British Journal of Nutrition (1995), **74**, 125–139

A randomized double-blind controlled calcium supplementation trial, and bone and height acquisition in children

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(*Received 8 August 1994 – Revised 10 November 1994 – Accepted 6 December 1994*)

There is limited information relating Ca intake to bone and height acquisition among Oriental children who consume little or even no milk. The present controlled study investigated the acquisition of bone mass and height of Chinese children with an initial Ca intake of approximately 567 mg/d who were supplemented to about 800 mg/d. Eighty-four 7-year-old Hong Kong Chinese children underwent an 18-month randomized, double-blind, controlled Ca-supplementation trial. The children were randomized to receive either 300 mg elemental Ca or a placebo tablet daily. Bone mass of the distal one-third radius was measured by single-photon absorptiometry, lumbar spine and femoral neck were determined using dual-energy X-ray absorptiometry. Measurements were repeated 6-monthly. Baseline serum 25-hydroxycholecalciferol concentration and physical activity were also assessed. Baseline Ca intakes of the study group and controls were respectively 571 (SD 326) and 563 (SD 337) mg/d. There were no significant differences in baseline serum 25-hydroxycholecalciferol concentration ($P = 0{\cdot}71$) and physical activity ($P = 0{\cdot}36$) between the study and control groups. After 18 months the study group had significantly greater increases in lumbar-spinal bone mineral content (20·9 *v.* 16·34%; $P = 0{\cdot}035$), lumbar-spinal area (11·16 *v.* 8·71%; $P = 0{\cdot}049$), and a moderately greater increment in areal bone mineral density of the radius (7·74 *v.* 6·00%; $P = 0{\cdot}081$) when compared with the controls. The results confirm a positive effect of Ca on bone mass of the spine and radius but no effects on femoral-neck and height increase. A longer trial is warranted to confirm a positive Ca effect during childhood that may modify future peak bone mass.

Calcium: Childhood: Bone

Several population studies (Matkovic *et al.* 1979; Chan, 1991; Fehily *et al.* 1992; Hu *et al.* 1993) have consistently shown that an adequate Ca intake in early life is associated with attainment of a greater adult peak bone mass. Achievement of a higher peak bone mass is considered to be the best prophylactic measure to prevent the risk of developing osteoporotic fractures later in life. Two recent controlled Ca intervention trials among Caucasian children and adolescents (Johnston *et al.* 1992; Lloyd *et al.* 1993) have together demonstrated that giving Ca supplements to children whose usual Ca intakes have already reached the US recommended dietary allowances (RDA; National Research Council, 1989) could further enhance bone-mineral deposition. It has been suggested that the peak bone mass of an average Chinese adult is in general smaller in comparison to that of an average Caucasian adult (Garn *et al.* 1964; Hu *et al.* 1993; Lee, 1993). It follows that skeletal Ca accretion during the years of growth in Chinese children may be lower than that of their Caucasian counterparts (Lee, 1993; Matkovic & Illich, 1993). There have been few

* For reprints.

published studies on the relationship between Ca intake and bone acquisition among Oriental children and adolescents. Until recently our research group has reported that the bone mass of pre-school Chinese children was positively associated with Ca intakes over the past years (Lee *et al.* 1993*a*), and that pre-school children from mainland China with a mean habitual Ca intake of 245 mg/d had a bone mass 14% less than that of their counterparts from Hong Kong whose mean habitual Ca intake was 540 mg/d (Lee *et al.* 1993*b*). Over the last few decades the food habits of Hong Kong Chinese people have been influenced substantially by the West, and there is an increased consumption of cow's milk among Hong Kong Chinese children. More than 40% dietary Ca in 5-year-old children is derived from milk, with mean Ca intake rising to approximately 550 mg/d (Lee *et al.* 1993*a*). However, there is a lack of information on the rate of bone acquisition when Chinese children accustomed to such a habitual level of Ca intake are brought up to the RDA of the Western industrialized countries (German Society of Nutrition, 1981; National Research Council, 1989).

The present report describes a randomized, double-blind, placebo-controlled Ca-supplementation trial undertaken for 18 months to determine its effects on bone mineral acquisition in the distal radius, lumbar spine (L2–L4) and proximal femoral neck and on height increment of Hong Kong Chinese children.

SUBJECTS AND METHODS

Study sample

Healthy Hong Kong Chinese children (*n* 109; sixty-three boys and forty-six girls) aged 7 years participated in the trial. The study children came from a longitudinal growth study (Leung & Lui, 1989; Lee *et al.* 1993*a*); these normal cohort children in the longitudinal study have been followed up since they were recruited at birth in 1984. The cohort children were 7 years of age when they entered into the current trial. All the children in the present trial had been growing normally since birth, none of them had had any recent metabolic disorder or incident of fractures which might directly or indirectly affect bone metabolism.

Calcium supplementation

The study children were randomly allocated to the study group (*n* 54; thirty-two boys and twenty-two girls) and the placebo group (*n* 55; thirty-one boys and twenty-four girls). The controlled trial lasted for 18 months. $CaCO_3$ in the form of cherry-flavoured chewable tablets was used as the Ca supplement (Tums-Ex; Smithkline Beecham, Weybridge, Surrey). Each tablet contained 300 mg elemental Ca. The placebo used was a Ca-free sucrose tablet. The colour, shape and taste of the placebo were similar to those of the Ca supplement. The placebo tablets were also produced by the same drug manufacturer. The study children were required to take one tablet daily immediately after breakfast. The appropriate number of tablets were dispensed to the parents for home use every 6 months during the trial. Compliance was calculated by counting the number of tablets left in the returned bottles. Subjects, parents and field workers were blinded to the design of the trial. Throughout the trial, two research nurses monitored the compliance every 1–2 months by encouraging the children to comply with the instructions.

Assessments of dietary intake

Dietary intake was assessed at baseline and repeated after 12 months by a research dietitian (WTKL) using the method of dietary history, cross checked with a quantitative food-frequency questionnaire and 24 h recall. Details of the method have been described in a previous study (Lee *et al.* 1993*a*). Portion sizes were either in multiples or sub-multiples of

the actual food item, e.g. one egg, 1/4 of an apple, one chicken leg, or in household measures, e.g. three Chinese tablespoons, 1·5 teaspoons, one 250 ml glass or one 250 ml Chinese rice bowl. The average weight of either a standard portion of a food item (large, medium or small size) or a food item in household measures had been weighed out and standardized. Nutrient intake was calculated using a computerized food table with food items compiled from appropriate food tables (US Department of Health, Education and Welfare 1972; Paul & Southgate, 1978; Watt & Merrill, 1983; Pennington, 1989; Institute of Nutrition and Food Hygiene, 1991). The children were also reminded to maintain usual eating patterns throughout the trial.

Weight and height measurements

Unclothed weight of the children was measured using a Seca electronic balance (Model 707; Vogel & Halke GmbH & Co., Hamburg, Germany). Standing height without shoes was measured using a stadiometer (Technical Services Unit, Chinese University of Hong Kong). Weight and height were evaluated every 6 months throughout the 18-month trial.

Bone mineral measurements

Bone mineral contents of three skeletal sites, namely, distal one-third radius, lumbar spine (L2–L4) and femoral neck of the proximal femur, were evaluated 6-monthly during the trial.

Bone mineral content (BMC) and bone width (BW) at the distal one-third radius of the non-dominant arm were evaluated by single-photon absorptiometry (SPA; Cameron & Sorenson, 1963; Sorenson & Cameron, 1967; Cameron *et al.* 1968) using a Norland 2780 bone absorptiometer (Norland Corp., Fort Atkinson, WI, USA). The BMC:BW ratio is an areal density derived by dividing BMC by BW. The detailed measurement procedures and limitations of the technique have been reported previously (Prentice *et al.* 1990; Lee *et al.* 1993*a*,*b*). Two scans were performed and an average value was taken. The source for the absorptiometer is ^{125}I with a maximum strength of 7400 MBq which has to be replaced every 6 months. A surface dose of 40 μSv is absorbed by the tissue in duplicate measurements. The radiation dose received by each subject in the present study was less than 10% of a conventional X-ray examination of the forearm (DePriester *et al.* 1991). Bone mineral measurement was performed by the same observers throughout the trial.

Lumbar spinal (L2–L4) bone mineral content (LSBMC), lumbar spinal L2–L4) bone mineral areal density (LSBMD), femoral-neck bone mineral content (FNBMC) and femoral-neck bone mineral areal density (FNBMD) of the proximal femoral neck were determined in antero-posterior position by dual-energy X-ray absorptiometry (DEXA; XR-26. Norland Corp.). The principles of DEXA and the accuracy and precision of the technique have been reported previously (Genant *et al.* 1989; Sartoris & Resnick, 1989; Ho *et al.* 1990). The radiation that the subject actually absorbed was low (< 30 μSv/scan; Genant *et al.* 1989). Lumbar spine (L2–L4) was scanned in the antero-posterior position. The child's upper legs were bent at the hip and the lower legs were elevated using a support to minimize lumbar lordosis during spine measurements. BMD in g/cm^2 is derived by dividing BMC (g) by the projected area of the bone. However, this measure is not a true volume density because the anterio-posterior depth and thus the volume of vertebrae cannot be determined by DEXA. BMD is a means to normalize BMC for the size of the vertebrae. To measure the proximal femoral neck in antero-posterior position, a foot support was used to maintain a 20° inward rotation of the legs to compensate for femoral neck anteversion. The left femoral neck was measured throughout the present study.

Quality assurance tests for both instruments (SPA and DEXA) were performed by daily calibration against the respective standard phantoms provided by the manufacturer. For

Table 1. *Table of categorized daily activities for the assessment of physical activity*

Category no.	Examples of activities
1	Sleeping and lying down
2	Sitting: eating, writing, reading and watching T.V.
3	Standing: standing, queuing, having shower and dish washing
4	Walking less than 4 km/h, shopping and walking up and down staircase
5	Light household work: sweeping floor, window washing and walking at 4–6 km/h
6	Leisure and non-competitive sports activities in a recreation environment: hill walking, table tennis, leisure cycling and running around at home
7	Competitive sports or activities of higher intensity: jogging, track and field, swimming, tennis and gymnastics

the technique of SPA, precision errors incurred in two successive scans without repositioning of the subjects were 2·1% and 2·29% for BMC and BW respectively. Intra-class correlations between scans with subjects repositioning for BMC and BW were 0·937% and 0·906% respectively (Lee *et al.* 1993*a*). The precision of the positioning technique using DEXA has been studied in ten healthy young adult volunteers measured five times, each time with repositioning; the coefficients of variation were 1·4% and 2% for the lumbar spine (L2–L4) and femoral neck respectively. To minimize inter-observer variability in the measurements, one research nurse was assigned to perform the measurements throughout the trial.

Assessment of physical activity

Physical activity has been shown to be associated positively with a higher bone density (Slemenda *et al.* 1991*b*; Grimston *et al.* 1993). The amount of physical activity of the study children was assessed using a 3 d physical activity record which was adapted from Bouchard *et al.* (1983). At least 3 d including a weekend day are required to evaluate usual activities (Saris, 1985; Durnin, 1990). In the present study a 3 d physical activity assessment period comprised two school days, and a physical education lesson had to be included in it, while the remaining day had to be a weekend day. The 24 h in a day were divided into ninety-six periods of 15 min each. Usual activities were categorized into a seven-grade scale with respect to the intensity of the activity as modified from Bouchard *et al.* (1983) (Table 1). The importance of maintaining usual activities in the study period was emphasized to the children and their parents. The activity record was administered by the parent, the children helped their parents to fill in the activity record. Record keeping started when the child woke up in the morning and continued until he/she went to the bed in the evening. The record was filled in every 1–2 h by the parent unless the child attended school. After school the child helped recall his/her physical activities in school with the aid of a class timetable or with assistance from the teacher. A category number (grade 1–7) corresponding to a dominant type of activity during a 15 min interval was recorded. The categorized activities between grades 4 and 7 were used for calculation; the total number of hours the child spent on these activities was averaged over the 3 d period.

Evaluation of serum 25-hydroxycholecalciferol concentration

Hong Kong is situated in the region of sub-tropical climate, and there is an abundance of sunshine throughout the year. A recent study has shown that the serum 25-hydroxycholecalciferol concentration of young children in this region is normal (Leung *et*

al. 1993). The study children at this age go outdoors regularly. Hence, they should obtain most of their vitamin D by exposure to the sun. In the present study, twenty children were randomly selected to evaluate the average serum concentration of 25-hydroxycholecalciferol (25-OHD). Serum level of 25-OHD was evaluated at the commencement of the trial in December (winter) using a competitive protein assay as described previously (Woo *et al.* 1990; Lee *et al.* 1994*a*). Venous blood (2 ml) was drawn from the antecubital vein for the 25-OHD assay. The serum was separated from the blood. 25-OHD was extracted from the serum using acetonitrile and then separated with a SepPak C-18 cartridge (Waters Associates, Milford, MA, USA) and the extract was analysed by a competitive protein-binding assay using a commercial kit (Amersham International, Little Chalfont, Bucks).

The study was approved by the Ethics Committee of the Faculty of Medicine, Chinese University of Hong Kong. Informed consent was obtained from the parents.

Statistical analysis

Descriptive statistics (mean and SD) were summarized for all variables. Owing to the small sample size or non-normal distribution of some variables the Mann–Whitney U test was used to compare mean differences in serum 25-OHD concentration, baseline characteristics between the drop-outs as well as the successful participants, and the mean percentage increases in bone measures and body size between the study and placebo groups. Otherwise, two-tailed Student's t test was used throughout the report. Multiple regression analysis with enter and stepwise procedures was used to control for baseline variables to predict the treatment effect on the net increase in lumbar spinal BMC over the trial. Diagnosis of residuals was performed and leverage points were identified. The level of significance (two-tailed) was $P \leqslant 0{\cdot}05$. Statistical analysis was performed using SPSS/PC, Version 4·0, SPSS Inc., Chicago, IL, USA.

RESULTS

Eighty-seven out of 109 children completed the trial, of whom three were rejected subsequently: one boy emigrated out of Hong Kong after 12 months of the trial, two girls moved house and lost contact for the last follow-up session. The success rate of the trial was 77% (n 84). Mean compliance (tablet count) of the eighty-four children who successfully completed the trial was 82%, the rate of the compliance was higher in the first year (84%) and then dropped to 77% in the last 6 months of the trial. There was no significant difference in the mean compliance between the study and control groups throughout the trial (80 (SD 15) *v.* 84 (SD 16)%; $P = 0{\cdot}29$). Twenty-two out of 109 children withdrew in the first 12 months of the trial: thirteen children disliked the flavour of the tablets, six children complied poorly and therefore withdrew subsequently, whereas three children had stomach upsets after taking the tablets.

The mean baseline dietary Ca intake of the eighty-four successful participants was 567 (SD 330) mg/d. Table 2 compares the baseline characteristics of the study and control groups of the eighty-four successful participants. There were no significant baseline differences in dietary intake, body size and bone measures between the study and control groups ($P > 0{\cdot}05$). Table 3 compares the baseline characteristics of boys and girls of the eighty-four successful participants. After 12 months, dietary assessment was repeated; there were no significant differences between the study and control groups with respect to the intake of Ca (548 (SD 237) *v.* 580 (SD 238) mg/d; $P = 0{\cdot}54$), protein (72·0 (SD 22·0) *v.* 78·1 (SD 26·4) g/d; $P = 0{\cdot}25$), and energy (6599 (SD 1518) *v.* 7214 (SD 1848) kJ/d; $P = 0{\cdot}10$).

Table 2. *Baseline characteristics of study and control groups of 7-year-old Hong Kong Chinese children taking part in an 18-month calcium-supplementation trial**

(Mean values and standard deviations)

	Study group (*n* 44)		Control group (*n* 40)	
	Mean	SD	Mean	SD
Calcium intake (mg/d)	571	326	563	337
Protein intake (g/d)	73·2	19·0	78·3	24·8
Energy intake				
(kJ/d)	6711	1648	7188	1858
(kcal/d)	1604	394	1718	444
Weight (kg)	22·0	4·9	21·4	3·3
Height (m)	1·203	0·053	1·194	0·043
Distal 1/3 radius				
BMC (g/cm)	0·375	0·056	0·382	0·043
BW (cm^2)	0·818	0·081	0·828	0·077
BMC:BW ratio (g/cm^2)	0·457	0·039	0·463	0·045
Lumbar spine (L2–L4)				
LSBMC (g/cm)	11·78	1·82	11·91	1·67
LSA (cm^2)	27·12	2·9	26·38	2·47
LSBMD (g/cm^2)	0·483	0·053	0·490	0·046
Femoral neck				
FNBMC (g/cm)	1·19	0·27	1·22	0·21
FNA (cm^2)	2·20	0·43	2·24	0·31
FNBMD (g/cm^2)	0·543	0·063	0·544	0·055
Physical activity (h/d)†	2·7	0·9	2·9	0·9

BMC, bone mineral content; BW, bone width; LSBMC, lumbar spinal bone mineral content; LSA, lumbar spinal projected area; LSBMD, lumbar spinal bone mineral density; FNBMC, femoral neck bone mineral content; FNA, femoral neck projected area; FNBMD, femoral neck bone mineral density.

* There was no significant difference between the study and control groups with respect to the baseline variables ($P > 0·05$), using 2-tailed Student's *t* test. For details of procedures, see pp. 126–129. Values are for the eighty-four participants who successfully completed the trial.

† Study group, *n* 40; control group, *n* 34 (for subjects who completed a 3 d record of physical activity).

In assessment of physical activity, seventy-four out of eighty-four records of physical activity were accepted, while the remaining ten records were rejected because they were incomplete. The average time that the study children spent on physical activity (grade 4–7) was 2·8 (SD 0·9). There was no significant difference in the amount of time spent on physical activity between the study and control groups ($P = 0·36$; Table 2). Mean serum 25-OHD concentration of the twenty randomly selected children was 33·69 (SD 7·74) ng/ml. Mean serum 25-OHD concentrations of the study and control groups were respectively 34·82 (SD 8·23) ng/ml (*n* 10), and 32·57 (SD 7·48) ng/ml (*n* 10). There was no significant difference in serum 25-OHD concentration between the study and control groups ($P = 0·71$). The mean serum 25-OHD concentration of the twenty children was 3-fold above the biochemical diagnostic index for vitamin D deficiency (10 ng/ml; Grindulis *et al.* 1986). Hence, the vitamin D nutritional status of the study children in the current study was adequate.

The baseline characteristics of the twenty-five drop-outs or rejected cases were compared with those of the eighty-four children who completed the trial. There were no significant differences in dietary intake, weight, height, and bone measures between the withdrawals and the eighty-four children who completed the trial ($P > 0·05$). As compliance was best in the first 6 months (85%), and the majority of the withdrawals occurred during the

Table 3. *Baseline characteristics of 7-year-old Hong Kong Chinese boys and girls taking part in an 18-month calcium-supplementation trial**

(Mean values and standard deviations)

	Boys (*n* 48)		Girls (*n* 36)		
	Mean	SD	Mean	SD	*P*†
Calcium intake (mg/d)	645	369	466	239	0·008
Protein intake (g/d)	79·2	22·6	71·1	20·6	NS
Energy intake					
(kJ/d)	7473	1657	6247	1661	0·001
(kcal/d)	1786	396	1493	397	
Weight (kg)	22·6	4·5	20·6	3·5	0·034
Height (m)	1·204	0·047	1·192	0·050	NS
Distal 1/3 radius					
BMC (g/cm)	0·389	0·049	0·365	0·048	0·034
BW (cm^2)	0·845	0·077	0·795	0·072	0·003
BMC:BW ratio (g/cm^2)	0·460	0·043	0·460	0·040	NS
Lumbar spine (L2–L4)					
LSBMC (g/cm)	11·87	1·56	11·81	2·0	NS
LSA (cm^2)	27·26	2·85	26·10	2·38	0·05
LSBMD (g/cm^2)	0·478	0·041	0·497	0·041	NS
Femoral neck					
FNBMC (g/cm)	1·28	0·23	1·12	0·23	0·002
FNA (cm^2)	2·32	0·31	2·09	0·41	0·004
FNBMD (g/cm^2)	0·549	0·061	0·536	0·057	NS
Physical activity (h/d)‡	2·7	0·9	3·0	0·8	NS

BMC, bone mineral content; BW, bone width; LSBMC, lumbar spinal bone mineral content; LSA, lumbar spinal projected area; LSBMD, lumbar spinal bone mineral density; FNBMC, femoral neck bone mineral content; FNA, femoral neck projected area; FNBMD, femoral neck bone mineral density.

* Values are for the eighty-four participants who successfully completed the trial. For details of procedures, see pp. 126–129.

† Group means were compared using the two-tailed Student's *t* test.

‡ Study group, *n* 44; control group, *n* 30 (for subjects who completed a 3 d record of physical activity).

second 6 months of the trial, comparisons were carried out to examine whether there was any difference in response to the treatment effect between the drop-outs and the eighty-four children who completed the trial in the first 6 months. The differentials in weight, height and bone measures between the drop-outs and the successful participants were compared. In the study group, height increment was slightly higher among the drop-outs (*n* 11) when compared with the successful participants (*n* 44) (3·6 (SD 0·5) *v.* 3·2 (SD 0·6)%; $P = 0·036$), whereas the increment of radial BMC:BW of the drop-outs was lower than that of the successful participants (2·3 (SD 5·3) *v.* 6·1 (SD 4·0)%; $P = 0·033$). Otherwise, variables did not differ significantly. On the other hand, among the controls the percentage increment of the LSBMD was significantly lower in the drop-outs (*n* 14) than in the successful participants (*n* 40) (2·8 (SD 2·9) *v.* 5·6 (SD 3·9)%; $P = 0·027$).

Due to an electronic breakdown of the DEXA instrument during the 12th month of follow-up, there were no data available for the lumbar spine and the femoral neck regions in the 12th month follow-up session. Table 4 shows bone mineral acquisition, weight and height increases of the eighty-four successful participants after 18 months. The study group had significantly greater gains in the LSBMC (20·95 *v.* 16·34%; $P = 0·035$), and LSA (11·16 *v.* 8·71%; $P = 0·049$) when compared with the control group. The ratios of percentage

Table 4. *Percentage gains in bone measures, weight and height of 7-year-old Hong Kong Chinese children during participation in an 18-month calcium-supplementation trial**

(Mean values and standard deviations)

	Study group (*n* 44)		Control group (*n* 40)		
	Mean	SD	Mean	SD	*P*†
Distal 1/3 radius					
BMC	15·92	7·24	14·95	6·09	0·53
BMC:BW	7·74	5·06	6·00	4·98	0·08
BW	7·59	5·54	8·58	5·62	0·32
Lumbar spine (L2–L4)					
LSBMC	20·95	7·45	16·34	7·34	0·035
LSA	11·16	4·8	8·71	5·0	0·049
LSBMD	8·82	5·14	7·01	4·55	0·20
Femoral neck					
FNBMC	24·19	36·1	23·42	21·3	0·37
FNA	14·21	34·3	12·57	18·4	0·78
FNBMD	9·03	5·43	9·62	6·59	0·76
Weight	24·4	6·8	25·6	7·9	0·46
Height	8·4	0·8	8·4	1·1	0·92

BMC, bone mineral content; BW, bone width; LSBMC, lumbar spinal bone mineral content; LSA, lumbar spinal projected area; LSBMD, lumbar spinal bone mineral density; FNBMC, femoral neck bone mineral content; FNA, femoral neck projected area; FNBMD, femoral neck bone mineral density.

* Values are for the eighty-four participants who successfully completed the trial. For details of procedures, see pp. 126–129.

† Group means were compared using the Mann–Whitney U test.

increase in LSBMC to percentage increase in LSA of both the study group (1·58, SD 2·7) and the control group (1·12, SD 3·2) were not statistically different ($P = 0·325$). The results of analysis imply that in both the study and control groups, LSBMC and its projected vertebral area (LSA) expand with a similar ratio but at different rates. This may explain in part why LSBMD was not significantly different between the study and control groups (8·82 *v.* 7·01 %; $P = 0·2$). On the other hand, in the study group there was a greater increase in radial BMC:BW (7·74 *v.* 6·0 %; $P = 0·081$) when compared with the controls. There were no significant differences in the differentials of FNBMC ($P = 0·37$) and FNBMD ($P = 0·76$) between the study and control groups. In addition, there were no significant differences in the percentage gain of weight ($P = 0·46$) and height ($P = 0·92$) between the study and control groups.

Further analysis was attempted to examine whether there was a true treatment effect (Ca supplement or placebo) on the net increase in LSBMC. Multiple regression analysis was employed to examine whether the observed treatment effect on LSBMC was confounded by variables such as baseline weight, height, LSA, dietary intakes of Ca and protein, and sex. Since there was a strong positive correlation between baseline height and weight (r 0·75, $P < 0·0001$) as well as between baseline Ca intake and protein intake (r 0·50, $P < 0·0001$), weight and protein intake were not used in the regression model, to avoid collinearity. When all the five independent variables were entered into a single model (Table 5), only treatment effect (partial r 0·32, $P = 0·0041$) and baseline Ca intake (partial $r -0·29$, $P = 0·0089$) were found to be statistically significant for predicting the net increase in LSBMC. An alternative multiple regression model using a stepwise approach gave similar results, with treatment effect (partial r 0·32, $P = 0·003$) and baseline Ca intake (partial r

Table 5. *Predicting variables included in a single multiple regression model to predict net increase in lumbar spinal bone mineral content of 7-year-old Hong Kong Chinese children during an 18-month calcium-supplementation trial*

Independent variable	Partial correlation coefficient	*P*
Treatment effect	0·32	0·0041
LSA (cm^2)	−0·087	0·44
Height (m)	0·13	0·23
Baseline calcium intake (mg/d)	−0·29	0·0089
Sex	0·11	0·32

LSA, lumbar spinal projected area.

−0·26, $P = 0·016$) being the two significant independent variables. Furthermore, in the stepwise procedure, no interaction was found between treatment effect and baseline Ca intake ($P = 0·30$). The results suggest that the significant treatment effect had not been confounded by the variables examined.

It is interesting to find that baseline Ca intake was negatively correlated with the net increase in LSBMC in multiple regression analysis. Further diagnosis of the residuals was performed. In the scatter plot between baseline Ca intake and the net percentage change in LSBMC there were three data points which had negative percentage changes in LSBMC. When one of the three points with the greatest Cook's distance (0·147) was removed from the analysis, baseline Ca intake became non-significant ($P = 0·11$), whereas the treatment effect remained significant ($P = 0·0057$). Furthermore, when all three data points with Cook's distance between 0·119 and 0·147 were excluded from the analysis, baseline Ca intake was also non-significant, and treatment effect remained significant ($P = 0·022$). Therefore, these three data points behaved like leverage points (Kleinbaum *et al.* 1987). However, an outlier should only be deleted provided that a cause, such as an error, is identified (Draper & Smith, 1981). In the present study there was no reason to suspect that these three data points were erroneous. Therefore, these data points were still included in the analysis, and the results of analysis should be interpreted with caution. In summary, treatment effect was consistently demonstrated to be a significant determinant to predict the change in LSBMC after the 18-month trial. Based on the results of statistical analysis there is no convincing evidence to support the hypothesis that the net increase in LSBMC after the trial period was negatively correlated with baseline Ca intake.

DISCUSSION

The results of the current study confirm the results of other supplementation trials which show that an increased Ca intake in children has a beneficial effect on bone mineral acquisition (Johnston *et al.* 1992; Lloyd *et al.* 1993). To our knowledge there have been no published intervention studies to evaluate the effects of increased Ca intake *per se* on skeletal accretion and height increase in Chinese children whose mean dietary Ca intake was about 567 mg/d. Children in the study group received an extra 246 mg/d supplemental Ca (after correcting for the compliance of 82%) in addition to the basal dietary intake of 567 mg/d throughout the trial. There were extra gains in LSBMC (4·6%) and LSA (2·5%)

when the study group children were compared with the controls. The additional gains in LSBMC and LSA in the study group were both 28% greater than those in the placebo group. The differential in radial BMC:BW ratio between the study and control groups also approached significance ($P = 0{\cdot}081$). Ca supplementation undertaken for 18 months had no marked beneficial effect on the differentials of FNBMC and FNBMD ($P > 0{\cdot}05$). There was also no additional benefit on height increment. In short, the results of the 18-month Ca trial showed that increasing Ca intake *per se* to approximately 800 mg/d enhanced vertebral bone acquisition remarkably in Chinese children but it had no beneficial effects on proximal femoral neck. Furthermore, Ca supplementation did not enhance height increment.

The technique of DEXA measures bone mass of both the trabecular bone (25%) within the vertebral body and the cortical bone (75%) of the posterior processes per unit projected area of the vertebrae (in g/cm^2) (Ott, 1991). The technique cannot quantify true volume density because the antero-posterior depth and thus the volume of the bone cannot be determined by DEXA. During growth, both the vertebral bone mass and its volume increase; the results of the present study show that the ratios of percentage increase in LSBMC and LSA were similar in both the study and control groups, but the rates of increase in LSBMC and LSA in the study group were significantly greater than those in the control group. Such phenomena suggest that Ca supplementation increases both the bone mass and bone area of the vertebrae. Due to the inherent technical limitation of DEXA, the evaluation of areal bone density (i.e. LSBMD) is influenced by the increase in the size of the vertebrae, the values of both bone mass and bone area increase in the absence of any marked change in bone density. Such an observation has been reported by other workers (Gilsanz *et al.* 1988; Glastre *et al.* 1990; Katzman *et al.* 1991). Gilsanz *et al.* (1991) used the technique of quantitative computed tomography (QCT), which measures the actual volume of the bone (in g/cm^3), to study the longitudinal increase in LSBMD between black and white adolescent girls before and after puberty. After puberty the increase in bone density of black girls significantly exceeded that of white girls (34 *v.* 11%). Such a magnitude of racial discrepancy in bone density was found to be higher than other reports in which LSBMD was determined by DEXA (Glastre *et al.* 1990; Bell *et al.* 1991; Southard *et al.* 1991). The authors attributed the greater discrepancy in vertebral growth found in their study to the methods used in quantifying bone density (Gilsanz *et al.* 1991).

The present trial demonstrated that Ca supplementation in 7-year-old Chinese children had no beneficial effect on height increase. The result was similar to that of our recent report on age-matched children from China (Lee *et al.* 1994*b*). By using identical Ca supplements and dosages in a group of Chinese children with an average dietary Ca intake of about 275 mg/d over an 18-month trial, no significant differences in the percentage increases of height were found between the study and control groups (7·16 (SD 0·12) *v.* 7·19 (SD 0·12)%; $P = 0{\cdot}40$) (Lee *et al.* 1994*b*). The results from our previous and present randomized controlled trials consistently indicate that supplementation with Ca of children habituated to Ca intakes lower than those of their Western counterparts does not affect height gain. Furthermore, the magnitudes of the mean percentage gains in height in both trials after 18 months were very similar as expressed in the significance levels. These findings also agree well with those of Johnston *et al.* (1992) and Lloyd *et al.* (1993). Studies in the early part of this century reported that children and adolescents supplemented with milk had increases in height (Leighton & Clark, 1929; Bureau of Statistics, 1970). However, such increases in height after milk supplementation could be confounded by the fact that milk, although a good source of Ca, is also a rich source of energy and protein for growth and development. Clinical studies have shown that linear growth of children may be stunted by chronic insufficiency of energy and protein (Kirschner *et al.* 1978). Thus, using a Ca salt may

be superior to using milk to test whether increasing Ca intake *per se* enhances height increment among children and adolescents.

Repeated dietary assessment 12 months after the initial assessment showed that there were no significant differences in the intakes of Ca, protein and energy between the study group and the controls. In fact, the variations of mean Ca intake in the respective groups were small over a 12-month period. Therefore, it is reasonable to assume that Ca intakes of the study and control groups were comparable at the end of the trial. The study cohort, like other children in Hong Kong, were under pressure to achieve good academic performance in school. The cohort children usually had to spend several hours a day after school on homework, some even attended private tuition classes after school. There were only about two physical education lessons per week in school. Furthermore, most of the families lived in high-rise blocks of apartments and there was limited space for recreational activities. During the limited leisure time, most children usually chose to have sedentary types of activity such as watching television and playing electronic games. These may explain why the time spent on physical activity was less than 3 h a day. Therefore, the time spent by individuals on physical activity probably did not vary a great deal over the 18-month period. In addition, during each 6-monthly interview each child was asked about their physical health and lifestyle, but none of them reported that they had had a significant change in daily activity. Therefore it is reasonable to assume that the physical activity of children in the study and control groups was similar 18 months later.

The mean serum level of 25-OHD of the twenty-two randomly selected children was in the normal range even though the blood was taken for assay in winter. The climate of Hong Kong is sub-tropical with plenty of sunshine throughout the year. The study children went outdoors regularly each day; they should have obtained most of their vitamin D by exposure to the sun. A recent study on serum 25-OHD in Hong Kong children also showed no evidence of sub-optimal vitamin D nutritional status (Leung *et al.* 1993). Therefore, vitamin D nutritional status of the study children in the present trial should not have been a limiting factor in bone mineralization. As vitamin D nutritional status and the levels of physical activity were not statistically different between the study group and the control group, these two factors should not be the confounders in affecting the variation in bone acquisition between the study and control groups. The compliance rate in the present trial was somewhat higher when compared with those of Johnston *et al.* (1992) (72–74%) and Lloyd *et al.* (1993) (64–77%). The relatively high compliance rate achieved (82%) might be attributable to the effort of our research nurses to encourage the children's compliance regularly throughout the trial, and the co-operative efforts of the parents to supervise their children at home to take the tablets.

In two prospective Ca supplementation trials, in Caucasian children (Johnston *et al.* 1992) and adolescents (Lloyd *et al.* 1993), mean Ca intakes of the subjects were comparable with or even higher than the US RDA (National Research Council, 1989). Johnston *et al.* (1992) reported a Ca trial conducted for 3 years in Caucasian identical twins. After the trial, among twenty-two pairs of prepubertal twins with a mean age of 7·4 (SD 1·5) years the gain in the radial BMC:BW ratio of the study group was significantly greater (by 5·1%) than that of the control group. Comparing the results of our current study with those of Johnston *et al.* (1992) for the first 18 months of the trial, although baseline mean radial BMC:BW of the subjects in the current study (0·46 (SD 0·04) g/cm^2; n 84) was slightly higher than that of the twins study (0·42 (SD 0·06) g/cm^2; n 44; $P < 0·001$) (Johnston *et al.* 1992; Slemenda, 1993), the total Ca intake in the Ca-supplemented group of the present study (habitual diet: 567 mg/d, supplement: 246 mg/d) was almost half the intake when compared with the twins study (894 mg/d from diet, 718 mg/d from the supplement). It is interesting to note that after the 18-month trial the Hong Kong study children were less

responsive than the twin counterparts in terms of net gain in radial BMC:BW (1·74 *v.* 3·6%). However, both the current study and the twins study consistently demonstrate that Ca supplementation has little effect on bone acquisition in the proximal femoral neck. Recently, Lloyd *et al.* (1993) reported another Ca trial, with ninety-four 12-year-old Caucasian girls. The girls in the study group, with a basal dietary Ca intake of 1016 mg/d, were supplemented with 354 mg Ca/d for 18 months, and the LSBMD of the study group increased significantly by 2·9% when compared with the controls. However, the study group had a pronounced, but not significant, net gain in LSBMC (4·7%) when compared with the controls. Although the study children in the current trial were younger than those of Lloyd *et al.* (1993), and the mean habitual Ca intake of the children in the present study was approximately half that reported by Lloyd *et al.* (1993), the net percentage increase in LSBMC in the study group of the present Ca study was similar to that reported by Lloyd *et al.* (1993).

To conclude, in an 18-month controlled Ca-supplementation trial in which 300 mg elemental Ca or a placebo tablet was administered daily to a group of Chinese children whose mean basal Ca intake was approximately 567 mg/d, the study group had significantly greater increases in lumbar spinal BMC (20·9 *v.* 16·34%; $P = 0·035$) and lumbar spinal area (11·16 *v.* 8·71%; $P = 0·049$) when compared with the control group. The study group also showed a moderate increase of radial BMC:BW (7·74 *v.* 6·00%; $P = 0·081$). However, Ca supplementation had no beneficial effects on the increments of femoral-neck bone mass and height in the study children.

It has been suggested that a persistently higher Ca intake from childhood to early adulthood may lead to a higher peak bone mass (Matkovic, 1992). Genetic inheritance accounts for 70–80% of the attainment in peak bone mass (Pocock *et al.* 1987; Slemenda *et al.* 1991*a*; Morrison *et al.* 1994); whereas body build (Lloyd *et al.* 1992; Turner *et al.* 1992), including lean body mass (Davee *et al.* 1992); physical activity (Slemenda *et al.* 1991*b*; Grimston *et al.* 1993); dietary intake, including Ca (Fehily *et al.* 1992; Hu *et al.* 1993), protein (Orwoll, 1991; Metz *et al.* 1993), P (Metz *et al.* 1993) and Na (Sabto *et al.* 1984; McParland *et al.* 1989); smoking (Mazess & Barden, 1991; Paganini-Hill *et al.* 1991); and alcohol consumption (Fehily *et al.* 1992) are known modifiable environmental factors that may determine the remaining 20–30% variation in peak bone mass. The amount of peak bone mass varies among different ethnic groups at skeletal maturity too. Black Africans have a relatively high amount of bone mass compared with Caucasians (Gilsanz *et al.* 1991; McCormick *et al.* 1991), whereas there is some evidence that Orientals (Garn *et al.* 1964; Hu *et al.* 1993) have a relatively low bone mass when compared with Caucasians (Mazess & Cameron, 1974). Since hereditary and environmental factors during skeletal growth would modify the attainment of future peak bone mass, a longer term controlled Ca trial extending from childhood until the early 20s may be required to confirm any substantial benefit of a higher Ca intake on peak bone mass achievement. Furthermore, studies of the rates of bone mineral accretion and factors contributing to peak bone mass in non-Caucasian children habituated on low-Ca diets are scanty. Therefore, more research is warranted in indigenous people to identify factors contributing to peak bone mass in different populations.

The clinical trial was supported in part by the UPGC Earmarked Research Grant (CUHK 51/91) of the Research Grant Council, Hong Kong. We express our gratitude to all the study children and their parents for their active and co-operative participation in the study. We acknowledge the invaluable advice of Professor S. J. Oppenheimer, Department of Paediatrics, Chinese University of Hong Kong, and Dr Ann Prentice of the MRC Dunn Nutritional Laboratory, Cambridge, at the start of the study. Thanks are also due to

Smithkline Beecham, Weybridge, Surrey for providing tablets of $CaCO_3$ and the placebo for this clinical trial. This paper forms part of the thesis of W.T.K.L. for partial requirements for the doctoral degree at the Chinese University of Hong Kong.

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Printed in Great Britain