Dietary Calcium Source Influences Body Composition, Glucose Metabolism and Hormone Levels in a Mouse Model of Postmenopausal Obesity

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Abstract. *Background: The prevalence of obesity has risen dramatically, with postmenopausal women particularly prone to increased adiposity. Epidemiologic data suggest that dietary calcium, particularly from dairy products, can decrease weight gain. The aim of this study was to evaluate effects of different calcium sources in a mouse model of postmenopausal obesity. Materials and Methods: Ovariectomized C57BL/6 mice were randomized to either low-fat (LF) or high-fat (HF) diets containing either calcium phosphate from non-fat dried milk and whey mineral concentrate (dairy) or calcium carbonate (supplement). Results: Dairy, but not supplement, decreased weight gain and percent body fat in HF mice, with no effect on food consumption. Dairy improved insulin resistance and glucose tolerance, while supplement increased bone mineral density in LF mice. Dairy had no effect on bone. Conclusion: The beneficial effects of dietary calcium on body weight and bone health after menopause may be significantly influenced by other dietary components.*

Obesity rates continue to increase and represent a major public health problem in the US (1). The prevalence of obesity among American women aged 20 years and older increased from 15.6% in 1960 to 34% in 2000 (1). Obesity results from significant increases in white adipose tissue, which acts as a major secretory organ that releases a number of hormones and adipokines, including leptin, adiponectin, resistin, monocyte chemoattractant protein (MCP)-1 and

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plasminogen activator inhibitor (PAI)-1 (2). Obesity is characterized by a chronic state of pro-inflammation (3), and the elevated production of inflammation-related adipokines is believed to be important in the development of a number of chronic diseases linked to obesity, particularly type 2 diabetes, metabolic syndrome and many cancers (2).

Although energy balance is the most critical factor in body weight regulation (4), an inverse relationship between dietary calcium and adiposity has been found in some human and animal studies (5). It has also been shown that high dietary calcium modulates energy metabolism and adipose tissue cytokine production (6). Dietary calcium is required for many biological processes (7), and calcium-rich diets are correlated with a decreased risk of disease (8). Several studies suggest that high dietary calcium decreases obesity, serum triglyceride values and insulin resistance (9, 10). In the Coronary Artery Risk Development in Young Adults (CARDIA) Study, these beneficial effects were observed in overweight, but not normal weight, subjects (11). Furthermore, several studies demonstrated that dairy-containing foods exert a substantially greater effect in weight regulation than supplemental (calcium carbonate) sources of calcium (12). In recent animal studies, a diet high in calcium from non-fat dry milk decreased body weight and fat content in male Wistar rats (13), and yoghurt supplementation decreased weight gain but did not affect insulin sensitivity in male mice fed a moderate-fat diet (14). Because dairy products are generally fatty foods, it is difficult to study the effects of dairy in the context of both low dietary fat and high dietary fat. In addition, the concentration of milk components is not always constant; whey proteins, sugar and even calcium content are known to fluctuate depending on many factors (15), which increases the challenge of simultaneously controlling calcium source and concentration together with fat content and protein composition in the diet.

Dietary calcium is also known to be critical for the development and maintenance of bone density (16).

However, in some studies the effects of dairy foods versus supplemental calcium on bone health are inconsistent. Budek *et al*. found that, when adjusted for protein intake, high intake of dairy actually decreased markers of bone turnover (17), while the Nurses' Health Study found no protective effect of milk consumption in fracture risk (18).

These studies suggest that calcium consumed as supplemental calcium or as dairy products may have differential effects on energy metabolism and bone health. Although the risks of obesity and of osteoporosis increase after menopause (18), little is known about the effects of dietary calcium on postmenopausal obesity and metabolism. The ovariectomized C57BL/6 mouse, in which surgical removal of the ovaries mimics the loss of ovarian estrogens after menopause, is the model of choice for investigating issues related to postmenopausal calcium malabsorption (19). However, there are no animal studies examining the effect of different sources of dietary calcium, such as calcium from dietary calcium supplements (usually calcium carbonate) and calcium from dairy (mainly as calcium phosphate and in combination with other dairy components), on body composition and metabolism in an animal model of postmenopause.

In the present study the effects of two different sources of calcium (supplement and dairy) on body composition, bone mineral density and endocrine parameters are compared in a model of postmenopausal obesity.

Materials and Methods

Animals and diets. Six-week-old ovariectomized C57BL/6 mice (n=108) and a group of 12 sham-ovariectomized mice, in which surgery was performed but the ovaries were not removed, were obtained from Charles River Laboratories, Inc. (Animal Production Area, NCI-Frederick, Frederick, MD, USA) and placed on a chow diet. All mice were individually housed, consumed food and water *ad libitum*, and were on a 12 h light/dark cycle. One week after arrival, ovariectomized mice were randomly assigned (n=18 per group) to either a high-fat (HF, 46 kcal% fat) or a low-fat (LF, 10 kcal% fat) diet varying in calcium amount and source: control, with ~0.6% (by weight) calcium as calcium carbonate; supplement, with ~2% calcium as calcium carbonate; or dairy, providing ~2% calcium as calcium phosphate through the use of non-fat dry milk and TruCal® D50, a whey mineral concentrate (Glanbia Nutritionals, Inc., Monroe, WI, USA). All experimental diets were formulated by Research Diets, Inc. (New Brunswick, NJ, USA) and were comparable within dietary fat categories except for the amount and source of calcium and source of protein (Table I). The dietary groups were categorized as follows: 1) LF control; 2) LF supplement; 3) LF dairy; 4) HF control; 5) HF supplement; or 6) HF dairy. The sham-ovariectomized mice were fed the HF control diet for the duration of the study and served as an additional control group. All animal protocols were approved by the University of Texas at Austin Institutional Animal Care and Use Committee and carried out in compliance with all guidelines and regulations. Throughout the 20-week study, mice consumed diets *ad libitum*;

feed intake for all groups was measured three times a week and body weight was measured weekly.

At weeks 5 and 10 of the study, after a 10-hour fast with access to water *ad libitum*, mice were lightly anesthetized with isofluorane for collection of a 150-μL blood sample *via* the retro-orbital sinus. Whole blood was allowed to clot at room temperature for 30 min prior to centrifugation at $1000 \times g$ for 10 min, and the serum was stored at –80°C for analysis.

After euthanasia, carcasses were stored at –20°C. Fat weight, lean weight, bone mineral density (BMD) and bone mineral content (BMC) were determined using dual energy X-ray absorptiometry (DXA) (GE Lunar Piximus II, Madison, WI, USA) (20). Each carcass was scanned three times and the average values were used for analysis.

Glucose tolerance and insulin tolerance tests. To determine the effects of different sources of calcium on glucose regulation and insulin sensitivity, glucose tolerance tests (GTT) were performed at week 13 and insulin tolerance tests (ITT) at week 14 in a randomly selected subset of mice from each group. GTT was performed after overnight fasting by administration of 20% glucose (2 g/kg body weight IP); ITT was performed at noon after a 6-hour fast by IP injection of insulin (0.75 U/kg body weight) (21). For both tests, blood samples were taken from the tail and analyzed for glucose using an Ascencia Elite XL 3901G glucose analyzer (Bayer Corporation, Mishawaka, IN, USA). Glucose levels were determined at baseline, 15, 30, 60 and 120 min after injection of glucose or insulin.

Serum hormone. Insulin, leptin, adiponectin, resistin, MCP-1 and PAI-1 were measured in serum collected at week 10 of the study using mouse adipokine LINCOplex™ Multiplex Assays (Millipore, Inc., Billerica, MA, USA). Insulin-like growth factor (IGF-1) was measured by radioimmunoassay in serum collected at weeks 5 and 10 (Diagnostic Systems Laboratories; Webster, TX, USA).

Statistical analysis. Values are presented as mean±standard error (S.E.). Statistical analyses were performed within each dietary fat category (*i.e*., HF or LF), except as noted. Repeated measures and one-way analysis of variance (ANOVA) using Tukey's Honestly Significant Difference comparison were used to assess the effects of diet on mean weekly body weight, kcal consumption and serum hormone analyses. Adiponectin values were subjected to a square root transformation before analysis to achieve a normal distribution. Body composition data were analyzed using either ANOVA or analysis of covariance (ANCOVA) with body weight as covariate. Repeated measures analysis was used to evaluate glucose and insulin tolerance tests. For all tests SPSS software was used (SPSS Inc., Chicago, IL, USA), and $p<0.05$ was considered statistically significant. Data were examined for outliers, which were defined as values outside the mean±three times the standard deviation; these outliers were excluded from analysis.

Results

Ovariectomized mice fed the HF diets consumed more kilocalories and consistently weighed more than the mice fed the LF diets throughout the 20-week study (Figure 1). Mean kilocalorie consumption was slightly higher for mice in the HF diet; however, it did not significantly differ within the

Table I. *Diet formulations*.

	Low-fat control	Low-fat supplement	Low-fat dairy	High-fat control	High-fat supplement	High-fat dairy
Diet #	D12450B	D06030301	D06030302	D12451	D06030303	D06030304
Ingredient (g)						
Casein	200.0	200.0	115.0	200.0	200.0	115.0
L-Cystine	3.0	3.0	1.7	3.0	3.0	1.7
Corn Starch	315.0	315.0	315.0	72.8	72.8	72.8
Maltodextrin	35.0	35.0	35.0	100.0	100.0	100.0
Sucrose	350.0	350.0	241.0	172.8	172.8	64.0
Soybean Oil	25.0	25.0	25.0	25.0	25.0	25.0
Lard	20.0	20.0	18.5	177.5	177.5	176.0
Milk, Non-fat, Dry			200.0			200.0
TruCal D50			50.0			50.0
Calcium Carbonate	5.5	41.5	5.5	5.5	41.5	5.5
Total[1]	1055.05	1091.05	1108.25	858.15	894.15	911.55
g (g%)						
Protein	179.0 (17.0)	179.0 (16.4)	178.8 (16.1)	179.0 (20.9)	179.0 (20.0)	178.8 (19.6)
Carbohydrate	710.0 (67.3)	710.0 (65.1)	710.0 (64.1)	355.6 (41.4)	355.6 (39.8)	355.8 (39.0)
Fat	45.0 (4.3)	45.0 (4.1)	45.1 (4.1)	202.5 (23.6)	202.5 (22.6)	202.6 (22.2)
Fiber	50.0 (4.7)	50.0 (4.6)	50.0 (4.5)	50.0 (5.8)	50.0 (5.6)	50.0 (5.5)
kcal (kcal %)						
Protein	716 (18)	716 (18)	715 (18)	716 (18)	716 (18)	715 (18)
Carbohydrate	2840 (72)	2840 (72)	2840 (72)	1422 (36)	1422 (36)	1423 (36)
Fat	405 (10)	405 (10)	406 (10)	1823 (46)	1823 (46)	1823 (46)
Total	3961 (100)	3961 (100)	3961 (100)	3961 (100)	3961 (100)	3962 (100)
kcal/g	3.8	3.6	3.6	4.6	4.4	4.3
g/kg diet						
Calcium	6.1	20.5	20.6	6.1	20.5	20.6
Phosphorous	4.6	4.6	12.3	4.6	4.6	12.3
Potassium	6.0	6.0	9.6	6.0	6.0	9.6

[1]All diets contained 50.0 g Cellulose, BW200; 10.0 g S10026 Mineral Mix; 13.0 g Dicalcium Phosphate; 16.5 g Potassium Citrate; 10.0 g V10001 Vitamin Mix; 2.0 g Choline Bitartrate; and 0.05 g FD&C dye.

dietary fat categories ($p>0.05$) (Figure 1A). Nevertheless, dairy decreased the rate of weight gain, but in the HF mice only, with no effect on body weight with the LF diet (Figure 1B). Among the HF ovariectomized groups, body weights were similar until week 15 of the study, when a significant inhibition of weight gain was observed associated with dairy consumption compared to HF control. At the end of the study (week 20), mice in the HF dairy group weighed significantly less compared to both the control ($p=0.035$) and supplement groups ($p=0.043$). The effect of calcium on body weight was independent of the fat content in the diet, and there was no interaction between dietary fat level and calcium on body composition (Table II). Fat mass was significantly decreased in the LF dairy group compared to the LF control ($p=0.04$) and supplement ($p<0.001$) groups; in addition, lean mass was significantly increased in the LF dairy group compared to the LF control ($p=0.006$) and supplement ($p=0.02$) groups (Table II). Percent body fat was

significantly decreased in the animals consuming dairy when compared to control for both the HF ($p=0.011$) and LF ($p=0.004$) diets and also decreased compared to supplement for the LF diet ($p<0.001$).

It was found that supplement and dairy had different effects on bone mineral content and density, and the effects of supplement or dairy on BMC and BMD were independent of diet and diet effects on body weight (Table II). BMC was significantly higher in both the HF and LF supplement groups compared to the respective dairy groups ($p<0.05$). The LF supplement mice had significantly higher BMD compared to both control ($p=0.001$) and dairy ($p=0.001$), whereas dairy had no effect on BMD compared to the respective control ($p>0.05$), regardless of the dietary fat category.

LF and HF groups responded differently to glucose and insulin tolerance tests, administered during weeks 13 and 14 of the study, respectively (Figures 2 and 3). The effect of dietary fat and/or mean body weight was stronger in these
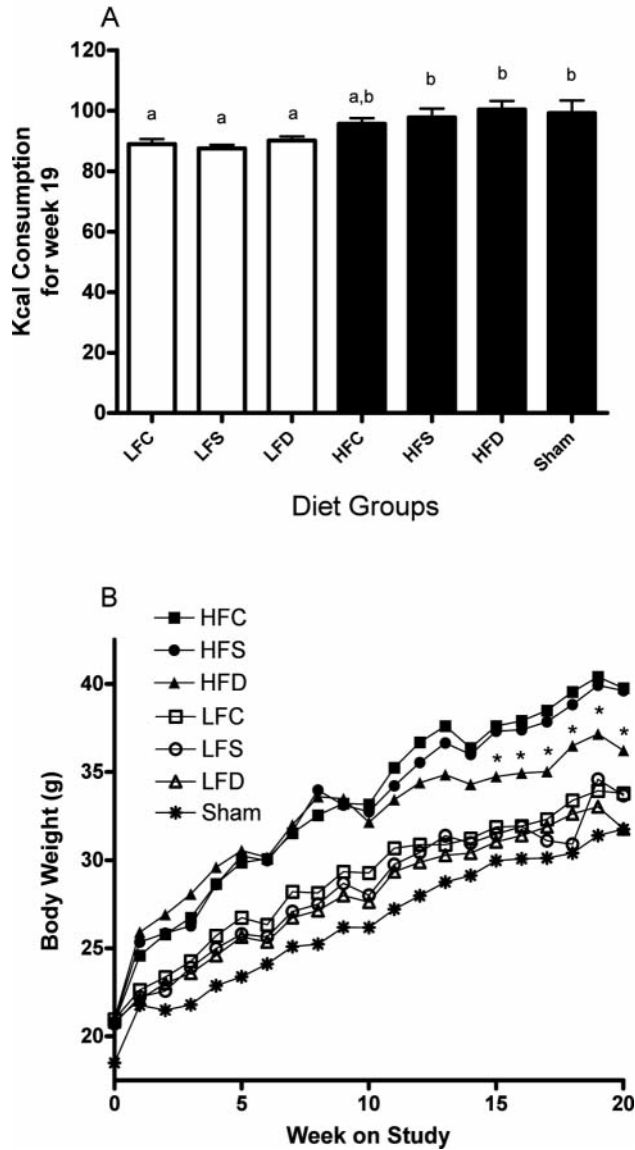
A

B

Figure 1. *Mean kilocalorie consumption (A) and body weights (B) for all groups. For the duration of the study mice consumed food and water ad libitum. Food intake and body weights were recorded weekly. The sham surgery (non-ovariectomized) group consumed the HF control diet. HFC, high-fat control diet; HFS, high-fat supplement diet; HFD, high-fat dairy diet; LFC, low-fat control diet; LFS, low-fat supplement diet; LFD, low-fat dairy diet.* [a,b]*Bars with different superscript letters are significantly different (p<0.05.) *Significantly different from the HF control diet (p<0.05).*

tests than that of either type of calcium. Collectively, glucose clearance was better in the LF groups compared to the HF groups. Supplement had no effect on glucose tolerance or insulin sensitivity in either the LF or HF diets. The dairy regimen improved the response to the GTT and ITT in the LF diet but not in the HF diet. Specifically, the LF dairy group exhibited the fastest glucose clearance compared to LF control and LF supplement diet categories ($p=0.009$) by

repeated measures analysis, and at the end of the test this group also displayed improved glucose tolerance ($p=0.026$) and insulin sensitivity. In contrast to the improved glucose tolerance seen with dairy in the LF diet, mice consuming the HF dairy diet were more glucose intolerant ($p=0.022$) and insulin resistant ($p=0.027$) compared to the HF control.

Serum hormone levels at week 10 of the study were also differentially affected by diet (Table III). Leptin levels in the HF control were higher than in the LF groups but were surprisingly low in the HF supplement and HF dairy groups, with no difference in serum leptin between the HF supplement and HF dairy groups. In the HF groups, serum resistin was also decreased in supplement and dairy compared to the HF control ($p=0.033$ and $p=0.012$, respectively), with no difference in resistin values between supplement and dairy. No differences were found in serum leptin or resistin levels between the LF groups. Dairy increased adiponectin levels in mice on the HF diet ($p=0.006$) but not the LF diet, compared to the respective control. In contrast, supplement, while not statistically different from dairy, had no effect on adiponectin levels in mice on the HF diet but surprisingly decreased adiponectin in mice on the LF diet ($p=0.022$). No significant differences were found in serum insulin, MCP-1 or PAI-1 levels in ovariectomized mice in either dietary fat category (data not shown). IGF-1, a protein hormone with insulin-like effects on growth and metabolism, was measured in serum of these mice at weeks 5 and 10 of the study. By a repeated measures analysis, IGF-1 did not have a major effect among the groups and there was no significant interaction between IGF-1 and group within each dietary fat category (data not shown).

Ovariectomy had significant effects on most of the parameters measured. Although the sham surgery (non-ovariectomized) group consumed the HF control diet, the ovariectomized mice on even the LF diets generally weighed more than the sham surgery group throughout the study, and the ovariectomized mice on the HF diets weighed considerably more (Figure 1). The ovariectomized mice on the HF control diet also had greater fat mass and percent body fat and lower BMC and BMD compared with the sham surgery group (Table IV). In correlation with fat mass, serum leptin and resistin levels were higher and adiponectin levels tended to be lower in the ovariectomized mice compared with the sham surgery group on the same HF control diet (Table IV). The sham surgery group also displayed better glucose tolerance than any of the HF ($p=0.007$) or LF groups ($p<0.05$) (Figure 2) but responded less well to the insulin tolerance test than the LF dairy group ($p<0.05$) (Figure 3).

Discussion

This study compared the effects of supplemental calcium and dairy calcium on body weight and composition, glucose and insulin metabolism, and serum adipokines in a mouse model

Table II. *Effects of diets on body composition and bone characteristics in ovariectomized C57BL/6 mice.*

Group	Body weight[1] (g)	Fat mass[1] (g)	Lean mass[1] (g)	Percent body fat[1]	Bone mineral content[2] (g)	Bone mineral density[2] (g/cm2)
LF control	29.9 (0.8)[a,b]	13.2 (0.8)[a]	16.7 (0.4)[a]	43.6 (1.7)[a]	0.47 (0.014)[a,b]	0.0480 (0.0005)[a]
LF supplement	32.1 (0.9)[b]	15.1 (1.0)[a]	17.0 (0.4)[a]	46.4 (2.1)[a]	0.52 (0.024)[b]	0.0512 (0.0007)[b]
LF dairy	28.8 (0.9)[a]	10.2 (0.7)[b]	18.6 (0.5)[b]	34.8 (1.6)[b]	0.41 (0.012)[a]	0.0480 (0.0005)[a]
HF control	38.1 (1.3)[a,b]	21.4 (1.3)[a]	16.7 (0.5)[a]	55.5 (2.1)[a]	0.49 (0.022)[a,b]	0.0474 (0.0005)[a]
HF supplement	39.1 (1.5)[a]	21.8 (1.4)[a,b]	17.3 (0.6)[a]	54.9 (2.0)[a,b]	0.50 (0.026)[b]	0.0494 (0.0007)[a]
HF dairy	34.9 (1.4)[b]	17.5 (1.2)[b]	17.3 (0.5)[a]	49.5 (1.8)[b]	0.45 (0.017)[a]	0.0474 (0.0006)[a]
ANOVA/ANCOVA[3]						
p (Dietary Fat)	<0.0001	<0.0001	0.4217	<0.0001	0.4788	0.1012
p (Calcium)	0.0907	0.0002	0.0322	<0.0001	0.0070	<0.0001
p (Fat*Calcium)	0.5792	0.7785	0.2063	0.2598	0.1570	0.4657

[1]Means and standard errors obtained from ANOVA within dietary fat category, n=16-18; [2]Means and standard errors obtained from ANCOVA within dietary fat category with body weight as the covariate, n=16-18; [3]*p*-values obtained from two-way ANOVA or ANCOVA for all diets; [a,b]Values within a dietary fat category with different superscripts are significantly different from each other, *p*<0.05.

Table III. *Effects of diets on serum hormones in ovariectomized C57BL/6 mice.*

Group[1]	Leptin (ng/mL)	Resistin (ng/mL)	Adiponectin (μg/mL)
LF control	9.0 (1.1)[a]	4.1 (0.5)[a]	34.9 (5.0)[a]
LF supplement	11.3 (1.5)[a]	4.5 (0.4)[a]	21.8 (1.0)[b]
LF dairy	10.0 (1.6)[a]	4.2 (0.3)[a]	25.5 (0.8)[a,b]
HF control	18.9 (2.8)[a]	8.8 (0.8)[a]	20.9 (2.5)[a]
HF supplement	7.9 (0.6)[b]	5.9 (0.9)[b]	26.8 (2.1)[a,b]
HF dairy	7.6 (0.5)[b]	5.4 (0.6)[b]	32.3 (2.4)[b]
ANOVA[2]			
p (Dietary Fat)	0.2835	<0.0001	0.6612
p (Calcium)	0.0034	0.0251	0.3377
p (Fat*Calcium)	<0.0001	0.0083	0.0012

[1]Means and standard errors obtained from ANOVA, n=10 for all groups [2]*p*-values obtained from two-way ANOVA for all diets; [a,b]Values within a dietary fat category with different superscripts are significantly different from each other, *p*<0.05.

Table IV. *Effects of ovariectomy on body composition and serum hormones in C57BL/6 mice fed HF control diet[1].*

Variable	Ovariectomized[2] (S.E.)	Sham[3] (S.E.)	*p*[4]
Body weight (g)	38.1 (1.3)	32.0 (2.0)	0.0097
Fat weight (g)	21.4 (1.3)	14.8 (1.8)	0.0042
Lean weight (g)	16.7 (0.5)	17.2 (0.4)	0.7544
Percent body fat	55.5 (2.1)	44.5 (3.3)	0.0067
BMC (g)	0.49 (0.022)	0.58 (0.025)	0.0055
BMD (g/cm2)	0.0474 (0.0005)	0.0556 (0.0007)	<0.0001
Leptin (ng/mL)	18.9 (2.8)	3.0 (0.7)	0.0003
Resistin (ng/mL)	8.8 (0.8)	2.2 (0.3)	<0.0001
Adiponectin (μg/mL)	20.9 (2.5)	33.8 (6.9)	0.0952

[1]Data for ovariectomized mice from Tables II and III; [2]n=17 for body composition variables; n=10 for serum hormones; [3]n=10; [4]*p*-value from *t*-test.

of postmenopausal obesity. The results support the hypothesis that consumption of dairy influences body composition by decreasing the rate of weight gain. In the present study, kilocalorie consumption did not differ within the respective dietary fat categories, and yet with the HF diet differences in body weight and body composition were evident in the mice consuming the dairy diet. As has been shown in some previous studies (22), decreased body weight in the HF dairy group was associated with decreases in fat mass and percent body fat rather than lean tissue. In the LF dairy group fat mass and percent body fat were also decreased compared to the LF control, but the decreased body fat mass was partially offset by increased lean mass, resulting in no significant effect on body weight.

Mechanisms by which dietary calcium may affect body weight are not understood. Among the mechanisms suggested for an effect of dietary calcium on body weight is a reduced Ca^{2+} influx into the adipocyte, mediated by calcium suppression of 1,25-dihydroxyviatmin D production and resulting in an increase in lipolysis and thermogenesis and hence weight loss (23). Alternatively, calcium is known to bind to lipids, and this binding contributes to increased fecal fat and decreased fat absorption (24). In the present study, however, while dairy significantly affected body composition, calcium supplement was ineffective and had no effect on
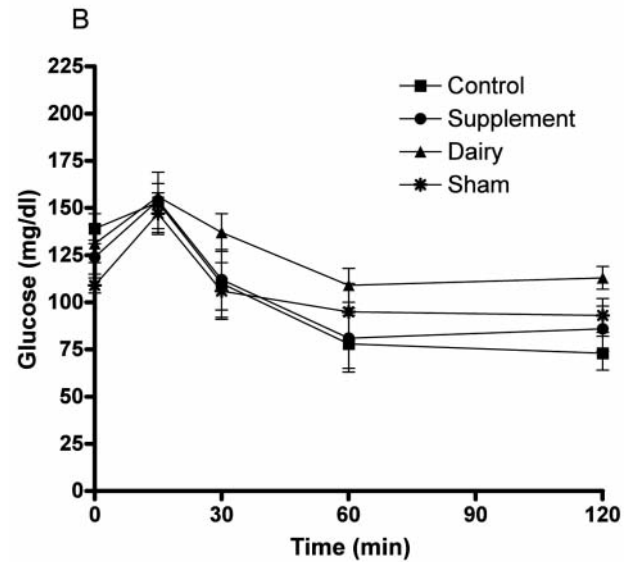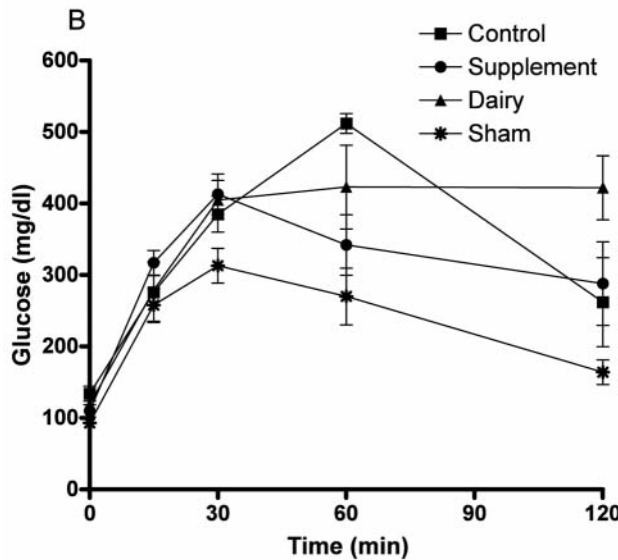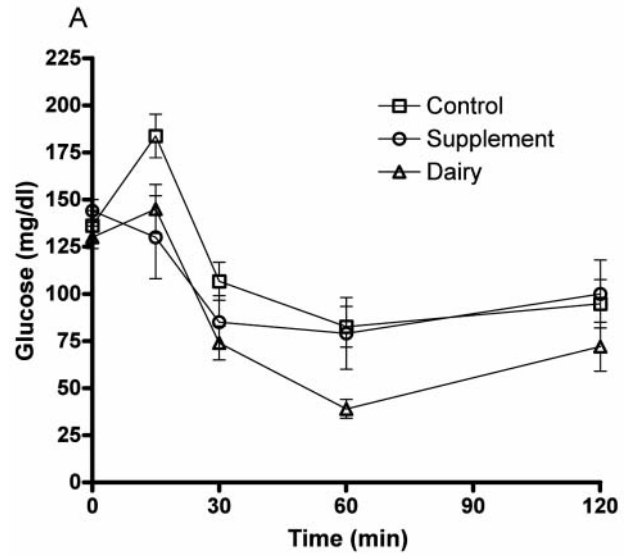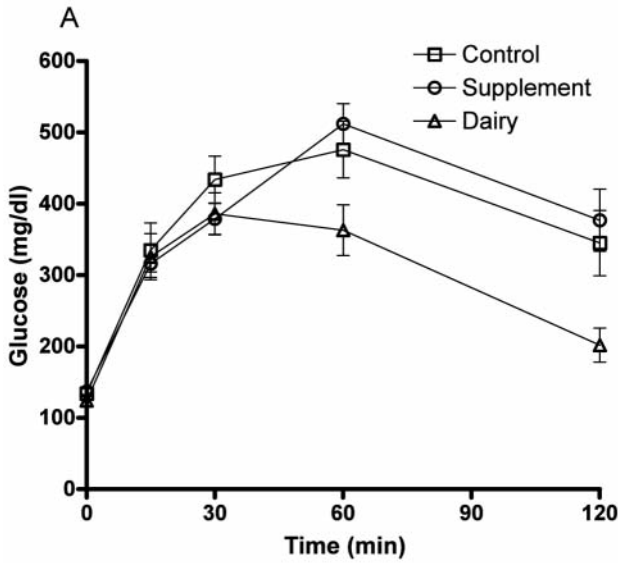
Figure 2. *Effect of diets on glucose tolerance. Mice were fasted overnight during week 13 on study and glucose tolerance tests (GTT) performed as described in Materials and Methods. (A) Ovariectomized mice fed LF control, supplement and dairy diets. (B) Ovariectomized mice fed HF control, supplement and dairy diets, and sham surgery (non-ovariectomized) mice fed HF control diet. Values are mean±S.E.; n=10 mice per group.*

Figure 3. *Effect of diets on insulin responsiveness. Mice were fasted for 6 hr during week 14 on study and insulin tolerance tests (ITT) performed as described in Materials and Methods. (A) Ovariectomized mice fed LF control, supplement and dairy diets. (B) Ovariectomized mice fed HF control, supplement and dairy diets, and sham surgery (non-ovariectomized) mice fed HF control diet. Values are mean±S.E.; n=10 mice per group.*

body weight, fat mass, lean mass or percent body fat in either the HF or LF diets. Therefore, the beneficial effects of dairy on body weight may have been due to components other than calcium, since similar effects were not observed in the supplement groups consuming the same amount of calcium but as calcium carbonate. Some studies suggest that whey peptides, calcitropic hormones, and/or sphingolipids in milk

may be responsible for the body weight-lowering effect of dairy in humans (22). Calcium in milk is usually associated with protein and, therefore, the calcium content is dependent on the protein content of the milk (15). Moreover, milk composition from cows is not constant, and proteins, fat, lactose, vitamins and minerals in milk may vary depending on many factors. In the present study, the study diets were

formulated to ensure that they contained the same amounts of protein and calcium, allowing for comparison of dietary calcium sources under both HF and LF conditions. However, the source of the protein in the dairy diets differed from that in the control and calcium supplement diets, in that non-fat dried milk was substituted for a portion of the casein component. Therefore, the dairy diets had a different amino acid composition because of the contribution of the whey proteins in milk. Compared to other dietary proteins, whey contains the highest concentrations of the essential branched-chain amino acids (BCAAs), especially leucine (25). Unlike other amino acids, BCAAs are not metabolized in the liver, and dietary intake directly affects circulating levels and availability in peripheral tissues. Furthermore, leucine stimulates protein synthesis at the level of translational initiation, both through the mammalian target of rapamycin (mTOR) cellular signaling pathway and through an mTOR-independent kinase (26). Given the pivotal role of mTOR signaling in obesity and its impact on a number of chronic diseases (27), further study of the possible contributions of amino acid composition to the beneficial effects of dairy consumption on body composition is warranted.

Calcium and bone metabolism in mammals is regulated by the vitamin D receptor–1,25-dihydroxyvitamin D complex (28), and dietary calcium, especially dairy calcium, is known to regulate the level of 1,25-dihydroxyvitamin D, the active form of vitamin D (18). In rodents this control varies with strain (29), and in C57BL/6 mice calcium metabolism is highly dependent on vitamin D status (30). This study focused on dietary calcium sources without altering basal vitamin D status. Because vitamin D levels or calcium absorption rates could not be assessed in these animals, it is not known whether calcium metabolism differed between dairy and supplement. However, this study showed that the presence of either dairy or supplemental calcium did not affect food intake and that, in the case of dairy, the beneficial effects on body composition may have been due to components other than calcium. Future studies should address this issue and identify possible mechanisms responsible for the differential effects of dairy and supplement calcium observed in this study.

Several studies attribute optimal bone health to consumption of milk and dairy products (8,31). The U.S. Department of Health and Human Services 2005 Dietary Guidelines for Americans recommends 3 servings of milk products per day to increase BMD. Recent evidence supports the idea that dietary calcium, primarily from dairy, plays a major role in preventing osteoporosis and loss of BMD in healthy adults (31, 32). However, there is much less evidence and a lack of agreement in the literature regarding the role of dairy after menopause, when calcium absorption is decreased independently of vitamin D status (33). These results suggest that dairy calcium may not be the first choice

when targeting bone health after menopause. In this model of postmenopausal obesity dairy did not improve BMD, in contrast to supplement calcium, which improved BMD in the LF diet. This supports findings from other studies concluding that the emphasis on dairy to improve bone health may be overstated (22). However, BMD was significantly lower in all diet groups compared with the sham (non-ovariectomized) mice, signifying once more the protective role of ovarian hormones in the maintenance of bone density.

The effect of consumption of dairy products on insulin and metabolism continues to be a center of debate (34, 35). Data from the Epidemiological Study on the Insulin Resistance Syndrome (DESIR), showed a beneficial effect of dietary calcium on insulin levels in women (9). Inverse associations between dairy consumption and type 2 diabetes have been reported previously and have been attributed to low-fat dairy products only (36). In the present study it was observed that dairy was effective in improving glucose tolerance and insulin sensitivity, but only with the LF diet, while calcium supplement had no effect. The beneficial effects of dairy on glucose metabolism may have been due to the different amino acid composition contributed by the whey proteins, as BCAAs, and leucine in particular, have been linked to maintenance of glucose homeostasis (25). In contrast, the HF dairy diet increased glucose intolerance and insulin insensitivity. These results suggest that either fat in the diet or increased adiposity hinders the dairy modulation of glucose and insulin metabolism in mice. Future studies isolating this effect will be needed to expand the characterization of the influence of dairy consumption on metabolism.

This study showed a clear interaction between dietary calcium and dietary fat/increased adiposity on several endocrine markers associated with insulin resistance and body weight. Leptin and resistin are two adipose-secreted proteins known to play a role in metabolism, and their serum levels increase with increased adiposity. In addition, resistin may contribute to insulin resistance, and some reports indicate that resistin may be a determinant factor in the progression to type 2 diabetes mellitus in obesity (2). Resistin may also be a link between inflammation and insulin resistance, as has been shown in rodents (37), although the human data are not conclusive. Adiponectin is another of the key adipokines related to body weight and metabolism; however, serum levels of adiponectin decrease with increased body weight, and leptin and adiponectin appear to exert opposite effects in metabolism. Recent studies have focused on the leptin-adiponectin ratio as a potential marker for metabolic diseases, including type 2 diabetes (38). In the present study, dietary calcium, regardless of source, decreased leptin and resistin and increased adiponectin, but only in the HF diet. The effect of calcium supplement on adiponectin levels in the HF diet was not significant, while in the LF diet calcium supplement actually decreased adiponectin in this study. However, the

differences in adipokine levels with different sources of calcium did not correlate with improvements in glucose tolerance or insulin sensitivity. These results suggest that, while dietary calcium may help counteract some of the deleterious effects of obesity on these endocrine markers, the beneficial effect of dairy on glucose metabolism observed in the LF mice may not be related to circulating levels of these hormones.

Given the dramatic increase in obesity rates in recent decades, the prevalence of postmenopausal obesity can be expected to increase as the current cohort of women enters menopause. Postmenopausal obesity is a well-known risk factor for several chronic diseases, including type 2 diabetes, metabolic syndrome and some cancers. This study in an animal model of postmenopausal obesity lends support to the relevance of dairy consumption and dietary calcium in the maintenance of body weight and composition, but more work is needed to clarify their roles.

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