Effects of Dairy Calcium Supplementation on Adiposity Plasma Leptin and Glucose in Obese Postmenopausal Women

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Authors’ contributions

This work was carried out in collaboration between all authors. Author DHF conducted of the study. Author CJLK assisted in data interpretation and supervised first drafts of study findings and provided editorial review of the manuscript. Author WLB oversaw all of the statistical analyses of the study and editorial review of the manuscript. Author TAD performed as screening participants for the study and collection of all clinical data. After the medical examination, he cleared the volunteers to participate in the study. Author AF did significant editing of the manuscript. Author ZRCM was principal investigator of the study. Author ZRCM developed and implemented the research protocol and editorial review of the manuscript. All authors read and approved the final manuscript.

ABSTRACT

The inclusion of low or non-fat dairy products which additional calcium in the diet may promote increased weight loss and improve insulin resistance. Therefore supplementing dairy products to obese subjects on a caloric restricted diet may be a useful strategy to enhance weight loss and improve insulin resistance. We therefore tested the short-term effects of supplementing 56 overweight or obese (body mass index [BMI] >26 kg/m²) post menopausal women on a caloric restricted diet (1,400 kilocalories [kcal]) with two levels of dairy as yogurt on body composition, blood insulin, leptin and glucose concentration. The group consuming four supplemented dairy...
servings (DS-4) group were provided ~1400 mg Ca/day, and the group consuming two supplemented dairy servings (DS-2) were provided ~800 mg Ca/day. Over the 3-months daily energy intake averaged 51% carbohydrate, 20.7% of protein and 27.6% of fat for both groups. At 3 months, the DS-4 group demonstrated decreased weight (87.7 to 86.2 kg, \(P=0.001\)), BMI (33.5 to 32.8 kg/m\(^2\), \(P = 0.001\)), total fat (36.1 to 34.7 kg, \(P < 0.001\)), trunk fat (18.3 to 17.6 kg, \(P < 0.001\)). There were non-significant decreases in plasma glucose (74.7 to 71.1 mg/dl, \(P = 0.494\)), leptin (32.5 to 31.3 µg/L, \(P = 0.231\)) and insulin. For the DS-2 group there was decreased weight (86.4 to 84.4 kg, \(p<0.02\)), BMI (32.5 to 31.8 kg/m\(^2\), \(P=0.002\)), total fat (37.3 to 35.4 kg, \(P=0.003\)), trunk fat (17.1 to 16.5 kg, \(P = 0.27\)) and plasma leptin (27.8 to 25.2 µg/L, \(P=0.114\)). The DS-2 group demonstrated a surprising and significant increase in the fasting blood glucose with a marginal significant increase in insulin resistance as measured by HOMA at 3 months. We observed a significant treatment effect between the DS-2 and DS-4 groups for: % energy from fat (\(P=0.025\)), % energy from protein (\(P =0.047\)) and leptin (\(P = 0.044\)). Our study demonstrated the expected weight loss with caloric restriction but a paradoxical increase in blood glucose levels with dairy supplementation provided to maintain baseline calcium intake. Increasing dairy supplementation abrogated this small increase in fasting blood glucose and insulin resistance. The benefits of dairy calcium supplementation may be dependent on both the dose and the context of over all caloric intake.

**Keywords:** Hormones; calcium; weight loss; body composition; dual x-ray absorptiometry; human study.

**ABBREVIATIONS**

- BIA : Bioelectrical Impedance Analysis
- BMI : Body Mass Index
- Ca : Calcium
- CHO : Carbohydrate
- CV : Coefficients of Variance
- DXA : Dual x-ray Absorptiometry
- DS-2 : Dairy 2 Servings
- DS-4 : Dairy 4 Servings
- HOMA-IR : Homeostatic Model Assessment Insulin Resistance
- Kcal : Kilocalories
- Kg : Kilograms
- NPQ : Nutrition Profile Questionnaire
- PAQ : Physical Activity Questionnaire

**1. INTRODUCTION**

Obesity is a pandemic health problem [1] associated with increased risk for several diseases, (e.g., heart disease, diabetes and other metabolic disorders). We now have an understanding that regulatory mechanisms for body weight and total body fat are achieved in part by signals which act primarily as determinants of satiety to self-limit the size of meals [2]. These short term signals have distinctive functions that differ from the long term regulators of energy homeostasis, i.e., insulin and leptin, which respond to energy consumed over prolonged periods of time to maintain body weight within narrow limits [2]. However, the short term signals can be overridden by the long term regulating signals. That is, the short term versus long term regulators have separate and distinctive functions.

The animal study of Shi et al. [3] demonstrated that low dietary calcium increased blood glucose and insulin and these increases were prevented by medium and high dairy calcium diets [3]. It was hypothesized that a diet low in dietary calcium could exhibit a diabetegenic effect.

Several human studies have provided evidence for an effect of dietary dairy products in increasing insulin sensitivity and modulating weight gain [4-7]. The Coronary Artery Risk Development in Young Adults (CADIA) study of young overweight adults, black and white, demonstrated that dietary patterns characterized by increased dairy consumption had an inverse association with insulin resistance in their study population. The longer term implications were that a high dairy diet could reduce risk for type 2 diabetes and cardiovascular disease [5]. Following on these studies, other research groups have provided evidence that dairy calcium functions as a short term signal for regulating insulin sensitivity.

Harkness and Boony [8] proposed that low serum calcium and vitamin D may be a risk factor for abnormalities in glucose homeostasis. The avenue for this control of glucose may involve more than one pathway, as extracellular calcium appears to be necessary for insulin-stimulated
leptin secretion and uptake or release from endogenous stores [9].

Zemel et al. [7] reported that in the case of obese African-Americans on either a low (500 mg/day) or high (1200 mg/day) dairy calcium diet, there was weight maintenance and the high calcium dairy group had a reduction in circulating insulin. Others have not confirmed that dairy calcium reduces circulating insulin [10] or insulin and leptin [11,12].

In the current study we hypothesized that weight loss combined with a dairy supplement which increases dietary calcium in postmenopausal women would decrease body fat, body weight, plasma glucose and leptin concentrations, and homeostatic model of assessment - insulin resistance (HOMA-IR) compared to weight loss in women maintained on their baseline calcium intake. No previous study has explored weight loss and hormone response to dairy calcium in overweight/obese postmenopausal women.

2. EXPERIMENTAL METHODS

2.1 Subjects

The study was a prospective, 3 month randomized, parallel trial on the effect of non-fat/low fat dairy calcium combined with energy restriction in overweight/obese postmenopausal women. The study by Cordero-MacIntyre et al. [13] was used to calculate the power and percentage of estimated weight loss in the current study.

In the current study, eighty-six overweight/obese postmenopausal women were recruited through mail (volunteers had participated in previous studies and had indicated willingness to be notified if other studies were conducted at Loma Linda) and flyers were posted in Loma Linda and Riverside, CA in a variety of areas including clinics, beauty stores, post offices and churches. Subjects were interviewed by the research coordinator and the decisions regarding suitability for participation in the study were made in consultation with the principal investigator.

Initial screening was conducted through telephone interviews and group meetings with the investigators during which the study was explained, consent forms signed, and brief medical, physical activity, and diet histories were obtained. A physician obtained a more extensive medical history during follow-up sessions, along with a physical examination, and cleared the participants for participation in the study.

The inclusion criteria were as follows: age (37-75 and either surgically or naturally postmenopausal); sex (female); BMI (>25 kg/m²), stable diabetes status (known to have diabetes type II for no more than five years); stable medication for at least 3 months and dietary habits (omnivores and lacto-ovo-vegetarians).

Exclusion criteria included: vegans; < 2 servings/day of dairy products, participation in any weight loss program, history of active alcohol and drug abuse, impaired mental condition, current glucocorticoid therapy use, history of hypersensitivity to dairy products present in the research diet, clinically relevant cardiovascular disease, pacemaker, hepatic, neurologic, endocrine; or other major systemic disease.

A meeting was held for all recruited subjects in which the trial was again explained in full detail. Subjects were consented and then evaluated by the study physician. Qualified subjects were instructed to discontinue use of any calcium supplements two weeks before the beginning of the study. All participants were provided with instructions on maintaining a caloric restricted diet to provide 1400 kcal per day. Participants were then randomized using a randomization table [14]. Starting randomly in the table each subject was assigned to either group (DS-4) or group (DS-2) depending on whether the random number was even or odd. Participants in the DS-4 group (~1400 mg/day of Ca) were instructed to consume the equivalent of four 8 oz servings of plain yogurt and the DS-2 group (~800 mg/day of Ca) to consume an equivalent of two 8 oz servings (each 350 mg of calcium) plain yogurt daily. The participants were allowed to have any non/low fat dairy products as long as they maintained the dietary calcium requirements of this study.

Blood, anthropometrics (height, weight, waist and hip circumferences), bioelectrical impedance analysis (BIA) and dual X-ray absorptiometry (DXA) were performed at baseline before the intervention and again at 3 months. Classes were offered once a month to reinforce compliance. These classes covered skills in changing eating habits, stress management, exercise and nutrition guided by the recommendations of the American Diabetes Association [15]. The diet provided carbohydrate (CHO) 50-55%, protein 15-20% and fat <30%
content. The study was approved by Loma Linda University and the University of Connecticut Institutional Review Boards.

2.2 Total Dietary and Dairy Intake

Dietary intake was measured by Nutrition Profile Questionnaires (NPQ), (Health Awareness Series, Wellsource Inc. of Clackamas, OR), once at baseline and again at 3 months. Three 24-hr recalls were collected from the participants by trained individuals during the intervention (2 weekdays and one weekend during the 3 months). Calcium intake was assessed using the NPQ food frequency questionnaire that was administrated once at baseline and again at 3 months. Subjects also noted their daily dairy intake on forms provided, which included the serving size, type of dairy, flavor and amount. A booklet with most of the dairy products with amounts of calcium, calories, and fat was given to each volunteer to help them make correct choices and estimate their daily calcium intake with different dairy products. The coefficients of variance (CV) for the macronutrients and calcium consumed for all participants were % Energy Carbohydrates: 0.171, % Energy Fat: 0.277, % Energy Protein: 0.231, and % Dairy Calcium: 0.621.

Physical Activity Questionnaires (PAQ), (Health Awareness Series, Wellsource Inc. of Clackamas, OR), which volunteers were trained to fill out at the first interview, were completed once a month. The PAQ included questions regarding the type, frequency and intensity of the different forms of exercise. Examples of calorie restricted menus with DS-4 versus DS-2 were handed out to the participants on the meeting day when the registered dietitian explained how to reduce calories and include dairy.

2.3 DXA Protocol and Quality Control of DXA Data

Whole body and regional scans were made with a QDR 4500-A densitometer (Hologic Inc., Waltham, MA). Certified technicians using standard subject positioning and data acquisition protocols carried out whole body scans. The scan time was approximately three minutes, and radiation exposure was 1.5 mrems (0.00015 sieverts). Follow-up scans were performed at 3 months using the standard protocol. The same trained technicians, using software version 8.24a, performed the analysis of the scans at baseline and again at three months [16].

2.4 Anthropometry

Anthropometric measurements including height, weight, and circumferences (waist, hip) were made to further characterize body shape and composition and muscle and fat distribution. All anthropometric measurements were made according to the Anthropometric Standardization Reference Manual [17]. Each of the measurements was taken three times by trained research assistants and these were then averaged. Trained individuals were assigned to carry out each of the anthropometric measurements. For each subject the measurements at baseline and at 3 months were carried out by the same research assistant. The CVs for body composition measurements were as follows: weight= 0.207; BMI= 0.195; waist circumference= 0.154; trunk fat= 0.367; and total body fat= 0.305.

2.5 Plasma Insulin

Plasma insulin was measured by radiommmunoassay using guinea pig antibodies to the porcine $^{125}$I-insulin (Linco Research Inc., St. Louis, MO) radioactive tracer. For this assay, the normal fasting range is 5-15 µU/ml and the difference between duplicate results of a sample should be <10%. The plasma samples and a human insulin standard were incubated with antibody and tracer for 4 hours at room temperature. Antibody-bound insulin was precipitated by a second antibody (goat anti-guinea pig gamma globulin), 10% guinea pig serum and polyethylene glycol. The precipitated complex was counted in a gamma counter. Insulin was calculated using a standard curve [18]. The CV for insulin concentrations was 0.767.

2.6 Plasma Leptin

Plasma leptin was measured using a competitive-binding radioimmunoassay (Linco Research Inc., St. Louis, MO). The mean leptin values (BMI ranges 18-25) in lean women is reported as 7.4±3.7 µg/L.

2.7 Plasma Glucose

Plasma glucose concentrations were determined in duplicate with an enzymatic method using kits from Wako Diagnostics [19]. The normal values for fasting glucose are 70-110 mg/dL. Plasma glucose samples were added to the working solution mixed well, and incubated for 5 minutes
absorbance was measured at 505 nm. The CV for plasma glucose was 0.179.

2.8 Homa-IR

The homeostatic model assessment (HOMA) is used to estimate insulin resistance and β-cell function [20]. The equation for insulin resistance was derived by use of the glucose-insulin product, divided by a constant: glucose (mmol/L) × insulin (mIU/L) / 22.5.

2.9 Statistical Analyses

All data were entered into a computer master file and analyzed using SPSS V12.0 (SPSS, Inc. Chicago, IL) for Windows. Double entry of data and cross-checking was carried out to assure error-free data. Generalized linear mixed models (SAS Inst., Cary, NC, version 9.4, – Proc Mixed) were also used to evaluate the treatment effect (DS-4 calcium group versus DS-2 calcium group) regarding changes in weight and other anthropometric measurements over time because of the repeated measures design. We used paired t-tests to compare three-month group means ± standard deviations (SD) to baseline group means ± SD within groups. All of the variables analyzed with the t-test were within acceptable limits of the underlying assumption of following a Gaussian distribution using the Kolmogorov-Smirnov normality test [21]. All t-test analyses were done 2-tailed. The p-values from a comparative non-parametric Wilcoxon Signed-rank test did not differ appreciably from those of the t-tests. The chi-square test was used to compare the distribution of the demographics of the SD-2 calcium group to the DS-4 calcium group at baseline. Analysis of variance was used to compare the mean weight loss ± SD among dairy tertiles. P-values < 0.05 were considered to be statistically significant.

3. RESULTS

Fifty-six of the original 86 subjects completed this 3 month intervention. Table 1 shows the demographics of the subjects in both groups. Both the DS-4 and DS-2 groups had a randomized sample size of 28. This table further shows the distribution of the subjects’ ages, races, education levels, occupations, hormone replacement therapies, and dietary habits for each group. The results demonstrate that the randomization adequately balanced these characteristics between the two study groups. Table 2 shows the average macronutrient and calcium intake from dairy products for the subjects in both groups at baseline and during the 3 months of the intervention. Macronutrient intakes for both groups during the intervention were consistent with their habitual diets. During the intervention the subjects were in compliance with the guidelines for macronutrient intake. For both groups combined, the mean macronutrient composition during the intervention was 51% of calories from carbohydrates, 27.6% of calories from fat and 20.7% of calories from proteins. At baseline both groups’ intakes of dairy products was >2 servings/day (~800 mg calcium). During the 3 month intervention the subjects had good compliance with the amount of dairy intake depending on their assigned group. The DS-4 group consumed ~1400 mg calcium daily and the DS-2 group consumed ~800 mg calcium daily. Both groups had a significant reduction in calorie intake (p<0.01) over the 3-month follow-up as expected because of the calorie restricted diets, whereas only the DS-4 group had a statistically significant increase in dietary calcium intake in the same follow-up period.

Table 3 shows anthropometric data for subjects in both groups during the intervention. Weight, BMI, and total body fat showed a significant reduction over time for both groups. Trunk fat was significantly decreased in the DS-4 group but not in the DS-2 group.

Table 4 shows the concentrations of glucose, insulin and leptin in both groups during the intervention. The DS-2 group had a significant increase in plasma glucose at 3 months while there were no other significant changes in the other parameters over time in either group, aside from the marginally significant increase in insulin resistance (HOMA-IR) in the DS-2 group.

Table 5 shows the distribution of change in dairy calcium intake (3 months - baseline) for randomly selected study subjects separated into approximate tertiles. The cut points established from the unabridged frequency distribution at approximately 33% and 66% were zero and ~500 mg respectively of additional calcium intake during the intervention. There were 31.4% of the randomly selected subjects in the < 0 (i.e. negative) group which means that these 11 subjects (8 DS-2 calcium and 3 DS-4 calcium groups) decreased their intake of calcium at 3 months compared to baseline which was in opposition to study protocol. The middle group of 37.1% subjects consumed between 0-500 mg of
additional dairy calcium per day above baseline and the highest tertile of 31.4% subjects consumed >500 mg of additional dairy calcium per day above baseline.

**Table 1. Demographics of study participants**

<table>
<thead>
<tr>
<th>Variable</th>
<th>DS-2 Group (%)</th>
<th>DS-4 Group (%)</th>
<th>p-value (^{1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>5 (17.9)</td>
<td>5 (17.9)</td>
<td>0.26</td>
</tr>
<tr>
<td>50-59</td>
<td>19 (67.9)</td>
<td>14 (50.0)</td>
<td></td>
</tr>
<tr>
<td>60+</td>
<td>4 (14.3)</td>
<td>9 (32.1)</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>19 (67.9)</td>
<td>19 (67.9)</td>
<td>0.92</td>
</tr>
<tr>
<td>Hispanic</td>
<td>4 (14.3)</td>
<td>4 (14.3)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>3 (10.7)</td>
<td>4 (14.3)</td>
<td></td>
</tr>
<tr>
<td>All others</td>
<td>2 (7.1)</td>
<td>1 (3.6)</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school or less</td>
<td>4 (14.3)</td>
<td>4 (14.3)</td>
<td>0.69</td>
</tr>
<tr>
<td>Some college/Trade school</td>
<td>11 (39.3)</td>
<td>14 (50.0)</td>
<td></td>
</tr>
<tr>
<td>bachelors, masters, doctorate</td>
<td>13 (46.4)</td>
<td>10 (35.7)</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trade/Finance</td>
<td>3 (10.7)</td>
<td>3 (10.7)</td>
<td>0.99</td>
</tr>
<tr>
<td>Health care/medical</td>
<td>12 (42.9)</td>
<td>13 (46.4)</td>
<td></td>
</tr>
<tr>
<td>Services (secretary, operator, hair stylist)</td>
<td>8 (28.6)</td>
<td>7 (25.0)</td>
<td></td>
</tr>
<tr>
<td>other (retired, homemaker)</td>
<td>5 (17.9)</td>
<td>5 (17.9)</td>
<td></td>
</tr>
<tr>
<td>Hormone replacement therapy *</td>
<td>19 (67.9)</td>
<td>18 (64.3)</td>
<td>0.78</td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>9 (32.1)</td>
<td>10 (35.7)</td>
<td></td>
</tr>
<tr>
<td>Diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lacto-ovo-vegetarian</td>
<td>3 (10.7)</td>
<td>5 (17.9)</td>
<td>0.45</td>
</tr>
<tr>
<td>Non-vegetarian</td>
<td>25 (89.3)</td>
<td>23 (82.1)</td>
<td></td>
</tr>
</tbody>
</table>

* herbal over the counter hormone replacement therapy; \(^{1}\) p-values were created using the chi-square statistics

**Table 2. Habitual (Baseline)\(^{1}\) and intervention diet (3 months)\(^{2}\)**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Total calories (^{1}) (Kcals)</th>
<th>% energy CHO</th>
<th>% energy fat</th>
<th>% energy protein</th>
<th>Dairy calcium (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DS-4 (n=28)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1934.4±573</td>
<td>51.5±5.8</td>
<td>25.0±7.0</td>
<td>22.9±5.0</td>
<td>827.8±453.6</td>
</tr>
<tr>
<td>3 Months</td>
<td>1248.6±546</td>
<td>54.3±7.8</td>
<td>25.7±7.4</td>
<td>19.7±4.2</td>
<td>1340±494</td>
</tr>
<tr>
<td>(p)-value (^{2})</td>
<td>(P = 0.003)</td>
<td>(P = 0.345)</td>
<td>(P = 0.814)</td>
<td>(P = 0.074)</td>
<td>(P = 0.012)</td>
</tr>
<tr>
<td><strong>DS-2 (n=28)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2092.8±491.8</td>
<td>49.3±9.6</td>
<td>31.9±6.6</td>
<td>18.6±4.6</td>
<td>835.9±612.7</td>
</tr>
<tr>
<td>3 Months</td>
<td>1353.1±499</td>
<td>49.3±9.5</td>
<td>30.0±8.5</td>
<td>20.4±5.1</td>
<td>861±721</td>
</tr>
<tr>
<td>(p)-value (^{2})</td>
<td>(P = 0.001)</td>
<td>(P = 0.995)</td>
<td>(P = 0.298)</td>
<td>(P = 0.273)</td>
<td>(P = 0.904)</td>
</tr>
<tr>
<td>treatment effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(p)-value (^{3})</td>
<td>(P = 0.628)</td>
<td>(P = 0.229)</td>
<td>(P = 0.025)</td>
<td>(P = 0.047)</td>
<td>(P = 0.151)</td>
</tr>
</tbody>
</table>

\(^{1}\) mean ± standard deviation; \(^{2}\) p-values created using the paired t-test; \(^{3}\) p-values created using the linear mixed methods for repeated measures
Table 3. Weight 1 (kg), BMI 1 (kg/m²), Waist circumference 1 (WC cm), Trunk fat 1 (kg), and Total body fat 1 (kg)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>WC (cm)</th>
<th>Trunk fat (%)</th>
<th>Trunk fat (kg)</th>
<th>Total fat (%)</th>
<th>Total body fat (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS-4 (n=28)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>87.7±16.0</td>
<td>33.5±5.8</td>
<td>98.7±12.9</td>
<td>42.6±4.2</td>
<td>18.3±4.4</td>
<td>42.4±3.3</td>
<td>36.1±7.0</td>
</tr>
<tr>
<td>3 Months</td>
<td>86.2±15.6</td>
<td>32.8±5.7</td>
<td>98.5±13.0</td>
<td>41.2±5.3</td>
<td>17.6±4.4</td>
<td>41.2±4.3</td>
<td>34.7±6.9</td>
</tr>
<tr>
<td>( p)-value(^2)</td>
<td>( P = 0.001 )</td>
<td>( P &lt; 0.001 )</td>
<td>( P = 0.269 )</td>
<td>( P &lt; 0.001 )</td>
<td>( P = 0.216 )</td>
<td>( P &lt; 0.001 )</td>
<td></td>
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<tr>
<td>DS-2 (n=28)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>86.4±18.9</td>
<td>32.5±6.6</td>
<td>95.4±14.9</td>
<td>39.0±7.3</td>
<td>17.1±7.1</td>
<td>41.1±4.8</td>
<td>37.3±12.4</td>
</tr>
<tr>
<td>3 Months</td>
<td>84.4±19.6</td>
<td>31.8±6.9</td>
<td>92.2±15.6</td>
<td>38.3±8.2</td>
<td>16.5±7.6</td>
<td>40.4±5.7</td>
<td>35.4±13.4</td>
</tr>
<tr>
<td>( p)-value(^2)</td>
<td>( P = 0.002 )</td>
<td>( P = 0.002 )</td>
<td>( P = 0.363 )</td>
<td>( P = 0.27 )</td>
<td>( P = 0.243 )</td>
<td>( P = 0.003 )</td>
<td></td>
</tr>
<tr>
<td>Treatment effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( p)-value(^3)</td>
<td>( P = 0.83 )</td>
<td>( P = 0.623 )</td>
<td>( P = 0.299 )</td>
<td>( P = 0.311 )</td>
<td>( P = 0.20 )</td>
<td>( P = 0.438 )</td>
<td>( P = 0.995 )</td>
</tr>
</tbody>
</table>

1 mean ± standard deviation;  
2 \( p\)-values created using paired t-test;  
3 \( p\)-values created using linear mixed methods for repeated measures

Table 4. Glucose (mg/dl)\(^1\), Insulin (µu/mmol)\(^1\), Insulin resistance (HOMA-IR)\(^{1,2}\) (mmol/L) and Leptin (µg/L)\(^1\)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Glucose (mg/dl)</th>
<th>Insulin (µu/mmol)</th>
<th>HOMA-IR (mmol/L)</th>
<th>Leptin (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS-4 (n=28)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>74.7±31.9</td>
<td>21.9±13.3</td>
<td>4.1±3.4</td>
<td>32.5±9.9</td>
</tr>
<tr>
<td>3 Months</td>
<td>71.1±12.2</td>
<td>19.6±17.1</td>
<td>3.5±3.4</td>
<td>31.3±9.6</td>
</tr>
<tr>
<td>( p)-values(^3)</td>
<td>( P = 0.494 )</td>
<td>( P = 0.477 )</td>
<td>( P = 0.27 )</td>
<td>( P = 0.231 )</td>
</tr>
<tr>
<td>DS-2 (n=28)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>63.1±17.5</td>
<td>16.8±11.2</td>
<td>2.7±2.2</td>
<td>27.8±9.9</td>
</tr>
<tr>
<td>3 Months</td>
<td>76.0±13.7</td>
<td>16.2±9.1</td>
<td>3.1±2.1</td>
<td>25.2±11.1</td>
</tr>
<tr>
<td>( p)-values(^3)</td>
<td>( P &lt; 0.001 )</td>
<td>( P = 0.559 )</td>
<td>( P = 0.050 )</td>
<td>( P = 0.114 )</td>
</tr>
<tr>
<td>Treatment effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( p)-values(^4)</td>
<td>( P = 0.206 )</td>
<td>( P = 0.149 )</td>
<td>( P = 0.126 )</td>
<td>( P = 0.044 )</td>
</tr>
</tbody>
</table>

1 mean ± standard deviation;  
2 HOMA-IR: \( IR = \frac{\text{glucose mmol/L} \times \text{insulin µu/L}}{22.5} \)  
3 \( p\)-values created using paired t-test  
4 \( p\)-values created using linear mixed methods for repeated measures
Table 5. Tertiles of dairy calcium change (3 Months-baseline) intake for randomly selected subjects and weight loss (3 Months-baseline). (n=35)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tertiles of calcium change (mg)</th>
<th>DS-2 sample size (n) and %</th>
<th>DS-4 sample size (n) and %</th>
<th>Percent total</th>
<th>Mean ± standard deviation for weight loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy tertiles</td>
<td>&lt;0</td>
<td>(8) 44.4%</td>
<td>(3) 17.6%</td>
<td>31.4</td>
<td>-1.09±2.7</td>
</tr>
<tr>
<td></td>
<td>0-500</td>
<td>(8) 44.4%</td>
<td>(5) 29.4%</td>
<td>37.1</td>
<td>-1.69±3.2</td>
</tr>
<tr>
<td></td>
<td>&gt; 500</td>
<td>(2) 11.1%</td>
<td>(9) 52.9%</td>
<td>31.4</td>
<td>-2.00±2.3</td>
</tr>
</tbody>
</table>

*p-value = 0.025 based on analysis of variance*

Although not statistically significant, likely because of the small sample size, there was an increased weight loss trend as the tertile of additional calcium intake from baseline increased during the intervention which may be clinically important.

The physical activity questionnaire that was completed by participants on a monthly basis during the intervention illustrated that the participants were non-compliant in keeping their usual physical activity and intensity during the intervention.

4. DISCUSSION

In this randomized 3 month weight reduction trial we tested the effects of two levels of dairy supplementation in obese postmenopausal women that either maintained their baseline dietary calcium intake of ~ 800 mg Ca/day (DS-2) or provided an increased dietary calcium of ~1400 mg Ca/day (DS-4) intake. We demonstrated a statistically significant weight loss in both groups. The observed significant reductions for both groups in weight, BMI and total body fat are consistent with previous studies [10-12,22-30]. We found an unexpected and highly significant increase in the blood glucose level in the DS-2 group and marginal insulin resistance despite weight loss.

The resulting blood glucose in the DS-2 group was small and consistent with levels found in the DS-4 at the 3 month period. The finding of an increase in the plasma glucose levels with a dairy supplement and increase in insulin resistance is consistent with a recent study by Tucker et al. [31].

The Tucker study found that women ingesting ~2 servings of dairy had an increase in insulin resistance compared to those ingesting lower servings. In our study increasing dairy intake to 4 servings of yogurt may have provided additional dairy factors such as whey protein and/or calcium to improve insulin resistance and abrogate the increase in plasm glucose. The beneficial effects of whey protein is supported by several studies, in which whey may have an insulinotropic effect and improve postprandial glycemic response [32-39]. The composition of protein in milk-based yogurt is primarily casein and whey proteins, which comprise approximately 80 and 20%, respectively, of the total protein fraction [40].

In this trial, the DS-4 group had a significant reduction in trunk fat and this result is consistent with recent data demonstrating an effect of higher dairy calcium intake in decreasing in trunk fat [11,23,30,41-46]. A possible mechanism in the development and increase in abdominal obesity is the proposed role of autocrine production of cortisol in adipose tissue [7]. Based on this, the effect of calcium on trunk fat loss could reflect adipocyte loss. Human adipose tissue expresses significant 11ß-hydroxysteroid dehydrogenase-1 (11ß-HSD-1) [47,48], producing cortisol from cortisone. The enzyme 11ß-HSD-1 expression is increased in visceral tissue more than in subcutaneous adipose tissue [45,47,48]. It has been determined that 11ß-HSD-1 is elevated in obese individuals [45]. Additionally, there is reportedly central obesity in white adipose tissue of mice with selective over-expression of 11ß-HSD-1 [49,50]. Furthermore, Kotelevstev et al. [51] demonstrated that homozygous 11ß-HSD-1 knockout mice were protected from central obesity.

Supporting the thesis that increased dietary calcium may inhibit 11ß-HSD-1 and proving an interaction of calcitriol and cortisol obesity, Zemel et al. [52,53] showed that 1,25-dihydroxyvitamin D reduces 11ß-HSD-1 expression and cortisol production in human adipocytes. Since HC diets are known to suppress 1,25-dihydroxyvitamin D levels, we suggest that loss of central adiposity on high dairy diets may mirror suppression of...
1,25-dihydroxyvitamin D levels and thus lead to decreased cortisol production because of the reduced expression of 11ß-HSD-1. This would result in attenuated local glucocorticoid action, particularly in the trunk region by visceral adipocytes [3]. This warrants further investigation.

There was a clinically important trend for reduced plasma glucose, insulin, and leptin concentrations and insulin resistance after 3 months in the HC group. In contrast, in the LC group there was a significant increase in glucose over time coupled with an increase in insulin resistance. The increases in glucose concentration and insulin resistance are consistent with the findings of Pereira et al. [5] who documented an inverse relationship between dietary intake of dairy and the development of obesity and abnormal glucose tolerance in overweight young adults. Additionally, Shi et al. [3] provide more supporting evidence from an animal model. Transgenic mice expressing agouti in adipose tissue under the control of a P2 promoter were evaluated. The mice were randomized to four different groups of calcium intake. The low calcium group exhibited increased blood glucose and hyperinsulinemia. Thus, a diet low in dietary calcium exhibited a diabetogenic effect in the transgenic mouse model.

In the current study, the analysis of subjects in tertile categories with reference to supplemented dairy calcium intake on a daily basis compared to baseline intakes revealed a trend for increased weight loss with increased dairy calcium intake. These results are consistent with studies [7,11,23,30,41-46,54-57] that have shown that higher intake of calcium is associated with increased weight loss.

Only a few studies have been conducted with dairy calcium to determine the effects of dairy calcium on plasma hormones (glucose, insulin and leptin) [5-7,55] and to our knowledge there are no reports for the effect of dairy calcium on hormones in postmenopausal women. Studies that have explored dairy calcium intake and hormones provide conflicting results. There are studies that demonstrate a decrease in glucose, insulin and insulin resistance with increased dietary intake [5-7,54]. On the other hand, others demonstrated a positive correlation of increased dietary calcium and hormones [4]. Furthermore, some studies have reported that there is no effect of increased dietary calcium on plasma glucose and insulin or insulin resistance [10-12]. In summary, we report that in a population of obese/overweight postmenopausal women supplementing their diet with dairy which provided a total estimated calcium intake of ~1400 mg/day (i.e. DS-4) was successful in the reduction of trunk fat and plasma glucose, while dairy calcium and caloric restriction decreased weight, BMI, total body fat, and insulin and leptin during the intervention. In contrast, the supplemented group ingesting 800 mg/d of dairy calcium (i.e. DS-2) in combination with caloric restriction provided significant increases in plasma glucose and insulin resistance. In conclusion, the hypothesis that a combination of caloric restriction (1,400 kcal) in combination with either ~ 800 mg Ca/day or ~ 1400 mg Ca/day of dairy calcium would have different effects on body and plasma hormones (insulin, leptin) and plasma glucose concentrations is accepted.

The main limitation of this study was the sample size because of the high rate of subject drop outs. Withdrawal rates of this magnitude are not unusual in studies that require a change in diet composition and quantity. We initiated the study with 86 subjects, but 30 subjects withdrew from the study citing inability to follow the prescribed diet for their group assignment on the basis of varied personal reasons. The demographics of this withdrawal group were not different in general from the subjects completing the study: (age distribution (p=0.41); race/ethnicity (p=0.70); education (p=0.50); occupation (p=0.06) [dropouts tended to be more in the “services” or “other” categories]; HRT (p=0.95); diet (p=0.11); BMI (p=0.12). However, with this limitation, our findings in this overweight/obese post-menopausal population of women illustrate the effect of caloric restriction coupled with dairy calcium in improving hormonal profiles and promoting weight reduction.

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COMPETING INTERESTS
Authors have declared that no competing interests exist.

REFERENCES