Dairy products do not lead to alterations in body weight or fat mass in young women in a 1-y intervention\textsuperscript{1–3}

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\textbf{ABSTRACT}

\textbf{Background:} Previous results suggested that increased intake of dairy calcium is associated with reduced weight and fat mass.

\textbf{Objective:} The purpose of this study was to determine whether long-term increases in consumption of dairy calcium alter body weight and fat mass in young, healthy women.

\textbf{Design:} We used a randomized, 1-y intervention for dairy calcium. Subjects were 155 young (aged 18–30 y), healthy, normal-weight women with intake of dietary calcium < 800 mg/d and energy intake \( \leq 2200 \text{ kcal/d}. \) Women were randomly assigned to 1 of 3 groups: 1) control: continue established dietary intake; 2) medium dairy: substitute dairy products to achieve intake of calcium of \( =1000–1100 \text{ mg/d and maintain isocaloric intake}; \) 3) high dairy: substitute dairy products to achieve intake of calcium of 1300–1400 mg/d and maintain isocaloric intake. The main outcome measures were 1-y changes in body weight (in kg) and fat mass (in kg). One hundred thirty-five women completed the trial.

\textbf{Results:} Mean intakes of calcium during the intervention were 742.4 ± 321.5, 1026.4 ± 311.3, and 1131.29 ± 337.2 mg/d for the control, medium-dairy, and high-dairy groups, respectively (\( P < 0.0001 \)). No significant differences were observed in the mean 1-y change in body weight between the control, medium-dairy, and high-dairy groups (0.8 ± 2.8, 0.7 ± 3.0, and 1.5 ± 4.1 kg, respectively; \( P = 0.45 \)). No significant differences were observed in the mean 1-y change in fat mass between the control, medium-dairy, and high-dairy groups (−0.5 ± 2.5, 0.3 ± 2.7, and 0.5 ± 3.5 kg, respectively; \( P = 0.26 \)).


\textbf{KEY WORDS} Body weight, fat mass, dairy, calcium, intervention

\textbf{INTRODUCTION}

The prevalence of obesity has rapidly increased in the past 20 y and has become a national and global epidemic (1). It has been estimated that overweight and obesity cost the United States an annual \$117 billion (2). Monetary costs as well as health costs are related to obesity. It is a risk factor for certain chronic diseases, such as heart disease, cancer, stroke, and diabetes, and weight loss is known to reduce the risk (1). Furthermore, because of the rapid rise in obesity, it is likely that environmental factors, such as diet, play a significant role (3, 4). Although much effort has been devoted to studying the effects of macronutrients on weight control, the role of micronutrients has not been studied as well. It has been proposed that dietary calcium, in particular, may play a role in weight regulation (5).

Increasing evidence in cellular, animal, and human models supports an inverse relation between calcium and weight. Those studies showed that higher intakes of calcium are associated with weight loss, with some showing specificity to fat mass (6–18). For example, in a 2-y exercise intervention trial in 54 young (aged 18–31 y), healthy women, calcium intake, adjusted for energy, was negatively associated with changes in body weight and fat mass, specifically in women whose energy intake was at or below the overall group mean of 1876 kcal/d (13). Other studies have been reanalyzed that, similar to this exercise intervention study, were not originally designed to study body fat mass. The results of those studies have supported the potential effect of calcium intake on body fat across a wide age range from children (8) to postmenopausal women (9) and in both men (16) and women (6, 9, 10, 12, 13, 16). The effect has also been noted in African American men (16) and women (7). Finally, the effect of calcium may be particularly beneficial during weight loss, and dairy products may enhance the effect of calcium on weight loss (11, 17, 18). However, because of the secondary analyses, the relatively small number of studies, and the conflicting results, the relation between the intake of calcium or dairy products and the modifications in body fat or weight remain unclear. For example, a recent review by Barr (19) reported that trials designed to study bone status, without including individual energy intake in the analysis of the trials, showed no relation of weight or body fat to intake of calcium or dairy products. In another example, calcium supplementation during a weight-loss intervention in premenopausal and postmenopausal women (\( n = 100 \)) was shown to have no significant effect on body weight or fat mass (20). Finally, no relation was observed between the intake of calcium or dairy and the accumulation of body fat or body mass index (BMI) in nonobese premenarcheal girls, aged 8–12 y (\( n = \)).


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The regulation of serum calcium by parathyroid hormone (PTH) and 1,25 dihydroxyvitamin D \( [1,25(OH)_2D] \) after changes in dietary calcium has been proposed to mediate the effects of calcium on fat mass (16). The vitamin D metabolite, 1,25(OH)_2D, and PTH are the principal hormonal regulators of calcium homeostasis. A low-calcium diet with subsequent lower serum calcium concentration leads to the increased production and release of PTH into the circulation. Once released, PTH promotes bone resorption, stimulates renal calcium reabsorption, and stimulates the renal enzyme, 25 hydroxyvitamin D-1 hydroxylase, that catalyzes the conversion of 25 hydroxyvitamin D to 1,25(OH)_2D. The 1,25(OH)_2D produced by the kidney and released into the serum acts on intestine, bone, and kidney to regulate calcium homeostasis. In addition to the classically defined function of regulating serum calcium, both PTH and 1,25(OH)_2D have been shown to increase the amount of intracellular calcium in adipocytes, which can lead to a decrease in lipolysis and an increase in lipogenesis through increases in the amount of fatty acid synthase in the cell (16). This shift in lipid utilization may lead to an accumulation of fat. Results of a clinical trial also suggest that changes in PTH are positively correlated with changes in fat mass in young women whose intake of calcium, adjusted for energy, was negatively related to body weight and fat mass (22). However, the role of PTH and 1,25(OH)_2D in the regulation of fat mass and body weight by dietary calcium has not been fully elucidated, and other mechanisms might also explain the effect (5).

The purpose of this study was to determine the effects of a 1-y intervention of dairy calcium on changes in body weight and fat mass in healthy women, aged 18–30 y. The hypothesis tested was that a long-term increase in calcium intake would lead to a decrease or less of an increase in body weight and fat mass through a suppression in PTH and 1,25(OH)_2D.

SUBJECTS AND METHODS

Subjects

Young, healthy women, aged 18–30 y, were solicited through flyers, radio announcements, direct mailings, and information booths located outside residential hall cafeterias. The control and intervention subjects were recruited in parallel. The Purdue University Institutional Review Board approved the study. All participants provided written, informed consent. Women with a food-frequency questionnaire, which included supplement information (23), and who were willing to consume dairy products, were invited to participate. Daily intake of calcium and energy was evaluated further with a food-frequency questionnaire, which included supplement information, (24) before inclusion in the study and random assignment. Exclusion criteria included 1) intake of energy > 2200 kcal/d; 2) intake of chronic medication that interfered with calcium metabolism; 3) pregnancy or lactation within the previous 6 mo; 4) irregular menses; 5) self-reported lactose intolerance; 6) malabsorptive, bone, kidney, or hormonal disorders that might affect calcium metabolism; 6) bone or muscle disorders; 7) being >20% overweight or >15% underweight according to the Metropolitan Life Insurance Tables (25); 8) eating disorder; and 9) high alcohol consumption (>2 drinks/d).

Anthropometric, fitness, and body-composition measurements

Measurements were taken at baseline and at 12 mo during days 3 to 11 of the menstrual cycle (follicular phase), between 0700 and 1100, after a 12-h fast. With subjects in light clothing and no shoes, we used a calibrated balance scale (HealthOMeter Inc, Bridgeview, IL) to measure weight (in kg) and a wall-mounted stadiometer (Holtain Ltd, Crymych, United Kingdom) to measure height (in cm). We also measured resting heart rate (bpm) while subjects were seated and postexercise heart rate (bpm) immediately after a 3-min step test, both at the radial artery. The 3-min step test, designed for this age range (26), was administered with the use of a tape-recorded 96-bpm metronome. Estimated \( V_{O2max} \) (expressed as mL · kg\(^{-1}\) · min\(^{-1}\)) was calculated from both resting heart rate and postexercise heart rate. Total body fat mass (in kg) and lean mass (in kg) were measured with dual energy X-ray absorptiometry (software version 4.3e; Lunar Corp, Madison, WI). Body weight (in kg) and body-composition measures (in kg) are expressed as total change at 12 mo from baseline (12 mo – baseline).

Assessment of dietary intake and other lifestyle factors

Daily intake of calcium (in mg) was assessed for all subjects by 3-d food records at baseline and at 3, 6, 9, and 12 mo. Dietary records were reviewed and analyzed by one trained nutritionist who used the Nutrition Data System (NDS) for Research, version 4.04, Food and Nutrient Database 28 (Minneapolis). More than 94% of the subjects returned food records at 6 and 12 mo. Three-day physical activity records (27) were collected from all subjects at baseline and at 3-mo intervals throughout the study to assess energy expenditure (in kcal/d). Briefly, participants were counseled to record activity in 15-min time periods throughout the day with the use of an activity code that defined 9 categories. The categories ranged from 1 (lying down, 0.26 kcal · kg\(^{-1}\) · min\(^{-1}\)) to 9 (intense work or activity, 1.95 kcal/kg per 15 min). Thus, an estimate of 24-h energy expenditure can be calculated, based on the results. Lifestyle questionnaires assessed previous and current medical history and medication use, including oral contraceptive use, at baseline and at 12 mo for all subjects.

Laboratory methods

Blood samples were collected at baseline and at 12 mo during days 3 to 11 of the menstrual cycle (follicular phase), between 0700 and 1100 after a 12-h fast. After collection, blood samples were immediately centrifuged at 12 000 \( \times \) g for 10 min at 4 °C, and serum was stored at −80 °C. Serum concentration of PTH was measured by a two-site immunoradiometric assay, which assessed biologically intact 84 amino acid chain of PTH with the use of the Allegro Intact PTH Immunoassay (Nichols Institute, San Clemente, CA). Serum 1,25(OH)_2D was extracted with acetonitrile and partially purified with a C18 silica cartridge. Serum 1,25(OH)_2D was assessed with the use of a competitive protein-binding assay that was based on calf thymus receptor (INCSTAR Co, Stillwater, MN).

Intervention protocol for dairy calcium

After completion of baseline testing, participants were randomly assigned into 1 of 3 groups: 1) control group (maintain...
current dietary consumption); 2) medium-dairy group (1000–1100 mg Ca/d from dairy); and 3) high-dairy group (1300–1400 mg Ca/d from dairy). Random assignment was stratified by the subject’s oral contraceptive use and energy intake, such that equal numbers of women in each energy decile (1200–1299, 1300–1399, 1400–1499, etc) were randomly assigned to each treatment group.

Participants randomly assigned into the dairy groups received individual dietary counseling by trained nutritionists and were instructed to increase intake of daily calcium by substituting dairy products rich in calcium, with an emphasis on nonfat and low-fat milk. Participants were given a pocket-sized pamphlet with a comprehensive list of substitutions. To maintain isocaloric intake and equivalent dietary fat, participants were instructed to remove other dietary components to approximate the added dairy intake of energy and fat. Each subject was counseled by a dietician on appropriate substitutions according to an analysis of their food records and on how to maintain a daily record of their added intake of dairy products and substitutions. For each day, subjects recorded the type and number of servings of dairy foods added and the corresponding foods subtracted. This daily record of dietary intake and foods removed from the diet to maintain iso-caloric balance was returned monthly by participants in the intervention groups to assess compliance. The logs were checked by a nutritionist to determine whether adequate increases in dairy intake were achieved and whether appropriate substitutions were recorded. If discrepancies were found, the participant was contacted and retrained to the dietary protocol. Thus, regular contact with the participants and dietary counseling were provided throughout the duration of the study. Subjects randomly assigned into the control group were instructed to make no changes to their food records verified their compliance.

Statistical analyses

Descriptive and univariate statistics were assessed for all variables. A general linear model (GLM) was used for the analyses. The primary outcome variables were total body weight (in kg) and fat mass (in kg). Explanatory variables included group (control, medium dairy, high dairy), PTH, and 1,25(OH)2D. Because both PTH and 1,25(OH)2D were not normally distributed, a log transformation of these variables was used in the analyses. For both PTH and 1,25(OH)2D were not normally distributed, a log transformation of these variables was used in the analyses. For both PTH and 1,25(OH)2D were not normally distributed, a log transformation of these variables was used in the analyses.

RESULTS

After baseline testing, 155 participants were randomly assigned, and 135 completed the 1-y protocol. Reasons for withdrawal (n = 20) included 1) loss of interest or time constraints (n = 13), 2) a move from the area (n = 4), 3) pregnancy (n = 2), and 4) death resulting from unrelated causes (n = 1). The overall retention rate was 87%. No significant baseline differences (age, weight, BMI, fat mass, lean mass, calcium intake, and energy intake) were found between participants who discontinued the study protocol and participants who remained in the intervention.

No significant differences were observed between groups in baseline characteristics for participants who completed the study (Table 1). Additionally, no significant differences in fasting serum PTH (in ng/mL), log PTH, 1,25(OH)2D (in ng/mL), and log 1,25(OH)2D were observed between groups at baseline (Table 2). Mean daily dietary calcium was significantly greater in the intervention groups than in the control group throughout the intervention (months 3, 6, 9, and 12) (Figure 1). Grand mean calcium intakes during the intervention were 742.4 ± 312.5 mg/d (control group), 1026.4 ± 311.3 (medium-dairy group), and 1131.29 ± 337.2 mg/d (high-dairy group) (P < 0.0001, ANOVA). Mean daily energy intake did not differ by group at each time period throughout the study (groups combined, month 3: 1578.3 ± 466.6, month 6: 1599.3 ± 446.2, month 9: 1591.2 ± 413.1, month 12: 1604.0 ± 457.4 kcal/d; Figure 2).

Table 1: Baseline characteristics of the subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group (n = 42)</th>
<th>Medium-dairy group (n = 45)</th>
<th>High-dairy group (n = 48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>20.1 ± 2.4</td>
<td>20.2 ± 2.4</td>
<td>20.1 ± 2.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.9 ± 5.9</td>
<td>165.4 ± 6.8</td>
<td>166.8 ± 7.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>60.9 ± 10.7</td>
<td>63.9 ± 11.7</td>
<td>62.4 ± 8.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.1 ± 3.1</td>
<td>23.3 ± 3.9</td>
<td>22.4 ± 2.6</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>17.8 ± 7.3</td>
<td>20.2 ± 8.6</td>
<td>18.0 ± 6.2</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>39.4 ± 4.7</td>
<td>39.5 ± 3.8</td>
<td>40.3 ± 4.8</td>
</tr>
<tr>
<td>Calcium intake (mg/d)</td>
<td>695 ± 263</td>
<td>727 ± 269</td>
<td>693 ± 281</td>
</tr>
<tr>
<td>Energy intake (kcal/d)</td>
<td>1678 ± 443</td>
<td>1659 ± 548</td>
<td>1752 ± 438</td>
</tr>
<tr>
<td>Energy expenditure (kcal/d)</td>
<td>2758 ± 618</td>
<td>2838 ± 553</td>
<td>2682 ± 459</td>
</tr>
</tbody>
</table>

Table 2: Baseline fasting serum parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D [1,25(OH)2D] values

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group (n = 41)</th>
<th>Medium-dairy group (n = 44)</th>
<th>High-dairy group (n = 48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH (ng/mL)</td>
<td>23.9 ± 12.62</td>
<td>25.7 ± 16.0</td>
<td>26.9 ± 13.0</td>
</tr>
<tr>
<td>Median</td>
<td>20.9</td>
<td>25.3</td>
<td>22.6</td>
</tr>
<tr>
<td>25th quartile</td>
<td>11.3</td>
<td>15.3</td>
<td>16.5</td>
</tr>
<tr>
<td>75th quartile</td>
<td>27.9</td>
<td>38.2</td>
<td>29.1</td>
</tr>
<tr>
<td>log PTH</td>
<td>3.0 ± 0.5</td>
<td>3.1 ± 0.6</td>
<td>3.2 ± 0.5</td>
</tr>
<tr>
<td>1,25(OH)2D (ng/mL)</td>
<td>42.7 ± 17.1</td>
<td>35.3 ± 15.5</td>
<td>38.4 ± 17.4</td>
</tr>
<tr>
<td>Median</td>
<td>41.0</td>
<td>41.0</td>
<td>39.6</td>
</tr>
<tr>
<td>25th quartile</td>
<td>35.9</td>
<td>32.5</td>
<td>35.8</td>
</tr>
<tr>
<td>75th quartile</td>
<td>48.7</td>
<td>47.3</td>
<td>50.1</td>
</tr>
<tr>
<td>log 1,25(OH)2D</td>
<td>3.7 ± 0.5</td>
<td>3.4 ± 0.6</td>
<td>3.5 ± 0.7</td>
</tr>
</tbody>
</table>

1 All values are ± SD. There were no significant differences between the groups at baseline (general linear model).

2 x ± SD (all such values).
only subjects who were compliant to the study protocol guidelines were included in the analyses. Compliance guidelines required that 1) subjects in all groups maintain a mean daily energy intake ≤ 2200 kcal/d over the study period, 2) subjects in the control group maintain a daily calcium intake similar to baseline (no increase > 200 mg/d over the study period), and 3) subjects in the intervention groups increase daily calcium intake (> 200 mg/d) over the study period.

Influential variables [age (y), race (white, African American, and Asian), oral contraceptive use (yes or no), serum log PTH, log 1,25(OH)₂D, energy expenditure (kcal/d), physical fitness (VO₂max), and energy intake (kcal/d)] were included in the GLM model that was designed to test for a group assignment effect. Similar to the results described earlier, no significant group effect on the mean 1-y change in body weight or body fat mass was observed when these influential variables were included in the model.

As previously noted, it has been hypothesized that one of the mechanisms by which calcium intake inhibits accumulation of body fat mass is by suppression of serum PTH and 1,25(OH)₂D. In a GLM model, group assignment did not significantly predict the mean log PTH at 1 y (control: 3.2 ± 0.42; medium dairy: 3.4 ± 0.52; high dairy: 3.2 ± 0.53) or did it predict the mean 1-y change in log PTH (control: 0.19 ± 0.60; medium dairy: 0.28 ± 0.60; high dairy: 0.05 ± 0.52). Group assignment did not significantly predict the mean log 1,25(OH)₂D at 1 y (control: 3.5 ± 0.33; medium dairy: 3.5 ± 0.29; high dairy: 3.5 ± 0.31) or did it predict the mean 1-y change in log 1,25(OH)₂D (control: −0.25 ± 0.37; medium dairy: −0.15 ± 0.40; high dairy: −0.24 ± 0.33). When baseline or 1-y change in log PTH or log 1,25(OH)₂D was added separately to the GLM model after group assignment, the results remained similar, with no significant group effect observed for the mean 1-y change in body weight and fat mass.

**DISCUSSION**

To our knowledge, no published clinical trial has been specifically designed to examine the effect of a diet high in dairy calcium on changes in body weight and fat mass in young, healthy, normal-weight women. Although much of the published clinical data supports a negative relation between calcium intake and body fat mass (6–18), others have found little evidence for an effect of dairy products or calcium supplementation in reducing body weight or fat mass (19, 27, 28). Because of conflicting published data, limited longitudinal clinical studies, and the increase in obesity and its strong environmental component, there is a critical need to better understand the effect of a high-calcium diet on the regulation of body weight.

Previously, this laboratory showed that calcium intake, controlled for energy, negatively predicts a significant change in body fat mass in a 2-y exercise intervention trial in 54 young, healthy women (13). Results from the current study showed that a year-long increase in calcium intake in young, healthy women did not lead to alterations in body weight or fat mass. An explanation for the discrepancy in results from these 2 studies is not readily apparent but may be related to differences in the current study compared with the prior study because 1) the subjects were younger (x ± SD, previous study: 24.6 ± 3.3 y; current study: 20.1 ± 2.4 y); 2) the length of study was shorter (previous study: 2 y; current study: 1 y); or 3) the subjects are more physically fit (x ± SD maximal oxygen consumption, previous study: 28.2 ± 5 mL·kg⁻¹·min⁻¹; current study:
39.8 ± 3.6 mL·kg⁻¹·min⁻¹). However, results from the current study are similar to a randomized, controlled dairy calcium intervention with a 12-mo follow-up conducted in pubertal girls (n = 48) (28). The objective of that study was to test the effect of calcium supplementation with dairy products on bone and body composition (28). Similar to the results from the current study, no differences were observed in serum 1,25(OH)₂D values between the 2 groups at the beginning or end of the study, and increased intake of dairy foods was not associated with weight gain or increased body fat (28). Phillips et al (21) also showed no relation between calcium intake and body composition in a nonobese adolescent population studied longitudinally over 3 y. Another recently published study, producing similar results to this study, showed that calcium supplementation during a weight-loss intervention in premenopausal and postmenopausal women (n = 100) had no significant effect on body weight or fat mass (20).

An analysis of the subjects’ 3-d diet records indicated that, overall, the intervention was successful (ie, the intervention groups achieved concentrations of mean daily dietary calcium that were significantly greater than the control group, and no significant differences were observed between groups in the mean daily energy intake). However, at each time point throughout the intervention, the medium- and high-dairy groups had slightly higher, although not statistically significant, mean energy intakes than did the control group. During the intervention, the mean ± SD for energy intake was 1558.4 ± 383.3, 1671.3 ± 345.1, and 1606.1 ± 317.1 kcal/d for the control, medium-dairy, and high-dairy groups, respectively. If this slight difference in energy intake between the control and intervention groups represented a lack of appropriate substitution by the intervention groups, it is possible that a weight loss in the intervention groups over a year’s time would be prevented. Note that, although these subjects received careful diet record counseling by trained nutritionists at the beginning and throughout the duration of the study, diet records are known to be poor indicators of actual intake (29); therefore, the results from the dietary analysis may not be a true reflection of the subjects’ actual intake. However, the results remained similar when only subjects who were compliant with the study protocol guidelines were included in the analyses.

With the use of the SD (3.3 kg) of weight changes for participants who completed the current study, a sample size of 45 per group provides 80% power to detect a difference between treatment groups of 2.5 kg fat mass and 90% power to detect a difference of 2.9 kg fat mass over a year-long intervention period for dairy products. In a previous uncontrolled observational study (13) with female subjects selected with similar age and weight exclusionary criteria, a linear equation predicted that fat mass (20). The results of the current study showed that dairy products do not promote gains in body weight or fat mass. Although a study conducted in a controlled environment might yield more accurate results (ie, capturing and controlling for daily variations in these influential lifestyle factors), one might argue that the results of this study better reflect what might occur in response to a public health recommendation to increase dairy calcium aimed at young women. In addition to these strengths, there are limitations worth noting. First, assessment of compliance with the intervention relied on self-reporting diet records, known to be poor indicators of actual energy intake (29); therefore, the results from the dietary analysis may not be a true reflection of the subjects’ actual intake. However, the results remained similar when only subjects who were compliant with the study protocol guidelines were included in the

analyses.

DT and RML were responsible for the design, data collection, and management of this study. PAL, CWG, and MSE were responsible for the data collection and program compliance. GPM performed the statistical analyses. MP contributed to the design, sample analysis, and interpretation of results. CWG was primarily responsible for writing the manuscript, and all authors contributed to writing and amendment of the manuscript. None of the authors had any conflict of interest.
REFERENCES