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School-milk intervention trial enhances growth and bone mineral accretion in Chinese girls aged 10–12 years in Beijing

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A 2-year milk intervention trial was carried out with 757 girls, aged 10 years, from nine primary schools in Beijing (April 1999 – March 2001). Schools were randomised into three groups: group 1, 238 girls consumed a carton of 330 ml milk fortified with Ca on school days over the study period; group 2, 260 girls received the same quantity of milk additionally fortified with 5 or 8 μg cholecalciferol; group 3, 259 control girls. Anthropometric and bone mineralisation measurements, as well as dietary, health and physical-activity data, were collected at baseline and after 12 and 24 months of the trial. Over the 2-year period the consumption of this milk, with or without added cholecalciferol, led to significant increases in the changes in height (≥0·6 %), sitting height (≥0·8 %), body weight (≥2·9 %), and (size-adjusted) total-body bone mineral content (≥1·2 %) and bone mineral density (≥3·2 %). Those subjects receiving additional cholecalciferol compared with those receiving the milk without added 25-hydoxycholecalciferol had significantly greater increases in the change in (size-adjusted) total-body bone mineral content (2·4 *v.* 1·2 %) and bone mineral density (5·5 *v.* 3·2 %). The milk fortified with cholecalciferol significantly improved vitamin D status at the end of the trial compared with the milk alone or control groups. It is concluded that an increase in milk consumption, e.g. by means of school milk programmes, would improve bone growth during adolescence, particularly when Ca intake and vitamin D status are low.

Milk supplement: Adolescent bone growth: Vitamin D: Ca

A commonly held view is that the greater the mass of bone mineral acquired by the time bone growth ceases, the lower the risk of osteoporotic bone fractures in later life. One possible influence on peak bone mass could be the quantity of milk consumed during the years of bone growth in adolescence. Intervention trials with white adolescent girls given milk or other dairy products have indeed shown gains in total bone mineral content (BMC), total bone mineral density (BMD) and lumbar spine density compared with unsupplemented control subjects (Chan *et al.* 1995; Cadogan *et al.* 1997). Other supplementation studies with Ca salts have found similar positive effects on BMC and areal BMD at different bone sites in pre-pubertal children and in adolescents (Matkovic *et al.* 1990; Johnston *et al.* 1992; Lloyd *et al.* 1993; Lee *et al.* 1994; Bonjour *et al.* 1997; Dibba *et al.* 2000). However, no consistent relationship has been found between the dietary supply of milk or Ca and the external dimensions of bone that would be reflected in changes in body height. In most studies there was no significant relationship between milk or Ca intake and change in height during growth.

A cross-sectional survey in Beijing of 1248 Chinese girls, aged 12–14 years, found that the average intake of Ca was 360 mg/d, of which only 21 % was obtained from dairy products (Du *et al.* 2001, 2002). As well as having a low intake of Ca, about 9 % of these girls also had a sub-clinical deficiency of vitamin D (plasma 25-hydroxycholecalciferol (25(OH)D) concentration <12·5 nmol/l) in summer, while in winter as many as 45 % of the surveyed children were sub-clinically deficient in vitamin D. The only nutritional factor associated with higher bone mass was milk intake, independently of intakes of Ca, protein or vitamin D (Du *et al.* 2002).

The implementation of a milk supplementation trial was therefore considered to be justified in this population of Beijing girls. No intervention study of this type had previously been done in Chinese pre-pubertal children or adolescents. Such an intervention study could also provide information on whether there was any effect of milk intake on the growth of bone during adolescence (and consequent effect on body height) and on mineral accretion (i.e. size-adjusted BMC) as well as on the rate of growth. If vitamin D-fortified milk were also to be included in a

Abbreviations: BA, bone area; BMC, bone mineral content; BMD, bone mineral density; PA, physical activity; PTH, parathyroid hormone; UHT, ultra-heat treated; 25(OH)D, 25-hydroxycholecalciferol.

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supplementation trial with adolescents who had a persistently low or deficient vitamin D status, the ability of a small increase in dietary vitamin D intake to affect either vitamin D status or bone growth could be tested.

Materials and methods

Subjects and study design

Healthy girls (*n* 757), aged 10 years, from nine primary schools in urban Beijing participated in a randomised controlled trial over 2 years. The nine schools were randomly assigned to three study groups, matched for the socio-economic background of the students. The subjects were recruited in April 1999 and were each assessed to be free of any disease that might affect bone development. Written informed consent was obtained from the parents of all subjects. The study was approved by the ethics committees of both the University of Sydney and the Institute of Nutrition and Food Hygiene of the Chinese Academy of Preventive Medicine (now the Chinese Centre for Disease Control and Prevention).

The three study groups were:

Group 1 (milk+Ca): 238 girls were each provided with 330 ml ultra-heat-treated (UHT) milk, which had been fortified to contain the equivalent amount of Ca (560 mg) found in 500 ml cows' milk. A carton of milk containing 560 mg Ca was consumed by each subject every school day for 24 months. After correcting for weekends and holidays, when no intervention milk was consumed, the average daily intake from this dietary supplement over the 24-month study period was 144 ml milk (245 mg Ca).

Group 2 (milk+Ca+vitamin D): 260 girls were each provided on school days with 330 ml UHT milk fortified with Ca as for group 1, but also containing 5 or 8 μg cholecalciferol. The average daily consumption, after correcting for weekends and holidays, was 144 ml intervention milk (245 mg Ca and 3·33 μg cholecalciferol) over the 24-month study period.

Group 3: A control group of 259 girls received no supplementary milk and consumed their habitual diets over the 24-month study period.

Each milk supplement was supplied in colour-coded UHT cartons with the identity of the supplement being unknown to both subjects and investigators during the course of the study.

The UHT milk was specially developed and produced for this project by Murray Goulburn Co-operative Co. Limited, Brunswick, Victoria, Australia. The composition of the UHT milk is given in Table 1. The milk was fortified with a Ca salt, derived from fresh milk whey, to give a total Ca content of 560 mg/330 ml. The added milk Ca salt (Natra-Cal), prepared by Murray Goulburn Co-operative Co. Limited, contained (g/kg): Ca 240, phosphate 520, lactose 50, protein 50, fat 10, other inorganic salts 20, water 110. The vitamin D (Vitamin $D_3$100 CWS/A) used to fortify the milk for group 2 was obtained from Roche Pty Ltd (Sydney, Australia). Six batches of milk supplement were manufactured and transported by air or sea from Australia to Beijing. The quality of each batch was assessed, in terms of the specified nutrients, before being used in the study. At each school, the milk was distributed by a health worker to the student in charge of the trial in each class. The subjects received the milk supplements in the morning, either before lessons began or during the first break. Each daily supplement was consumed by the end of the last morning break under the supervision of the teacher in charge. The student in charge of distributing the milk supplements kept records of compliance and these were checked regularly by project staff.

Table 1. Composition of the milk supplements (Mean values and standard deviations)

	Milk for group 1		Milk for group 2	
Nutrient	Mean	SD	Mean	SD
Total Ca (mg/l)	1700	170	1700	170
Total protein (g/l)	30	3	30	3
Fat (g/l)	30	3	30	3
Lactose (g/l)	≤50		≤50	
Sucrose (g/l)	20		20	
Cholecalciferol (μg/l)	None added		15*	
			24†	2†

* In first two of the six batches of milk.
† In last four of the six batches of milk.

The various measurements on each subject were made at the start of the trial (baseline), mid-trial (after 12 months) and at the end of the trial (after 24 months). At two additional times during the summer months dietary and physical activity (PA) data were collected to determine whether there were any seasonal variations.

Dietary assessment and physical activity

The baseline dietary intakes at the start of the study were determined using a 7 d recall technique. A 3 d recall (Thursday, Friday and Saturday) procedure was used for the remaining four periods of dietary assessment. Chinese measures of bowls and spoons of standard size were used to quantify food items with the assistance of a set of food measure models. The raw and cooked food conversion factors were re-checked on the basis of those used in an earlier study (Du *et al.* 2001). Chinese food composition tables and data entry program were used to calculate nutrient intakes. The vitamin D content of food was estimated from the UK food composition tables, with an adjusted decrease in the values for vitamin D in eggs and in fortified fresh milk based upon local analyses of these foods (Holland *et al.* 1991; Institute of Nutrition and Food Hygiene, 1991; He *et al.* 1997). Dietary records for baseline, mid-trial and end-trial time points were checked by interviewing the subjects and then by cross-checking with the data from a questionnaire, which sought details about the intake of dairy products over the previous 6 months. The consumption of edible oil (kg/month per family) was obtained from a questionnaire for parents at the time of baseline measurements and on each occasion of dietary recall thereafter.

PA over the previous 6 months was estimated by means of a questionnaire, and data for the baseline, mid-trial and

end-trial time points were obtained by interview. The two main indicators were 'spare-time PA' and 'school PA score' for out-of-school and school physical education respectively. More than fifteen categories of spare-time PA (min/week) were used, including walking, running, cycling, rope-skipping, the Chinese game of shuttlecock-kicking etc. in out-of-school hours and in class and lunch-time breaks. The school PA score is a comprehensive evaluation made once per term by the school physical education teachers. It includes performance and activity in a range of sports and PA during school physical education classes. The range of scores is from 1 to 4 where 1 corresponds to excellent and fully active (Du *et al.* 2002).

Bone mass and body composition

BMC, bone area (BA) and BMD were measured at the distal and proximal forearm of the non-dominant arm (results not shown) and in the whole body using dual-energy X-ray absorptiometry (Norland XR-36 densitometer; Norland, Fort Atkinson, WI, USA). Measurements were made at the Department of Nuclear Medicine, No. 304 Army Hospital, Beijing, at three time points (initial baseline, mid-trial and end-trial). The densitometer had a variation in precision of $<1{\cdot}0\,\%$ for the measured bone sites at standard speed and a variation in accuracy of $<1{\cdot}0\,\%$ with reference to a hydroxyapatite phantom. A quality assurance test was performed with the phantom each day and the quality assurance records confirmed that all the measurements had been conducted within the accepted specifications of the densitometer. Two experienced technicians performed the measurements throughout the study under the supervision of the department director. Total body measurements were made on a sample of only half the subjects, selected in a randomised fashion.

Biochemistry

Overnight fasting blood samples and a second voiding morning urine sample were taken from each subject at school during March and April (late winter) at the three monitoring intervals of baseline, mid-trial and end-trial. Plasma and serum were prepared and stored with the urine samples at -20°C in the Institute of Nutrition and Food Hygiene, Beijing, before being shipped in solid CO_2 to Australia for analysis at the University of Sydney and at the Children's Hospital at Westmead, Sydney. Laboratory analyses were done in random sample order and without knowledge of the intervention group from which they had come.

Plasma 25(OH)D concentration was measured by a competitive protein binding assay, modified after Mason & Posen (1977). Three quality control samples of low, medium and high 25(OH)D concentrations were included in each assay. The inter-assay and intra-assay CV were 12·4 and 4·2 % respectively. Vitamin D deficiency was defined as a plasma 25(OH)D concentration $\leq 12{\cdot}5$ nmol/l (Du *et al.* 2001). Serum intact parathyroid hormone (PTH) was measured by an immunometric technique (Diagnostic Products Corporation, Los Angeles, CA, USA). The reference range for serum PTH was 12–72 ng (1·3–7·6 pmol)/l, which was the central 95 % range for adults, with a median value 32 pg/ml. The total Ca concentration in plasma and urine was determined using an arsenazo III spectrophotometric method (Cobas MIRA Roche Diagnostica, Basle, Switzerland). The reference range for plasma Ca was 2·10–2·60 mmol/l. Total plasma Ca concentration was not corrected for albumin concentration. Urinary creatinine was measured by the Beckman Clinical Systems (Synchron CX5; Beckman Coulter Inc., Fullerton, CA, USA) enzymatic method. Urine Ca and creatinine concentrations were expressed as Ca:creatinine ratio (mmol/mmol). Plasma constituents indicating bone formation and resorption activities were also measured and will be reported elsewhere.

Other measurements

A questionnaire at the beginning of the study was used to collect information from the subjects and their parents on health history, medication, socio-economic status, pattern of dairy product consumption and their past and present intakes of Ca and vitamin D tablets. Information on the consumption of dairy products and of Ca or vitamin D tablets was also collected at interview at the mid-trial and end-trial monitoring periods.

Body weight was measured to the nearest 0·1 kg using electronic weight scales (Thinner; Measurement Specialities, Fairfield, NJ, USA), with subjects wearing light clothing and no shoes. Height and sitting height were measured to the nearest 0·001 m using a body-height measuring device (TG-III; No. 6 Machinery Plant, Beijing, China). Any clinical symptoms or signs of rickets were noted at the time of physical examination. Bone age to the nearest 0·25 year was determined using a Chinese standard (National Sports Commitee, 1992); a conventional hand and wrist radiograph was taken at the same hospital where bone mineral measurements were being made. The date of menarche was recorded; breast and pubic hair development were assessed using the Tanner staging criteria (Tanner, 1962; Adelaide Children's Hospital, 1989).

To obtain indirect information on the possible supply of vitamin D from sunlight, the exposure of each subject to UV was estimated at the time of blood collection using semi-quantitative personal badge dosimeters (Sherwood Skincare Ltd, Nottingham, UK). A badge was attached to the outer clothing surface at the top of the shoulder and this remained in place for 1 d. A new badge was applied each day for seven consecutive days. The average daily exposure of each subject to the UVB wavelength range (mJ/cm^2) was estimated by visual comparison of the colour of the exposed badge with a standard colour reference range.

Statistical analyses

All data sets were checked for statistical normality and any extreme values were removed according to a defined set of criteria. Descriptive statistics were made on all variables at the baseline and end-trial measurement points. Differences in continuous variables between the study groups at the baseline time point were tested by ANOVA and by Bonferroni *post hoc* methods. Repeated measures ANOVA was used to compare supplementation effects at the end-trial

time point. Differences in frequency data between groups at the same period were analysed by χ^2 test, and α 0·017 was used as the statistically significant level for further comparison of a 2×3 table. The McNemar test was applied to detect any change in rate over time.

To investigate any proportional effects of discrete variables (such as supplementation group), the continuous variables (such as bone and anthropometric measurements) were converted to natural logarithms. The basic model for examining any effect of supplementation was:

$$\ln(\text{value at the end of the study}) = k + a \times \ln(\text{value at baseline}) + b \times (\text{group}),$$

where k is a constant and a is the slope of the relationship between ln (value at end of the study) and ln (value at baseline). The supplemented groups were coded as 1 and the control group was coded as 0. The coefficient b (multiplied by 100) is the difference between the supplemented and the control groups in the percentage change from baseline values to the end of the study values, after adjusting for baseline values.

For the size-adjusted BMC, body and bone size variables including BA, body weight and height were all adjusted by inclusion in the model as the mean values and the differences at the baseline and at the end-trial time points, after conversion of the data to natural logarithms. Other variables that might influence BMC, such as stage of puberty, were also included in the regression analysis (Dibba *et al.* 2000). Unless otherwise indicated, any differences (two-tailed) at the 0·05 level were regarded as significant. All statistical analysis was done using SPSS software (SPSS Inc., Chicago, IL, USA).

Results

Of the original 757 subjects, 698 completed the 2-year trial, with a drop-out rate of 7·8 %. There were fifty-nine subjects (twenty-nine, eighteen and twelve from groups 1, 2 and 3 respectively) who did not continue until the end of the trial. Of these, thirty-three were excluded because of non-compliance with the supplementation (failure to drink the milk for ≥ 4 d for any reason), twenty were lost because of changing schools, five withdrew because of concerns about venepuncture and one withdrew because of skin allergy. No significant differences were observed in the anthropometric and other measured characteristics between those who left the study and those who completed it. Of the 698 subjects who completed the trial, full data sets from 681 subjects were included in the final analysis. Overall compliance amongst those who completed the intervention study was close to 100 %, with only a few subjects on any one day consuming < 330 ml of the milk provided as determined by strict supervision in the classroom and the maintenance of consumption records.

The physical characteristics and nutrient intakes of the subjects at the start of the intervention period and after 24 months (end-trial) are presented in Table 2. At the baseline, the mean Ca, vitamin D, protein and P intakes per d for the whole sample (681 subjects) were 430 (SD 158) mg, 0·8 (SD 0·6) μg, 52 (SD 13) g and 800 (SD 222) mg respectively. These values represented 43·1, 9·0 and 80·9 % of the adequate intake or reference nutrient intake of the Chinese reference dietary intakes for Ca, vitamin D and protein respectively for children aged 10 years (Chinese Nutrition Society, 2000). However, the protein intake of these girls in Beijing is greater than that recommended for Western countries because of assumed lower digestibility of protein derived mainly from plant-based diets. For example, for 10-year-old girls weighing 34 kg in the UK the reference dietary intakes for protein would be about 44 g (Department of Health, 1991), which is only 85 % of the average protein intake of the Beijing girls in the present study. The results in Table 2 also show no significant differences in average background milk consumption between the control and milk-supplemented groups at the start or end of the study. However, in a separate dietary investigation by food-frequency questionnaire on dairy product consumption (results not shown), there was an indication that those receiving milk at school did decrease their background intake of milk somewhat. Nevertheless, total milk consumption in groups 1 and 2 was substantially greater than that in group 3 as a result of the intervention.

There were no other significant differences in the physical characteristics or in the nutrient intakes between the three groups, except for energy intake, which was slightly higher in the control group 3 than in group 1. The absolute values for energy intake were underestimated, since the consumption of edible oil was not included in the dietary recall assessment. The mean values for edible oil consumption per family were not significantly different between the groups (3·1 (SD 1·8), 3·0 (SD 1·6) and 3·1 (SD 2·3) kg/month for groups 1, 2 and 3 respectively at baseline). No differences were found in bone age between the three groups (9·9 (SD 1·0), 9·9 (SD 1·1) and 10·1 (SD 1·0) years respectively). The variable socio-economic characteristics of the subjects and occupations of the fathers and mothers were similar between the three groups. The average family income and the percentage of income used for purchasing food were not significantly different between the three groups.

Nutrient intakes at the beginning of the study were determined by use of a 7 d dietary recall, whereas 24 months later the dietary recall was collected over 3 d. The change from 7 to 3 d was made because of difficulty in maintaining the interest of the 10-year-old subjects in accurately recalling their food consumption over a 7 d period. To ensure that this did not introduce errors when comparing the two nutrient intake surveys, a comparison was made of 3 d data and 7 d data in the original baseline survey. No significant differences were found in energy, protein or vitamin D intakes; however, Ca and P intakes were significantly greater in the 3 d data period (Table 3). Nevertheless, both the shorter-term and longer-term methods of dietary assessment confirmed that the background Ca intake was low (400–500 mg/d).

After 24 months of intervention, the liquid milk and Ca intakes were higher in the two supplementation groups, and the vitamin D intake was higher in the milk+Ca+-vitamin D group (group 2) compared with group 1 and the group 3 control values, because of the supplementation. Supplementation increased the Ca:P ratio (0·77 for group 1

Table 2. Physical characteristics and nutrient intakes of Beijing girls in the milk supplementation study‖
(Mean values, standard deviations and 95 % CI)

	Baseline									24 months								
	Group 1 (M+Ca)			Group 2 (M+Ca+VitD)			Group 3 (control)			Group 1 (M+Ca)			Group 2 (M+Ca+VitD)			Group 3 control		
	Mean	SD	95 % CI	Mean	SD	95 % CI	Mean	SD	95 % CI	Mean	SD	95 % CI	Mean	SD	95 % CI	Mean	SD	95 % CI
Subjects (*n*)	207			240			234			207			240			234		
Age (years)	10·1	0·4		10·1	0·3		10·0	0·3		12·1	0·4		12·1	0·3		12·0	0·3	
Height (m)	1·404	0·065		1·411	0·069		1·407	0·062		1·538	0·065		1·541	0·066		1·529	0·062	
Weight (kg)	33·9	7·2		33·5	7·0		33·4	6·6		45·5	9·4		45·3	9·3		43·5	8·4	
BMI (kg/m^2)	17·1	2·8		16·8	2·6		16·8	2·6		19·1	3·2		19·0	3·3		18·5	3·0	
Tanner breast staging (%)																		
I	42·4		36·1, 48·7	40·6		34·3, 46·9	45·7		39·2, 52·2	2·0		0·1, 3·9	7·1		3·8, 10·4	5·1		2·2, 8·0
II	49·3		43·0, 55·6	49·8		43·3, 56·3	49·6		43·1, 56·1	29·8		23·4, 36·2	35·1		28·9, 41·3	28·2		22·3, 34·1
III–V	8·3		4·5, 12·1	9·6		5·8, 13·4	4·7		1·9, 7·5	68·2		61·7, 74·7	57·7		51·5, 64·0	66·6		60·4, 72·8
Menstruating (%)	1·5		−0·2, 3·2	2·3		0·4, 4·2	0·4		−0·4, 1·2	50·5†		43·5, 57·5	48·4†		41·9, 54·9	39·2†		32·8, 45·6
UV exposure (mJ/cm^{2}UV/B)	19·8	7·6		22·0	5·5		20·0	5·9		29·2	13·6		27·6	12·4		24·7	13·7	
Spare-time PA (min/week)	364	327		349	340		430	324		251	155		271	157		227	185	
Milk intake (g/d)	113	89		113	95		135	101		251‡	106		251‡	110		134	118	
Ca intake (mg/d)	418·2	145·3		418·1	162·5		455·3	166·1		649·2‡	173·4		660·6‡	177·7		457·5	197·3	
P intake (mg/d)	780·1	219·5		792·5	214·1		830·6	232·5		837·9	221·8		923·2	242·5		803·3	241·8	
Vitamin D intake (μg/d)	0·827	0·550		0·894	0·644		0·915	0·637		0·607§	0·675		3·934‡	0·439		0·639	0·721	
Energy intake (kJ/d)	5527*	1351		5715	1364		5883	1301		5573	1515		5899	1648		5732	1548	
Protein intake (g/d)	52·4	16·1		53·1	15·2		55·9	17·9		53·5	14·5		56·9	15·3		53·5	15·4	

(M+Ca); milk with Ca; (M+Ca+VitD) milk with Ca and vitamin D; PA, physical activity.
Mean value was significantly different from that of the control group at baseline (ANOVA): *P=0·024.
Mean values were significantly different between the three groups at 24 months (χ^2 test): †P=0·039 (further comparisons showed: group I *v.* control P=0·018; group 2 *v.* control P=0·051; both P values did not reach significance level of α 0·017).
Mean values were significantly different from those of the control group at 24 months (repeated measures ANOVA): ‡P<0·01.
Mean value was significantly different from that of group 2 at 24 months (repeated measures ANOVA): §P<0·01.
‖ For details of subjects, milks and procedures, see Table 1 and p. 160.

Table 3. Comparison of nutrient intakes obtained by 7 d and 3 d dietary recall baseline data for eighty-nine subjects randomly selected from nine schools†
(Mean values and standard deviations)

Nutrient intake	7 d data		3 d data			Correlation coefficient (*r*)	
	Mean	SD	Mean	SD	*P*		*P*
Energy (kJ/d)	5703	1255	5845	1312	0·059	0·848	<0·001
Protein (g/d)	54	15	55	14	0·171	0·784	<0·001
Ca (mg/d)	433	153	470*	174	<0·001	0·883	<0·001
P (mg/d)	810	206	849*	214	0·003	0·837	<0·001
Vitamin D (g/d)	0·9	0·7	0·9	0·7	0·716	0·826	<0·001

Mean values were significantly different from those of 7 d data: *$P<0·05$.
† For details of subjects and procedures, see Table 1 and p. 160.

and 0·72 for group 2 compared with 0·57 for the control group 3; $P<0·0005$, repeated measures ANOVA). There was a trend towards earlier onset of menstruation among the supplemented girls. There were no significant differences in Tanner breast staging and school PA score (χ^2 test), and body height, weight, UV exposure and spare-time PA between the three groups (repeated measures ANOVA).

Bone mineral and biochemical measurements are shown in Table 4. There were no differences in total body BMC, areal BMD and BA between the three groups at baseline. The concentrations of plasma 25(OH)D, serum PTH and plasma Ca were also comparable between the groups at the start of the study. Baseline urine Ca:creatinine ratio was significantly higher in group 1 than in the control group 3 (0·17 (SD 0·16) and 0·08 (SD 0·08) mmol/mmol respectively; $P=0·01$).

After 24 months the mean total body BA of the two supplemented groups was less than that of the controls. In contrast, the mean values for total body BMC and areal BMD were higher for the supplemented girls compared with the control values. However, none of the differences in mean values for BA, BMC or BMD was statistically significant, as determined by repeated measures ANOVA. Likewise, there were no significant differences in these bone measurements when compared between the two supplementation groups. At 24 months the average plasma 25(OH)D concentration of girls who had received vitamin D-fortified milk (group 2) was more than double that of girls in the milk-supplemented group 1 and the control group 3 (17·9 (SD 9·0), 47·6 (SD 23·4) and 19·4 (SD 10·2) nmol/l for groups 1, 2 and 3 respectively; $P<0·0005$). Compared with control values, serum PTH, plasma Ca and urine Ca:creatinine ratio all tended to be lower in girls in the two supplemented groups compared with the control values. Bone measurements at mid-trial (12 months) did not show any significant difference and the results are not presented here.

Another way to assess the effects of milk supplementation on the growth of bone is to compare the percentage changes from baseline, rather than the absolute values, between the study groups (Table 5). When the relative percentage changes were expressed in this way, the increases in height, sitting height and body weight after 2 years in the girls in the two supplemented groups were significantly greater compared with the girls in the control group 3.

The total body percentage increases in BMC were also greater in the two supplemented groups than in the controls. Size-adjusted BMC confirmed a 1·2 and 2·4 % greater gain in group 1 and group 2 respectively. The vitamin D-supplemented group 2 had a significantly different greater gain of 1·3 % in size-adjusted BMC than the group 1 subjects, who had received the milk supplement without vitamin D. Compared with control values, group 1 girls had a 3·2 % and group 2 girls a 5·3 % greater gain in areal total body BMD. There was again a significant difference between the two supplementation groups (2 % greater gain in the group 2 girls who had received vitamin D-fortified milk). Of all the measurements at mid-trial (12 months), group 2 had significantly greater percentage increases only in total body BMD and in body weight compared with the control values. Any differences for any of the measured variables between group 1 and the control values were not significant at mid-trial and the results are not presented here.

There were significantly fewer girls in group 2 (receiving vitamin D in the supplement) with biochemical vitamin D deficiency (plasma 25(OH)D $<12·5$ nmol/l) than those in group 1 and in the control group 3. The deficiency rates were 16·9, 2·6 and 17·6 % at 12 months and 19·6, 4·1 and 16·0 % at 24 months for groups 1, 2 and 3 respectively. The deficiency rates were also lower at 12 and 24 months in the group 2 girls than at the baseline measurement for this group (15·2 %; $P<0·01$, McNemar test). Milk supplementation also significantly lowered the proportion of subjects with elevated serum concentration of PTH (>7·6 pmol/l) at 24 months. In comparison with 51·2 % of control subjects who had elevated serum PTH values, only 27·9 % of those in group 1 ($P=0·002$) and 17·7 % of those in group 2 ($P<0·0005$) had elevated PTH values at the end of the supplementation trial.

Discussion

The purpose of the present intervention study was to examine the effects of providing a dietary supplement of a small volume of milk to Chinese pre-pubertal girls in Beijing; these girls were on a persistently low-Ca diet and had a high prevalence of vitamin D deficiency (Du *et al.* 2001). The milk, which had been fortified with additional Ca (and also with cholecalciferol in one treatment group), was consumed on school days over a 2-year period. This supplement, when averaged over the time of the

Table 4. Bone measurements and biochemical variables of Beijing girls in the milk supplementation trial§
(Mean values and standard deviations)

	Baseline						24 months‖					
	Group 1 (M+Ca)		Group 2 (M+Ca+VitD)		Group 3 (control)		Group 1 (M+Ca)		Group 2 (M+Ca+VitD)		Group 3 (control)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Subjects (*n*)	111		113		122		111		113		122	
Total body:												
BMC (g)	1359·4	211·6	1341·0	199·2	1361·0	203·6	1875·6	281·3	1867·0	255·0	1845·4	268·4
BA (cm^2)	1952·2	171·5	1953·4	188·7	1943·2	170·5	2520·2	189·9	2501·1	187·8	2543·2	237·0
BMD (g/cm^2)	0·693	0·057	0·684	0·046	0·698	0·054	0·743	0·084	0·746	0·077	0·726	0·083
Plasma 25(OH)D (nmol/l)	17·7	8·7	20·6	8·8	19·1	7·4	17·9†	9·0	47·6**	23·4	19·4	10·2
Serum PTH (pmol/l)	3·73	2·11	4·33	3·82	4·24	2·14	6·68	3·03	5·64	3·34	8·19	6·30
Plasma Ca (mmol/l)	2·53	0·24	2·51	0·23	2·47	0·16	2·55**	0·34	2·68**	0·30	2·95	0·16
Urine Ca:creatinine (mmol/mmol)	0·17‡	0·16	0·14	0·17	0·08	0·08	0·07	0·06	0·07	0·08	0·12	0·16

(M+Ca); milk with Ca; (M+Ca+VitD), milk with Ca and vitamin D; BMC, bone mineral content; BA, bone area; BMD, bone mineral density; 25(OH)D, 25-hydroxycholecalciferol; PTH, parathyroid hormone.
Mean values were significantly different from those of control group 3 at 24 months: **$P<0·01$.
Mean value was significantly different from that of group 2 at 24 months: †$P<0·01$.
Mean value was significantly different from that of the control group 3 at baseline (ANOVA): ‡$P<0·01$.
§ For details of subjects, milks and procedures, see Table 1 and p. 160.
‖ There were significant main effects between baseline and 24 months ($P<0·0005$) and significant interaction of year × group ($P<0·05$) except for total BA for all variables (repeated measures ANOVA).

Table 5. Effect of milk supplementation on percentage changes in height, weight and bone measures of Beijing girls‡
(Mean values with their standard errors)

	Percentage change (24 months minus baseline)						Adjusted percentage difference in change§								
	Group 1 (M+Ca)‖		Group 2 (M+Ca+VitD)‖		Group 3 (control)‖		Group 1 minus group 3 (M+Ca) − control			Group 2 minus group 3 (M+Ca+VitD) − control			Group 2 minus group 1 (M+Ca+VitD) − (M+Ca)		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*P*	Mean	SE	*P*	Mean	SE	*P*
Height (m)	0·95**	0·001	0·93*	0·001	0·087	0·001	0·007	0·001	<0·0005	0·006	0·001	<0·0005	−0·002	0·002	0·3
Sitting height (m)	0·92**†	0·002	0·087*	0·002	0·078	0·002	0·012	0·002	<0·0005	0·008	0·2	<0·0005	−0·004	0·002	0·04
Weight (kg)	34·6*	0·7	35·9**	0·8	30·8	0·6	2·9	0·007	<0·0005	3·7	0·7	<0·0005	0·8	0·8	0·3
Total body:															
BMC (g)	38·4	0·8	39·7*	0·8	35·9	0·8	1·8	0·8	0·03	2·6	0·8	0·002	0·8	0·8	0·3
Size-adjusted BMC¶							1·2	0·5	0·012	2·4	0·5	<0·0005	1·3	0·5	0·006
BA (cm^2)	29·5	0·9	28·5	0·8	31·3	1·0	−1·0	0·9	0·2	−1·8	0·9	0·04	−0·8	0·7	0·3
BMD (g/cm^2)	7·0*	0·6	8·9**	0·6	3·9	0·6	3·2	0·8	<0·0005	5·3	0·8	<0·0005	2·0	0·8	0·009

(M+Ca), milk with Ca; (M+Ca+VitD), milk with Ca and vitamin D; BMC, bone mineral content; BA, bone area; BMD, bone mineral density.
Mean values were significantly different from those of control group 3 (ANOVA): *$P<0·01$, **$P<0·0005$.
Mean value was significantly different from that of group 2 (ANOVA): †$P<0·05$.
‡ For details of subjects, milks and procedures, see Table 1 and p. 160.
§ Adjusted for baseline (see p. 162).
‖ Subjects for anthropometry (*n*): group 1 207, group 2 113, group 3 234; subjects for bone mineral measurements (*n*): group 1 111, group 2 113, group 3 122.
¶ Adjusted for baseline BMC, BA, height weight and menstruating (see p. 164).

trial, provided an additional 245 mg Ca/d on top of the background Ca intake, which averaged between 418 and 455 mg/d. Hence, the children receiving the milk supplement were consuming on average between 54 and 59 % more Ca/d than those in the control group who received no supplement.

Not surprisingly, the group of children (group 2) who received on average 3·33 μg cholecalciferol/d in the milk supplement had a significantly higher vitamin D status after 24 months (plasma 25(OH)D 47·6 (SD 23·4) nmol/l) than those in the control group, whose mean plasma 25(OH)D was 19·4 (SD 10·2) nmol/l. Although this intake of vitamin D from the milk supplement was less than the 5 or 10 μg/d often recommended for growing children, it was a substantially higher intake than the approximately 1 μg/d usually consumed in food by children in Beijing (Du *et al.* 2001).

Another biochemical measurement that was significantly affected by the milk supplement was the concentration of total Ca in plasma. Both the supplemented groups had significantly lower mean plasma Ca levels than the unsupplemented controls at the end of the trial (Table 2). If the fall in plasma Ca was caused by increased excretion of Ca in urine, then an increase in urine Ca:creatinine ratio would occur. This might be obscured by a possible increase in creatinine excretion related to increased lean tissue mass in the milk-supplemented groups. However, as there was no apparent difference in the urinary Ca:creatinine ratio between the three groups at the end of the trial, the lower concentration of Ca in blood was compatible with an increased rate of Ca incorporation into bone in the two milk-supplemented groups. Research on bone growth in white girls indicates that the maximum rate of Ca accretion of 300–400 mg/d in the skeleton occurs at about 12 years of age (Matkovic *et al.* 1994). In the current study, the mean dietary Ca intakes of the Beijing girls at this age were about 650 and 450 mg/d for the supplemented and control groups respectively; hence, the somewhat lower mean plasma Ca concentrations of the supplemented groups, and the somewhat elevated mean serum PTH concentrations of all groups at the end of the trial (Table 4), would be expected from the known rate of Ca accretion by bone at this stage of growth.

Because of the considerable individual variability in growth characteristics at the onset of puberty, it is not surprising that no significant differences were found in the group mean values of any of the physical measurements related to bone. However, when the mean percentage changes from baseline values for each subject were compared, the effects of providing supplementary milk became apparent (Table 5). The percentage changes showed that the children who had received the milk supplement had significant increases in bone mineralisation (BMC and BMD) and in height, sitting height and body weight relative to the control group. Thus, consumption of the milk supplement appeared to have directed more Ca into growing bone with the effect of increasing bone mineralisation and increasing the size of those bones that determined the increase in height. This phenomenon has not been readily found in previous studies (Fraser, 1988).

An alternative explanation for the effects of supplementation might be that the milk was promoting growth simply by supplying additional energy and protein to children who were marginally deficient in these nutrients. This possibility would need exploration by means of data analysis beyond the scope of the present paper. However, on average the supplement only increased the daily intake of protein by about 8 % and of energy by about 5 %, whereas the increase in Ca intake was more than 50 %.

One association with the additional milk consumption was an increase in the proportion of girls attaining menarche by the end of the 24-month intervention trial. Although this increase did not reach statistical significance when comparing each supplemented group with the control values (Table 2), it is possible that accelerated pubertal development may have contributed to the increased BMC of subjects in the milk-supplemented groups. However, when the data were adjusted for menarcheal status, the effects of the milk supplement on bone were still apparent (Table 5). A stratified analysis by menarcheal status showed that the effects of the milk supplementation on anthropometric and bone mineral measurements were independent of menarcheal status, with the proviso that the differences in percentage change in height, weight, BMC and BMD between the three groups were greater after menarche (Table 6). The mechanism for an earlier onset of menarche in the milk-supplemented groups is not known, but it should be noted that the increased intake of milk had no effect on the Tanner stage of pubertal development (Table 2). The onset of menarche and the anatomical changes of puberty are clearly related but they are not chronologically interdependent.

One possible outcome of this intervention trial was the testing of the hypothesis that a low Ca intake increases the metabolic destruction of 25(OH)D and reduces vitamin D status when vitamin D input is low. Such an effect has been clearly demonstrated in experimental rats on low-Ca diets (Clements *et al.* 1987). In a previous survey of Beijing children, a positive association between Ca intake and vitamin D status had been found (Du *et al.* 2001). However, it was apparent in the intervention study that the vitamin D status of the children in group 1 (supplemented with milk without vitamin D), who received at least 50 % more Ca than those in the control group, was just as low as that of the control group at the end of the trial. As the end-trial time point was in March/April, it is possible that the very low supply of vitamin D from solar irradiation during the previous winter had still been insufficient to allow an effect of Ca intake on vitamin D status to be evident. Nevertheless, in both groups given the supplementary milk, either without vitamin D (group 1) or fortified with vitamin D (group 2), there was a significant decrease in the percentage of subjects with elevated serum PTH concentrations and there were significant increases in the anthropometric and bone mineralisation measurements. This suggests either that Ca from milk or some other component from milk was having the effect on bone development, independently of vitamin D status. It is also possible that the supplementary milk, not fortified with vitamin D, had prolonged the period in winter when adequate vitamin D status had been maintained and that this

Table 6. Effect of milk supplementation on percentage changes in height, weight and bone measurements of Beijing girls by menarcheal status‡

(Mean values with their standard errors)

	Before menarche: percentage change (24 months minus baseline)						After menarche: percentage change (24 months minus baseline)					
	Group 1 (M+Ca)§		Group 2 (M+Ca+VitD)		Group 3 (control)§		Group 1 (M+Ca)‖		Group 2 (M+Ca+VitD)‖		Group 3 (control)‖	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Height (m)	0·097***	0·002	0·095**	0·001	0·089	0·001	0·093**	0·002	9·0	0·2	8·3	0·2
Sitting height (m)	0·090***	0·002	0·085*	0·003	0·077	0·002	0·095***	0·002	8·9*	0·2	8·1	0·2
Weight (kg)	34·8*	1·1	33·2	1·2	31·0	0·8	34·4*†	0·9	38·8***	1·2	30·6	1·0
Total body:												
BMC (g)	36·2	1·1	36·3	1·2	34·9	1·1	40·5	1·2	43·2**	1·1	37·1	1·0
BA (cm^2)	31·8	1·3	30·0	1·2	33·3	1·5	27·6	1·1	27·3	1·1	28·3	1·3
BMD (g/cm^3)	3·5	0·8	5·0**	0·7	1·6	0·7	10·2*†	0·6	12·7***	0·7	7·1	0·7

(M+Ca), milk with Ca; (M+Ca+VitD), milk with Ca and vitamin D; BMC, bone mineral content; BA, bone area; BMD, bone mineral density.
Mean values were significantly different from those of control group 3 (ANOVA): *$P<0·05$, **$P<0·01$, ***$P<0·0005$.
Mean values were significantly different from those of group 2 after menarche (ANOVA): †$P<0·05$.
‡ For details of subjects, milks and procedures, see Table 1 and p. 160.
§ Subjects for anthropometry (*n*): group 1 102, group 2 123, group 3 143; subjects for bone mineral measurements (*n*): group 1 52, group 2 53, group 3 68.
‖ Subjects for anthropometry (*n*): group 1 102, group 2 123, group 3 85; subjects for bone mineral measurements (*n*): group 1 58, group 2 53.

had contributed to the beneficial effect of this particular supplement. Even though both supplemented groups produced significant improvements in percentage change in bone mineralisation and growth, those in group 2 receiving the vitamin D-fortified milk had a significantly greater percentage increase in size-adjusted BMC and in BMD than those in group 1, who received milk but with no additional vitamin D. Hence in the present study, the effect of vitamin D on bone can be interpreted as mainly an effect on promoting the supply of dietary Ca for bone mineralisation. Even with a low vitamin D status, the additional Ca from the milk supplement still caused significant improvements in the bone measurements.

These findings lend weight to the proposal that the provision of a dietary milk supplement through school milk programmes, particularly when background Ca intake and vitamin D status are low, would improve bone growth and development during adolescence.

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