

The effect of short-term calcium supplementation on biochemical markers of bone metabolism in healthy young adults

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The influence of Ca supplementation of the usual diet for 14 d on biochemical markers of bone turnover was investigated in healthy young adults aged 21–26 years. In a crossover study, eighteen subjects (five male and thirteen female) were randomly assigned to their self-selected diet (about 22 mmol Ca/d) or their self-selected diet with a 20 mmol/d Ca supplement (about 40 mmol Ca/d) for 14 d followed by crossover to the alternative diet for a further 14 d. During each dietary period fasting morning first void urine samples (last 3 d) and fasting blood serum samples (morning of twelfth day) were collected. Ca supplementation reduced urinary excretion of pyridinoline (14%) and deoxypyridinoline (16%) (biochemical markers of bone resorption) but had no effect on biochemical markers of bone formation (serum osteocalcin and bone-specific alkaline phosphatase; EC 3.1.3.1). It is concluded that Ca supplementation of the usual diet in young adults suppresses bone resorption over a 2-week period. If sustained, this could result in suppression of the bone remodelling rate and an increase in bone mass over time. The findings of this short-term study with a relatively small number of young adults highlight the need for a longer-term intervention study of the effect of increased Ca intake on bone mass in this age group.

Calcium: Bone: Biochemical markers: Young adults

There is evidence that bone mass in early adult life (peak bone mass; PBM) is an important factor influencing bone mass and fracture susceptibility in later life (Hui *et al.* 1989; Melton *et al.* 1990; Hansen *et al.* 1991). Therefore, achievement of optimal PBM is critical to delaying or preventing osteoporosis (Matkovic *et al.* 1979; Jackman *et al.* 1997).

Adequate Ca intake is required in early life for bone development and inadequate dietary Ca during the critical growth and building periods may result in failure to achieve maximum PBM at maturity (Matkovic *et al.* 1990). A number of studies have shown that increasing Ca intake, from foods or supplements, above the usual dietary intake enhances the rates of increase in bone mineral content and bone mineral density in children and adolescents (Fehily *et al.* 1992; Johnston *et al.* 1992; Lloyd *et al.* 1993; Lee *et al.* 1994, 1995, 1996; Chan *et al.* 1995; Slemenda *et al.* 1997). The higher Ca intake, if sustained, may lead to a higher PBM at maturity (Institute of Medicine, 1997).

There is evidence to suggest that there is potential for gain in bone mass in young adults for about 10 years after longitudinal bone growth has stopped, although there is uncertainty about the age at which PBM is achieved at different sites (Katzman *et al.* 1991; Matkovic *et al.* 1994; Institute of Medicine, 1997). Teegarden *et al.* (1995) reported that 92% of total body bone mineral was present

at age 17.9 years and 99% at age 26.2 years. Recker *et al.* (1992) observed an average increase in total body bone mineral content of 1.2% per year during the third decade of life, slowing with age, and with median gains of 5.9% and 4.8% for lumbar and forearm bone mineral content respectively, during the decade.

The estimate of daily Ca requirement for young adults, based on maximal Ca retention in bone calculated from balance studies, is about 1000 mg (Matkovic & Heaney, 1992; Institute of Medicine, 1997). However, many young adults, especially women, fail to achieve this level of intake (Gregory *et al.* 1990; Irish Nutrition and Dietetic Institute, 1990; Van Dokkum, 1995; Institute of Medicine, 1997). This suggests that Ca intake may be a limiting factor for development of bone mass in some individuals in this age group.

There is very little information on the effect of dietary Ca intake on bone mass in young adults. Recker *et al.* (1992), in a longitudinal study of women in their early 20s, estimated that increasing Ca intake from 220 to 2106 mg/d resulted in gains in spinal bone mineral density from –1.05 to 16.41% when physical activity levels were kept constant.

The rates of bone formation and bone resorption, the balance of which ultimately determines bone mass, may be assessed by measurement of biochemical markers (Eastell

Abbreviations: Cr, creatinine; Dpyr, deoxypyridinoline; PBM, peak bone mass; Pyr, pyridinoline.

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et al. 1993). The urinary excretion rates of pyridinium crosslinks of collagen have been shown to be specific and sensitive indices of bone resorption (Eyre, 1992), and serum osteocalcin and serum bone-specific alkaline phosphatase (EC 3.1.3.1) have been shown to be specific markers of bone formation (Delmas, 1992).

Shapses *et al.* (1995) reported that short-term (5 d) changes in dietary Ca intake influenced the excretion of collagen crosslinks in healthy young adult subjects. However, 5 d may not allow sufficient time for adaptation to a new level of Ca intake, as suggested by the findings of Dawson-Hughes *et al.* (1993) who showed that Ca-regulating hormone levels and fractional Ca retention required up to 2 weeks to stabilize after a change in dietary Ca intake.

Thus, the objective of the present study was to investigate the effect of increasing Ca intake above the usual dietary intake on biomarkers of bone metabolism in healthy young adults over a 2-week period.

Methods

Subjects

Eighteen young healthy adults (thirteen females and five males, mean age 23.3 (range 21–26) years) were recruited from among a group of postgraduates at University College, Cork. The mean height, weight and BMI for the group are shown in Table 1. Males were taller (1.77 m) and heavier (73.7 kg) than females (1.67 m and 61.7 kg respectively) but their mean BMI was similar to that of females. There were no significant differences between males and females in baseline levels of urinary and serum biochemical indices (see p. 440) or baseline dietary intakes of energy, protein, fibre, P, Ca, K and Mg. The subjects were healthy, without any history of bone or articular disease, and with no intake of medicine that could affect bone or cartilage metabolism. Additional exclusion criteria included chronic illness or taking nutritional supplements. Subjects were requested to avoid vigorous exercise and excessive alcohol intake for the duration of the study.

Ethical considerations

Before participation in this study, all subjects signed an informed consent document approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals.

Design

The study consisted of a crossover trial of the effect of 'unsupplemented' (22 mmol (880 mg) Ca/d) or

'Ca-supplemented' (40 mmol (1600 mg) Ca/d) diets for 14 d on biochemical markers of bone turnover in healthy young subjects (Fig. 1).

The Ca intervention trial was designed in two periods, each of 14 d, in which the Ca intakes were about 22 mmol Ca/d (self-selected Ca intake) or about 40 mmol Ca/d (self-selected Ca intake + 20 mmol Ca supplement). Subjects were randomly assigned to the unsupplemented or Ca-supplemented diet regimens for 14 d followed by crossover to the alternative dietary regimen for a further 14 d (Fig. 1). During the 14 d supplementation period, each subject received, in addition to their usual diet, 800 mg elemental Ca/d, as Ca lactate gluconate and CaCO₃ (Sandolcal[®] 400, Sandoz Pharmaceuticals, Surrey, UK) in two equal doses of 400 mg Ca, each morning and evening, so as to maximize absorption (Heaney, 1991). Subjects were instructed to collect fasting first void urine samples between 07.00 and 09.00 hours for the last 3 d of each treatment period and for 3 d during the week immediately preceding the trial (baseline). The 24 h diet recalls for each individual were also obtained on each of these days. In addition, after an overnight fast, a blood sample (10 ml) was taken at 09.00 hours on the morning of the twelfth day of each dietary period and during the week immediately preceding the trial (baseline).

Dietary analysis

Nutrient intakes were estimated from 24 h diet recall data for food consumption using the *McCance and Widdowson's The Composition of Foods* (Paul & Southgate, 1978) database and updated versions (Microdiet, Salford, Greater Manchester, UK).

Collection and preparation of samples

Subjects were supplied with suitable collection containers for urine samples and asked to collect fasting first void morning urine samples between 07.00 and 09.00 hours each day for the last three consecutive days of each dietary period. Portions of urine were stored at -20° from the morning of collection until required for analysis. Blood was collected by venepuncture into vacutainer tubes and was processed to serum which was immediately stored at -70° until required.

Experimental techniques

Urinary pyridinoline and deoxypyridinoline. Each of the daily urine samples from an individual collected over three consecutive days was analysed separately in duplicate using a three-step procedure. Urine was first hydrolysed with an equal volume of 12 M-HCl at 110° for 18 h, the crosslinks were then extracted by CF1 cellulose chromatography with the use of an internal standard (acetylated pyridinoline, MetraBiosystems Ltd., Wheatley, Oxon., UK) and were measured using a reversed-phase HPLC method with fluorescence detection (Colwell *et al.* 1993). The acetylated pyridinoline was used in accordance with the method as described by Calabresi *et al.* (1994) and Robins *et al.* (1994). The crosslinks contents of urine samples were quantitated by external standardization using a commercially

Table 1. Characteristics of the group of healthy young adult volunteers (*n* 18) selected for the calcium intervention trial (Mean values with their standard deviations and ranges)

	Mean	SD	Range
Age (years)	23.3	1.2	21–26
Height (m)	1.70	0.04	1.57–1.85
Weight (kg)	65.1	7.6	54–79
BMI (kg/m ²)	22.3	2.4	19.1–25.8

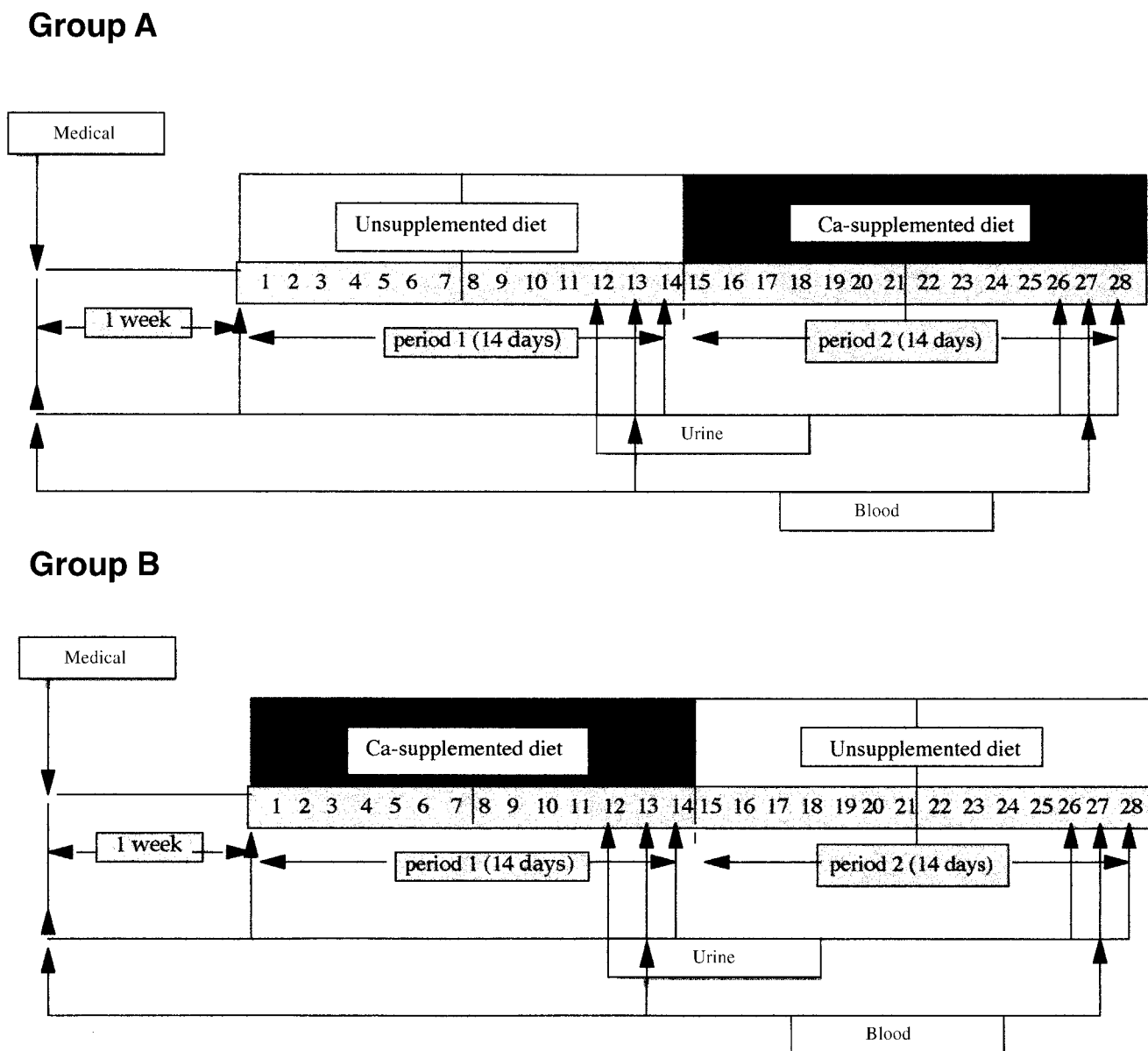


Fig. 1. Design of the study.

available pyridinoline/deoxypyridinoline HPLC calibrator (MetraBiosystems Ltd.). The mean content of the pyridinium crosslinks in the three urine collections was used to represent the individual's excretion in that dietary period. The intra-assay CV for pyridinoline (Pyr) and deoxypyridinoline (Dpyr) measured as the variation between ten chromatograms obtained between column regenerations as described by Colwell *et al.* (1993) were 6% and 7% respectively. Interassay variation was avoided by analysing all samples from an individual in the same run.

Urinary creatinine. Fresh daily urine samples were analysed in duplicate by a colorimetric procedure using a diagnostic kit (catalogue no. 124 192, Boehringer Mannheim GmbH, Mannheim, Germany). The intra- and interassay CV were 3.4 and 6.3% respectively.

Urinary calcium and magnesium. Ca and Mg were analysed in duplicate in urine samples by atomic absorption spectrophotometry (Pye-Unicam Atomic Absorption Spectrophotometer, Model SP9, Cambridge, Cambs., UK) after appropriate dilution with LaCl_3 solution (5 g/l, BDH Ltd., Poole, Dorset, UK). A range of Ca and Mg standards was used to obtain Ca and Mg calibration curves. The intra- and interassay CV for Ca were 2.6 and 7.9%, and for Mg were 3.4 and 8.3% respectively.

Serum osteocalcin and bone-specific alkaline phosphatase. Serum bone-specific alkaline phosphatase levels were measured in serum samples using a recently developed ELISA (MetraBiosystems Ltd.). The intra-assay CV was 4.7%. Serum osteocalcin levels were measured in serum samples using an ELISA (BRI-Diagnostics, Dublin,

Table 2. Mean daily nutrient intakes from the final 3 d during unsupplemented and calcium-supplemented dietary periods for healthy young adults (*n* 18)†

Nutrient	Unsupplemented diet		Ca-supplemented diet	
	Mean	SD	Mean	SD
Energy (kcal)	2211	504	2086	524
Energy (kJ)	9255	2108	8722	2192
Protein (g)	69.1	20.0	65.0	20.4
Fibre (NSP)‡ (g)	16.5	5.2	15.7	5.2
Ca (mmol)	21.6	7.6	40.1***	7.2
P (mmol)	42.1	11.2	42.0	12.4
K (mg)	2651	972	2617	740
Mg (mg)	268	68	262	76

Mean value was significantly different from that for the unsupplemented diet, ****P* < 0.001. (Student's *t*-test). The value represents 20.1 mmol Ca from the diet and 20 mmol Ca from supplements.

† For details of subjects and procedures, see Table 1 and pp. 438–440.

‡ Total NSP measured according to the method of Englyst & Cummings (1988).

Republic of Ireland). The intra-assay CV was 10.2%. Interassay variation for both serum osteocalcin and bone-specific alkaline phosphatase was avoided by analysing all samples from an individual in the same run.

Serum calcium and magnesium. Both Ca and Mg were analysed in duplicate in serum samples according to previously described methods (Trudeau & Freier, 1967; Pesce & Kaplan, 1987). The intra-assay CV were 3.2 and 3.0% respectively. There was no interassay variation as all samples from an individual were analysed in the same run.

Statistical analysis. Data are presented as means with their standard deviations. Data for all variables were normally distributed and allowed for parametric tests of significance. Comparisons of means for nutrient intakes during unsupplemented and Ca-supplemented periods were made using paired Student's *t* tests. Changes in the

different biochemical indices during the unsupplemented and Ca-supplemented periods were analysed by the appropriate analysis for a 2 × 2 crossover trial with continuous data as described by Jones & Kenward (1989) in which two-sample *t* tests are used to test hypotheses about direct treatment effects (i.e. Ca supplementation) and carry-over effects.

Results

Dietary Ca intakes, excluding the contribution from Ca supplementation, were similar during the unsupplemented and Ca-supplemented dietary periods (Table 2). Similarly, there were no significant differences in the dietary intakes of energy, protein, fibre, P, K or Mg between the unsupplemented and Ca-supplemented dietary periods (Table 2).

The effects of increasing Ca intake from 22 mmol/d to 40 mmol/d for 14 d on serum and urinary biochemical variables are shown in Table 3. Urinary creatinine (Cr) concentration, urinary Mg/Cr and serum Ca and Mg concentrations were unaffected by dietary Ca intake.

Urinary excretion of Ca/Cr increased significantly (*P* < 0.001) and there was a significant decrease (*P* < 0.05) in urinary Pyr/Cr excretion (14.0%) and urinary Dpyr/Cr excretion (16.1%) when subjects were switched from the unsupplemented to the Ca-supplemented diet.

There were no significant differences in the serum levels of biochemical markers of bone formation (serum bone-specific alkaline phosphatase or serum osteocalcin) between the unsupplemented and Ca-supplemented dietary periods.

Discussion

The present study was carried out in young adults aged between 21 and 26 years in whom bone accretion is likely to

Table 3. Urinary and serum biochemical variables in healthy young adults (*n* 18) during unsupplemented and calcium-supplemented dietary periods*

Diet...	Unsupplemented (22 mmol Ca/d)		Ca-supplemented (40 mmol Ca/d)		<i>P</i> value†
	Mean	SD	Mean	SD	
Urine:					
Creatinine (Cr) (mmol/l)	13.4	5.5	13.0	5.6	0.736
Ca (mmol/mmol Cr)	0.25	0.09	0.42	0.17	< 0.001
Mg (mmol/mmol Cr)	0.30	0.05	0.29	0.07	0.637
Pyr (nmol/mmol Cr)	42.9	12.9	36.9	10.1	0.044
Dpyr (nmol/mmol Cr)	12.4	3.4	10.4	3.0	0.032
Serum:					
Ca (mmol/l)	2.54	0.30	2.58	0.26	0.130
Mg (mmol/l)	0.83	0.16	0.83	0.14	0.965
B-Alkphase (U/l)	23.6	9.4	23.9	7.5	0.845
Osteocalcin (μg/l)	9.7	5.7	10.5	4.9	0.531

Pyr, pyridinoline; Dpyr, deoxypyridinoline; B-Alkphase, bone-specific alkaline phosphatase.

* For details of subjects and procedures, see Table 1 and pp. 438–440.

† Direct treatment effect (i.e. Ca supplementation) was analysed for each biochemical index by two-sample *t* tests of within-group differences after testing for carry-over effects. No significant carry-over effects were found.

be ongoing (Recker *et al.* 1992; Teegarden *et al.* 1995). The results from the study show that supplementation of the usual diet (22 mmol (882 mg) Ca/d) with additional Ca (20 mmol (800 mg)/d) for a 2-week period resulted in a significant reduction in the urinary excretion of Pyr/Cr (about 14%) and Dpyr/Cr (about 16%), indicating a reduction in the rate of bone resorption.

Shapses *et al.* (1995) found that urinary pyridinium crosslink excretion was reduced when subjects were given a Ca supplement (total Ca intake, 41.1 mmol (1643 mg)/d) compared with usual Ca intakes (14.7 mmol (589 mg)/d) in a group of healthy young adults. However, the size of the reduction (33% for Pyr and Dpyr) was much greater than that observed in the present study. This could be due to the short duration (5 d) of the study which may not have been sufficient to allow full adaptation of Ca metabolism to the change in Ca intake. Dawson-Hughes *et al.* (1993) showed that when Ca intake was reduced from 50 mmol to 7.5 mmol/d fractional Ca retention and serum parathyroid hormone and 1,25 dihydroxycholecalciferol increased, urinary Ca excretion decreased, and all had reached a plateau by the second week, but not by the first week, after the diet change. The duration of the present study (14 d) was chosen to allow an adequate period for adaptation of Ca metabolism to a change in dietary Ca intake. It is also possible that the greater reduction in urinary pyridinium crosslink excretion observed by Shapses *et al.* (1995) was related to the lower mean usual Ca intake (14.7 mmol (589 mg)/d) of the subjects in their study compared with that (22 mmol (882 mg)/d) in the present study.

The effect of Ca supplementation on bone resorption in the present study is likely to be due to the increased Ca intake increasing plasma Ca leading to a suppression of plasma parathyroid hormone and, consequently, a reduction in the stimulus for bone resorption due to inhibition of osteoclastic activity (Rubinacci *et al.* 1996).

The suppression of bone resorption observed in the present study, if sustained, would be expected to result in suppression of the bone remodelling rate and a measurable increase in bone mass over time (Heaney, 1994). It is unclear from this 2-week study how long suppression of bone resorption is sustained, and the possibility of long-term adaptation cannot be excluded. However, McKane *et al.* (1996) showed that postmenopausal women who were maintained on a supplemented Ca intake (60.2 mmol (2414 mg)/d) had significantly reduced urinary Dpyr excretion for up to 3 years compared with age-matched subjects on their usual Ca intakes (20.4 mmol (815 mg)/d). Furthermore, Johnston *et al.* (1992) and Slemenda *et al.* (1997) showed that serum levels of osteocalcin, a biochemical marker of bone remodelling, were significantly reduced for up to 3 years in prepubertal children who were supplemented with Ca over that time.

There has been considerable discussion about whether the gain in bone mass resulting from Ca supplementation is maintained, either in whole or in part, after Ca supplementation is withdrawn and about the significance of the remodelling transient. The term 'remodelling transient' is used to describe the suppression of bone remodelling which is seen with Ca supplementation and which results in a one-time initial gain in bone mass over the first 3–12 months

after increasing Ca intake (Frost, 1973). It is transient in so far as much, if not all, of the gain in bone mass appears to be sustained only for the duration of Ca supplementation. This has been observed on follow-up of some Ca supplementation trials in children, which showed that differences in bone mass between subjects and controls disappeared after supplementation ceased (Lee *et al.* 1996, 1997; Slemenda *et al.* 1997), although not all studies show this (Bonjour *et al.* 1997). However, this one-time gain in bone mass resulting from higher Ca intake may produce a higher PBM if the higher Ca intake is maintained (Institute of Medicine, 1997).

While the rate of bone resorption was significantly reduced by increased Ca intake in the present study, there was no alteration in the rate of bone formation (serum osteocalcin and bone-specific alkaline phosphatase). This may be due to the short duration of the study since bone formation, although coupled to resorption, is separated in time by approximately 6 weeks (Eriksen *et al.* 1984).

The findings of the present study in young adults suggest that the mean dietary Ca intake of the group (22 mmol (882 mg) Ca/d) may not be optimal for maximal rate of bone development. This is consistent with the estimate of daily Ca requirement of approximately 1000 mg for this age group, based on maximal Ca retention in bone (Matkovic & Heaney, 1992; Institute of Medicine, 1997).

In conclusion, Ca supplementation of the usual diet in young adults suppresses bone resorption for up to 2 weeks and, if sustained, could result in a suppression of bone remodelling rate and an increase in bone mass over time. This suggests that there is a potential for gain in bone mass in young adults whose usual dietary Ca intakes are significantly below 1000 mg/d through increasing Ca intake. This highlights the need for a longer-term intervention study of the effect of increased Ca intake on bone mass in this age group.

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