Original Article

Effects of two years' milk supplementation on size-corrected bone mineral density of Chinese girls

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Much existing data on the effects of calcium or milk products on bone mineral accretion are based on bone mineral content (BMC) or areal bone mineral density (aBMD), neither of which accounts for changing bone size during the growing period. The aim of this study was to investigate the effects of 2-year milk supplementation on total body size-corrected BMD in Chinese girls with low habitual dietary calcium intake. Chinese girls aged 10 years were randomised, according to their school, to receive calcium fortified milk (Ca milk), or calcium and vitamin D fortified milk (CaD milk) for two years or act as unsupplemented controls. Dual-energy X-ray absorptiometry total body bone measures were obtained from 345 girls at baseline and 2 years. Size-corrected total body and regional BMD was calculated as: $BMDsc = BMC/BA^{pc}$, where pc was the regression coefficient of the natural logarithm transformed total body BMC and bone area. After 2 years, both supplemented groups had significantly greater gain in BMDsc of total body (3.5-5.8%, $p < 0.05$) and legs (3.0-5.9%, $p < 0.05$) than did the control group. Milk supplementation showed positive effects on bone mineral accretion when accounting for the changing skeletal size during growth. The effects were mainly on the lower limbs.

Key Words: size-corrected BMD, milk products, calcium, vitamin D, Chinese girls

INTRODUCTION

The pubertal years are a critical period for linear growth and bone mineral accretion. Dietary calcium intake and vitamin D status are commonly viewed as important nutritional factors influencing bone mineral accretion during the growing years. Prospective studies have shown positive effects of dietary supplementation with calcium and dairy products on bone mineral acquisition in children and adolescents from different countries with background calcium intakes ranging from 280 to 980 mg/d.[1-10] However, most of these studies are based on bone mineral content (BMC) or areal bone mineral density (aBMD) measurements, neither of which accounts for changing bone size during the growth period.

In a school milk intervention study in Beijing girls aged 10 years at baseline, we showed that after 2 years, the two groups who received a milk supplement (milk fortified with calcium with/without vitamin D) every school day had significantly greater increases than the control group in total body areal BMD (by 3.2-5.3%) and height (by 0.6-0.7%).[11] The milk fortified with vitamin D also significantly improved vitamin D status compared to the milk alone or control groups (plasma 25(OH)D concentrations 47.6 ± 23.4 vs 17.9 ± 9.0, 19.4 ± 10.2 nmol/L, $p < 0.0005$).[11] The aim of the present analysis was to investigate the effects of 2-year milk supplementation on size-corrected total body and regional BMD, which accounts for the change in size during the growth period, in Chinese girls aged 10 years at baseline with low habitual dietary calcium intake.

SUBJECTS AND METHODS

Subjects and study design

Seven hundred and fifty-seven urban Beijing girls aged 10 years participated in a 2-year school milk intervention trial. They were from nine randomly selected schools in one district in urban Beijing. Subjects were randomised into three groups according to their schools ensuring that the schools in the different groups had comparable socio-economic circumstances. There were 238 girls in the Ca milk group receiving 330 ml calcium fortified UHT milk per school day, while 260 girls in CaD milk group received 330 ml calcium and vitamin D fortified UHT milk per school day, and 259 girls in the control group received no supplementary milk and consumed their habitual diet during the study period. The UHT milk for this project was specially formulated by Murray Goulburn Co-operative Co.

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Ltd. (VIC, Australia), to comply with both Chinese and Australian food regulations. The milk was fortified with a milk calcium salt (NatraCal) to give a total calcium content of 560 mg (the equivalent of the calcium in 500 ml regular milk) within a volume of 330 ml. This was in order to keep the supplementation milk within the amount readily consumed on each occasion and to avoid any problems associated with milk overload. Each carton of milk (330 ml) also contained 10 g of fat, 10 g of protein, and for the milk used in CaD milk group, 5-8 μg added vitamin D as well. A detailed description of the composition of the milk supplements and the added milk calcium salt has been given elsewhere[11]. After correcting for weekends and holidays, when no intervention milk was consumed, the average daily supplementation over the 24 months was 144 ml milk, containing 245 mg calcium, and for CaD milk group, 3.33 μg vitamin D. The study was carried out with the approval of the Ethics Committees of the University of Sydney, Australia, and the Institute of Nutrition and Food Hygiene of the Chinese Academy of Preventive Medicine (now the Chinese Center for Disease Control and Prevention). A consent form in Chinese was signed by the parents of all the study participants.

Bone mineral measurement

Complete baseline and 2 years total body bone mineral assessment data were obtained from a subset of 345 randomly selected subjects (110, 112, 123 girls from Ca milk, CaD milk and control groups, respectively). Total body BMC, bone area (BA) and BMD were measured by dual-energy X-ray absorptiometry (DXA) using the same Norland XR-36 densitometer (Norland, Fort Atkinson, WI, USA) at baseline and 2 years. Regional BMD of head, chest, midriff, pelvis, arms and legs was derived from the DXA total body bone scan. Software version 3.94 was used. The densitometer had a variation in precision of <1.0% for the measured bone site at standard speed. A daily quality assurance test was performed over the study period using a manufacturer-supplied hydroxyapatite phantom, and the accuracy error was less than 1.0%. The scan and analysis were performed by the same two technicians throughout the study.

Other measurements

Health histories of the subjects and family members and family socio-economic status were obtained by a general information questionnaire at baseline. The following assessments were made at baseline and 24 months: body weight by an electronic scale (Thinner, WI, USA), height and sitting height by body and sitting height measures (TG-III, Beijing, China), dietary intakes by 7-day unweighed food record (24-hour recall diary for seven days) at baseline and 3-day food record at 24 months (number of days were reduced at 24 months due to subject fatigue), and pubertal stage of breast and pubic hair development according Tanner's definitions of the five stages of puberty[12]. Date of menarche was recorded.

Statistical analysis

Descriptive statistics are reported as mean ± SDs, and differences as mean ± SEs for all variables, unless otherwise indicated. Baseline values between the three groups were compared using analysis of variance (ANOVA) and chi-square test, as appropriate. Size-corrected total body and regional BMD was calculated as: BMDsc = BMC/BA^{pc}, where pc was the regression coefficient of the natural logarithm transformed BMC and BA. To allow for clustering by school, adjusted analyses were conducted using the linear mixed model, with school defined as a random effect.[13] In order to determine power relationships between the continuous variables and proportional effects of discrete variables, all continuous variables were converted to natural logarithms. Outcomes were analyzed by adjusting for the baseline value and intervention group. As the adjusted analyses are based on the natural log-transformed outcome variables, the differences between each of the intervention groups and the control group were calculated as percentage differences. The supplementation effects were also analyzed at the individual level, with a multiple regression model with backward elimination. The significance level for test statistics was set at $p < 0.05$. All data were analyzed by SPSS 15 (SPSS Inc, Chicago, IL, USA).

RESULTS

The characteristics of subjects at baseline are shown in Table 1. No significant differences were observed among the three groups in any of the variables listed. At baseline, the average calcium intake was 436 ± 168 mg/day for all subjects. This is only slightly more than half of the Adequate Intake (800 mg/day) of the Chinese DRIs for this age group.[14] The average vitamin D intake was 0.9 μg/day, which represented only 9.0% of the Recommended Nutrient Intake (10 μg/day) of the Chinese DRIs for this age group.[14]

With supplementation, calcium intakes in the two supplemented groups became: Ca milk 650 ± 173 mg/day; CaD milk 672 ± 186 mg/day, while those of controls were 453 ± 196 mg/day ($p < 0.001$). Vitamin D intakes were also significantly higher in the CaD milk group than those of the other two groups (CaD milk 3.9 ± 0.4 μg/day, Ca

Table 1. Baseline characteristics of subjects

	Ca milk (n = 110)	CaD milk (n = 112)	Control (n = 123)
Age (y)	10.1 ± 0.4	10.1 ± 0.4	10.1 ± 0.3
Height (cm)	141 ± 6.4	142 ± 7.1	141 ± 6.7
Weight (kg)	34.3 ± 7.4	33.1 ± 6.7	33.7 ± 7.0
% at Tanner breast stage			
1	36	39	48
2	55	47	47
3	9	14	5
% at Tanner pubic hair stage			
1	95	92	96
2	5	7	3
3	0	1	1
% post-menarche	1.7	1.9	1.8
Ca intake (mg/d)	422 ± 146	422 ± 165	454 ± 171
Vitamin D intake (μg/d)	0.8 ± 0.6	0.9 ± 0.6	0.9 ± 0.6

Values are mean ± SD or percentage as stated.

milk 0.6 ± 0.7 μg/day, controls 0.6 ± 0.7 μg/day, $p < 0.001$).

In repeated-measures analyses, there were significant group and time interactions for total body, legs and arms size-corrected BMD, indicating treatment effects on these variables (Table 2). Over the two years, total body and legs size-corrected BMD increased in the CaD milk group, decreased in the control group and remained unchanged in the Ca milk group (Table 2). Arms size-corrected BMD increased in both supplemented groups, but not in the control group (Table 2). Pelvis and midriff size-corrected BMD increased in all three groups (Table 2).

Further analysis accounting for baseline values and clustering by school showed that after 2 years, both supplemented groups had significantly greater gain in total body size-corrected BMD (3.5-5.8%, $p < 0.05$) and legs size-corrected BMD (3.0-5.9%, $p < 0.05$) than did the control group (Table 3). Both supplemented groups also had significantly greater gain in arms size-corrected BMD (2.5-2.3%, $p < 0.05$) than did the control at individual level analysis, but not after accounting for clustering by school (Table 3).

DISCUSSION

The present study found that in Chinese girls aged 10 years at baseline, 2 years of milk supplementation (fortified with either calcium alone or both calcium and vitamin D) had positive effects on bone mineral accretion when accounting for the changing skeletal size during growth. The effects were mainly on the lower limbs.

Previous studies have shown that during peak growth, there may be a dissociation between linear growth and bone mineralization, which leads to a drop in size-corrected BMD.[15] The present study showed that in Chinese girls, from age 10 to 12 years, there was a decrease in total body size-corrected BMD in the unsupplemented control group, whereas the total body size corrected BMD remained unchanged or increased in the groups receiving milk supplementation. This finding suggested that increasing calcium intake during the peak growth period may help to reduce the extent of the drop in bone mineral density.

A few studies investigated the sensitivity to calcium or milk products supplementation of different bone regions in growing children, and the results were not consistent.

Table 2. Total body and regional size-corrected BMD during the study

		Ca milk (n = 110)	CaD milk (n = 112)	Control (n = 123)
Total body BMDsc ($mg/cm^{2.528}$)†	Baseline	94 ± 7	93 ± 5	95 ± 6
	24 mo	94 ± 10	95 ± 10§	92 ± 11§
Arms BMDsc ($mg/cm^{2.250}$)†	Baseline	200 ± 29	193 ± 22	197 ± 27
	24 mo	205 ± 24§	202 ± 22§	199 ± 21
Legs BMDsc ($mg/cm^{2.490}$)†	Baseline	145 ± 14	143 ± 11	146 ± 14
	24 mo	144 ± 19	146 ± 16§	140 ± 18§
Pelvis BMDsc ($mg/cm^{3.082}$)‡	Baseline	47 ± 5	46 ± 4	47 ± 5
	24 mo	49 ± 8	49 ± 7	49 ± 8
Midriff BMDsc ($mg/cm^{1.586}$)‡	Baseline	1659 ± 342	1585 ± 332	1584 ± 337
	24 mo	1794 ± 463	1803 ± 446	1760 ± 499

Values are mean ± SD. †Significant interactions between group and time (two-factor repeated-measures ANOVA with interaction), $p < 0.05$. ‡Significant main effect of time (two-factor repeated-measures ANOVA with interaction), $p < 0.05$. §Significantly different from baseline (one-factor repeated-measures ANOVA), $p < 0.05$.

Table 3. Effects of milk supplementation on total body and regional size-corrected BMD

		Adjusted percentage difference in outcome at 2 years relative to control group†		Adjusted percentage difference in outcome at 2 years relative to control group allowing for clustering by school‡	
		Mean (95% CI)	*p*	Estimate (95% CI)	*p*
Total body BMDsc	Ca milk	3.6 (1.8, 5.4)	<0.001	3.5 (1.0, 5.9)	0.01
	CaD milk	5.8 (4.0, 7.6)	<0.001	5.8 (3.4, 8.2)	0.001
Arms BMDsc	Ca milk	2.5 (0.1, 4.8)	0.04	2.5 (-0.9, 6.0)	0.13
	CaD milk	2.3 (0, 4.7)	0.05	2.3 (-1.1, 5.8)	0.16
Legs BMDsc	Ca milk	3.3 (1.2, 5.3)	0.002	3.0 (0, 6.1)	0.05
	CaD milk	6.0 (4.0, 8.1)	<0.001	5.9 (2.7, 9.2)	0.003
Midriff BMDsc	Ca milk	-1.5 (-6.5, 3.5)	0.55	-1.5 (-6.5, 3.4)	0.54
	CaD milk	3.2 (-1.7, 8.2)	0.20	3.2 (-1.7, 8.2)	0.20
Pelvis BMDsc	Ca milk	0.4 (-3.0, 3.9)	0.81	0.4 (-4.1, 4.8)	0.85
	CaD milk	1.7 (-1.7, 5.1)	0.32	2.0 (-2.5, 6.5)	0.34

For analysis at individual level, $n = 110$ (Ca milk), 112 (CaD milk), 123 (control). For analysis at cluster level, $n = 3$/group. †Analysis at individual level with multiple regression model, adjusted for baseline value (see Subjects and Methods for details). ‡Analysis at cluster level with linear mixed model, adjusted for baseline values and clustering by school (see Subjects and Methods for details).

Bonjour *et al.*[7] and Cadogan *et al.*[8] found that calcium supplementation had a stronger impact on BMD at the radius, hip and legs, where cortical bone is predominant, than at the lumbar spine, where more trabecular bone is present, whereas two other studies[5,9] showed that the effects were more pronounced in the lumbar spine. The difference could be due to the difference in subjects' age, pubertal status and background calcium intake and type of supplementation calcium or milk products. Our results showed that in peri-pubertal Chinese girls, after accounting for change in skeletal size during growth, the effects of milk supplementation were mainly on the legs.

Among the main strengths of the study is its randomized, controlled design. The limitation of the study is that the effects of milk supplementation on regional bone mineral accretion were examined using data derived from total body bone scan. Precision of this method is not as high as those measuring a specific site (i.e. hip, spine). Another limitation is that because of practical and ethical considerations and other physical constraints, only 9 schools participated in the study, and subjects were randomly assigned according to their schools. When the analysis was adjusted for the cluster design, some of the significant effects found in the randomized group analysis, including the effects of on arms size-corrected BMD became obscured because of the reduced effective sample size and power. This limited the interpretation of such findings.

In conclusion, in Chinese peri-pubertal girls, milk supplementation showed positive effects on bone mineral accretion when accounting for the changing skeletal size during growth. The effects were mainly on the lower limbs.

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AUTHOR DISCLOSURES

Kun Zhu, Heather Greenfield, Xueqin, Qian Zhang, Guansheng Ma, Xiaoqi Hu, Chris T Cowell and David R Fraser, no conflicts of interest.

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