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Original Article

A Co-Twin Study of the Effect of Calcium Supplementation on Bone Density During Adolescence

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Abstract. The effect of calcium supplementation on bone mineral density (BMD) was evaluated in female twin pairs aged 10–17 years with a mean age of 14 years. Forty-two twin pairs (22 monozygotic, 20 dizygotic; (including one monozygotic pair from a set of triplets) completed at least 6 months of the intervention: 37 pairs to 12 months and 28 pairs to 18 months. BMD was measured by dual-energy X-ray absorptiometry (DXA). In a double-blind manner, one twin in each pair was randomly assigned to receive daily a 1000 mg effervescent calcium tablet (Sandocal 1000), and the other a placebo tablet similar in taste and appearance to the calcium supplement but containing no calcium. Compliance (at least 80% tablets consumed), as measured by tablet count, was 85% in the placebo group and 83% in the calcium group over the 18 months of the study, on average increasing dietary calcium to over 1600 mg/day. There was no within-pair difference in the change in height or weight. When the effect of calcium supplementation on BMD was compared with placebo at approximately 6, 12 and 18 months, it was found that there was a 0.015 ± 0.007 g/cm^2 greater increase in BMD (1.62 ± 0.84%) at the spine in those on calcium after 18 months. At the end of the first 6 months there was a significant within-pair difference of 1.53 ± 0.56% at the spine and 1.27 ± 0.50% at the hip. However, there were no significant differences in the changes in BMD after the initial effect over the first 6 months. Therefore, we found an increase in BMD at the spine with calcium supplementation in females with a mean age of 14 years. The greatest effect was seen in the first 6 months; thereafter the difference was maintained, but there was no accelerated increase in BMD associated with calcium supplementation. The continuance of the intervention until the attainment of peak bone mass and follow-up after cessation of calcium supplementation will be important in clarifying the optimal timing for increased dietary calcium and the sustained, long-term effects of this intervention.

Keywords: Adolescence; Bone density; Calcium supplementation; Twins

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Introduction

Osteoporotic fractures occurring in later life are attributed in part to low bone mass and are thought to be related to the level of peak bone mass achieved by early adulthood [1]. This peak adult bone mass is thought to provide a sufficient reserve of bone mineral so that bone loss accompanying the menopause and subsequent aging does not result in low bone density, which is associated with increased risk of fractures. The age at which peak bone mass is attained and the determinants of peak bone mass are controversial [2,3]. Although one study has observed slightly higher mean bone mineral density in early adulthood compared with the late teenage years [4] there is evidence that peak bone mass is achieved much earlier, at about 16 years in

females, after which there is no appreciable increase in bone mass [5,6]. It is also clear that early adolescence is the period when the most rapid increase in bone density is seen [5,6] and where possibly the greatest effects of interventions may be seen.

Genetic factors account for most of the cross-sectional variation in age-adjusted bone density in adults [7]; however, the contribution of genetic factors during early life has yet to be characterized. Although genetic factors may account for up to 80% of the variation in adult bone mass, environmental factors such as diet, exercise and smoking may still exert an effect on bone density [8,9]. Dietary calcium has been implicated in the determination of peak bone mass [10] and calcium supplementation during childhood and adolescence has been shown to have a positive effect on bone density in intervention studies [11–13].

We conducted a randomized co-twin, placebo-controlled, double-blind intervention study to measure the effect of increased calcium intake on bone density in adolescence around the age at which peak bone mass is attained. This twin design controls for age, sex and for all or part of the genetic and common environmental factors shared by a pair.

Materials and Methods

Subjects and Protocol

Female twin pairs aged 10–17 years enrolled with the Australian National Health and Medical Research Council (NHMRC) Twin Registry were approached. Of the 55 pairs who commenced the calcium intervention study, 9 pairs dropped out within 1 month, and a further 4 pairs took less than 4 months of supplements, leaving 42 pairs who completed at least 6 months of the intervention and are reported in this analysis. The study was approved by the Ethics Committee on Research and the Board of Medical Research of the Royal Melbourne Hospital, and by the NHMRC Twin Registry. For each pair informed consent was obtained from both twins and at least one of their parents.

All twins were measured for height, weight and blood pressure, and completed questionnaires to assess their medical history, use of medication, physical activity and usual nutrient intake (particularly calcium). Twins completed a 4-day food record, which included 3 weekdays and one weekend day, recorded in household measures with the option of using scales if preferred. Calcium (mg/day) was calculated from the food records using the dietary analysis program Diet 3 (Xyris Software). Physical activity was determined over the previous 3 months using a short questionnaire in which hours of sport per week and hours of walking per week were split into four categories (Table 1). Bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry (DXA) using a QDR 1000W instrument (Hologic, Waltham, MA), and measurements were taken at the lumbar spine (L2–L4), total hip, neck of femur, Ward's triangle and forearm at 12-monthly intervals. Total body bone mineral content (BMC), forearm BMD, as well as total body soft tissue composition in terms of fat mass and lean mass, were measured by DXA using Hologic software (version 6.10) only at baseline and in some twins at the 12-month interval; therefore only baseline measurements are included. Whereas BMD at the hip and spine was measured at each time point of 6 months. For twins who had reached menarche, densitometry was usually performed within 12 days following the onset of a normal menstrual period to minimize the possibility of irradiation during pregnancy.

In a double-blind manner, one twin in each pair was randomly assigned to receive daily a 1000 mg effervescent calcium tablet (Sandocal 1000) containing calcium carbonate (0.8 g per tablet) and calcium lactate gluconate (5.23 g per tablet) and the other twin a placebo tablet similar in taste, appearance and composition to the calcium supplement but containing no calcium. The Sandocal tablets and placebo were kindly provided by Sandoz, Australia. Four-day food records (quantified in household measures) and 2-day activity records were completed on study entry. Every 3 months, when twins returned to the centre, they completed a 4-day food record, and a brief questionnaire to elicit any changes in physical activity. Tablet counts were performed to assess compliance during the preceding period.

Statistical Methods

Mean differences and within-pair differences in continuous variables at separate time points, and changes in variables between time points, were assessed by *t*-tests. The possible influences of differences and/or changes in covariates on the above means were assessed by multiple linear regression, using the statistical package GLIM. For example, following Hopper and Seeman [9], let $Y_{ij} = a_0 + a_1X_{1ij} + \ldots + a_qX_{qij} + E_{ij}$ be the bone density of twin i [i = 1 (calcium), 2 (placebo)] at time j (j = 0, 1, 2), where each X represents a different covariate measured on twin i at time j and E_{ij} is measurement error and effects specific to the twin and time point. The key statistic of this study is D_j, which is the within-pair difference (calcium − placebo) in change in bone density with time j (j = 1, 2) from baseline (time 0). Therefore $D_j = (Y_{1j} - Y_{10}) - (Y_{2j} - Y_{20}) = a_1D_{1j} + \ldots a_qD_{qj} + E_{0j}$, where $D_{kj} = (X_{k1j} - X_{k10}) - (X_{k2j} - X_{k20})$, where k = 1, . . . , q. Let X_1 represent calcium supplementation, so that $X_{11j} = 0$ if $j = 0$ and 1 if j = 1 or 2, and $X_{12j} = 0$ for all j. Then $D_{1j} = X_{11j} = 1$, so by regressing D_j on $D_1, \ldots, D_{qj}$ the effect of calcium supplementation relative to placebo, a_1, is the intercept term. Proportions in the two groups according to different categories of a variable were compared using the usual contingency table chi-square analyses.

Results

Of the 42 pairs (22 monozygotic, 20 dizygotic; including one monozygotic pair from a set of triplets) who had at least 6 months of intervention, 37 completed a total of 12 months and 28 pairs 18 months. Subjects discontinued the study for the following reasons: disliked the taste of the supplements, found the requirements of the study too demanding, changes in family circumstances and (in one individual) perceived gastrointestinal side effects of the calcium supplement.

The baseline characteristics are shown in Table 1. Unpaired *t*-tests indicated that there were no differences between groups. Importantly, the group means in baseline BMD and in lean mass were within 1%, and in BMC within 2.5%, and none of these differences were significant. All pairs were matched for menarchial status at baseline and 74% (31 pairs) were post-menarchial.

Table 1. Baseline characteristics of 42 twin pairs (22 monozygotic, 20 dizygotic)

	Placebo supplement	Calcium supplement
Age (years)	14.0 (2.6)	14.0 (2.6)
Weight (kg)	47.8 (12.2)	48.7 (12.6)
Height (cm)	154.2 (10.9)	154.9 (10.9)
Menarche	Yes 31, No 11	Yes 31, No 11
Lean mass (kg)	34.43 (7.33)	34.73 (7.63)
Fat mass (kg)	10.97 (5.48)	11.62 (5.80)
Total bone mineral content (g)	1543.52 (76.31)	1583.74 (82.79)
Percentage sporting activities (h/week) (*n*=78)	0–1: 8% 2–3: 39% 4–7: 35% >7: 18%	0–1: 15% 2–3: 18% 4–7: 39% >7: 28%
Percentage walking (h/week) (*n*=78)	0–1: 16% 2–3: 39% 4–7: 37% >7: 8%	0–1: 28% 2–3: 51% 4–7: 16% >7: 5%
Calcium intake (mg)[a] (*n*=60)	692.3 (253.7)	776.1 (318.7)

Values are the mean (SD)
[a]Four-day food record.

Compliance (at least 80% tablets consumed), as measured by tablet count, did not differ between the groups. It was 85% in the placebo group and 83% in the calcium-supplemented group over the 18 months of the study. Of those allocated placebo, 81% took at least 80% of their tablets and of those allocated calcium, 79% took at least 80%. During the first 6 months tablet compliance was 88% and 87%, in the second 6 months it was 87% and 84%, and in the third 6 months it was 79% and 78%, respectively. Therefore on average dietary calcium intake increased to more than 1600 mg/day.

The group means and the within-pair differences in changes from baseline, at 6-monthly intervals are shown in Tables 2 and 3 and analysed by paired *t*-tests. There was an increase in height, weight and BMD in both groups. There was no within-pair difference in the change in height or weight. During the first 6-month interval calcium supplementation was associated with a greater increase in BMD at the spine (0.0146 ± 0.005 g/cm^2, or 1.5%; $p<0.01$) and at the total hip (0.0113 ± 0.004 g/cm^2, or 1.3%; $p<0.01$) (Table 3; Fig. 1). This effect was independent of age. From baseline to 18 months there was a greater within-pair difference in change in BMD detected at the spine (0.0154 ± 0.007 g/cm^2; $p<0.05$) but not at the hip (0.0062 ± 0.038 g/cm^2; $p>0.05$) and femoral neck (0.0064 ± 0.009 g/cm^2; $p>0.05$). When the effect of calcium supplementation

Table 3. Percentage within-pair differences of changes in BMD at 6, 12 and 18 months

BMD (g/cm^2)	Within-pair differences of change (calcium − placebo)		
	0 to 6 months (42 pairs)	6 to 12 months (37 pairs)	12 to 18 months (28 pairs)
Lumbar spine	1.53±0.56**	0.26±0.50	0.25±0.58
Total hip	1.27±0.50*	−0.06±0.50	−0.32±0.63
Femoral neck	1.12±0.68†	1.30±0.79†	−0.01±0.92

Values are the mean ± SEM.
**$p<0.01$, *$p<0.05$, †$p<0.1$.

Table 2. Measurements at baseline and at the first, second and third 6-month intervals

	Baseline (42 pairs)		First interval: 6 months[a] (42 pairs)		Second interval: 12 months[b] (37 pairs)		Third interval: 18 months[c] (28 pairs)	
	Placebo	Calcium	Placebo	Calcium	Placebo	Calcium	Placebo	Calcium
Weight (kg)	47.8 ±1.9	48.7 ±1.9	50.0 ±1.8	50.8 ±2.0	53.1 ±1.8	53.3 ±1.8	55.7 ±1.9	56.7 ±1.7
Height (cm)	154.2 ±1.7	154.9 ±1.7	156.0 ±1.6	156.8 ±1.6	159.4 ±1.2	160.2 ±1.3	162.0 ±1.2	162.2 ±1.3
Total hip BMD (g/cm^2)	0.884±0.020	0.886±0.021	0.904±0.020	0.918±0.021	0.931±0.019	0.947±0.020	0.968±0.019	0.979±0.021
Lumbar spine BMD (g/cm^2)	0.901±0.025	0.898±0.026	0.925±0.024	0.937±0.027	0.960±0.023	0.975±0.025	1.001±0.027	1.017±0.028
Femoral neck BMD (g/cm^2)	0.810±0.018	0.805±0.018	0.820±0.017	0.824±0.017	0.834±0.018	0.846±0.017	0.871±0.019	0.877±0.017

Values are the mean ± SEM.
Measurement intervals (years): [a]0.56 ± 0.01; [b]0.58 ± 0.39; [c]0.67 ± 1.44.

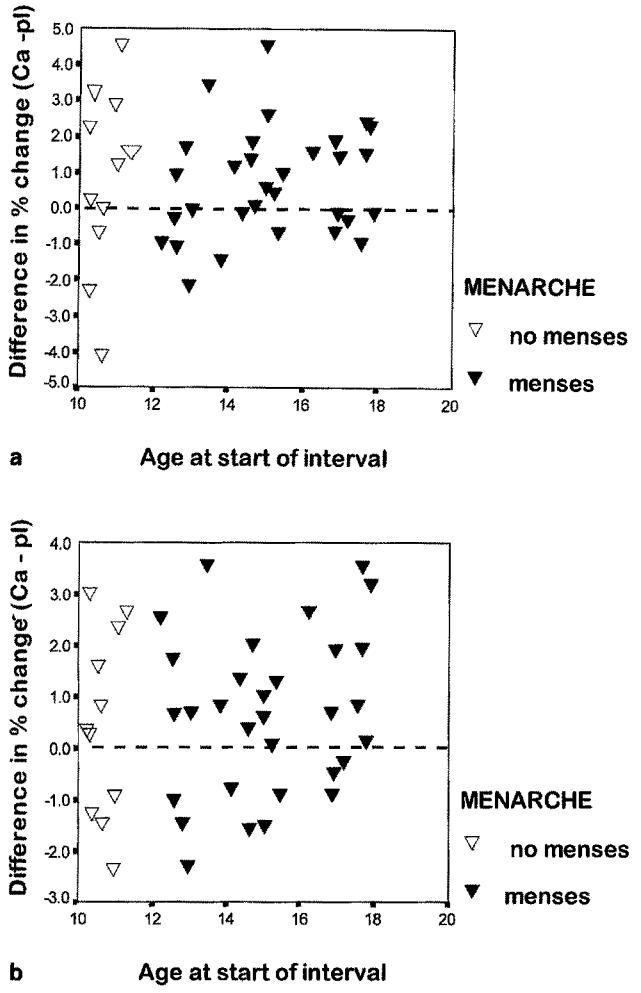

Fig. 1. Percentage difference in change in bone mineral density (BMD) (calcium twin − placebo twin) at the lumbar spine (**a**) and the total hip (**b**) at the end of the first 6-month interval versus age at commencement of the study.

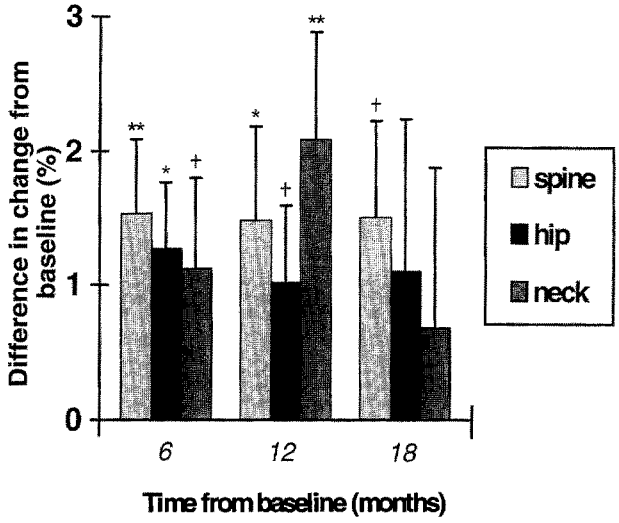

Fig. 2. Percentage difference in change in bone mineral density (BMD) from baseline (calcium twin − placebo twin) at the lumbar spine, total hip and femoral neck at the end of 6, 12 and 18 months (vertical bars represent SEM) $^{**}p<0.01$, $^{*}p<0.05$ $\dagger p<0.1$ percentage difference compared with baseline.

on bone density was assessed compared with placebo over the combined three time intervals, each of approximately 6 months (phase 1, 0.56 ± 0.01 months; phase 2, 0.58 ± 0.39 months; phase 3, 0.67 ± 1.44 months), there were no differences in the within-pair changes in BMD after the first 6 months, i.e. between 6 months and 18 months (Fig. 2). This finding was confirmed by a statistical modelling approach (GLIM) using the cumulative time intervals (first 6 months, 42 intervals), where significantly greater increases in BMD were seen at the lumbar spine (1.53 ± 0.56%; $p<0.05$), and hip (1.27 ± 0.50%; $p<0.05$) at the end of the first 6-month interval only. This effect was significantly only for spinal BMD (0.78 ± 0.34%) over the whole 18 months (107 intervals). No covariates were shown to be associated with the within-pair differences in change in BMD and there was no pronounced variation in the results after adjusting for any covariates (age, menarchial status, body weight, height, dietary calcium intake).

There were no within-pair differences in the changes between calcium-supplemented and placebo-supplemented groups in bone area after 6 months (total hip, −0.012 ± 0.25 cm^2; femoral neck, −0.11 ± 0.05 cm^2; spine, 0.40 ± 0.24 cm^2). Similarly there were no within-pair differences in the changes between 6 and 12 months (total hip, −0.48 ± 0.41 cm^2; femoral neck, −0.05 ± 0.04 cm^2; spine, −0.17 ± 0.16 cm^2). All twins were matched for menarchial status and when twins were divided according to whether they had reached menarche before or during the study, 31 pairs were post-menarchial [mean (SD) age 15.1 (1.8) years] and 11 pairs were pre-menarchial [mean (SD) age 10.6 (0.4) years]. No significant effect of calcium on bone density was evident in those 11 twin pairs who were premenarchial at the commencement of the study [difference in change in lumbar spine BMD, calcium − placebo, 0.012 ± 0.011 g/cm^2 (1.69 ± 1.53%), total hip BMD 0.077 ± 0.008 g/cm^2 (0.98 ± 1.09%) and femoral neck BMD (0.86 ± 1.35%)], whereas a significant effect was seen in the 31 pairs who were postmenarchial [difference in change in lumbar spine BMD, calcium − placebo, 0.157 ± 0.005 g/cm^2, $p<0.01$ (1.48 ± 0.54%), total hip BMD 0.0125 ± 0.005 g/cm^2, $p<0.02$ (1.37 ± 0.56%) and femoral neck BMD 0.010 ± 0.007 g/cm^2 (1.21 ± 0.79%) (NS)]. Age at commencement of the study did not predict response to calcium supplementation. Using the statistical modelling approach with cumulative time intervals, the response at the spine from baseline to 6 months was 1.7 ± 1.5% (NS) greater on calcium supplementation compared with those on placebo in twins aged 10–11 years, which was not different from the 1.5 ± 0.5% change in those aged 14–17 years.

Discussion

These results indicate that calcium supplementation was effective in increasing bone density at the spine and hip in adolescent females across the age range 10–17 years. This positive effect of calcium supplementation on bone density was similar to that found in the study by Johnston et al. [11], which was a double-blind co-twin control calcium intervention in 45 monozygotic pairs of male and female twins, with a mean age of 10 years at baseline. Johnston et al. found that over 3 years bone density increased by 5.1% (95% confidence interval 1.5% to 8.7%) more at the mid-shaft radius, and 2.8% (95% confidence interval 1.1% to 4.5%) more at the lumbar spine, in the supplemented twins. On subdivision according to puberal status, there was no intervention effect in pairs who were post-puberty (4 pairs) at baseline, or who passed through puberty during the study (19 pairs). In contrast, we have found an increase in bone density with calcium supplementation of 1.3% at the hip and 1.5% at the spine after 6 months, despite the fact that 74% of our subjects had already achieved menarche. The use of twins in our study ensured that the twins were likely to be well matched for skeletal maturity. Moreover there were no twins discordant for pubertal status as indicated by onset of menses. The lack of any effect post-puberty in the Johnston et al. study may have been due to a number of factors, such as a reduction in power to detect an effect with smaller numbers at varying stages of puberty and a different effect between boys and girls. Data from cross-sectional studies [2,3,15] indicate that the timing of the development of peak bone density differs between boys and girls and that girls have a steeper rise in bone density, particularly at the spine [3], at about age 12 years, whereas the greatest increase in bone density in boys occurs later at about age 13 years. The rate of increase is not as great as that in girls [14]. Gordon et al. [2], as well as finding that the increase in lumbar spine BMD was more pronounced and earlier in girls than boys, found that this spurt of increased bone density contributed 51% of peak bone mass in girls, whereas in boys the contribution was only 15%. In a subsequent study conducted by Lloyd et al. [15] where they supplemented girls from age 14 to 16 years who had previously completed a calcium supplementation study commencing at 11 years [12], they found that those who were taking placebo for 4 years gained 765 g bone mineral whereas those taking calcium supplement (500 mg/day) for 4 years gained 852 g, suggesting that there is still an effect of calcium supplementation on bone density after the onset of puberty.

We were unable to detect a significant effect on bone density in our premenarchial twins, but this was not unexpected due to the small numbers. Although there was a positive effect of calcium supplementation on bone density in our subjects aged from 10 to 18 years, it may be that the greatest effect is seen in younger girls, but our 11 pairs of premenarchial twins do not provide sufficient power to detect such an effect in this younger age group. In contrast, other studies assessing the effects of calcium supplementation in younger girls (mean age 7–11 years) [11–13] have demonstrated a greater effect on bone density in younger girls of 2.8–3.1% at the lumbar spine compared with 1.6% in this study.

Although in the United States milk is fortified with vitamin D and calcium is added to many more foods, including cereals and fruit juices, the mean baseline dietary intake reported for twins in the study by Johnston et al. [11] of 874 mg for girls compares with 692 mg (food record) reported by twins in our study. These figures indicate a similar dietary intake of calcium in both studies, given the considerable limitations of dietary calcium assessment [16]. It is recognized that calcium intake can vary widely on a daily basis in this age group [17] and that precise assessment of daily dietary calcium is impossible.

Compared with the US studies, dietary vitamin D intake in this study would have been smaller, as there is no fortification of food with vitamin D (except for margarine) in Australia as endogenous synthesis is thought to be sufficient in the sunny climate.

Data from calcium balance studies indicate an intake threshold level below which skeletal accumulation is a function of intake and above which skeletal accumulation is constant irrespective of further increases in intake [18]. Adolescents (9–17 years) have been found to retain more calcium than either children (2–8 years) or young adults (18–30 years) [19]. In another calcium balance study, adolescent girls (mean age 13 years) achieved a strikingly positive calcium balance of 326 $\pm$ 107 mg/day with a calcium intake of 1332 mg/day compared with adults (mean age 22 years), who averaged a positive calcium balance of only 73 $\pm$ 104 mg/day on the same intake [19]. This increased retention of calcium is thought to be due to increased efficiency of calcium absorption, which is feed-back-regulated according to physiological demands for calcium. Therefore, it does appear that although the greatest increase in bone density may be seen before puberty, there is a smaller significant effect after puberty, particularly before the achievement of peak bone mass where calcium retention is greatest. It is likely that the period of adolescence is the time of life where the potential is greatest to increase bone density in the long term. It may be that the initial positive effect of calcium supplementation on bone density, without a continued incremental effect, is a reflection of the increasing calcium intake reaching a threshold level: once this level is achieved, there is no additional effect on bone density. A recent double-blind calcium intervention study in Chinese schoolchildren [13] (with an estimated mean calcium intake of 280 mg/day) supplemented with 300 mg/day caclium produced a similar effect on bone density as that found by Johnston et al. [11] where the calcium intake was 894 mg/day supplemented with 718 mg/day. Despite the threefold greater intake than the Chinese study the effects on bone density were remarkably similar.

Considering the large differences in calcium intake it is interesting that there were similar increases in bone density: 3.14% [13] versus 3.6% [11]. These data,

together with the results from our study, where the effect on bone density is evident within the first 6 months with no further incremental effect, are consistent with the hypothesis that the effect of calcium supplementation may be to reduce bone remodelling, leading to a modest increase in bone density by decreasing the remodelling space.

Slemenda et al. [20] have reported that in the 1-year follow-up of their 45 twin pairs after discontinuation of supplementation, the significance of the differences between the twins was no longer evident, with the rate of gain of bone mineral density being marginally higher in the placebo group. The authors suggest that calcium supplementation produces its effects through suppression of bone remodelling and postulate that continued calcium supplementation may be required to maintain any beneficial effects. Another possibility is that calcium exerts its effect through stimulation of bone remodelling during this phase of rapid growth, leading to a sustained increase in peak bone mass. Bonjour et al. [21] have demonstrated an increase in bone size in 7- to 9-year-old girls supplemented with food products fortified with calcium who were on a low dietary calcium intake. We found no significant differences in changes in bone area between baseline to 6 months versus 6 to 12 months between those on calcium and those on placebo, indicating that any changes in bone density due to calcium may only be transient, by filling of the remodelling space only. However, there was a trend for a greater difference in bone area at the spine between 0 and 6 months versus 6 and 12 months in the group on calcium. It is also likely that environmental factors such as exercise and current nutritional status influence peak bone density and any response to calcium supplementation. We found that the greatest effect was seen in the first 6 months (1.5% increase at the spine) and there was no significantly greater increase in bone density from 6 to 12 months and from 12 and 18 months. There was also a reduction in the number of twins completing 18 months (28 pairs) compared with those completing 6 months (42 pairs). However, 28 pairs of twins provide 80% power to detect a change in the bone density of 1% at the lumbar spine, indicating that the absence of an incremental effect of calcium supplementation in bone density after 6 months was not related to lack of power to detect any such effect. There was a slight decline in compliance over this time period, but at the end of the 18 months the average intake of calcium supplements was still high at 78%. It should be noted that the effective calcium dose adjusted for body mass or skeletal mass decreased with time in our study, as occurs in any study involving growing adolescents. This effect provides another potential mechanism for diminishing response to calcium supplements through the study.

Conclusions

In agreement with two recent calcium supplementation studies that found a positive effect on bone density in growing females [11,12], we found at 18 months an increase in bone density at the spine with calcium supplementation in females with a mean age of 14 years. The effect was seen in the first 6 months; thereafter, there was no accelerated increase in bone density. The subsequent analysis after 3 years. Follow-up and continuance of the study until the attainment of peak bone mass will be important in clarifying the size of the intervention effect, the optimal timing for increased dietary calcium and the sustained, long-term effects of this intervention.

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