hypogonadism with fused epiphyses treatment with testosterone results in increases in forearm bone density without changes occurring in vertebral trabecular density.²³ These data support the concept that appendicular bone, such as the forearm, might be more responsive than axial bone, such as the spine or hip, to changes in circulating concentrations of testosterone.

In conclusion, the predictors of bone mineral density of the appendicular and axial skeleton were studied in a group of normal men. There was a strong positive relation between dietary calcium and bone mineral density of the lumbar spine and of the three regions of the proximal femur. In contrast with the axial skeleton, bone mineral density at appendicular sites was predicted by an index of free serum testosterone. These data suggest differential effects of environmental influences on the different sites in the adult male skeleton. Furthermore, the data show that dietary calcium intake has an important role in the determination or maintenance, or both, of the adult male axial skeleton and suggest that this issue requires further examination in women.

This research was supported by the National Health and Medical Research Council, the Garvan Research Foundation, and the New South Wales Dairy Corporation.

- Gallagher JC, Melton LJ, Riggs BL, Bergstrath E. Epidemiology of fractures of the proximal femur in Rochester, Minnesota. *Clin Orthop* 1980;150:163-71.
- Naessen T, Parker P, Persson I, Zack M, Adami HO. Time trends incidence rates of first hip fracture in the Uppsala health care region, Sweden, 1965-1983. *Am J Epidemiol* 1989;130:289-99.
- Eisman JA, Kelly PJ, Sambrook PN, Kempster S. Peak adult bone density greater in women than in men. *Journal of Bone and Mineral Research* 1989;4(suppl 1):Abstract 1188.
- Pocock NA, Eberl S, Eisman JA, *et al*. Dual-photon bone densitometry in normal Australian women: a cross-sectional study. *Med J Aust* 1987;146:293-6.
- Hassager C, Gotfredsen A, Jensen J, Christiansen C. Prediction of body composition by age, height, weight, and skin fold thickness in normal adults. *Metabolism* 1986;35:1081-4.
- Angus RM, Sambrook PN, Pocock NA, Eisman JA. A simple method for assessing calcium intake in caucasian women. *J Am Diet Assoc* 1989;89:209-14.
- Nordin BEC. Diagnostic procedures in disorders of calcium metabolism. *Clin Endocrinol* 1978;8:55-67.
- Anderson DC. Sex hormone binding globulin. *Clin Endocrinol* 1974;3:69.
- Wickham CAC, Walsh K, Cooper C, *et al*. Dietary calcium, physical activity, and risk of hip fracture: a prospective study. *Br Med J* 1989;299:889-92.
- Riggs BL, Wahner HW, Melton LJ, Richelson LS, Judd HL, O'Fallon WM. Dietary calcium intake and rates of bone loss in women. *J Clin Invest* 1987;80:979-82.
- Stevenson JC, Lees B, Cust MP, Ganger KF. Determinants of bone density in normal women: risk factors for future osteoporosis. *Br Med J* 1989;298:924-8.
- Cooper C, Barker JDP, Wickham C. Physical activity, muscle strength, and calcium intake in fracture of the proximal femur. *Br Med J* 1988;297:1443-6.
- Holbrook TL, Barrett-Connor E, Wingard DL. Dietary calcium intake and risk of hip fracture: 14 year prospective population study. *Lancet* 1988;ii:1046-9.
- Matkovic V, Dekanic D, Siminovic I, *et al*. Bone status and fracture rates in two regions of Yugoslavia. *Am J Clin Nutr* 1979;32:540-9.
- Picard D, Ste-Marie LG, Coutu D, *et al*. Premenopausal bone mineral content relates to height, weight, and calcium intake during early adulthood. *Bone and Mineral* 1988;4:299-309.
- Block JE, Freidlander AL, Brooks GA, Steiger P, Stubbs HA, Genant H. Determinants of bone density among athletes engaged in weight-bearing and non-weight-bearing activity. *J Appl Physiol* 1989;67:1100-5.
- Kanders B, Dempster D, Lindsay R. Interaction of calcium nutrition and physical activity on bone mass in young women. *Journal of Bone and Mineral Research* 1988;3:145-9.
- Fresta C, Ruzza G, Mioni R, *et al*. Osteoporosis and decline in gonadal function in elderly men. *Horm Res* 1984;19:18-22.
- McElduff A, Wilkinson M, Ward P, Posen S. Forearm mineral content in normal men: relationship to weight, height, and plasma testosterone concentrations. *Bone* 1988;9:281-3.
- Finkelstein JS, Klibanski A, Neer RM, Greenspan SL, Rosenthal DL, Crowley WF. Osteoporosis in men with idiopathic hypogonadotrophic hypogonadism. *Ann Intern Med* 1987;106:354-61.
- Stepan JJ, Lachman L, Zverina J, Pacovsky V, Baylink D. Castrated men exhibit bone loss: effect of calcitonin treatment on biochemical indices of bone remodeling. *J Clin Endocrinol Metab* 1989;69:523-7.
- Greenspan SL, Oppenheim DS, Klibanski A. Importance of gonadal steroids to bone mass in men with hyperprolactinemic hypogonadism. *Ann Intern Med* 1989;110:526-31.
- Finkelstein JS, Klibanski A, Neer RM, *et al*. Increases in bone density during treatment of men with idiopathic hypogonadotrophic hypogonadism. *J Clin Endocrinol Metab* 1989;69:776-83.

(Accepted 9 March 1990)