

Effects of dairy compared with soy on oxidative and inflammatory stress in overweight and obese subjects^{1–3}

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ABSTRACT

Background: We recently showed that calcitriol increases oxidative and inflammatory stress; moreover, inhibition of calcitriol with high-calcium diets decreased both adipose tissue and systemic oxidative and inflammatory stress in obese mice, whereas dairy exerted a greater effect. However, these findings may be confounded by concomitant changes in adiposity.

Objective: The objective of this study was to evaluate the acute effects of a dairy-rich diet on oxidative and inflammatory stress in overweight and obese subjects in the absence of adiposity changes.

Design: Twenty subjects (10 obese, 10 overweight) participated in a blinded, randomized, crossover study of dairy- compared with soy-supplemented eucaloric diets. Two 28-d dietary periods were separated by a 28-d washout period. Inflammatory and oxidative stress biomarkers were measured on days 0, 7, and 28 of each dietary period.

Results: The dairy-supplemented diet resulted in significant suppression of oxidative stress (plasma malondialdehyde, 22%; 8-isoprostane-F_{2x}, 12%; $P < 0.0005$) and lower inflammatory markers (tumor necrosis factor- α , 15%, $P < 0.002$; interleukin-6, 13%, $P < 0.01$; monocyte chemoattractant protein-1, 10%, $P < 0.0006$) and increased adiponectin (20%, $P < 0.002$), whereas the soy exerted no significant effect. These effects were evident by day 7 of treatment and increased in magnitude at the end of the 28-d treatment periods. There were no significant differences in response to treatment between overweight and obese subjects for any variable studied.

Conclusion: An increase in dairy food intake produces significant and substantial suppression of the oxidative and inflammatory stress associated with overweight and obesity. This trial was registered at clinicaltrials.gov as NCT00686426. *Am J Clin Nutr* 2010; 91:16–22.

INTRODUCTION

Obesity is associated with subclinical chronic inflammation (1, 2). Adipose tissue is an active secretory organ that releases numerous peptides and cytokines into circulation (3, 4). Obesity alters the balance among these such that enlarged adipocytes produce a more proinflammatory cytokines pattern (5, 6); this dysregulation of adipocytokine (adipokine) production contributes to the development of metabolic disorders associated with obesity.

Nutrient overload and obesity increase reactive oxygen species (ROS) generation and oxidative stress (7, 8), whereas caloric restriction and weight loss lead to a reduction in oxidative stress (8). Oxidative stress plays a significant role in the regulation of inflammatory status in adipose tissue. Previous studies showed

that oxidative stress is augmented in obesity, and the addition of oxidants suppresses the adipose tissue expression of adiponectin and increases the expression of inflammatory factors (2). Additionally, obesity is associated with increased inflammatory stress, which is attenuated with weight loss (9). These results indicate that a local increase in oxidative stress in accumulated fat causes dysregulated production of adipokines. Fat accumulation stimulates NADPH oxidase 4 (Nox4), which is a key factor in enzymatic cellular ROS production (10–12). ROS stimulates macrophage infiltration of adipose tissue via ROS-induced monocyte chemoattractant protein (MCP) production, which results in further promotion of local Nox4 expression and ROS production (13, 14), which indicates that both adipocytes and macrophages contribute to elevated oxidative stress in obesity.

Adipocyte ROS production is regulated, in part, by mitochondrial uncoupling status and cytosol Ca²⁺ signaling, and we have previously shown calcitriol to inhibit uncoupling protein 2 and to stimulate Ca²⁺ signaling in adipocytes (11). Accordingly, we have recently investigated the effects of calcitriol on oxidative and inflammatory stress in adipocytes; our data show that calcitriol (0.1–10 nM) stimulates ROS production in murine and human adipocytes via both Nox4-dependent and Nox4-independent mechanisms (11); stimulates inflammatory cytokine [eg, tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6)] expression and secretion; and inhibits anti-inflammatory cytokine [eg, adiponectin, interleukin-15 (IL-15)] expression and secretion (12, 13). In addition, we have shown calcitriol to exert comparable effects on inflammatory cytokine production in macrophages and to stimulate macrophage-adipocyte cross-talk and inflammatory response in coculture (15).

Consequently, we recently evaluated the role of dietary calcium-induced suppression of calcitriol in the modulation of oxidative and inflammatory stress in a mouse model of diet-induced obesity and oxidative stress (12, 16) and observed that high-calcium diets suppressed both adipose tissue and systemic oxidative and inflammatory stress. Notably, the consumption of nonfat dry milk as the source of calcium resulted in significantly

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greater effects (16), which is consistent with the findings of previous studies of dairy foods compared with calcium in weight management (17). Furthermore, analysis of archival samples from previous clinical trials showed that an increase in dairy food intake results in suppression of circulating C-reactive protein and augmentation of adiponectin during weight loss and maintenance in obese subjects (16). However, these studies resulted in significant reductions in adiposity, which could lead to confounding, although the supporting cellular/mechanistic data indicate an effect independent of adiposity reduction. Accordingly, the objective of this study was to determine the acute effects of a dairy-rich diet on oxidative and inflammatory stress in overweight and obese subjects independently of any changes in adiposity.

SUBJECTS AND METHODS

Participants

Twenty otherwise healthy adults (14 men and 6 women), 10 of whom were overweight [body mass index (BMI; in kg/m²): 25.0–29.9] and 10 mildly obese (stage 1 obesity; BMI: 30.0–34.9), were recruited from the faculty, staff, and student populations of the University of Tennessee. The BMI of the overweight group was 28.0 ± 1.01 , and that of the obese group was 32.5 ± 1.10 . Enrolled subjects were 31.0 ± 10.3 y of age. To qualify, subjects must have maintained a stable weight with ≤ 3 kg weight loss in the 3 mo that preceded the study. Subjects were excluded for the following reasons: BMI < 25 or ≥ 30 (overweight subjects) or < 30 or ≥ 35 (obese subjects); type II diabetes that required the use of any oral antidiabetic agent and/or insulin; history/presence of significant metabolic disease, active gastrointestinal disorders, or eating disorders; adverse response to dairy foods; recent use of tobacco, pharmacotherapeutic agents, over-the-counter antiobesity agents, or psychotropic medication; recent initiation of an exercise program; recent initiation/change in hormonal birth control or hormone replacement therapy; or pregnancy/lactation.

Design

Two weight-maintenance diets were presented to the subject groups in a randomized crossover design. A soy-based placebo diet (500–600 mg calcium/d) was studied against a dairy diet (3 daily servings; 1200–1400 mg calcium/d, of which 1050 mg/d were derived from the dairy supplement, described below). Each diet was presented for 28 d, with measurements (described below) taken at baseline, day 7, and day 28 of each dietary period. Diets were presented in random order. Half the overweight and half the obese subjects received the dairy-free diet first and the other half received the dairy-adequate diet first. The 2 dietary periods were separated by a 28-d washout period. After washout the study was repeated, with subjects exposed to the other diet for an additional 28 d.

Dietary intervention in women commenced during the luteal stage (14–24 d) after the onset of menses to control for confounding of menstrual cycle and oxidative and/or inflammatory stress; this resulted in a longer, >28 -d washout for some subjects (up to 38 d) to start at the same point in the menstrual cycle for both phases of the crossover. This study was approved from an ethical standpoint by the Institutional Review Board of the University of Tennessee-Knoxville.

Diets

Two diets (dairy and soy) in the form of “smoothies” were administered 3 times per day throughout each 28-d treatment period. Each smoothie contained 170 kcal, 10 g protein, 1 g fat, and 30 g carbohydrate. Two of the daily smoothies were consumed by subjects at the clinic site and the third was taken for off-site consumption.

The dairy smoothies were milk based, with nonfat dry milk as the protein source, and contained 350 mg calcium per smoothie. The placebo smoothies were soy based and contained 50 mg calcium per smoothie. The soy protein isolate was used as the protein source in the placebo smoothies. For both dairy and placebo, subjects were provided a choice of fruit flavorings.

The 2 diets (smoothies plus other foods consumed) were constructed to provide comparable amounts of macronutrient and fiber that approximated the average consumption in the United States (fat, $\approx 35\%$ of total kcal; carbohydrates, $\approx 49\%$; protein, $\approx 16\%$; fiber, 8–12 g/d). Maintenance levels of caloric intake were determined from measurement of resting metabolic rate (RMR) via indirect calorimetry, as described below (in RMR/substrate oxidation). Total daily energy expenditure and maintenance energy requirements were calculated as 1.2 – $1.4 \times$ RMR, depending on amount of physical activity.

Nutritional supplements were not permitted. Caffeine intake was maintained at a constant level per subject based on baseline assessment. Subjects were given individual instruction, counseling, and assessment from the study dietitian regarding dietary adherence. All subjects maintained complete diet and physical activity diaries throughout the study.

Anthropometric measurements

Body weight and waist circumference were measured at baseline and day 28 of each dietary period, with subjects wearing street clothes with no shoes, outerwear, or accessories. Body weight was measured with a calibrated scale. Height was measured with a wall-mounted stadiometer. Waist circumference was measured in the standing position with measurements obtained midway between the lateral lower rib margin and the ileac crest. Two measurements were taken mid-exhalation and the average was recorded.

Body composition

Total fat mass, trunk fat mass, and percentage lean and fat mass were assessed via dual-energy X-ray absorptiometry at baseline and day 28 of each dietary period. A LUNAR Prodigy dual-energy X-ray absorptiometry system (GE Healthcare, Madison, WI) maintained and calibrated by LUNAR staff annually was used. A spine phantom was assessed every day to determine whether any drift in the machine occurred, followed by the daily calibration block; spine phantom variation was $<3\%$ throughout the study.

Blood pressure

Blood pressure and heart rate measurements were taken after the patient had been seated in an upright position in a chair for ≥ 5 min with the arm supported at heart level. Blood pressure was measured with an appropriately sized cuff with the use of a standard calibrated sphygmomanometer on the same arm for

every measurement. Three readings, one or more minutes apart, were taken. The average of the last 2 values was reported. If these readings differed by >10 mm Hg (systolic or diastolic pressure), additional readings were taken until there were 2 successive determinations within 10 mm Hg. The average of these 2 was reported. Blood pressure and heart rate measurements were taken at baseline, day 7, and day 28.

ROS/oxidative stress

Plasma malondialdehyde and plasma 8-isoprostane- F_{2x} (8-epi prostaglandin F_{2x}) were measured at baseline, day 7, and day 28. Malondialdehyde was measured with the use of a fluorometric assay based on the method of Yagi (18), and plasma 8-isoprostane F_{2x} was measured by enzyme-linked immunosorbent assay (Assay Designs, Ann Arbor, MI).

Inflammatory markers and cytokines

Plasma samples were collected at baseline, day 7, and day 28. IL-6, IL-15, MCP-1, adiponectin, TNF- α , and C-reactive protein concentrations in plasma were determined by enzyme-linked immunosorbent assay (Assay Designs; Linco Research, St Charles, MO; and Bioscience, San Diego, CA).

Additional biochemical variables

Plasma glucose was determined with the use of a glucose oxidase method and insulin via standard radioimmunoassay with a commercially available kit (Linco Research). Parathyroid hormone concentrations were determined with the use of a commercial immunoradiometric assay, and calcitriol (1,25-hydroxyvitamin D_2) was determined via standard radioimmunoassay (Nichols Institute Diagnostics, San Juan Capistrano, CA) at baseline and day 28. Fasting lipid profiles (cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride) were assessed by standard clinical techniques in the clinical laboratory.

Biomarker assay variability

Intraassay variation for the oxidative stress biomarkers, inflammatory stress biomarkers, and calcitrophic hormones noted above ranges from 4.2% to 7.8% and interassay variation ranges from 6.8% to 9.2%.

RMR/substrate oxidation

RMR was determined by indirect calorimetry with the use of the open circuit technique between 0600 and 1000 h after a 12-h fast and 48-h abstention from exercise. RMR was used with a physical activity correction to calculate energy needs for accurate formulation of each individual's diet prescription. Respiratory gas exchange was measured via the ventilated hood technique with the use of a SensorMedics Vmax 29n metabolic cart (Sensor Medics, Anaheim, CA). All measures were obtained after a 12-h fast and ≥ 48 h abstention from exercise.

After a urinary void, subjects rested quietly in the supine position for 30 min in a semidarkened, thermoneutral (21–24°C) room. Subjects had a clear, ventilated respiratory canopy placed over the head, remained in a quiet, supine position, and breathed normally until steady state was reached (normally 20–45 min).

Criteria for a valid RMR is ≥ 15 min of steady state, as defined by <10% fluctuation in minute ventilation and oxygen consumption and <5% fluctuation in respiratory quotient. Readings were taken for the steady-state period for determination of resting energy expenditure and for a total of 30 min under these basal conditions for measurement of basal substrate respiratory quotient. Metabolic rate was calculated with the use of the Weir equations (19). Respiratory quotient was calculated as carbon dioxide production/oxygen consumption (20).

A 24-h urine sample was collected for nitrogen analysis for each assessment to calculate nonprotein respiratory quotient and, subsequently, substrate oxidation. Quality control included calibration of the gas analyzers before each measurement with the use of known gases of 95% O_2 and 5% CO_2 .

Statistical analysis

Changes from baseline values were computed for all biomarkers from each dietary period. These data were analyzed with the use of a doubly multivariate repeated-measures analysis, which evaluates multiple dependent variables measured across more than one time point. This method is multivariate both in the number of variables and in the number of measurements. Our analysis evaluated differences between treatments (dairy or soy), obesity status (overweight or obese), and treatment order for more than one endpoint (the 8 oxidative and inflammatory stress biomarkers) measured over multiple time points (days 7 and 28). Although more complex than the familiar analysis of variance or Bonferroni-corrected multiple tests, the doubly multivariate repeated-measures analysis is a superior approach because it accounts for confounding correlations among responses while it tests for differences between groups. The analyses were conducted with the use of SAS, version 9.1.3 (SAS Institute, Cary, NC), and all summary statistics are presented as mean \pm SD.

RESULTS

There were no significant changes in body weight, body fat, trunk fat, or lean mass during either dietary treatment, which was consistent with expectations because of the short study duration. There was no significant effect of order of diet presentation for any of the variables studied (with the exception of MCP-1, as described below). There was no significant effect of sex, but this study was not sufficiently powered to test a sex effect. Each dietary period had its own baseline measurements, and there was no significant difference between the 2 baselines for any variable studied, as shown in **Table 1**. There were no effects of dietary treatment, treatment order, or obesity status on parathyroid hormone concentrations. Calcitriol concentrations decreased significantly in response to the dairy supplement (from 59 ± 7 pg/mL at baseline to 43 ± 9 pg/mL on day 28, $P < 0.01$), whereas there was no significant change in response to the soy supplement (56 ± 8 compared with 59 ± 9 pg/mL, NS).

The effect of dietary treatments on oxidative biomarkers in overweight compared with obese individuals on each study day is summarized in **Table 2**. The dairy-supplemented diet resulted in significant attenuation of oxidative stress, as shown by significant decreases in both malondialdehyde (22%, $P < 0.05$) and 8-isoprostane F_{2x} (12%, $P < 0.02$), whereas the soy supplement exerted no effect. The dairy diet lowered both biomarkers by day

TABLE 1
Baseline values for outcome variables¹

	Baseline of dietary phase I	Baseline of dietary phase II
Body weight (kg)	92.34 ± 9.50	91.64 ± 9.29
Body fat (kg)	31.76 ± 7.08	31.38 ± 7.63
Trunk fat (kg)	17.81 ± 4.43	17.50 ± 5.09
Malondialdehyde (nmol/L)	4.7 ± 0.3	4.1 ± 0.2
8-Isoprostane F _{2x} (pg/mL)	57.6 ± 6.3	56.0 ± 5.7
TNF-α (pg/mL)	463.6 ± 43.6	446.1 ± 53.9
C-reactive protein (μg/mL)	33.6 ± 12.5	26.2 ± 13.5
IL-6 (pg/mL)	83.0 ± 20.7	90.0 ± 23.8
MCP-1 (pg/mL)	102.4 ± 12.4	102.0 ± 13.3
Adiponectin (ng/mL)	7.1 ± 1.1	7.8 ± 1.1
IL-15 (pg/mL)	55.8 ± 11.5	65.7 ± 13.1

¹ All values are means ± SDs. TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; IL-15, interleukin-15. No significant difference between baseline values for the 2 dietary phases was observed for any of the variables shown.

7, with further significant decreases from day 7 to day 28. There was no significant effect of obesity status (overweight compared with obese) on either biomarker.

The effect of dietary treatments on inflammatory biomarkers in overweight compared with obese individuals on each study day is summarized in **Table 3**. The dairy supplement significantly decreased circulating TNF-α (15%) and IL-6 (13%) ($P < 0.01$ for both), whereas the soy diet exerted the opposite effect. These effects were evident at day 7 of each treatment and significantly increased in magnitude from day 7 to day 28. There was no significant effect of obesity status (overweight compared with obese) on either inflammatory biomarker. MCP-1 concentrations were significantly decreased by the dairy supplement (20%, $P < 0.004$) at day 7, with no change from day 7 to day 28, whereas the soy supplement elicited an increase in MCP-1 that was only evident at day 28. There was no effect of obesity status on the MCP-1 response to treatment. However, unlike other variables in this study, there was a significant effect of treatment order ($P = 0.0173$). Subjects who received dairy before soy exhibited a significant decrease, whereas those who received soy before dairy exhibited an increase in MCP-1 from baseline. C-reactive protein exhibited an overall treatment effect that resulted in

a significant decrease (57%) with the dairy supplement on day 28.

Diet was without significant effect on IL-15, although there was a nonsignificant increase on the dairy diet on days 7 and 28. The dairy supplement resulted in a significant increase in circulating adiponectin on day 28 (20%, $P < 0.002$), whereas the soy supplement resulted in a significant decrease on day 28. There was no significant effect of obesity status on either variable.

Treatment was without significant effect on blood pressure, total cholesterol, HDL cholesterol, or triglycerides. However, there was a significant decrease in LDL cholesterol (-8.9 mg/dL with the dairy supplement compared with $+2.9$ mg/dL with the soy supplement, $P < 0.05$) at 28 d of diet.

DISCUSSION

Data from this study demonstrate a role for dairy foods in the attenuation of the oxidative and inflammatory stress associated with excess adiposity. These effects precede any measurable changes in body composition and most were evident within 7 days of dietary change.

TABLE 2
Effects of dietary treatments on oxidative biomarker changes from baseline, by subject population and study day

Biomarker	Obesity status	Diet	Day	Change from baseline		Day	Change from baseline		<i>P</i> value (all subjects) ¹
				Diet	Day		Diet	Day	
Malondialdehyde (nmol/L)	Overweight	Soy	7	-0.11 ± 0.25 (10) ²	Soy	28	-0.16 ± 0.66 (10)	Diet, $P = 0.0117$; day, $P = 0.0257$; diet × day, $P = 0.0120$; diet × order, $P = 0.00138$; order and obesity, NS	
		Dairy	7	-0.40 ± 0.45 (10)	Dairy	28	-1.39 ± 1.10 (10)		
	Obese	Soy	7	-0.22 ± 0.31 (9)	Soy	28	0.10 ± 0.56 (9)		
		Dairy	7	-0.18 ± 0.73 (7)	Dairy	28	-0.55 ± 0.65 (8)		
	All	Soy	7	-0.16 ± 0.28 (19)	Soy	28	-0.03 ± 0.61 (19)		
		Dairy	7	-0.31 ± 0.57 (17)	Dairy	28	-1.02 ± 1.00 (18)		
8-Isoprostane F _{2x} (pg/mL)	Overweight	Soy	7	0.84 ± 11.74 (10)	Soy	28	6.17 ± 9.35 (10)	Diet, $P = 0.0478$; day, $P = 0.0124$; diet × day, $P = 0.0020$; day × order, $P = 0.0338$; day × obesity, $P = 0.0417$; day × order × obesity, $P = 0.0041$	
		Dairy	7	-1.79 ± 3.17 (10)	Dairy	28	-7.84 ± 6.10 (10)		
	Obese	Soy	7	-2.08 ± 5.29 (9)	Soy	28	-3.32 ± 5.09 (9)		
		Dairy	7	-2.45 ± 1.65 (7)	Dairy	28	-7.88 ± 6.04 (8)		
	All	Soy	7	-0.54 ± 9.14 (19)	Soy	28	1.67 ± 8.89 (19)		
		Dairy	7	-2.06 ± 2.61 (17)	Dairy	28	-7.86 ± 5.89 (18)		

¹ Obtained by using doubly multivariate repeated-measures analysis of change from baseline data for all subjects.

² Mean ± SD; *n* in parentheses (all such values).

TABLE 3Effects of dietary treatments on inflammatory biomarker changes from baseline, by subject population and study day¹

Biomarker	Obesity status	Diet	Day	Change from baseline		Change from baseline		<i>P</i> value (all subjects) ²
TNF- α (nmol/L)	Overweight	Soy	7	11.65 \pm 126.80 (10) ³	Soy	28	21.52 \pm 128.83 (10)	Diet, <i>P</i> = 0.0098; diet \times day, <i>P</i> = 0.0452; day \times order, <i>P</i> = 0.0197; diet \times day \times order, <i>P</i> = 0.0440; day \times order \times obesity, <i>P</i> = 0.0003
		Dairy	7	-28.93 \pm 45.22 (10)	Dairy	28	-47.60 \pm 99.72 (10)	
	Obese	Soy	7	55.39 \pm 89.31 (10)	Soy	28	146.45 \pm 108.13 (9)	
		Dairy	7	14.59 \pm 126.25 (7)	Dairy	28	-78.20 \pm 125.45 (8)	
	All	Soy	7	32.37 \pm 109.94 (19)	Soy	28	80.70 \pm 130.27 (19)	
		Dairy	7	-11.01 \pm 87.26 (17)	Dairy	28	-61.20 \pm 109.50 (18)	
MCP-1 (pg/mL)	Overweight	Soy	7	9.10 \pm 11.46 (10)	Soy	28	19.45 \pm 21.14 (10)	Diet, <i>P</i> = 0.0032; day, <i>P</i> = 0.0114; order, <i>P</i> = 0.0173; diet \times day, <i>P</i> = 0.0146; day \times order, <i>P</i> = 0.0113; obesity, NS
		Dairy	7	-7.06 \pm 10.13 (10)	Dairy	28	-7.33 \pm 16.26 (10)	
	Obese	Soy	7	-2.75 \pm 21.79 (9)	Soy	28	8.28 \pm 23.25 (9)	
		Dairy	7	-12.31 \pm 18.46 (7)	Dairy	28	-14.87 \pm 19.65 (8)	
	All	Soy	7	3.48 \pm 17.71 (19)	Soy	28	14.16 \pm 22.28 (19)	
		Dairy	7	-9.22 \pm 13.88 (17)	Dairy	28	-10.68 \pm 17.72 (18)	
IL-6 (pg/mL)	Overweight	Soy	7	-0.41 \pm 13.11 (10)	Soy	28	0.85 \pm 20.64 (10)	Diet, <i>P</i> = 0.0095; diet \times day, <i>P</i> = 0.0291; day \times obesity, <i>P</i> = 0.0354; day, order, and obesity, NS
		Dairy	7	-7.28 \pm 13.01 (10)	Dairy	28	-13.03 \pm 11.38 (10)	
	Obese	Soy	7	5.18 \pm 13.53 (9)	Soy	28	23.99 \pm 42.72 (9)	
		Dairy	7	-1.46 \pm 14.77 (7)	Dairy	28	-10.86 \pm 7.38 (8)	
	All	Soy	7	2.24 \pm 13.25 (19)	Soy	28	11.81 \pm 34.13 (19)	
		Dairy	7	-4.88 \pm 13.63 (17)	Dairy	28	-12.07 \pm 9.60 (18)	
CRP (ng/mL)	Overweight	Soy	7	0.65 \pm 3.37 (10)	Soy	28	4.17 \pm 15.04 (10)	Diet, <i>P</i> = 0.046; diet \times day, <i>P</i> = 0.042; day, order, and obesity, NS
		Dairy	7	14.77 \pm 37.77 (10)	Dairy	28	-26.39 \pm 7.03 (10)	
	Obese	Soy	7	-1.37 \pm 7.37 (9)	Soy	28	42.23 \pm 9.82 (9)	
		Dairy	7	-1.03 \pm 7.57 (7)	Dairy	28	-5.64 \pm 8.38 (8)	
	All	Soy	7	-0.99 \pm 5.47 (19)	Soy	28	4.19 \pm 12.49 (19)	
		Dairy	7	-9.11 \pm 29.54 (17)	Dairy	28	-17.17 \pm 52.50 (18)	
Adiponectin (ng/mL)	Overweight	Soy	7	-1.56 \pm 10.88 (10)	Soy	28	-1.93 \pm 14.47 (10)	Diet, <i>P</i> = 0.0015; diet \times day, <i>P</i> = 0.00048; day, order, and obesity, NS
		Dairy	7	3.03 \pm 8.71 (10)	Dairy	28	15.77 \pm 17.71 (10)	
	Obese	Soy	7	-0.92 \pm 9.42 (9)	Soy	28	-7.85 \pm 12.56 (9)	
		Dairy	7	1.06 \pm 6.41 (7)	Dairy	28	5.79 \pm 10.22 (8)	
	All	Soy	7	-1.25 \pm 9.93 (19)	Soy	28	-4.73 \pm 13.57 (19)	
		Dairy	7	1.83 \pm 7.76 (17)	Dairy	28	11.33 \pm 15.33 (18)	
IL-15 (pg/mL)	Overweight	Soy	7	1.93 \pm 8.20 (10)	Soy	28	1.44 \pm 6.17 (10)	Diet, day, order, and obesity, NS
		Dairy	7	45.62 \pm 143.25 (10)	Dairy	28	56.97 \pm 178.17 (10)	
	Obese	Soy	7	-0.91 \pm 1.72 (9)	Soy	28	-1.53 \pm 3.24 (9)	
		Dairy	7	1.40 \pm 1.82 (7)	Dairy	28	3.35 \pm 3.94 (8)	
	All	Soy	7	3.26 \pm 46.68 (19)	Soy	28	2.87 \pm 64.40 (19)	
		Dairy	7	27.41 \pm 109.76 (17)	Dairy	28	33.14 \pm 132.53 (18)	

¹ TNF- α , tumor necrosis factor- α ; MCP-1, monocyte chemoattractant protein-1; IL-6, interleukin-6; CRP, C-reactive protein; IL-15, interleukin-15.² Obtained by using doubly multivariate repeated-measures analysis of change from baseline data for all subjects.³ Mean \pm SD; *n* in parentheses (all such values).

It is well established that excess adiposity results in an increase in both ROS and inflammatory cytokine production and a corresponding suppression of antiinflammatory cytokine production (1–6). Adipocyte ROS production is modulated by mitochondrial uncoupling status and cytosolic Ca²⁺ signaling, both of which are regulated, in part, by calcitriol (11); calcitriol stimulates ROS production in both murine and human adipocytes (11, 12, 15, 16), which results in dysregulation of adipokines and a corresponding increase in inflammatory stress (12, 16). Accordingly, the reduction in calcitriol that results from the correction of suboptimal dietary calcium intakes is anticipated to decrease oxidative stress, and we have recently reported suppression of oxidative and inflammatory stress in mice fed high-calcium, compared with low-calcium, diets (12, 16). Similarly, retrospective analyses of archival plasma samples from obese subjects demonstrate that diets high in dairy foods resulted in significant reductions in C-reactive protein and corresponding increases in adiponectin (16).

Previous interventions in both mice and humans were potentially confounded by concomitant reductions in adiposity, whereas the present study avoided such confounding by virtue of the randomized-order crossover design, weight maintenance diets, short-term (7-d) measurements, and independent baseline measurements (before each dietary intervention), which were not significantly different from one another. Moreover, there were no significant changes in body weight or body fat over the 28-d measurement period for either dietary treatment. Consistent with these observations, recent data from the Prevención con Dieta Mediterránea (PREDIMED) trial of the effects of a Mediterranean diet on cardiovascular risk factors demonstrate a significant inverse relation between dairy food consumption and both C-reactive protein and ICAM-1 (intercellular adhesion molecule-1) concentrations, and this relation persisted after adjustment for BMI (21).

We previously showed that nonfat dry milk elicited a significantly greater suppression of inflammatory and oxidative stress than calcium carbonate in mice (16). Accordingly, the present

study was designed to compare the effects of nonfat dry milk and a nondairy, low-calcium source of protein (soy protein isolate); as such, it is not possible to determine the extent to which the observed effects were mediated by calcium or other components of the milk. However, it should be noted that other dairy components are anticipated to contribute to these effects. Milk contains angiotensin-converting enzyme inhibitory peptides (22, 23), whereas adipose tissue expresses all components of a fully functional renin angiotensin system (17). A large body of evidence shows that stimulation of this system promotes oxidative and inflammatory responses (24–28), whereas pharmacologic antagonism of this system suppresses oxidative stress (29, 30). Accordingly, it is likely that the angiotensin-converting enzyme inhibitory peptides found in milk may contribute to the observed suppression of oxidative and inflammatory stress.

The high concentration of leucine in milk may also play a significant role in the attenuation of adipose tissue oxidative and inflammatory responses. Leucine stimulates muscle protein synthesis and inhibits protein degradation (31), and recent data indicate that leucine also suppresses energy storage in adipose tissue while it stimulates fat oxidation in support of protein synthesis in skeletal muscle (32). Moreover, we have recently shown that leucine stimulates mitochondrial biogenesis in both adipocytes and skeletal muscle cells (33), with an associated increase in oxygen consumption and fatty acid oxidation in both cell types. This increase in energy utilization is likely to attenuate the oxidative and inflammatory stress that otherwise results from nutrient overload. In addition, we have shown the effects on mitochondrial metabolism to be mediated, in part, by sirtuin 1 (Sirt1) (33). Sirt1 activation of peroxisome proliferator-activated receptor coactivator-1 α not only drives an increase in mitochondrial energy metabolism but also results in the induction of antioxidant enzymes, such as manganese superoxide dismutase (34) and the inhibition of nuclear transcription factor- κ B (NF- κ B) activity (34, 35), thereby attenuating oxidative and inflammatory stress, respectively. Indeed, Sirt1 transgenic mice exhibit a significant reduction in NF- κ B activity and protection against the induction of IL-6 and TNF- α by a high-fat diet (34). Accordingly, leucine stimulation of Sirt1 is likely to contribute to the observed reductions in oxidative and inflammatory stress shown in the present study, because the milk-based smoothies contributed substantially more leucine than the soy-based smoothies (3192 compared with 1731 mg/d).

We observed no significant difference between overweight and obese subjects in any of the variables studied, either in terms of baseline measurement or response to the dietary treatments. Although increased adiposity is associated with increased oxidative and inflammatory stress, the relatively small difference in BMI between the overweight and obese groups (28.0 compared with 32.5) may have precluded detection of any differences in oxidative or inflammatory biomarkers in the modest number of subjects studied.

In summary, data from this study support our previous cellular and mouse data, because they demonstrate a significant suppression of oxidative and inflammatory stress on a dairy-supplemented diet, but not on a comparable soy-supplemented diet. The nature of the experimental design (randomized placebo-controlled crossover) and the successful blinding of the experimental interventions (flavored smoothies for the soy or dairy protein superimposed on an otherwise normal diet for each

individual) provide significant strength to this study and limit the likelihood of any confounding secondary to subject-related variability.

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The authors' responsibilities were as follows—MBZ and XS: study planning and direction; XS and TS: analyses; BW: statistical assessments; and MBZ and BW: manuscript writing. All authors participated in study interpretation. There were no conflicts of interest to report.

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