Calcium and vitamin D supplementation is associated with decreased abdominal visceral adipose tissue in overweight and obese adults¹–⁴

Jennifer L Rosenblum, Victor M Castro, Carolyn E Moore, and Lee M Kaplan

ABSTRACT
Background: Several studies suggest that calcium and vitamin D (CaD) may play a role in the regulation of abdominal fat mass.

Objective: This study investigated the effect of CaD-supplemented orange juice (OJ) on weight loss and reduction of visceral adipose tissue (VAT) in overweight and obese adults (mean ± SD age: 40.0 ± 12.9 y).

Design: Two parallel, double-blind, placebo-controlled trials were conducted with either regular or reduced-energy (lite) orange juice. For each 16-wk trial, 171 participants were randomly assigned to 1 of 2 groups. The treatment groups consumed three 240-mL glasses of OJ (regular or lite) fortified with 350 mg Ca and 100 IU vitamin D per serving, and the control groups consumed either unfortified regular or lite OJ. Computed tomography scans of VAT and subcutaneous adipose tissue were performed by imaging a single cut at the lumbar 4 level.

Results: After 16 wk, the average weight loss (~2.45 kg) did not differ significantly between groups. In the regular OJ trial, the reduction of VAT was significantly greater (P = 0.024) in the CaD group (-12.7 ± 25.0 cm²) than in the control group (-1.3 ± 13.6 cm²). In the lite OJ trial, the reduction of VAT was significantly greater (P = 0.039) in the CaD group (-13.1 ± 18.4 cm²) than in the control group (-6.4 ± 17.5 cm²) after control for baseline VAT. The effect of calcium and vitamin D on VAT remained highly significant when the results of the 2 trials were combined (P = 0.007).

Conclusions: The findings suggest that calcium and/or vitamin D supplementation contributes to a beneficial reduction of VAT. This trial is registered at clinicaltrial.gov as NCT00386672, NCT01563115.


INTRODUCTION
Although epidemiologic data and clinical trials suggest that a small daily increase in calcium or dairy products may result in annual losses in body weight and body fat (1–5), the possible utility of calcium-rich diets for weight loss remains controversial. Recently, a review of 15 randomized clinical trials examined the potential role of calcium D in the regulation of body weight and body fat (6). High-calcium diets were found to reduce weight gain and the accumulation of fat in adipocytes (7–9). Using the agouti mouse as a model, Zemel et al (7) proposed that increasing dietary calcium, associated with a reduction of PTH⁵, lowered adipocyte intercellular calcium, which was associated with reduced fatty acid synthase and increased adipose tissue lipolysis. Another proposed mechanism to explain a possible link of calcium to increased loss of body fat and weight is through increased oxidation of fat and thermogenesis via up-regulation of uncoupling proteins (7–11).

The effect of dietary calcium on body weight also may occur at the level of the gastrointestinal tract or through suppression of hunger. A meta-analysis concluded that dietary calcium had the potential to increase fecal fat excretion, which may be beneficial at preventing weight gain (12). In addition, suppression of hunger by calcium has been reported (13), and meal design studies have found that calcium plus vitamin D attenuates meal intake, prolongs between-meal intervals, and reduces food intake the following day (14).

Although vitamin D is well known for its essential role in bone metabolism and calcium homeostasis, increasing evidence indicates that the vitamin D endocrine system is linked to obesity. In adults, an inverse relation has been reported between adiposity and circulating 25(OH)D concentrations, the physiologically relevant marker of vitamin D status (15–20). Low circulating concentrations of 25(OH)D were independently associated with increased BMI and fat mass, in both children (21) and adults (3, 4, 19, 20). In addition, overweight and obese premenopausal women lost more body fat on a hypocaloric diet when their baseline vitamin D concentrations were higher (22). Nevertheless, prospective studies (23, 24) examining the effect of both calcium and vitamin D on body weight and/or abdominal fat have been inconclusive.

The purpose of this study, therefore, was to investigate the effect of calcium and vitamin D supplementation delivered in

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⁵ Abbreviations used: CaD, calcium and vitamin D; CT, computed tomography; ITT, intention-to-treat; OJ, orange juice; PTH, parathyroid hormone; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; 25(OH)D, 25-hydroxyvitamin D.

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orange juice (regular and reduced-energy) for 16 wk on loss of body weight and reduction of VAT in a population of overweight and obese adults participating in a weight-loss program. We hypothesized that a greater loss of body weight and VAT would occur with calcium and vitamin D supplementation.

SUBJECTS AND METHODS

Study design

We conducted 2 parallel, double-blind, placebo-controlled trials: 1 with regular OJ and 1 with reduced-energy (lite) OJ (Table 1). In each 16-wk trial, 171 participants were randomly assigned to 1 of 2 groups (control and treatment). Study design development and implementation were performed by researchers from MGH Weight Center, and the beverages were donated by the Beverage Institute for Health & Wellness. The primary outcome measure was a significant loss of body weight, and the secondary outcome measure was a reduction of VAT as assessed by CT.

Study population

Healthy overweight and obese men and women between the ages of 18 and 65 y who had a BMI (in kg/m2) between 25 and 35 were recruited for the study from the community by using local newspaper ads, posted flyers, hospital-based research recruitment e-mails, and online advertising. Most of the participants were white (non-Hispanic) women (Table 2).

Inclusion criteria

The participants were overweight (BMI: 25.0–29.9) or obese (BMI ≥ 30). They had to report a stable weight (within 5%) for ≥3 mo and be able to provide informed consent. Participants could not be pregnant and had to be willing to use contraception throughout the duration of the study.

Exclusion criteria

Participants were excluded if they were pregnant or had a history of diabetes mellitus, polycystic ovary syndrome, known parathyroid disease, sarcoidosis, substance abuse, or an active eating disorder. Participants were also excluded if they had participated in any other weight loss study within 90 d, used any medication within 6 mo of the study that could cause significant weight change, had a high calcium intake (>2 servings dairy products/d or took multivitamin or calcium supplements ≥3 times/wk) for 1 mo before the study, had a medical contraindication to taking calcium supplements, or had other significant medical or psychological illnesses considered by the investigators to interfere with trial participation. Any potential participant with a vitamin D deficiency [serum 25(OH)D < 10 ng/mL] on screening evaluation was also excluded.

During a telephone screening, a research coordinator determined that participants met the major inclusion and exclusion requirements and then sent a copy of the consent form to potential participants before the screening visit. At the screening visit, the study physician obtained informed consent from all subjects.

Methods

The 2 trials were performed sequentially by using identical protocols except for the type of OJ (regular or lite). Both the regular and lite OJ trials were designed to randomly assign 72 subjects (36 participants per group) to achieve a final sample size of 64 participants (32 participants per group), based on a 12% predicted dropout rate. Participants were recruited between July 2005 and May 2006 for the regular OJ trial and between May 2006 and December 2006 for the lite OJ trial. The inclusion and exclusion criteria, recruitment strategies, and study personnel were the same for both trials. Throughout both trials, investigators and participants were blinded as to the treatment group. The Institutional Review Board of Massachusetts General Hospital approved the study.

After the screening visit, participants were randomly assigned to control or treatment groups via a computer-generated randomization code. The study files were maintained in a locked file cabinet in a secured clinical office. Commercially available beverages were blind-packaged and provided in color- and number-coded containers by the manufacturer (Minute Maid) to maintain the double-blind study design. The blinded color coding of groups was broken after the completion of each trial. The regular-OJ and lite-OJ trials primarily differed only in the energy content of the 2 beverages; both included the same amount of CaD (Table 1). Each participant received a juice delivery every 2 wk.

In the regular trial, 88 eligible subjects were randomly assigned to drink 240 mL regular OJ (regular control group) or regular OJ fortified with 350 mg Ca and 100 IU vitamin D3 (regular CaD group) 3 times/d for 16 wk. Regular juice provided 110 kcal/240 mL serving (Table 1). Each participant received a juice delivery every 2 wk.

In the lite trial, 83 eligible subjects were randomly assigned to drink 240 mL of the reduced-energy (lite) OJ beverage (lite control group) or the lite OJ beverage fortified with 350 mg Ca and 100 IU vitamin D3 (lite CaD group) 3 times/d for 16 wk. The lite beverage provided 50 kcal/240 mL (Table 1). The energy content of the lite beverage was lowered by reducing the juice content, refortifying the major key nutrients to the level found in regular OJ except for folate, and using flavorings and non-

TABLE 1

Constituents of the regular and lite OJ beverages for the control and CaD-supplemented groups

<table>
<thead>
<tr>
<th>Nutrient per 240 mL</th>
<th>Regular OJ</th>
<th>Lite OJ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>CaD</td>
</tr>
<tr>
<td>Total energy (kcal)</td>
<td>110</td>
<td>110</td>
</tr>
<tr>
<td>Total carbohydrate (g)</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>450</td>
<td>450</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>20</td>
<td>350</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>Vitamin D3 (IU)</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Thiamine (mg)</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Folate (µg)</td>
<td>60</td>
<td>60</td>
</tr>
</tbody>
</table>

CaD, calcium and vitamin D; lite, reduced energy; OJ, orange juice.
nutritive sweeteners to maintain the taste profile. Current regulations do not allow for the addition of folic acid to juices.

The fortified beverages contained vitamin D3 and a combination of calcium phosphate and calcium lactate. We determined estimates of potential weight change over the 16-wk trial period from a study of healthy obese adults (mean BMI: 35 ± 4.1) (5). The estimated projected mean (±SD) weight changes at 16 wk were 4.3 ± 2.58 kg for the control group and 5.7 ± 1.6 kg for the CaD group for both trials. The regular and lite OJ trials were each powered to have an 80% likelihood of detecting a net difference of 1.4 kg (with an estimated 16-wk change of 2.0 kg between the 2 groups).

Participants in both the regular and lite OJ trials had 6 outpatient visits at the study center: a screening visit, a baseline visit (~2–4 wk later (week 0), and follow-up visits every 4 wk thereafter until week 16. A comprehensive physical examination that included a fasting laboratory evaluation was performed at the screening and final visits. At each visit, weight was measured on the same scale, which was calibrated monthly. A urine pregnancy test was performed at each visit for women of childbearing age. Height and waist and hip circumferences were measured at the screening and final visits. Waist circumference was measured with the patient standing, at a level midway between the iliac tubercle and lower lateral rib margin, and hip circumference was measured at the level of the iliac tubercle. At baseline and 16 wk, a CT scan of the abdomen was performed to assess visceral and subcutaneous fat compartments (a single slice at the level of the lumbar vertebra). The total abdominal cross-sectional area was computed by outlining the outer contour of the abdomen. A second outline of the back and abdominal wall musculature (inner contour) was used to define the area of subcutaneous fat. Intraabdominal fat mass was expressed as VAT and was defined as the area within the inner contour. These values were used to calculate the area of SAT, VAT, and total adipose tissue (25).

In both studies, physical activity and caloric reduction were standardized to avoid masking any intervention effect of calcium and vitamin D supplementation on weight loss and body-fat changes. At the screening visit, each participant met with a diettitian to establish his or her daily caloric intake goal. Individual goals were calculated to be 500 kcal less than the participant’s maintenance caloric requirement as estimated by the Harris-Benedict equation (26) and physical activity factors (light = 1.375, moderate = 1.55, very active = 1.725). The study diettitian instructed participants on how to limit dairy and dietary calcium intake to ≤2 servings/d and how to estimate calories consumed by using a 3-d food record (2 weekdays and 1 weekend day each week) to remain within the caloric intake goal. Subjects were given a step counter to record the number of steps taken daily and were instructed to use this device for ≥3 d each week. All participants were encouraged to aim for ≥10,000 steps/d.

At each monthly clinic visit, participants attended a group nutrition lesson focused on the principles of healthy eating and exercise. At that time, each participant’s 3-d food intake diaries

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### TABLE 2

Demographic and anthropometric characteristics of the control and CaD-supplemented groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regular OJ</th>
<th>CaD</th>
<th>Lite OJ</th>
<th>CaD</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>39 ± 14</td>
<td>40</td>
<td>43 ± 11</td>
<td>39</td>
<td>41 ± 12</td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>88</td>
<td>77</td>
<td>95</td>
<td>95</td>
<td>91</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>78.9</td>
<td>78.8</td>
<td>73.8</td>
<td>85.4</td>
<td>75.9</td>
</tr>
<tr>
<td>Black</td>
<td>10.5</td>
<td>9.1</td>
<td>9.5</td>
<td>9.8</td>
<td>10.1</td>
</tr>
<tr>
<td>Hispanic</td>
<td>2.6</td>
<td>6.1</td>
<td>7.1</td>
<td>0</td>
<td>5.1</td>
</tr>
<tr>
<td>Other</td>
<td>7.9</td>
<td>3.0</td>
<td>9.5</td>
<td>4.9</td>
<td>8.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>81.7 ± 10.0</td>
<td></td>
<td>79.9 ± 9.0</td>
<td>78.2 ± 10.9</td>
<td>80.2 ± 9.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.8 ± 2.8</td>
<td>30.0 ± 2.6</td>
<td>30.0 ± 2.7</td>
<td>30.0 ± 2.7</td>
<td>29.9 ± 2.7</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>91.7 ± 10.5</td>
<td>90.8 ± 7.7</td>
<td>90.3 ± 8.8</td>
<td>91.2 ± 8.9</td>
<td>92.0 ± 9.7</td>
</tr>
<tr>
<td>Total abdominal fat (cm³)</td>
<td>408.8 ± 128.6</td>
<td>448.7 ± 111.9</td>
<td>432.7 ± 111.1</td>
<td>429.4 ± 121.1</td>
<td>449.8 ± 111.2</td>
</tr>
<tr>
<td>SAT (cm²)</td>
<td>316.1 ± 92.4</td>
<td>357.0 ± 92.8</td>
<td>545.5 ± 88.1</td>
<td>337.3 ± 94.3</td>
<td>349.8 ± 88.9</td>
</tr>
<tr>
<td>VAT (cm³)</td>
<td>92.7 ± 61.5</td>
<td>91.7 ± 45.7</td>
<td>87.2 ± 55.5</td>
<td>92.2 ± 53.5</td>
<td>100.0 ± 65.7</td>
</tr>
</tbody>
</table>

* Differences between the control and experimental groups at baseline were tested by using unadjusted, independent-sample, 2-sided t tests. *Significantly different from the control value, *P* < 0.05. CaD, calcium and vitamin D; lite, reduced energy; OJ, orange juice; SAT, subcutaneous adipose tissue; V AT, visceral adipose tissue.

1 Mean ± SD (all such values).
and step-counter logs were also reviewed by the dietitian. Although the determination of dietary calcium and vitamin D intakes was not performed by analyzing food records, participants consumed <2 dairy product servings/d. Therefore, at time of study entry, calcium intake was estimated to be ~600–800 mg/d. During the 2 trials, control participants continued to receive ~600–800 mg Ca/d, and the treatment groups received an additional 1030 mg Ca/d from juice. Step-counter logs were reviewed, but steps were not totaled to estimate physical activity. The study coordinator and study physician assessed compliance with study beverage consumption and evaluated each participant for adverse effects and any need to adjust medications.

Statistical analyses

The primary outcome of interest was a significant loss of body weight, and the secondary outcome measure was a significant reduction of VAT. Comparisons of changes between the control and treatment groups were made by using unadjusted, independent-sample, 2-sided t tests by using SPSS 15 software (SPSS Inc). Change in body weight and VAT were adjusted for baseline variables found to be different between groups by using ANCOVA. Only participants who completed the studies were included in the data analysis. In addition, because the regular and lite studies followed the identical protocol and were essentially the same study, except for the small difference in caloric content of the beverages (60 kcal/240 mL more in the lite studies), data were also combined for analysis. A secondary analysis of baseline 25(OH)D concentrations compared with baseline and change of BMI, VAT, and SAT at 16 wk was performed by using ANCOVA. All differences were considered significant at the 2 level of P < 0.05.

RESULTS

In the regular OJ trial, we screened 130 subjects; 42 were determined to be ineligible and 88 were enrolled. Of these 88 subjects, 23 withdrew: 1 because of pregnancy, 1 because of a diagnosis of pancreatic cancer, and 21 for nonspecific reasons. Therefore, 65 subjects completed the regular OJ study (34 in the control and 31 in the CaD group). In the lite OJ trial, 123 subjects were screened; 40 were determined to be ineligible and 83 were enrolled. Of the 83 subjects, 17 withdrew: 1 because of flu, 1 because of pregnancy, 2 because of acid reflux, and 13 for nonspecific reasons. Therefore, 66 participants completed the lite OJ study (31 in the control group and 35 in the CaD group). (The CONSORT flow diagrams are available in an online supplement.) Subsequent to completion of the regular and lite OJ studies, an ITT analysis was performed due to the rather high participant dropout rate (20–24%) for unspecified causes. Because biochemical measurements and CT scans were obtained only at baseline and 16 wk, the ITT analysis was performed only for weight changes with all subjects who had at least one weight measurement carried forward.

Regular OJ trial

In the regular OJ trial, age, sex, weight, BMI, waist circumference, and VAT were not statistically different at baseline for the control and treatment groups (Table 2). However, the regular control group had significantly less total abdominal fat at baseline than did the regular CaD group (408.8 ± 128.6 cm² compared with 471.5 ± 109.3 cm²; P < 0.038; Table 2). At baseline, vitamin D concentrations and other biochemical variables were not significantly different (Table 3).

![Table 3: Biochemical variables at baseline in the control and CaD-supplemented groups](https://www.jci.org/articles/view/97115)
TABLE 4
Changes in anthropometric and body-fat variables from baseline to week 16 in the control and CaD-supplemented groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n = 34)</th>
<th>CaD (n = 31)</th>
<th>Control (n = 31)</th>
<th>CaD (n = 35)</th>
<th>Control (n = 65)</th>
<th>CaD (n = 66)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute body weight (kg)</td>
<td>−2.4 ± 3.5†</td>
<td>−2.2 ± 3.0†</td>
<td>−2.3 ± 2.9</td>
<td>−2.9 ± 3.8</td>
<td>−2.4 ± 3.2</td>
<td>−2.5 ± 3.3</td>
</tr>
<tr>
<td>Body weight (%)</td>
<td>−3.1 ± 4.4</td>
<td>−2.5 ± 3.2</td>
<td>−3.3 ± 3.8</td>
<td>−3.9 ± 4.7</td>
<td>−3.1 ± 4.0</td>
<td>−3.2 ± 4.1</td>
</tr>
<tr>
<td>Absolute BMI (kg/m²)</td>
<td>−0.94 ± 1.37</td>
<td>−0.80 ± 0.99</td>
<td>−1.11 ± 1.1</td>
<td>−1.3 ± 1.5</td>
<td>−1.0 ± 1.0</td>
<td>−1.1 ± 1.3</td>
</tr>
<tr>
<td>Absolute waist circumference (cm)</td>
<td>−2.2 ± 4.1</td>
<td>−2.2 ± 3.8</td>
<td>−2.8 ± 2.8</td>
<td>−3.7 ± 4.2</td>
<td>−2.4 ± 3.6</td>
<td>−3.0 ± 4.1</td>
</tr>
<tr>
<td>Total abdominal fat (cm²)</td>
<td>−25.5 ± 58.3</td>
<td>−32.3 ± 44.1</td>
<td>−31.6 ± 41.8</td>
<td>−41.9 ± 61.0</td>
<td>−25.3 ± 51.0</td>
<td>−37.6 ± 54.0</td>
</tr>
<tr>
<td>SAT (cm²)</td>
<td>−18.3 ± 48.4</td>
<td>−19.6 ± 34.3</td>
<td>−25.3 ± 31.6</td>
<td>−28.8 ± 50.9</td>
<td>−21.6 ± 41.1</td>
<td>−24.8 ± 44.1</td>
</tr>
<tr>
<td>VAT (cm²)</td>
<td>−1.3 ± 13.6</td>
<td>−1.27 ± 25.0²</td>
<td>−6.4 ± 17.5</td>
<td>−13.1 ± 18.4¹</td>
<td>−3.7 ± 15.7</td>
<td>−12.9 ± 21.8⁰</td>
</tr>
<tr>
<td>VAT (%)</td>
<td>−4 ± 19</td>
<td>−9 ± 13</td>
<td>−5 ± 20</td>
<td>−16 ± 18⁰</td>
<td>−5 ± 19</td>
<td>−13 ± 16⁰</td>
</tr>
</tbody>
</table>

¹ All values are means ± SDs. Changes in the anthropometric and body-fat variables between the control and experimental groups over time were made by using unadjusted, independent-sample, 2-sided t tests. ²⁰ Significantly different from the control value: *P < 0.05, **P < 0.01. CaD, calcium and vitamin D; lite, reduced energy; OJ, orange juice; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

2 ANCOVA was used to adjust for baseline total abdominal fat in the regular OJ trial. Differences in change in body weight were not significant (adjusted P = 0.755), and differences in the change in VAT remained significant (adjusted P = 0.02).

3 ANCOVA was used to adjust for baseline VAT in the Lite OJ trial. Differences in the change in VAT were significant (P = 0.039).

(Table 5). No significant changes from baseline to the end of the study were noted in other laboratory variables.

Lite OJ trial

In the lite OJ trial, baseline demographic and anthropometric characteristics (age, sex, weight, BMI, waist circumference, and VAT), vitamin D concentrations, and other biochemical variables were not significantly different between the control and CaD treatment groups (Tables 2 and 3).

After 16 wk, weight, BMI, and waist circumference had not changed significantly in the control and CaD groups (Table 4). In addition, the ITT analysis found no significant difference in weight change between the control and CaD groups (−2.1 ± 2.7 kg compared with −2.8 ± 3.7 kg, respectively; P = 0.3748). After 16 wk, however, VAT had changed significantly (P < 0.024) between the control (−5 ± 20%) and CaD (−16 ± 18%) groups (Table 4). In addition, we noted a trend toward a greater absolute reduction in VAT (control group: −6.4 ± 17.5 cm²; CaD group: −13.1 ± 18.4 cm²; P = 0.142). In the adjusted model, the change in VAT was significantly different between the 2 groups (P = 0.039) after baseline VAT was controlled for.

As in the regular OJ trial, the percentage change in vitamin D concentrations after 16 wk changed significantly (P = 0.015) between the control (−14 ± 41%) and CaD (11 ± 40%) groups (Table 5), which provided strong objective support for participant compliance with consuming the CaD-supplemented beverages. Other biochemical laboratory variables did not change significantly from baseline to the end of the study.

Combined trials

At baseline, the combined mean BMI was 30.4 ± 2.8, and the mean age was 40.0 ± 12.9 y. After 16 wk, we found a significant decrease in VAT in the CaD group when compared with the control group (control group: −3.7 ± 15.7 cm²; CaD group: −12.9 ± 21.8 cm²; P = 0.007). However, as for the data from the regular and lite trials, the combined data showed no

TABLE 5
Changes in biochemical variables from baseline to week 16 in the control and CaD-supplemented groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n = 34)</th>
<th>CaD (n = 31)</th>
<th>Control (n = 31)</th>
<th>CaD (n = 35)</th>
<th>Control (n = 65)</th>
<th>CaD (n = 66)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D (ng/mL)</td>
<td>0.3 ± 10.1</td>
<td>4.9 ± 15.0</td>
<td>−7.0 ± 14.4</td>
<td>2.1 ± 11.7***</td>
<td>−3.1 ± 12.7</td>
<td>3.4 ± 13.3***</td>
</tr>
<tr>
<td>Change in 25(OH)D (%)</td>
<td>3 ± 42</td>
<td>34 ± 75⁰</td>
<td>−14 ± 41</td>
<td>11 ± 40⁰</td>
<td>−5 ± 42</td>
<td>22 ± 60⁰</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>−0.5 ± 0.3</td>
<td>−0.1 ± 0.4</td>
<td>−0.01 ± 0.3</td>
<td>−0.05 ± 0.4</td>
<td>−0.03 ± 0.3</td>
<td>0.02 ± 0.4</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>9.5 ± 11.0</td>
<td>7.5 ± 12.8</td>
<td>4.6 ± 12.6</td>
<td>5.5 ± 12.2</td>
<td>7.2 ± 11.9</td>
<td>6.5 ± 12.4</td>
</tr>
<tr>
<td>TSH (μU/mL)</td>
<td>0.4 ± 1.5</td>
<td>−0.2 ± 1.2</td>
<td>0.12 ± 1.02</td>
<td>0 ± 0.7</td>
<td>0.3 ± 1.31</td>
<td>−0.07 ± 1.0</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>0.6 ± 7.8</td>
<td>0.8 ± 11.4</td>
<td>1.4 ± 12.4</td>
<td>0.03 ± 4.3</td>
<td>1.0 ± 10.1</td>
<td>0.4 ± 9.4</td>
</tr>
<tr>
<td>Insulin (μU/mL)</td>
<td>0.09 ± 3.6</td>
<td>0.2 ± 4.6</td>
<td>4.3 ± 23.9</td>
<td>−0.3 ± 24.3</td>
<td>2.0 ± 16.4</td>
<td>−0.5 ± 4.4</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>−2.0 ± 24.4</td>
<td>−1.07 ± 10.0</td>
<td>−4.9 ± 29.5</td>
<td>−3.5 ± 24.2</td>
<td>−3.8 ± 26.7</td>
<td>−6.9 ± 27.6</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>−1.7 ± 7.9</td>
<td>−2.9 ± 10.0</td>
<td>−4.9 ± 11.5</td>
<td>−0.3 ± 8.1</td>
<td>−3.2 ± 9.8</td>
<td>−1.5 ± 9.0</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>3.0 ± 44.5</td>
<td>−5.0 ± 27.9</td>
<td>−9.2 ± 36.1</td>
<td>−14.1 ± 36.4</td>
<td>−2.6 ± 41.0</td>
<td>−9.8 ± 32.7</td>
</tr>
</tbody>
</table>

¹ All values are means ± SDs. Changes in biochemical variables between the control and experimental groups over time were made by using unadjusted, independent-samples, 2-sided t tests. ²⁰ Significantly different from the control value: *P < 0.05, **P < 0.01. CaD, calcium and vitamin D; lite, reduced energy; OJ, orange juice; PTH, parathyroid hormone; TSH, thyroid-stimulating hormone; 25(OH)D, 25-hydroxyvitamin D.
significant weight change or other changes in anthropometric variables between groups (Table 4). Similarly, the ITT analysis yielded no difference in weight change between the control (−2.2 ± 3.0 kg) and CaD (−2.5 ± 3.3 kg) groups (P = 0.5783).

In the secondary analysis of the combined data, we found a significant negative relation between baseline 25(OH)D concentrations and baseline BMI (P = 0.011) and VAT (P = 0.026), but not with SAT (P = 0.765). However, after 16 wk, we did not find a significant association between baseline 25(OH)D and changes in BMI, SAT, or VAT.

DISCUSSION

The regular OJ and combined OJ trials showed that CaD supplementation reduced VAT without affecting the loss in body weight, other objective body measurements (eg, waist circumference), or other laboratory correlations with obesity (eg, blood lipids, glucose, and insulin concentrations). In the lite trial, there was a trend toward reduced VAT that was statistically significant in the adjusted model when baseline VAT was controlled for (P = 0.039). When the 2 trials were combined, CaD supplementation explained a >3-fold reduction in VAT as compared with the control. Thus, these randomized controlled trials provide further evidence of a role for calcium and vitamin D supplementation in the selective reduction of visceral fat and indicate that calcium and/or vitamin D may contribute to the regulation of lipid metabolism and/or fat distribution.

Despite the differences in caloric content of the regular and lite OJ beverage formulations, final weight loss and total intra-abdominal fat reduction were not significantly different between the 2 trials, which indicated that the minor calorie difference (ie, 60 kcal/240 mL) between the regular and lite OJ did not influence the study outcome. On average, participants lost ~5 lb (~2.25 kg) over 4 mo, consistent with the study goal to have a modest behavioral intervention so as not to mask any intervention effect. As in many other weight-loss studies, the weight loss varied among individuals; some participants lost very little weight and others losing a substantial amount. The participants’ compliance with the calorie and exercise goals of the study contributed to the variation and influenced the mean absolute body weight loss across all study groups, which ranged from −2.2 kg to −2.9 kg at the end of 16 wk.

Our data support those of other studies that failed to show an effect of calcium on weight loss (27–30). Prospective studies that have examined the effect of both calcium and vitamin D on body weight and/or abdominal fat have been inconclusive (23, 24). Calcium plus vitamin D had a small effect on the prevention of weight gain over 3 y (23). In contrast, high vitamin D (3400–11,400 IU/d) supplementation levels were not associated with significant changes in body weight, percentage fat mass, or waist-hip ratios in overweight and/or obese subjects (31, 32).

Baseline serum 25(OH)D concentrations in the regular and lite OJ trials did not predict subsequent weight loss at the end of 16 wk. Similarly, in the 2-y Dietary Intervention Randomized Controlled Trial (DIRECT), baseline 25(OH)D concentrations decreased significantly across the tertiles of baseline BMI; however, baseline concentrations of vitamin D and dairy calcium intake were not associated with subsequent weight loss (33).

Previously, studies have relied on BMI, body fat mass assessed by dual energy X-ray absorption, or bioelectric impedance to support a relation between 25(OH)D and measures of adiposity. More recently, studies such as ours have examined this relation by using volumetric quantification of SAT and VAT compartments. In the regular and lite OJ trials, our baseline 25(OH)D concentrations were inversely associated with baseline BMI and VAT but not with SAT. In other studies, volumetric quantification found SAT and VAT to be highly correlated and independently associated with 25(OH)D (34). Likewise, the third-generation Framingham Heart Study noted that higher BMI was associated with lower vitamin D concentrations (35). In addition, other investigators reported a strong negative correlation between 25(OH)D concentrations and CT measures of VAT in obese adolescents (36) and with VAT and SAT in young women (37).

Whereas CaD supplementation in the combined trials resulted in a significant reduction of VAT (CaD group: −13%; control group: −5%; P = 0.011), the reduction of VAT with CaD supplementation was not associated with changes in fasting glucose, insulin, or lipids at the end of 16 wk. Intraabdominal fat has been shown to be independently associated with elevated triglycerides, low HDL, hypertension, and glucose intolerance (35, 38), all of which are associated with the metabolic syndrome. Selective reduction of intraabdominal fat by vitamin D or calcium supplementation, alone or in combination with other interventions, could help improve signs of the metabolic syndrome (34). In contrast with our findings, supplementation with vitamin D for 6 mo led to significant improvement in insulin resistance (39), short-term megadoses of vitamin D improved postprandial insulin sensitivity in centrally obese but non-diabetic men (40), and daily intake of vitamin D or a vitamin D plus calcium yogurt drink for 12 wk improved glycemic status in type 2 diabetes (41).

Inadequate vitamin D may also promote greater adiposity through other metabolic effects, such as regulation of PTH and modulation of adipogenesis. Moderate to severe vitamin D deficiency leads to increased PTH, which may promote an increase in free intracellular calcium into adipocytes and, thereby, enhance lipogenesis (42). In our 2 trials, any potential participant with a vitamin D deficiency [serum 25(OH)D <10 ng/mL] on screening evaluation was excluded; therefore, the likelihood of participants having elevated PTH concentrations was minimized. Moreover, mean PTH values ranged from 36 to 44 pg/mL (well within the normal range of 10 to 55 pg/mL), and the observed reductions in VAT were not statistically correlated with changes in PTH in this study.

The strengths of the current study include the use of a double-blind, randomized clinical trial design and assessment of study compliance through monthly reviews of food diaries and step-counter logs. Likewise, expected increase in 25(OH)D concentrations in the CaD treatment groups support good participant compliance. In addition, volumetric quantification of SAT and VAT by using CT increased the ability to detect small changes in abdominal fat compartments. Combining the results of the 2 trials helped to demonstrate a highly significant effect of Ca and D on total VAT (P = 0.007). Furthermore, use of commercially available calcium and vitamin D orange juices increased the potential application of study findings to the general population.

Several limitations of the study should be noted. The recruitment of primarily white women limited the ability to generalize findings to men or other ethnic groups. We did not perform actual estimates of dietary calcium and vitamin D intakes or
calculations of physical activity based on step-counter logs. Each trial lasted for only 16 wk, and a longer study may have resulted in favorable improvements in biochemical indexes associated with the metabolic syndrome. The significant difference in total abdominal fat at baseline in the regular OJ trial was another limitation; however, ANCOVA adjustment confirmed that the decreased VAT of the CaD group remained significant. Although there was only a trend toward a significant VAT reduction in the lite OJ study, the VAT reduction remained significant in the adjusted model after baseline VAT was controlled for. Finally, the provision of both calcium and vitamin D limited the ability to clearly separate contributions of calcium and vitamin D to the observed reduction in VAT.

In summary, our results suggest that, in overweight and obese adults, a moderate reduction in energy intake and supplementation of calcium and vitamin D in juice beverages lead to a reduction in intraabdominal fat. A large portion of the population is deficient in vitamin D, and dietary calcium intake often does not meet current recommendations. Although more needs to be learned about the role of calcium and vitamin D in lipolysis, the data underscore the possible role of these 2 nutrients in fat metabolism and support a potential role of calcium and vitamin D in the preferential and beneficial reduction of VAT, which has been linked to several metabolic disorders.

The authors’ responsibilities were as follows—LMK: conceived and designed the research and was principally responsible for writing the manuscript and for the final content; ILR: designed the research and was principally responsible for conducting the research, analyzing the data, and writing the manuscript; CEM: contributed to writing the manuscript; and VMC: was principally responsible for conducting the research and the outcome of short-term weight management. J Clin Endocrinol Metab 2005;89:1313–20.

REFERENCES