Supplementation with calcium + vitamin D enhances the beneficial effect of weight loss on plasma lipid and lipoprotein concentrations\textsuperscript{1–3}

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ABSTRACT

Background: Adequate calcium intake can have a favorable effect on some metabolic variables.

Objective: The objective of the study was to determine the effects of daily calcium intake and of supplementation with calcium and vitamin D (calcium+D) during a weight-loss intervention on blood pressures, plasma lipid and lipoprotein concentrations, and glucose and insulin concentrations in low calcium consumers.

Design: Healthy, overweight or obese women (n = 63) with a daily calcium intake of <800 mg/d were randomly assigned in a double-blind manner to 1 of 2 groups: the group consuming 2 tablets/d of a calcium + vitamin D supplement (600 mg elemental calcium and 200 IU vitamin D/tablet) or the group consuming placebo; both groups observed a 700 kcal/d energy restriction. These 63 women then completed a 15-wk weight-loss intervention.

Results: Initial daily calcium intake was significantly correlated with plasma HDL cholesterol (r = 0.41, P < 0.001) and with 2-h postload glycemia (r = −0.29, P < 0.05) during an oral-glucose-tolerance test, independent of fat mass and waist circumference. After the 15-wk intervention, significantly greater decreases in total: LDL and LDL:HDL (P < 0.01 for both) and of LDL cholesterol (P < 0.05) were observed in the calcium+D group than in the placebo group. The differences in total:HD and LDL:HDL were independent of changes in fat mass and in waist circumference. A tendency for more beneficial changes in HDL cholesterol, triacylglycerol, and total cholesterol was also observed in the calcium+D group (P = 0.08).

Conclusion: Consumption of calcium+D during a weight-loss intervention enhanced the beneficial effect of body weight loss on the lipid and lipoprotein profile in overweight or obese women with usual low daily calcium intake. Am J Clin Nutr 2007;85:54–9.

KEY WORDS Caltrate, lipoproteins, glucose, insulin, blood pressure

INTRODUCTION

It was shown recently that overweight persons with low calcium and dairy consumption were at much greater risk of developing the metabolic syndrome over a 10-y follow-up than were overweight persons with high calcium and dairy consumption (1). This finding suggests that adequate calcium intake could exert a significant effect on the predisposition to a healthier metabolic profile similar to that of a macronutrient-balanced diet and regular physical activity (2, 3).

From a physiologic standpoint, metabolic deteriorations and their potential relation with calcium or dairy intake is an area of recent interest. Fujita and Palmieri (4) proposed the existence of a calcium paradox according to which a suboptimal bone calcium content would be associated with an increased calcium content of soft tissues. In accordance with this, Zemel et al (5) showed that a low-calcium diet results in a greater calcium content of the adipocyte. One consequence of the increase in intraadipocyte calcium content is an increase in lipogenesis in relation to lipid mobilization (5), which could explained the association reported by Melanson et al (6) between a low calcium intake and low fat oxidation in humans. In the Québec Family Study, this issue has been considered by examining the relation between usual calcium intake and the lipid and lipoprotein profile of healthy subjects. The main finding of this study was that a low calcium intake was significantly related to higher plasma concentrations of total and LDL cholesterol and the ratio of total to HDL cholesterol (total:HDL) in both sexes, independent of fat mass and waist circumference (7). However, the intervention by Zemel et al (8) showed that, despite the significant effect of calcium and dairy supplementation on body fatness during a weight-loss program, no significant change in plasma triacylglycerol or HDL and LDL cholesterol was induced by this supplementation. In addition, it was not clear in this study whether the reduction in systolic blood pressure and the increase in insulin sensitivity were direct effects of a high-dairy diet or the consequences of the significant weight and fat losses observed (8). Considering the equivocal and conflicting findings, we investigated this issue by testing in women with usual low calcium intake the effects of a long-term calcium supplementation on blood pressures and plasma cholesterol, glucose, and insulin concentrations. We hypothesized that a better metabolic profile would be seen with consumption of the calcium

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and vitamin D (calcium + D) supplement than with consumption of a placebo, independent of the improvements promoted by losses of body weight and fat mass.

SUBJECTS AND METHODS

Screening
Recruitment advertisements were made through diverse media sources. Because it was suggested that inadequate calcium intake can increase the risk of metabolic deteriorations (1, 4), we recruited for the current study women with a low calcium intake. Thus, a prescreening evaluation questionnaire on the usual intake can increase the risk of metabolic deteriorations (1, 4), we recruited for the current study women with a low calcium intake. This questionnaire was used to assess adequacy of usual calcium and vitamin D intake by the use of a prescreening evaluation questionnaire, which was as follows: daily intake of <800 mg Ca/d (assessed with the prescreening evaluation questionnaire), no use of calcium supplements within 30 d of screening, absence of menopause, stable body weight (body weight change <3 kg for 2 mo before intervention), body mass index (BMI; in kg/m²) between 27 and 40, <3 periods of 20 min of physical exercise/wk, general good health, no smoking, normal blood pressure values (<160/95 mm Hg), cholesterol concentrations not requiring pharmaceutical treatment, normal thyroid hormone concentrations, no use of medication that could affect body weight, no participation in another clinical trial within 6 mo of screening, and consumption of ≤5 cups coffee/d (1250 mL/d). Finally, breastfeeding or pregnant women whose status was verified by a urine-based pregnancy test could not participate in this study.

Participants who met the above inclusion criteria came to the laboratory for a screening evaluation during which blood samples were collected after a 12-h overnight fast and after 24 h without alcohol consumption and physical activity for the measurement of a biochemical profile; the method of keeping a 3-d dietary record was explained by a nutritionist (9). A computerized version of the Canadian Nutrition File (NUTRIFAQ software, version 0.99; Department of Nutrition and Food Sciences, Laval University, Quebec, Canada) was used to assess macronutrient and micronutrient contents of foods. Anthropometry
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Blood pressure
Systolic and diastolic blood pressures were measured in the right or the left arm supported at heart level of seated participants. Means of 3 measurements taken at 5, 8, and 11 min of rest were used.

Oral-glucose-tolerance test
A 75-g OGTT was performed in the morning after 12-h overnight fast. Blood samples were collected in tubes containing EDTA (Vacutainer tubes; Becton Dickinson, Franklin Lakes, NJ) through a venous catheter from an antecubital vein at −15, 0, 15, 30, 45, 60, 90, 120, 150, and 180 min for measurement of plasma glucose and insulin. Plasma glucose was measured enzymatically (12), whereas plasma insulin was measured by radioimmunoassay with polyethylene glycol separation (13). Plasma concentration of glucose at 120 min of the OGTT was measured (2-h postload glycemia).

Plasma lipid and lipoprotein profile
After a 12-h overnight fast, blood samples were collected from an antecubital vein into the Vacutainer tubes. Cholesterol and triacylglycerol concentrations were determined enzymatically in
plasma and lipoprotein fractions with an automatic immunoanalyzer (AU400; Olympus America Inc, Center Valley, PA), and enzymatic reagents were obtained from Olympus. Plasma lipoprotein fractions (VLDL, LDL, and HDL) were isolated by ultracentrifugation (14). The HDL-cholesterol fraction was obtained after precipitation of LDL cholesterol in the infranatant solution (d > 1.006g/mL) with heparin and MnCl₂ (15). The cholesterol and triacylglycerol contents of the infranatant solution were measured before and after the precipitation step.

Weight-loss intervention

Shortly after the baseline visit (at week 0), participants came to the laboratory to meet with a nutritionist who explained how to conform to the weight-loss intervention, which consisted of a targeted 700 kcal/d decrease in energy intake in addition to consumption of either the calcium +D supplement or the placebo. To maintain equilibrated macronutrient composition, the food exchange system for diabetics from the Association Diabète Québec (1993) was explained to the participants. The nutritionist also talked with the participants about planning daily meals and activities to easily follow the dietary guidelines. During this visit, treatment tablets (calcium +D or placebo) were given to participants. The morning after this visit, participants began energy restriction and the consumption of the treatment tablets for a period of 15 wk.

Participants met the nutritionist on 7 other occasions at 2-wk intervals. To assess compliance with the weight-loss program, each subject participated with the nutritionist in a count of the remaining treatment tablets and a 24-h dietary recall. During the interview, the participants also completed visual analogue scales evaluating satisfaction with the diet, hunger, and compliance with the treatment. Participants were also questioned about the difficulties they may have faced in trying to follow their dietary regimen, and solutions were found with the nutritionist to improve their compliance. Nutritional advice was always aimed at adjusting the energy intake of participants so that diet quality would be preserved. During these visits, the nutritionist also provided additional study products (placebo or calcium +D) if needed.

Statistical analysis

Statistical analyses were performed by using SAS software (version 9.1.2; SAS Institute, Cary, NC). A 2-factor analysis of variance with repeated measure on one factor (time) was used to assess the effects of treatment (calcium +D and placebo) and time and their interaction on all dependent variables. Change in these variables was calculated [(mean value week 15) − (mean value week 0)]. A 2-factor analysis of covariance was used to compare blood pressure and plasma variables between the calcium +D and placebo groups with change in fat mass and waist circumference as covariates. Simple Pearson correlations were computed in the total sample of women between initial calcium intake and the initial values of all dependent variables as an indication of the relation between the degree of metabolic deterioration and calcium intake. To adjust the analyses for confounding variables, simple correlations were repeated by using residual scores for all dependent variables derived from linear regression analysis after adjustment for fat mass and waist circumference as the independent variables. P < 0.05 was considered significant.

RESULTS

Characteristics of participants are shown in Table 1. Baseline physical characteristics were similar in the calcium +D and placebo groups. Significant effects of time between weeks 0 and 15 revealed a decrease in body weight, body mass index, waist circumference, and fat mass. No significant difference in changes in these variables was observed between groups.

Significant time × treatment interaction effects were found for total:HDL and LDL:HDL, showing a significant decrease in these variables in the calcium +D group only (Table 2). The analyses of covariance showed that these differences were independent of variations in fat mass and waist circumference. A significant time × treatment interaction for LDL cholesterol showed a greater decrease in this variable in the calcium +D group than in the placebo group (Table 3), but this significant difference did not remain after adjustments were made for fat mass and waist circumference changes. A tendency was observed for a smaller decrease in HDL cholesterol in the calcium +D group than in the placebo group. Moreover, plasma triacylglycerol concentrations decreased in the calcium +D group and increased in the placebo group, but the 2-factor analysis of variance found no significant time × treatment interaction for this variable. A significant effect of time was observed for systolic and diastolic blood pressure, HDL and LDL cholesterol, LDL:HDL, total cholesterol, and fasting plasma glucose and insulin.

Significant correlations were observed between initial calcium intake and HDL cholesterol and 2-h postload glycemia (Table 3). A trend was also observed for a correlation between calcium intake and total:HDL. Only the relation between initial calcium intake and HDL cholesterol remained significant after
Correlation coefficients for the associations between baseline calcium intake and baseline metabolic variables

**TABLE 3**

Correlation coefficients for the associations between baseline calcium intake and baseline metabolic variables

<table>
<thead>
<tr>
<th></th>
<th>Simple correlation (r)</th>
<th>Partial correlations (r) (adjusted for fat mass and waist circumference)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>P</strong></td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>−0.04</td>
<td>0.76</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.02</td>
<td>0.88</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.41</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total:HDL</td>
<td>−0.22</td>
<td>0.08</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.06</td>
<td>0.65</td>
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<tr>
<td>LDL:HDL</td>
<td>−0.20</td>
<td>0.12</td>
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<tr>
<td>Triacylglycerol</td>
<td>−0.06</td>
<td>0.67</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.19</td>
<td>0.13</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>−0.05</td>
<td>0.57</td>
</tr>
<tr>
<td>2-h Postload glycemia</td>
<td>−0.29</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Fasting insulin</td>
<td>−0.14</td>
<td>0.28</td>
</tr>
</tbody>
</table>

1 Calcium+D, calcium + vitamin D. Change values = [(mean values week 15) − (mean values week 0)]; 2-h postload glycemia = plasma glucose concentration at 120 min of the oral-glucose-tolerance test.

2 n = 29 and 32 for calcium+D and placebo groups, respectively.

3 x ± SD (all such values).

4 n = 31 for placebo group.

5 Significantly different from values in week 0, P < 0.01.

6 Significantly different from mean placebo values in week 15, P < 0.05.

7 Difference between groups remained statistically significant after adjustment for fat mass and waist circumference, P < 0.05.

8 n = 30 for placebo group.

DISCUSSION

This study is the first to report that, in overweight or obese women with low calcium intakes, supplementation with calcium and vitamin D improved blood lipid and lipoprotein during a weight-loss intervention. In a first series of analyses, we observed that daily calcium intake was independently associated with HDL-cholesterol concentration after adjustment for fat mass and waist circumference as confounding variables. These results, in addition to the previously reported significant inverse correlations between daily calcium intake and LDL cholesterol, total cholesterol, and total:HDL in the Québec Family Study after statistical corrections for the same confounding variables (7), suggest a beneficial influence of calcium intake on the lipid and lipoprotein profile.

The design of our study permitted us to validate the relation between calcium and the lipid and lipoprotein profile in women who are low calcium consumers, a status that is known to increase the risk of metabolic deterioration (1, 4). We observed that supplementation with calcium+D decreased total:HDL and LDL:HDL independent of the loss in total body fat mass and in fat mass located in the abdominal region that occurred during the weight-loss program. Moreover, the similarity between calcium+D and placebo groups with respect to macronutrient composition of the diet and alcohol consumption before and after the intervention (results not shown) suggests that these variables could not explain the changes observed in lipid profile. Therefore, in women who are low calcium consumers, supplementation with calcium+D could adjustments for fat mass and waist circumference, although a tendency was still observed for 2-h postload glycemia.
contribute to the improved cardiovascular risk profile that usually accompanies body weight loss (16–18).

Our results are concordant with other randomized controlled studies that have reported an effect of calcium on lipoprotein concentrations (19, 20), and they therefore strengthen the link between calcium and cholesterol metabolism which was not clear after the recent study of Zemel et al (8). Indeed, in that study, the effect of an energy-restricted diet providing either 400–500 mg Ca/d from dairy products (placebo group) or 1200–1300 mg Ca/d from an additional 800 mg calcium carbonate (high calcium group) or from an additional 3 servings of dairy products (high dairy group) had no effect on LDL- and HDL-cholesterol and triacylglycerol concentrations (8). One explanation for the difference in these results could be the different quantities of calcium given to participants in the 2 studies. In the study of Zemel et al, supplementation brought the total daily calcium intake of participants to 1200–1300 mg (8), whereas, in the current study, supplementation consisted of 1200 mg Ca/d that was added to the diet of participants who previously averaged an intake of 704 mg Ca/d. The greater quantity of calcium given in the current study may suggest that a daily calcium consumption that slightly exceeds the Dietary Reference Intakes (21) is necessary for individuals with usual inadequate calcium intake to experience a decrease in lipid and lipoprotein concentrations.

The decrease in lipid and lipoprotein concentrations observed in the current study could be due to several effects attributed to calcium intake, such as a reduction in fatty acid absorption and an increase in fecal fatty acid content, probably resulting from the formation of insoluble calcium-fatty soaps in the gut (22, 23). Such a decreased absorption of fat, especially saturated fat, would reduce the serum total and LDL-cholesterol concentrations (24, 25). Other properties attributed to calcium are the mineral’s capabilities to bind bile acids (26, 27), increase the conversion of cholesterol to bile acids, and thus increase cholesterol excretion (28). Moreover, an increase of intracellular calcium in hepatocytes was shown to stimulate microsomal triacylglycerols transfer protein (MTP) which is implicated in the formation and secretion of VLDL (29). In this regard, it has been shown that increasing dietary calcium suppresses the stimulation of calcium influx into adipocytes by calcitropic hormones that occur as a consequence of low calcium diets (5) and that stimulates lipolysis. Therefore, it is possible to speculate, first, that the increase in hepatocellular calcium would also be suppressed by increasing calcium intake and that the VLDL-induced increases in triacylglycerol and LDL cholesterol would be reduced (29). In addition, it is possible that the increased lipolysis resulting from a calcium-rich diet could favor lipid mobilization, as suggested by the positive relation and effect of calcium intake on fat oxidation (6, 30). Finally, from a statistical perspective, the beneficial effects of a calcium+D supplement on cholesterol concentrations observed in this study could be due to calcium as much as to vitamin D. However, current literature tends toward the attribution of a larger contribution from calcium because the reported effects of vitamin D on apolipoprotein gene expression (31–33) and cholesterol concentrations and ratio (34, 35) are still controversial.

Fasting plasma concentrations of glucose and insulin decreased significantly during the intervention, most likely as a consequence of the energy restriction–induced body weight and fat mass loss, because between-group effects were not significant. In their study, Zemel et al (8) found a significant decrease in the glucose area under the curve and in the plasma insulin concentrations in the high-dairy but not in the high-calcium groups. The greater loss in body weight and fat mass in the former group could, however, explain part of these differences, which would be concordant with the effect observed in our study and which could suggest that calcium per se does not significantly affect glucose profile in overweight or obese persons with normal glycemia during a weight-loss intervention. In that same weight-loss study, Zemel et al reported a significant reduction in systolic blood pressure in the high-dairy group, but not in the high-calcium group. We did not observe a significant difference between groups for the decrease in blood pressures in response to the intervention, which is concordant with the results of Bostick et al, who tested the effect of supplementation with 1000–2000 mg/d of elemental calcium on blood pressures (19).

The nonsignificant effect of elemental calcium in the above-mentioned studies raises the issue of the difference between the influence of calcium in supplements and that of foods containing calcium, such as dairy products, on the glucose and insulin profile and on blood pressures. Indeed, it was shown in the Coronary Artery Risk Development in Young Adults Study that abnormal glucose homeostasis incidence was decreased with increasing categories of dairy intake in overweight persons (1), which agrees with the significant inverse correlation observed in our study between initial calcium intake from dairy products and other dietary sources and the 2-h postload glycemia results. With respect to blood pressures, the concept of a hypotensive effect attributed to dietary calcium seems to have been widely accepted (35). Nevertheless, a recent and well-conducted meta-analysis reported that dietary and nondietary calcium supplementation interventions promoted an effect of lowering systolic and diastolic blood pressure, although the effect with the dietary intervention was nearly twice that with the nondietary intervention (36). Therefore, better understanding is still needed of whether the benefit of the calcium-induced improvement in the glucose and insulin profile and blood pressures can be attributed to dairy products, to calcium per se, or to both.

In conclusion, our results showed that consumption of a calcium+D supplement enhanced the beneficial effect of body weight loss on the lipid and lipoprotein profile in overweight or obese women with usual low calcium intake. Future research in this area should be oriented toward a better understanding of the effect of a usual insufficient calcium intake on the expected outcome of a supplementation with this mineral, more specifically with respect to its effect on glucose and insulin and blood pressure variables. In conclusion, it is suggested that in the clinical context of obesity treatment, calcium supplementation could be recommended in women with inadequate calcium intake to improve the cardiovascular disease risk profile.

We thank Claude Leblanc for his assistance with statistical analyses.

AT designed the study; GCM conducted the literature review, performed the statistical analyses, and interpreted the data; FA, JD, and SP were responsible for collecting the data; GCM wrote the draft of the manuscript, and AT, FA, JD, and SP participated in revising the manuscript. None of the authors had a personal or financial conflict of interest.

REFERENCES


CALCIUM+VITAMIN D AND CHOLESTEROL CONCENTRATIONS


Erratum


In the second sentence of the Results section of the abstract, “total:LDL” appears in error. The sentence should read as follows: “After the 15-wk intervention, significantly greater decreases in total:HDL and LDL:HDL (P < 0.01 for both) and in LDL cholesterol (P < 0.05) were observed in the calcium + D group than in the placebo group.

Erratum


The fourth author’s first name was misspelled. The correct spelling is “Chuanzhong.”

In addition, the numbers of premenopausal participants reported in Table 4 should be as follows: for High intake, the correct numbers for Ile/Ile + Ile/Val are 862/796, and those for Val/Val are 40/23; for Low intake, the correct numbers for Ile/Ile + Ile/Val are 878/853, and those for Val/Val are 46/25. All odds ratios and P values are correct.

REFERENCES