Long-term calcium supplementation may have adverse effects on serum cholesterol and carotid intima-media thickness in postmenopausal women: a double-blind, randomized, placebo-controlled trial"¹⁻³

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ABSTRACT

Background: Several studies have focused on the effects of calcium intake on serum lipid concentrations in postmenopausal women. However, many premenopausal women are taking calcium supplements in China. To our knowledge, no studies have assessed whether the effects of calcium supplementation on blood lipids are similar between premenopausal and postmenopausal women.

Objective: We assessed the effects of calcium supplementation on blood lipid concentrations in premenopausal and postmenopausal women with dyslipidemia.

Design: A total of 190 premenopausal women (30–40 y old) and 182 postmenopausal women (50–60 y old) with dyslipidemia were given 800 mg Ca/d or a placebo for 2 y in a double-blind, randomized, placebo-controlled trial. Blood pressure, fasting glucose and serum lipid concentrations, carotid intima-media thickness (CIMT), dietary nutrient intakes, and physical activity levels were determined at baseline and after 2 y.

Results: There was a significant interaction between calcium supplementation and menopausal status on serum cholesterol concentrations ($P < 0.001$) and CIMT ($P = 0.017$). Serum cholesterol concentrations and CIMT were significantly increased in postmenopausal women ($P < 0.01$) after 2 y. Serum triglyceride, low-density lipoprotein-cholesterol, and high-density lipoprotein-cholesterol concentrations were not affected after 2 y.

Conclusions: Calcium supplementation in postmenopausal women with dyslipidemia increases serum total cholesterol concentrations and CIMT. In postmenopausal women with dyslipidemia, calcium supplements should be prescribed with caution. This trial was registered at http://www.chictr.org.cn/ as ChiCTR-TRC-12002806. Am J Clin Nutr doi: 10.3945/ajcn.113.062844.

INTRODUCTION

Adequate calcium intake is critical for bone health. Calcium supplementation is recommended for postmenopausal women for the prevention and treatment of osteoporosis (1, 2); calcium supplements are commonly used by individuals >50 y of age. In addition to its pivotal role on bone health, the effects of calcium supplementation on nonskeletal health outcomes have received a lot of attention (3–5); of the effects, the impact of calcium intake on serum lipid concentrations is a subject of great interest.

Most of the studies focused on the effect of calcium intake on serum lipid concentrations have been conducted in postmenopausal women. However, many premenopausal women in China take calcium supplements. An inadequate calcium intake is a common health problem in China; the average daily calcium intake in China is ~ 391 mg, which represents only 41% of the recommended intake according to the Chinese Nutrition Society (6). Therefore, we compared the response to calcium supplementation between premenopausal and postmenopausal women. There are differences in hormonal concentrations, especially estrogen, between postmenopausal and premenopausal women (7, 8). Estrogen is involved in the regulation of blood lipid homeostasis (9, 10); an estrogen deficit is mainly responsible for increased risk of cardiovascular disease (CVD), metabolic syndrome, increased blood lipid concentrations, and increased carotid intima-media thickness (CIMT) in postmenopausal women (7). It is possible that estrogen interacts with calcium and changes its effects on blood lipid concentrations or regulation.

Studies that have focused on the effect of calcium intake on serum lipid concentrations have provided inconsistent results. Randomized, placebo-controlled, clinical trials have reported that supplementation with 1–2 g elemental Ca lowers total and LDL-cholesterol concentrations and increases HDL-cholesterol concentrations in postmenopausal women (11–15). In contrast, other studies have reported no effects (16–19). Note that these studies were mostly secondary analyses of studies that assessed the effect of calcium supplementation on osteoporosis or CVD. Some of these studies had small sample sizes (<20 subjects), consisted of short-term observations (<1 y), or failed to take into account sex and age effects. To assess the effect of calcium supplementation on blood lipid concentrations in women, long-term studies with adequate sample sizes should be performed.

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In this study, we carried out a randomized, placebo-controlled trial that included premenopausal and postmenopausal women with dyslipidemia over a period of 2 y. Subjects were randomly assigned to receive a placebo or calcium (800 mg/d). The primary outcomes were serum triglyceride, total cholesterol, HDL cholesterol, LDL cholesterol, and CIMT. Secondary outcomes were body weight, fasting blood glucose concentrations, blood pressure, and serum calcium concentrations.

**SUBJECTS AND METHODS**

**Recruitment**

We recruited subjects through local community health settings in Harbin, China. Women with dyslipidemia, premenopausal women between 25 and 35 y of age, and postmenopausal women between 50 and 60 y of age were recruited. Subjects were considered to have dyslipidemia if they met 2 of the following criteria: a fasting triglyceride concentration >1.7 mmol/L, fasting total cholesterol concentration >5.18 mmol/L, fasting LDL cholesterol >3.37 mmol/L, or fasting HDL cholesterol concentration <1.04 mmol/L. Exclusion criteria were as follows: the presence of any major diseases (eg, diabetes, hypertension, coronary artery disease, stroke, hypothyroidism or hyperthyroidism, renal or hepatic disease, or malignant or metabolic bone disease), regular medication use, which affected calcium or lipid metabolism, a serum 25-hydroxyvitamin D$_3$ concentration <20 nmol/L, the use of bisphosphonates or hormone therapy in the previous year, calcium or vitamin D$_3$ supplementation in the previous year, pregnant women, breastfeeding women, hysterectomized women, or perimenopausal period. Women were considered to be postmenopausal if ≥12 mo had passed since the last natural menstruation or if they had been previously subjected to a bilateral oophorectomy.

Subjects were screened over the telephone by trained research assistants and subsequently by medical officers during a clinical assessment that included a blood test for fasting glucose, blood lipid, and serum 25-hydroxyvitamin D$_3$ concentrations; a physical examination that included the measurement of blood pressure and BMI; and a questionnaire about lifestyle and disease history. A total of 200 premenopausal women and 200 postmenopausal women were recruited. The study was approved by the ethics committee of Harbin Medical University and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from subjects.

**Random assignment**

Eligible subjects entered a 1-mo run-in phase during which they took a placebo. Subjects who achieved ≥95% compliance (190 premenopausal women and 182 postmenopausal women) proceeded to the randomization step. Two research statisticians performed the random assignment. Subjects were randomly assigned to a calcium supplementation or placebo group by using software-generated random numbers within blocks of 2. Subjects and study staff were blinded to treatment allocation throughout the study.

**Intervention**

This study had a double-blind, randomized, placebo-controlled design. The study was performed at the Harbin Medical University, Harbin, China, between July 2008 and December 2010. The intervention lasted 2 y from January 2009 to December 2010. Subjects were given either 800 mg elemental Ca (1 tablet of calcium carbonate) or a placebo (1 tablet of maize starch) per day with supper. Subjects were asked to maintain their habitual diets and lifestyles during the intervention. Compliance was assessed by tablet count. Subjects were interviewed and supplied with tablets every 2 mo. The occurrence of any diseases during the intervention was recorded at each visit. Premenopausal women who experienced perimenopause or entered the postmenopausal period during the intervention were excluded from the study. Requirements for participants during the intervention were reinforced at each visit.

**Measurements and analyses**

Questionnaires and fasting blood collection were originally planned to be performed at baseline and 2 and 4 y. However, the trial was stopped after 2 y because we showed an increase in serum cholesterol concentrations in women supplemented with calcium. Therefore, we only obtained one measurement at the study endpoint, which prevented us from using intention-to-treat analyses. Instead, we used per-protocol analyses. Dietary intake (food and alcoholic beverages) was assessed by using a food-frequency questionnaire and estimated by using the Food Nutrition Calculator (V1.60; Chinese CDC). Physical activity level was assessed by using a physical activity questionnaire and calculated by using a formula from the American Institute of Medicine (20).

Total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, glucose, and calcium concentrations were measured by using a ROCHE Modular P800 Automatic Biochemical Analyzer (Roche Diagnostics). Blood pressure was measured with a mercurial sphygmomanometer stethoscope. Serum estradiol concentrations were measured by using an Electrochemiluminescence Immune Analyzer (Roche Diagnostics) at the second affiliated hospital of Harbin Medical University. Estradiol concentrations were measured on the third day of the menstrual period for premenopausal women.

CIMT was assessed at baseline and at the end of the 2-y intervention period by using a B-mode ultrasound at the second affiliated hospital of Harbin Medical University. Briefly, the right and left common carotid arteries in close proximity to the bulb were imaged in multiple longitudinal planes to obtain the best resolution of the intima-media thickness. The mean intima-media thickness was obtained by manually tracing the intima-media in the far wall of the artery for a 10-mm distance. All scans were performed by 2 trained ultrasound technicians. Technicians were blinded to the treatment allocation. Measurements were performed on 3 end-diastolic images and averaged. Before the trial began, a pilot study was performed to test the validity and reliability of the CIMT measurements. Forty subjects were randomly enrolled and examined twice for CIMT by a different technician at the same visit or by the same technician at a different visit. Interobserver and intraobserver variability were estimated by using the absolute difference between paired measurements and the CV. The CV for each pair of CIMT measurements was calculated according to the following formula:

\[
(\text{SD} \div \text{mean}) \times 100
\]

The average CV was the mean of 80 CVs. The interobserver difference was 0.005 ± 0.004 mm, the average CV was 3.20%, the intraobserver difference was −0.003 ± 0.004 mm, and the...
average CV was 2.99% (see Supplemental Table 1 under “Supplemental data” in the online issue).

Statistical analyses

Statistical analyses were performed with SPSS (version 13.01S; Beijing Stats Data Mining Co. Ltd). Data were expressed as means ± SDs. All P values were 2 tailed. Statistical significant was set at \( P \leq 0.05 \). Differences in categorical variables between the calcium and placebo groups were analyzed by using the chi-square test. Mean concentrations of continuous variables at baseline between the 2 groups were compared by using an independent-sample t test. Mean concentrations of continuous variables between the 2 groups after 2 y were tested by using an ANCOVA adjusted for their baseline values. An ANOVA was used to analyze the association of calcium supplementation, menopausal status, and their interactions with measured variables.

RESULTS

Subject characteristics and compliance

A total of 359 subjects including 185 premenopausal women (\( n = 92 \) in the placebo group; \( n = 93 \) in the calcium group) and 174 postmenopausal women (\( n = 88 \) in the placebo group; \( n = 86 \) in the calcium group) completed the study (Figure 1). A total of 13 subjects did not complete the study for the following reasons: 6 subjects could no longer be contacted, and 7 subjects withdrew from the study for personal reasons. Compliance was good; the rate of capsule intake was >95% in the groups.

Descriptive and biochemical characteristics and dietary intakes

No differences were observed in anthropometric measures, physical activity levels, fasting blood glucose, serum calcium, or other nutrient intakes between the 2 groups either at baseline or after 2 y (Table 2).

Blood lipid concentrations and CIMT

Baseline blood lipid concentrations and CIMT were not significantly different between placebo and calcium groups (Table 3). After adjustment for baseline serum total cholesterol, the serum total cholesterol concentration after 2 y increased significantly in the calcium group compared with that in the placebo group (\( P < 0.001 \)). Serum triglyceride, LDL cholesterol, and HDL cholesterol concentrations and CIMT were not significantly affected after 2 y. There was a significant interaction between calcium supplementation and menopausal status on serum cholesterol concentrations (\( P < 0.001 \)) and CIMT (\( P = 0.017 \)). The change of total cholesterol in premenopausal women and postmenopausal women after calcium supplementation was 0.04 ± 0.07 compared with 0.61 ± 0.21 mmol/L (\( P < 0.001 \)). The change of CIMT in premenopausal women and postmenopausal women after calcium supplementation was 0.0238 ± 0.0348 compared with 0.0615 ± 0.134 mm (\( P = 0.003 \)), respectively. Serum cholesterol concentrations and CIMT of postmenopausal women supplemented with calcium increased remarkably, whereas these indexes did not change significantly in premenopausal women supplemented with calcium (Figure 2).

DISCUSSION

To the best of our knowledge, this was the first double-blind, randomized, placebo-controlled study that assessed the effect of calcium supplementation on blood lipid concentrations and CIMT in premenopausal and postmenopausal women with hyperlipidemia over a 2-y period. Results revealed a significant interaction between calcium supplementation and menopausal status on serum cholesterol concentrations and CIMT. Serum cholesterol concentrations and CIMT were significantly increased in postmenopausal women supplemented with calcium but not in premenopausal individuals supplemented with calcium.

Several studies have focused on the effects of calcium supplementation on blood lipid concentrations in women (11–19). However, to our knowledge, no study has reported whether the effects of calcium supplementation on blood lipid concentrations are similar between premenopausal and postmenopausal women. The largest and longest study that assessed this association was the Women’s Health Initiative study, which involved 1191 women. Results of this study, which was designed to assess the major causes of morbidity and mortality in postmenopausal women, revealed that the daily supplementation of 1 g Ca plus 400 IU vitamin D3 for 5 y was not associated with changes in blood lipid concentrations in women (16). Another associated large trial was conducted with 223 healthy postmenopausal women; this study was primarily designed to assess the effect of calcium supplementation on bone fractures. Results revealed that women who received 1 g elemental Ca/d for 1 y had a significant decrease in LDL concentrations and an increase in HDL concentrations (11). Both studies were secondary analyses, and study
participants were not all dyslipidemic. In our study, 372 women with dyslipidemia were randomly assigned to 800 mg elemental Ca/d for 2 y to assess the effect of calcium supplementation on blood lipid concentrations. We showed that there was an interaction between calcium supplementation and menopausal status on serum cholesterol concentrations. Total cholesterol concentrations were significantly increased in postmenopausal women, but not in premenopausal women, supplemented with calcium. This result indicated that calcium supplementation may lead to higher cholesterol concentration in postmenopausal women than premenopausal women. To our knowledge, only 2 intervention trials should be conducted to assess any interactions between intracellular Ca\(^{2+}\), which is a secondary messenger, and calcium, which regulate cholesterol metabolism. In postmenopausal women, a decline in estradiol leads to a slight increase in serum cholesterol concentrations (8). Calcium supplementation contributes to an additional increase in total cholesterol concentrations. To the best of our knowledge, estrogens, especially estradiol, can regulate intracellular Ca\(^{2+}\) homeostasis in excitatory cells (29); however, little is known on the regulations of intracellular Ca\(^{2+}\) homeostasis by estradiol in nonexcitatory cells, such as hepatocytes, where cholesterol metabolism is active. Additional studies should be conducted to assess any interactions between intracellular Ca\(^{2+}\) homeostasis and estradiol concentrations in the regulation of cholesterol metabolism and the mechanism of action.

Circulating lipids represent an established independent risk factor for CVD (30, 31). We did not directly evaluate whether an increase in serum total cholesterol concentrations would increase risk of CVD, metabolic syndrome, and increased blood lipid concentrations in postmenopausal women (7). However, intracellular Ca\(^{2+}\), which is a secondary messenger, is involved in several regulation processes including lipid metabolism (26–28), which may be affected by dietary calcium intake. Because estradiol and calcium are involved in the regulation of lipid metabolism, there might be certain interactions between estradiol and calcium, which regulate cholesterol metabolism. In postmenopausal women, a decline in estradiol leads to a slight increase in serum cholesterol concentrations (8). Calcium supplementation contributes to an additional increase in total cholesterol concentrations. To the best of our knowledge, estrogens, especially estradiol, can regulate intracellular Ca\(^{2+}\) homeostasis in excitatory cells (29); however, little is known on the regulations of intracellular Ca\(^{2+}\) homeostasis by estradiol in nonexcitatory cells, such as hepatocytes, where cholesterol metabolism is active. Additional studies should be conducted to assess any interactions between intracellular Ca\(^{2+}\) homeostasis and estradiol concentrations in the regulation of cholesterol metabolism and the mechanism of action.

TABLE 2

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Placebo (n = 180)</th>
<th>Calcium supplementation (n = 179)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake (kcal/d)</td>
<td>2338.92 ± 259.45</td>
<td>2339.62 ± 234.61</td>
<td>0.979</td>
</tr>
<tr>
<td>Fat intake (g/d)</td>
<td>82.35 ± 21.12</td>
<td>83.19 ± 21.86</td>
<td>0.712</td>
</tr>
<tr>
<td>Carbohydrate intake (g/d)</td>
<td>316.98 ± 47.78</td>
<td>314.38 ± 45.62</td>
<td>0.590</td>
</tr>
<tr>
<td>Protein intake (g/d)</td>
<td>71.72 ± 16.96</td>
<td>76.12 ± 17.71</td>
<td>0.443</td>
</tr>
<tr>
<td>Cholesterol intake (mg/d)</td>
<td>335.59 ± 33.09</td>
<td>330.10 ± 30.81</td>
<td>0.299</td>
</tr>
<tr>
<td>Dietary calcium intake (mg/d)</td>
<td>495.65 ± 180.20</td>
<td>497.23 ± 182.09</td>
<td>0.934</td>
</tr>
<tr>
<td>Fiber intake (g/d)</td>
<td>23.67 ± 5.59</td>
<td>23.88 ± 4.81</td>
<td>0.708</td>
</tr>
</tbody>
</table>

\(^1\) All values are means ± SDs. Differences in daily intakes of nutrients at baseline between the 2 groups were analyzed by using the independent-samples t test. Differences in daily intakes of nutrients at 2 y between the 2 groups were analyzed by using ANCOVA adjusted for their baseline values, respectively.
risk of CVD in postmenopausal women supplemented with calcium. However, studies have reported that calcium supplementation increases risk of CVD, especially myocardial infarction, in postmenopausal women (32–36). This finding suggests that increased serum cholesterol concentrations contribute to an increase in risk of CVD during calcium supplementation. Therefore, CIMT was measured in our study. Several studies have shown that increased CIMT increases risk of future coronary artery disease and stroke (37–39). CIMT can be affected by serum cholesterol concentrations (40); CIMT has been reported to increase in patients with hypercholesterolemia (41, 42). Our results were in accordance with previous studies that CIMT was significantly increased in postmenopausal women with high serum cholesterol concentrations. This result suggested that an increase in total serum cholesterol concentrations may increase risk of CVD by increasing CIMT in postmenopausal women supplemented with calcium.

We could not exclude other potential factors that may affect CIMT in postmenopausal women supplemented with calcium. Serum calcium concentrations (2.25–2.75 mmol/L) are tightly regulated by vitamin D, calcitonin, and parathyroid hormone. Kärkkäinen et al. (43) reported that calcium supplementation resulted in a slight increase in postsupplemental blood calcium concentrations. This result was probably because supplemental calcium raises serum calcium in a more pronounced way than does dietary calcium, which may be absorbed more slowly. Serum calcium concentrations are positively correlated with serum cholesterol concentrations (44). Therefore, a perturbation in the postsupplemental serum calcium concentrations may disrupt the homeostasis of cholesterol and stimulate blood vessels. Whether this perturbation may result in blood vessel damage and increase CIMT should be further studied.

The strengths of our study included a primary design for determining blood lipid concentrations, a long follow-up period, and the assessment of interactions between calcium supplementation and menopausal status. However, there were certain limitations in our study. The follow-up period was only 2 y as a result of the negative calcium effects. Therefore, there was only one post-randomization measurement, which prevented the use of intention-to-treat analyses. Instead, per-protocol analyses were used. Per-protocol analyses may overestimate effects compared with intention-to-treat analyses. Therefore, we could not directly evaluate whether an increase in serum cholesterol concentrations would increase risk of CVD because of the relatively short intervention period. In addition, as reported in certain trials and animal studies, calcium supplementation has beneficial effects on circulating lipids; it is possible that calcium binds to fatty acids and bile acids in the intestinal lumen and leads to fat malabsorption (45, 46). Postprandial circulating calcium concentrations and urinary and fecal calcium and fecal lipid concentrations should be determined in future studies to assess beneficial and adverse effects of calcium supplementation.

In conclusion, results of our study revealed that there is a significant interaction between calcium supplementation and menopausal status on serum cholesterol concentrations and CIMT. Calcium supplementation in postmenopausal women with dyslipidemia increases serum total cholesterol concentrations and CIMT. Therefore, postmenopausal women with dyslipidemia should not consume calcium supplements. Premenopausal women should be cautious when prescribed calcium supplements.

### Table 3

Values of measured variables in dyslipidemic subjects at baseline and after 2 y

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Placebo (n = 180)</th>
<th>Calcium supplementation (n = 179)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.54 ± 0.69</td>
<td>5.56 ± 0.65</td>
<td>0.807</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.70 ± 0.67</td>
<td>1.69 ± 0.63</td>
<td>0.904</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.22 ± 0.16</td>
<td>1.23 ± 0.14</td>
<td>0.483</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.52 ± 0.63</td>
<td>3.57 ± 0.68</td>
<td>0.466</td>
</tr>
<tr>
<td>CIMT (^2) (mm)</td>
<td>0.738 ± 0.10</td>
<td>0.7354 ± 0.10</td>
<td>0.787</td>
</tr>
</tbody>
</table>

\(^1\) All values are means ± SDs. Differences in measured variables at baseline between the 2 groups were analyzed by using the independent-samples t test. Differences in measured variables at 2 y between the 2 groups were analyzed by using ANCOVA adjusted for their baseline values, respectively.

\(^2\) CIMT, carotid intima-media thickness.

**FIGURE 2.** Interaction between calcium supplementation and menopausal status in premenopausal women (n = 92 in the placebo group; n = 93 in the calcium-supplementation group) and postmenopausal women (n = 88 in the placebo group; n = 86 in the calcium-supplementation group). The change of total cholesterol in premenopausal women and postmenopausal women after calcium supplementation was 0.04 ± 0.07 compared with 0.61 ± 0.21 mmol/L, respectively. The change of CIMT in premenopausal women and postmenopausal women after calcium supplementation was 0.0238 ± 0.0348 compared with 0.0615 ± 0.134 mm, respectively. There was a significant interaction between calcium supplementation and menopausal status on serum cholesterol concentrations (P < 0.001) and CIMT (P = 0.017). CIMT, carotid intima-media thickness.
The authors’ responsibilities were as follows—SL, LN, and CS: designed the study; SL, LN, YL, LG, FY, YN, and YZ: conducted the study; SL and LN: performed the statistical analysis and wrote the manuscript; CS: had primary responsibility for the final content of the manuscript; and all authors: read and approved the final manuscript. None of the authors had a conflict of interest.

REFERENCES


