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Original Article

Calcium supplementation for 2 years improves bone mineral accretion and lean body mass in Chinese adolescents

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Objective: to study the effects of different doses of calcium intake on bone health and body composition in Chinese adolescents. Methods: a double-blind randomized controlled trial of calcium carbonate supplementation in 257 healthy adolescents aged 12-15 years old for 24 months. Subjects were randomly assigned to four groups receiving chewable calcium carbonate tablets providing elemental calcium at 63 mg/d, 354 mg/d, 660 mg/d, 966 mg/d, respectively. At the end of intervention, we reclassified 197 adolescents into 3 groups who had received actual doses of elemental calcium of 85 mg/d (Low dose), 230 mg/d (Medium dose) and 500 mg/d (High dose). We measured bone mineral content (BMC), bone mineral density (BMD) and body composition by dual-energy X-ray absorptiometry. Results: BMC and BMD of total body and lumbar spine were increased significantly in both males and females after intervention at all doses ($p<0.05$). In males, after supplementation, total body BMC in the medium and high dose groups (2464 g and 2437 g, respectively) was significantly higher than that in the low dose group (2321 g) after adjusting for age, pubertal development, BMI, physical activity and energy intake; in addition, lean body mass in the medium and high dose groups (49.1 kg and 48.8 kg, respectively) was significantly higher than that in the low dose group (46.7 kg) ($p<0.05$). There was no significant effect of calcium supplementation on bone mass or body composition in females. Conclusions: calcium supplementation more than 230 mg/d for two years can improve bone mineral accretion and lean body mass in Chinese male adolescents.

Key Words: adolescents, bone mineral accretion, calcium supplementation, body composition, China

INTRODUCTION

Adolescence is a period of rapid skeletal growth and nearly half of the adult skeletal mass is accrued during this period. Impaired achievement of bone mass in puberty is an important risk factor for the development of osteoporosis in later life.[1] Adolescence is therefore a window of opportunity for improving peak bone mass and reducing the risk of osteoporosis later in life.[2] Calcium intake may be an important modifiable factor influencing whether an individual attains the peak bone mass within their genetic potential.[3] Skeletal development may be at risk if calcium intake falls short of required levels.[4] Approximately 200 mg/d of calcium is accreted into the skeleton during childhood.[5]

Randomized controlled trials studied the effects of calcium or milk supplementation on bone mineral accretion and body composition in children and adolescents,[6,7] however, the results were still conflicting. It is now well accepted that bone mineral mass is largely controlled by familial and genetic factors. However, environmental factors, such as diet and lifestyle, are also important contributors to the population variance in bone mineral mass.[8] Some studies indicated that differences in calcium intakes, dietary pattern, calcium absorption and retention, physique and bone physiological structure and incidence rate of bone fracture might exist under different genetic and environmental backgrounds.[9-12] While most of these studies were carried out in western countries, it is necessary to study the effects of calcium intake in the Chinese population. There have been a few studies carried out in Chinese adolescents,[13-16] comparing either the effects of calcium intakes at two levels, or effects within short period which could not examine the long-term effect of calcium supplementation, and provided poor evidence with small sample sizes or wide age ranges. Therefore, it is important to investigate the effects of calcium supplementation on bone health and growth in Chinese adolescents, especially during growth spurt period.

The 2002 China National Nutrition and Health Survey (CNNHS) found that daily calcium intake was 376 mg for boys aged 14-17 of years, and 343 mg for their female

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counterparts,[17] which was only about 30 percent of current recommendation on calcium adequate intakes (1000 mg/d). The aim of this study was to evaluate the effects of calcium intake at different levels on bone mineral accretion and body composition in Chinese adolescents with low habitual calcium intake.

MATERIALS AND METHODS

We performed a double-blind randomized controlled trial (RCT) of 4 doses of calcium supplementation lasting for 24 months among 257 (135 boys and 122 girls) healthy adolescents aged 12-15 years. The adolescents were recruited from two junior high schools in Beijing. They were randomly assigned to one of four doses stratified by sex, and the doses of calcium carbonate per day provided elemental calcium of 63 mg/d, 354 mg/d, 660 mg/d or 966 mg/d, respectively. The calcium carbonates were given as three chewable tablets and were distributed by the teacher of each class every week in each term. To cover weekends and holidays, calcium carbonate tablets were given to participants beforehand by teachers. Trained investigators monitored compliance by calling participants and asking them to record the amounts they took in a diary. Compliance, defined as the number of tablets consumed relative to the number allocated, was monitored with the diary and by counting tablets remaining at the end of the intervention. We also asked participants to recall actual intake of tablets in an interview at the end of intervention. Actual calcium intake from supplementation was calculated based on these records. Subjects with adequate data were re-classified into three groups based on actual total calcium intake, including those from diet and calcium tablets supplied in this study, cut-off points were 500 mg/d and 800 mg/d. The average calcium intakes were 386 mg/d, 629 mg/d and 984 mg/d for Group 1, 2 and 3, respectively (Figure 1), while the average doses of calcium supplements were 85 mg/d, 230 mg/d, and 500 mg/d in the three groups, respectively.

All subjects completed questionnaires about medical and family fracture history. Subjects were excluded if they had a family history of bone diseases or arthropathy, taking medicines that could affect bone, cartilage or calcium metabolism, or if they refused to have blood taken. Data collection was conducted at baseline and after 24 months of supplementation. The study protocol and questionnaires used were approved by the Ethical Committee of the National Institute for Nutrition and Food Safety, Chinese Center for Disease Control and Prevention. A written consent form was obtained from each participant and his/her parents or guardian.

Bone mineral and body composition measurement

A Norland XR-36 (DXA) scanner (Norland, Fort Atkinson, WI) was used to measure the bone mineral content (BMC) and bone mineral density (BMD) at the total body and lumbar spine, as well as body composition of total body at baseline and 24 months. Quality control was performed every day during study period according to the manufacturer's instructions. The coefficient of variation (CV) value for repeated measurements was 0.56%-0.65%.

Assessment on physical activity level and dietary intake

Information on physical activity for the previous year was collected via validated questionnaire by trained interviewers at baseline and 24 months.[18] Energy expenditure and duration of physical activity were calculated. Dietary intake at baseline and 24 months was assessed with a consecutive 3-day food recall questionnaire (including

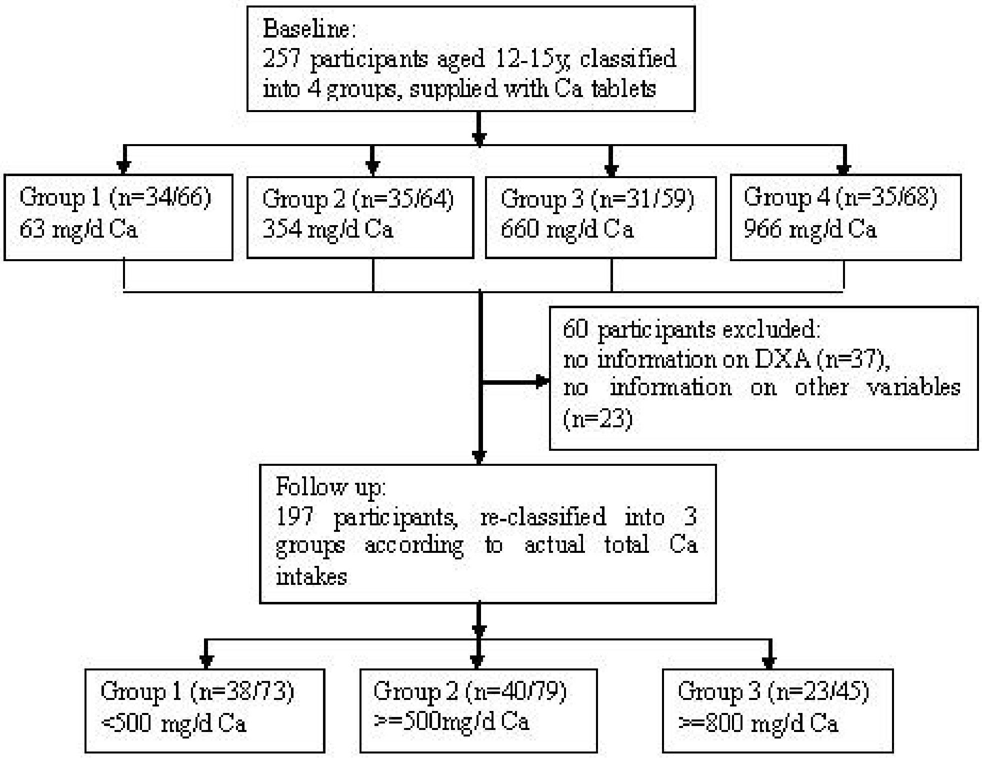

Figure 1. Flow chart of participation and classification in this study. (n=number of males / total number)

two weekdays and one weekend day). The Chinese Food Composition Tables were used to calculate nutrient intakes.[19] Calcium content in foods was determined by atomic absorption spectrophotometry in the China Food Composition Tables. Duplicate food samples were analyzed. Standard reference materials were obtained from the China National Center of Standard Material for quality control. The relative standard deviation was within 10%.

Anthropometric measurements and pubertal stage

Height and weight were measured in standing position in bare feet while wearing light clothing. Participants were weighed to the nearest 0.1 kg with an electronic digital scale (Thinner, Fairfield, WI). Height was measured by the same investigator. Subjects were measured in bare feet to the nearest 0.1 cm by stadiometer (TG-III Type, No. 6; Machinery Plant, Beijing, China). Body mass index (BMI) was calculated (kg/m^2). Female breast or male genitalia development and pubic hair development were ascertained according to Tanner's definitions of 5 stages of puberty during an interview.[20]

Plasma biochemical factors

Bone-specific alkaline phosphatase (BAP) was measured by Immunoenzymetric Assay (BIOSOURCE HOST EASIA kit, Europe BioSource). Tartrate resistant acid phosphatase (TRACP-5b) was measured by a radioimmunoassay (Bone TRAP Assay SB-TR201 kit, SBA Science, Finland).

Statistical analysis

Descriptive statistics were reported as means±SDs unless otherwise indicated. The differences between adolescents who completed this 2 years study and those lost to follow-up were analyzed with t-tests and Mann-Whitney U-tests. ANOVA, Kruskal-Wallis tests and chi-square test were used to compare differences in baseline characteristics between supplemented groups. The differences in bone mass, body composition and biochemical indicators between the 3 groups at the end of the intervention period were investigated by ANCOVA models to adjust for baseline values and for potential confounders. The factors potentially relate to bone mineral accretion of the total body were analyzed using stepwise regression. *P* values for entry and removal of terms into and out of the model were 0.10 and 0.15. All statistical analysis was done with SAS (SAS 8.2e for Windows, SAS Institute, Inc.).

RESULTS

A total of 197 subjects completed the two years of supplementation (101 boys, 96 girls), corresponding to a response rate of 77%. There were no significant differences between subjects who completed 2 years of supplementation and those were lost to follow-up except in dietary calcium intake (Table 1).

The male subjects in Group 3 had higher dietary intakes of energy (2211 kcal/d) and calcium (532 mg/d) and performed less physical activity (6.5h/w) compared with other groups (p<0.05). In females, Group 2 and Group 3 had higher dietary intakes of energy and calcium than their counterparts in Group 1. A higher proportion of females in Group 3 had a tanner stage ≥Ⅳ. There were no other significant differences between the three groups in

Table 1. Comparison on characteristics of subjects with adequate data and those without (means±SDs)

	Completed 2 years study (n=197)	Lost to follow-up (n=60)	*p*-value
Male (%)†	51.3	56.9	0.409
Age (y)	13.4±0.5	13.3±0.6	0.306
Height (cm)	157.1±8.1	158.6±7.7	0.154
Weight (kg)	48.3±11.6	49.1±10.4	0.608
Energy intake (kcal/d)	1804±565	1862±461	0.458
Dietary calcium intakes (mg/d)	380±163	297±124	<0.001
Time of physical activity (h/w) ‡	9.6 (13.1)	11.5 (18.6)	0.161
Energy expenditure of physical activity (kcal/w) ‡	2329 (3640)	2824 (5090)	0.222
Tanner stage of female breast/male genitailia ≥4 (%)†	39.0	47.2	0.224
Tanner stage of pubic hair ≥4 (%)†	12.8	22.2	0.059
First spermorrhea/menarche (%)†	56.6	51.4	0.444
Total body bone mineral content (g)	1870.8±350.2	1906.0±396.1	0.482
Total body bone density (g/cm^2)	0.769±0.089	0.779±0.074	0.418
Lumbar spine bone mineral content (g)	31.0±7.7	32.4±8.8	0.221
Lumbar spine bone density (g/cm^2)	0.782±0.139	0.789±0.132	0.699
Lean body mass (kg)	35.8±7.5	36.1±8.9	0.820
Percentage body fat (%)	24.5±8.4	24.0±10.0	0.719

Student t-test. † percentage, ‡Med (Q3-Q1), Mann-Whitney U-test.

Table 2. Comparison on characteristics among three groups (means±SDs)

	Male			*p*-value	Female			*p*-value
	Group 1	Group 2	Group 3		Group 1	Group 2	Group 3	
n	38	40	23		35	39	22	
Age (y)	13.5±0.5	13.6±0.5	13.5±0.5	0.801	13.4±0.6	13.2±0.5	13.3±0.5	0.525
Height (cm)	156.9±9.3	158.0±8.1	161.6±9.8	0.133	155.5±8.7	155.7±6.2	156.7±5.0	0.800
Weight (kg)	48.5±14.9	48.7±11.1	48.2±10.7	0.988	46.8±9.9	48.5±10.7	49.9±12.3	0.565
Energy intake (kcal/d)	1790±370	1818±428	2212±640	0.002*	1369±292	1541±350	1639 ±354	0.009*
Calcium intakes from food (mg/d)	311±67	397±127	532±250	<0.001*	290±84	401 ±131	430±159	<0.001*
Calcium intakes from tablets (mg/d)	76±60	232±130	496±298	<0.001*	95±74	229±119	508±231	<0.001*
Total Calcium intakes (mg/d)	387±17	629±16	1028±22	<0.001*	385±18	630±17	938±22	<0.001*
Time of physical activity (h/w)†	10.8(13.3)	12.4(11.5)	6.7(5.9)	0.008*	9.5(9.2)	10.2(7.2)	9.8(9.7)	0.935
Energy expenditure of physical activity (kcal/w)†	3557(5573)	3684(4924)	2409(2670)	0.081	2570(2930)	2358(2724)	2783(2201)	0.649
Tanner stage≥Ⅳ (%)‡	50	63	61	0.499	49	51	82	0.029*

†Med (Q3-Q1), Kruskal-Wallis Test. ‡Chi-square test

Table 3. Effect of calcium intervention on bone measures at lumbar spine and of total body (means±SDs)

		Male			Female		
		Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Total body BMC (g)	Baseline	1821±436	1872±357	1893±374	1864±348	1902±292	1885±265
	Change	500±135	592±165 †‡§	544±132 †‡§	308±133	314±129	323±136
Total body BMD (g/cm²)	Baseline	0.75±0.10	0.76 ±0.09	0.75±0.09	0.78±0.09	0.80±0.08	0.79±0.08
	Change	0.12±0.04	0.14±0.06	0.13±0.04	0.07±0.03	0.08±0.03	0.08±0.03
Lumbar spine BMC (g)	Baseline	28.97±8.74	29.43±6.74	30.79±7.81	32.57±8.93	32.79±6.57	32.07±6.38
	Change	12.00±3.85	12.93±4.03	12.24±3.92	6.56±3.80	6.87±3.37	5.81±3.22
Lumbar spine BMD (g/cm²)	Baseline	0.73±0.13	0.74±0.12	0.718±0.11	0.84±0.16	0.84±0.13	0.81±0.13
	Change	0.20±0.07	0.20±0.06	0.19±0.05	0.13±0.07	0.14±0.06	0.12±0.07

Comparison at baseline $p > 0.05$.
Comparison at 2 years later:
†adjusted for the baseline value, $p<0.05$
‡additional adjusted for age, pubertal development and BMI, $p < 0.05$
§additional adjusted for physical activity and energy intake, $p < 0.05$

both genders (Table 2).

BMC and BMD of total body and lumbar spine increased significantly in both males and females after intervention in each dose group ($p<0.05$). At baseline, no significant differences in BMC and BMD of total body and lumbar spine were found among the three groups. After two years of calcium supplements, total body BMC of males in Group 2 (2464 g) and Group 3 (2437 g) were significantly higher than that of their counterparts in Group 1 (2321 g), with or without adjustment for baseline value ($p<0.05$) (Table 3). The significant difference remained after additionally adjusting for age, pubertal development, BMI, physical activity and energy intake. The increments in total body BMC of male subjects after 2 years in Group 2 and Group 3 were higher than that of their counterparts in Group 1 (Figure 2) ($p<0.05$). No statistically significant differences in BMD at total body or lumbar spine were found between the groups after 2 years. There were no significant differences among groups in females.

Lean body mass and body weight in males and females increased significantly after supplementation in each dose group ($p<0.05$). At baseline, total body composition was not different in the 3 groups. After two years, lean body mass and body weight of male subjects in Group 2 (49.1 kg and 62.3 kg, respectively) and Group 3 (48.8 kg and 61.7 kg) were significantly higher than that of their counterparts in Group 1 (46.7 kg and 58.9 kg), adjusting for baseline value ($p<0.05$) (Table 4). This difference remained after additional adjustment for age, pubertal development, BMI, physical activity, and energy intake ($p<0.05$). Changes in lean body mass but not body fat and body fat percentage in Group 2 and Group 3 were significantly higher than that in Group 1 ($p<0.05$) (Figure 3). In females, there were no significant differences in changes of body composition among the groups over 2 years.

Significant increases were found with regard to body weight in both males and females after supplementation ($p<0.05$). At baseline, body weight of male subjects were 48.5 kg, 48.7 kg and 48.2 kg for Group 1, 2 and 3, respectively ($p>0.05$). After supplementation, body weight of male subjects in Group 2 (62.3kg) and Group 3 (61.7kg) were significantly higher than that of their counterparts in Group 1 (58.9kg) after adjustment ($p<0.05$). There were no significant differences among groups for height or biochemical indicators before and after intervention.

The factors which might be related to bone mineral accretion of the total body were analyzed using multivariable stepwise regression. We found that sex, pubertal stage, physical activity and calcium intakes were main factors associated with changes in total body BMC, with adjusted coefficient of determination $R_c^2=0.429$ (Table 5). Sex explained about 39% of variance in bone mineral accretion such that males having greater increases than females. Pubertal stage was negatively associated, and physical activity and calcium intakes were positively associated with total body BMC change.

DISCUSSION

This is the first report of a calcium supplementation study using more than 2 dose levels and lasting for 2 years in Chinese adolescents. This randomized, double-blind, controlled study demonstrated that calcium carbonate supplementation at doses more than 230 mg/d for 2 years enhances bone mineral accretion, lean body mass and weight of Chinese male adolescents with low habitual

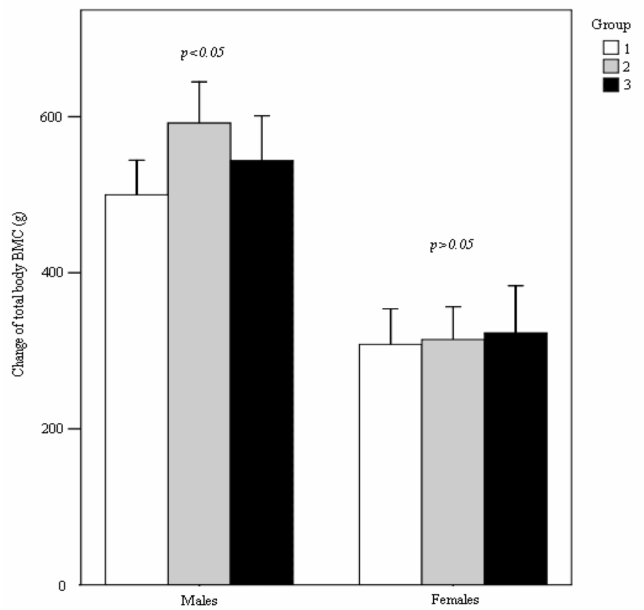

Figure 2. Relationship between calcium intakes and change in total body BMC in adolescents. Error bars: 95% CI. Adjusted for the baseline value, BMI, pubertal development, time of physical activity and energy intake.

Table 4. Effect of calcium intervention on body composition (means±SDs)

		Male			Female		
		Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Lean body mass (kg)	Baseline	38.3±10.1	38.5±7	38.7±8.1	32.9±5.7	32.8±4.8	33.8±5.3
	Change	8.4±4.8	10.5±4.1 †‡§	10.1±4.8 †‡§	2.2±2.7	2.3±3.5	2.2±2.7
Body fat (kg)	Baseline	9.9±6.3	9.9±5.8	9.4±4.5	13.6±4.9	15.3±6.8	15.8±7.5
	Change	1.2±2.5	1.7±2.6	2.3±3.5	4.3±3.3	3.4±2.8	4.2±3.0
Percentage of body fat (%)	Baseline	19.7±7.1	19.5±7.3	19.1±6.7	28.6±5	30.7±7.1	30.6±6.6
	Change	-1.4±4.4	-1.2±4.2	-1.1±5.1	4.5±4.1	3.5±4.8	4.5±4.2

Comparison at baseline P>0.05.
Comparison at 2 years later:
†adjusted for the baseline value, $p<0.05$
‡additional adjusted for age, pubertal development and BMI, $p<0.05$
§additional adjusted for physical activity and energy intake, $p<0.05$

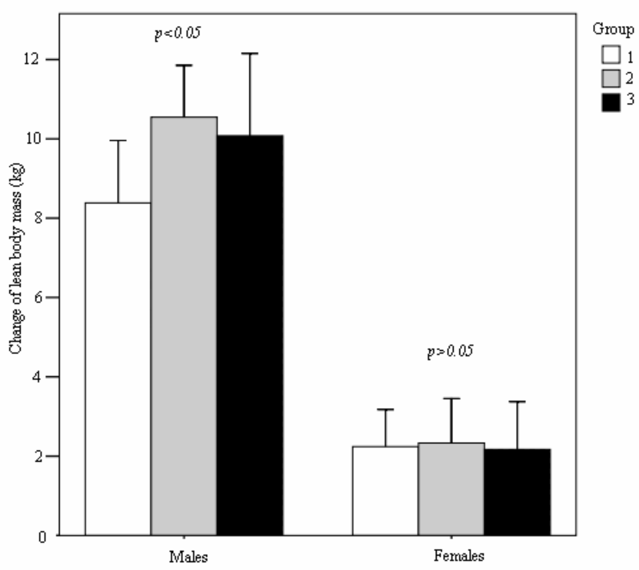

Figure 3. Relationship between calcium intakes and change of lean body mass in adolescents. Error bars: 95% CI. Adjusted for baseline value, pubertal development, time of physical activity and energy intake.

Table 5. Stepwise regression on influence factors for bone mineral accretion of the total body with

Variables	*β*	*p*	R^2	R_c^2
Gender (Females>males)	-0.607	<0.001	0.389	0.429
Tanner stage (Ⅳ + Ⅴ)>(Ⅰ −Ⅲ)	-0.181	0.001	0.026	
Time of physical activity	0.136	0.013	0.013	
Groups of calcium supplementation	0.115	0.036	0.012	

Independent variables were sex, groups of calcium supplementation, energy intakes, time of physical activity, dependent variable was change of total body BMC.
p-values for entry and removal of terms into and out of the model were 0.10 and 0.15

calcium intakes. Furthermore, sex, tanner stage and physical activity were potential factors for bone mineral accretion. This finding provides evidence for the development of calcium DRIs and dietary guideline for the Chinese adolescents.

A recent meta-analysis of randomized controlled trials of calcium supplementation showed that there was a slight effect of calcium supplementation on the upper limbs,[7] but raised doubts as to whether calcium supplementation in children benefits bone mineral density. However, in our study, dietary calcium intake of subjects was low 382 mg/day at baseline, and the total body BMC of male adolescents with low habitual calcium intakes benefited from calcium supplementation for 2 years of 230 mg/d and 500 mg/d. Our study may have demonstrated an effect because male subjects in this study were at the peak stage of bone mineral accretion since BMC velocity peak being at 13.3 years for boys.[21] Our results also show that tanner stage was one of the most important potential factors affecting bone mass gain. During puberty, bone turnover is increased and sex steroids, the GH-IGF-I axis and 1,25$(OH)_2$D played major roles in bone mass change.[22] Calcium intake in mid-puberty was strongly associated with young adult bone mass.[23] Similar results were found in white adolescents at 16-18 years old who were supplemented with calcium carbonate 600 mg per day for 13 months, and it has also been demonstrated that calcium supplementation increased skeletal growth.[24]

Gender was another important determinant of bone mineral accretion, yet no significant effects of supplementation were found in females in this study. The BMC velocity peak in girls is at 11.4 years, earlier than that in boys. Within three years on either side of peak BMC velocity, boys had consistently higher BMC and the BMC velocity compared with girls, and the discrepancy increased steadily through puberty.[21] Subjects in this study were 13-15 years old during the supplementation of two years, which might contribute to the result that males benefited from calcium supplementation and females did not.

Dietary calcium intake is a potential factor influencing weight gain and may reduce body weight,[25] however, the evidence in children is conflicting. A recent meta-analysis performed in children showed that no statistically significant effects of calcium supplementation were found on weight or body fat.[6] However, most studies on the effects of calcium or milk supplementation on body composition have been conducted in girls.[26-28] Our finding that calcium supplementation had no effect on body composition in females is consistent with this, but our data also suggest that male adolescents may benefit in terms of lean body mass and body weight from calcium supplementation. Similar significant results on lean body mass were found in white adolescents at 16-18 years of age.[24] Other studies found that peak bone mass could be influenced by many factors, such as heritability, nutrition, exercise, adequate solar exposure, etc.[29] Physical activity was another important determinant of BMC gain in adolescents in this study. This is consistent with studies showing that increases in weight-bearing physical activity or calcium intakes have positive effects on bone mass gains in children and adolescents; and that calcium supplementation increases the effect of physical activity on bone mineral acquisition.[30-32] In order to clarify the relationship between calcium intake and bone mass, we adjusted for physical activity in multivariate regression and we found that physical activity could explain 1.3% variance of bone mineral accretion.

Our study has several limitations. Firstly, we intended to include all 4 groups into the analysis, but loss of follow-up and low compliance to calcium carbonate tablets resulted in a small sample size in the highest 2 groups of calcium supplementation that the original design. In order to assess the effects of calcium supplements at multi-levels within small sample size, we have to perform a compliance-based analysis with participants being reclassified into 3 groups according to their actual calcium intake and thus failed to compare effects of calcium supplementation of 900 mg per day. Secondly, calcium intake from dietary of male subject in Group 3 was significantly higher than other groups after reclassification. So we also adjusted for energy intake in our analysis in order to control for potential confounders related to higher BMC and body composition. Thirdly, we used multiple days of 24 hour food recall to estimate within-person variability at baseline, 12 months and 24 months from baseline, the consecutive days for food recall investigation might not be independent of one and another. But a consecutive 3-day food recall method has been proved to provide valid estimation of nutrient intake, and was used in national nutrition survey in China in 1992 and 2002.[17,33]

In conclusion, calcium supplementation of more than 230 mg/d for two years can enhance total body bone mineral accretion and increase lean body mass in Chinese male adolescents with low habitual calcium intake but not in females. Sex, tanner stage, physical activity and calcium intakes are important determinants of bone mineral accretion in Chinese adolescents.

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AUTHOR DISCLOSURES

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Original Article

Calcium supplementation for 2 years improves bone mineral accretion and lean body mass in Chinese adolescents

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钙剂补充兩年改善中国青少年骨量沉积和瘦体重

目的：观察不同剂量钙剂补充对中国青少年骨量和体成分的影响。方法：采用双盲随机对照研究，对 257 名 12-15 岁健康青少年补充 24 个月的碳酸钙咀嚼片。研究对象随机分为 4 个组，分别补充元素钙每日 63 mg、354 mg、660 mg、及 966 mg。干预結束后，按照 197 名完成干预研究者的实际钙补充量分为 3 组，各组钙补充量为每日 85 mg (低钙组)、230 mg (中钙组) 和 500 mg (高钙组)。采用双能 X 线吸收仪测定骨矿物含量(BMC)、骨矿物密度(BMD)和体成分。结果：干预期间各组男女生全身和腰椎 BMC 和 BMD 显著增加 ($p<0.05$)。干预后，在调整年龄、青春发育、BMI、身体活动和能量摄入后，中钙组和高钙组男生的全身骨矿物含量(2464 g 和 2437 g)显著高于低钙组(2321 g)，而且这两组瘦体重(49.1 kg 和 48.8 kg)也显著高于低钙组(46.7 kg) ($p<0.05$)。钙补充對女生骨量和体成分没有显著影响。结论：为期两年，每日高于 230 mg 钙补充能改善中国男性青少年的骨量沉积和瘦体重增长。

關鍵字：青少年、骨量沉积、钙剂补充、体成分、中国