Dairy attentuates oxidative and inflammatory stress in metabolic syndrome[1–3]

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ABSTRACT

Background: Oxidative and inflammatory stress are elevated in obesity and are further augmented in metabolic syndrome. We showed previously that dairy components suppress the adipocyte- and macrophage-mediated generation of reactive oxygen species and inflammatory cytokines and systemic oxidative and inflammatory biomarkers in obesity.

Objective: The objective of this study was to determine the early (7 d) and sustained (4 and 12 wk) effects of adequate-dairy (AD) compared with low-dairy (LD) diets in subjects with metabolic syndrome.

Design: Forty overweight and obese adults with metabolic syndrome were randomly assigned to receive AD (3.5 daily servings) or LD (<0.5 daily servings) weight-maintenance diets for 12 wk. Oxidative and inflammatory biomarkers were assessed at 0, 1, 4, and 12 wk as primary outcomes; body weight and composition were measured at 0, 4, and 12 wk as secondary outcomes.

Results: AD decreased malondialdehyde and oxidized LDL at 7 d (35% and 11%, respectively; $P < 0.01$), with further decreases by 12 wk. Inflammatory markers were suppressed with intake of AD, with decreases in tumor necrosis factor-α at 7 d and further reductions through 12 wk (35%; $P < 0.05$); decreases in interleukin-6 (21%; $P < 0.02$) and monocyte chemoattractant protein 1 (14% decrease at 4 wk, 24% decrease at 12 wk; $P < 0.05$); and a corresponding 55% increase in adiponectin at 12 wk ($P < 0.01$). LD exerted no effect on oxidative or inflammatory markers. Diet had no effect on body weight; however, AD significantly reduced waist circumference and trunk fat ($P < 0.01$ for both), and LD exerted no effect.

Conclusion: An increase in dairy intake attenuates oxidative and inflammatory stress in metabolic syndrome. This trial was registered at clinicaltrials.gov as NCT01266330. *Am J Clin Nutr* 2011;94:422–30.

INTRODUCTION

Oxidative stress is augmented in obesity; the addition of oxidants suppresses the adipose tissue expression of adiponectin and increases the expression of inflammatory cytokines, whereas antioxidants exert the opposite effect (1–3). Moreover, the oxidative and inflammatory burden is further magnified in metabolic syndrome, which markedly increases the risk of diabetes and cardiovascular and cerebrovascular disease in obese adults (4–6). This observation may explain the disproportionate increase in coronary heart disease (CHD) risk that results when metabolic syndrome accompanies obesity, compared with uncomplicated obesity (7). It was reported that the oxidative and inflammatory burden was augmented in otherwise healthy obese subjects with metabolic syndrome compared with body mass index (BMI; in kg/m^2)–matched obese subjects without metabolic syndrome (8).

Although the effect of diet composition on the oxidative and inflammatory burden has not been well studied, data from the Nurses' Health Study indicate that a diet high in soft drinks, refined grains, and processed meat is positively associated with inflammatory biomarkers (9). Additional data from the Nurses' Health Study show an inverse relation between a "prudent" dietary pattern, which includes low-fat dairy products, and inflammatory biomarkers (10). Similar associations were reported in the Multi-Ethnic Study of Atherosclerosis (MESA) study (11), in a cross-sectional study of dietary patterns in middle-aged Iranian women (12), and in the ATTICA study of >3000 healthy Greek adults (13). Additionally, Miller et al (14) showed a dairy-rich diet to significantly reduce urinary isoprostanes in a 3-mo randomized controlled trial of the Dietary Approaches to Stop Hypertension (DASH) diet in overweight adults. In contrast, Wennersberg et al (15) observed no change in oxidative or inflammatory biomarkers in a 6-mo randomized trial of 3–5 dairy servings/d in patients with metabolic syndrome. However, the control group continued habitual consumption of ≈200 g milk/d, which possibly minimized the effects of the supplemental dairy.

We showed recently that calcitriol augments adipocyte and muscle oxidative and inflammatory stress, whereas dietary calcium–induced suppression of calcitriol decreases the oxidative and inflammatory burden in a mouse model of obesity (7, 16). Moreover, dairy exerted a significantly greater effect than calcium per se, and retrospective assessment of archival serum samples from prior clinical trials indicates a comparable protective effect of dairy in obese humans (17). We confirmed these findings in a recent randomized crossover trial in otherwise healthy overweight and obese adults, and showed that dairy-based smoothies, but not soy-based smoothies, significantly reduced circulating biomarkers of oxidative stress (malondialdehyde and 8-isoprostane $F_{2\alpha}$) and inflammation [tumor necrosis factor-α (TNF-α), interleukin-6, monocyte che-

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moattractant protein 1, and C-reactive protein (CRP)], and increased adiponectin (18). Accordingly, we conducted the present study to determine whether dairy-rich diets can similarly attenuate the elevated oxidative and inflammatory burden associated with metabolic syndrome; we studied early (7-d) and later (28- and 84-d) times to ascertain whether these effects occur before any diet-induced weight changes.

SUBJECTS AND METHODS

Participants

Forty overweight and obese adults (19 men and 21 women) with a diagnosis of metabolic syndrome as defined by National Cholesterol Education Program, Adult Treatment Panel III (NCEP ATP III) criteria (19) were recruited from the staff and student population of the University of Tennessee and the staff and patient population of the University of Tennessee Medical Center via posters, and from the Knoxville area via electronic and print advertisements in the *Knoxville*. The criteria included the presence of $\geq$3 of the following risk determinants: abdominal obesity (a waist circumference of $\geq$102 cm for men and $\geq$88 cm for women), high triglycerides ($\geq$150 mg/dL), low HDL cholesterol ($<$40 mg/dL for males and $<$50 mg/dL for females), elevated blood pressure ($\geq$130/$\geq$85 mm Hg), and high fasting glucose ($\geq$100 mg/dL). To qualify, subjects must have maintained a stable weight, with $\leq$3 kg weight loss in the 3 mo that preceded the study. Subjects were excluded for the following reasons: type II diabetes that required the use of any oral antidiabetic agent and/or insulin; active gastrointestinal disorders or eating disorders; adverse response to dairy foods; recent (past 12 wk) 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 parallel-group design. A low-dairy diet ($<$0.5 serving/d) was studied against an adequate-dairy diet ($>$3.5 servings daily). Each diet was presented for 84 d, with measurements (described below) taken at baseline and on day 7, day 14, day 28, and day 84. This study was approved from an ethical standpoint by the Institutional Review Board of the University of Tennessee-Knoxville; standardized informed consent was obtained from all subjects, and the research was conducted in accordance with the ethical standards outlined in the Helsinki Declaration. This study is registered at clinicaltrials.gov (registration ID no. NCT01266330).

Sample size

Sample size was estimated based on the following considerations. Our initial pilot data, which evaluated the effects of dairy on circulating adiponectin and CRP, showed significant effects with a sample size of 16–18/group. Power calculations based on the variability of the CRP data indicated a sample size of 7/group would be sufficient to detect a 20% difference in CRP and that a sample size of 9/group would be sufficient to detect a 20% difference in adiponectin. This suggested that a sample size of 10 per treatment (20 total) would provide sufficient power for the primary outcome variables. However, the present study includes a broader BMI range than the pilot studies (25.0–39.9 compared with 30–34.9), and a higher variability in baseline values for oxidative and inflammatory variables was consequently anticipated. Accordingly, the sample size estimate was increased 2-fold, to 40 (n = 20/group).

Diets

Subjects were randomly assigned to either a low-dairy ($<$0.5 dairy serving/d and $<$600 mg calcium/d) or adequate-dairy ($>$3.5 dairy servings/d, $\geq$1200 mg calcium/d) weight-maintenance diet. Maintenance levels of calorie intake were determined from the measurement of resting metabolic rate (RMR) via indirect calorimetry. Total daily energy expenditure and maintenance energy requirements were calculated as 1.2–1.4 times RMR, depending on the level of physical activity. The diets were constructed to provide amounts of macronutrient and fiber comparable to the approximate average consumption in the United States (fat, $\approx$35% of total kcal; carbohydrates, $\approx$49%; protein, $\approx$16%; and fiber, 8–12 g/d). Nutritional supplements were not permitted, and caffeine intake was maintained at a constant level (individualized for each patient, based on baseline assessment).

Subjects consuming the adequate-dairy diet received 3 daily servings of dairy, each of which provided 300–350 mg calcium and 8–10 g dairy protein. Dairy-derived calcium totaled 1000–1050 mg/d and dairy-derived protein totaled 28–35 g/d. The dairy products were provided to each subject based on individual choice from a standardized list, with adjustments made to the remainder of the diet to ensure that subject choices did not alter macronutrient intake. Two of the 3 dairy servings consumed daily by those on the adequate-dairy diet were milk and/or yogurt, to ensure sufficient consumption of whey proteins.

Subjects consuming the low-dairy diet were provided with 3 daily servings of prepackaged nondairy foods selected from a standardized list, which included a combination of luncheon meats (low-sodium varieties), soy-based luncheon meat substitutes, packaged fruit cups, granola bars, and peanut butter crackers. Each serving provided no more than 8g of protein and 50 mg calcium, and adjustments were made to the remainder of the diet to ensure that subject choices did not alter macronutrient intake. All other foods were purchased by the participants.

Subjects were given individual instruction, counseling, and assessment regarding dietary adherence. All subjects maintained complete diet and physical activity diaries throughout the study.

Compliance

Review of diet records and product package return was used to assess compliance with the protocol, as follows:

Low dairy: $\leq$600 mg calcium/d and $\leq$0.5 servings of dairy/d
Adequate dairy: $\geq$1000 mg calcium/d and $\geq$3.5 servings of dairy/d
All subjects: product package return (dairy or dairy substitute foods) to reflect $\geq$80% consumption of provided foods

Compliance was assessed weekly, and compliance with all criteria was required to consider a subject compliant within any given week. Subjects were considered to be compliant for the

entire study if weekly compliance criteria were met for 75% (ie, 9 wk) of the 12 active treatment weeks.

Anthropometric measures

Body weight and waist circumference were measured weekly, 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 midexhalation 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 on day 28 and day 84. A LUNAR Prodigy dual-energy X-ray absorptiometry system (GE Health Care, 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 $\geq$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, $\geq$1 min apart, were taken and the average of these 2 was reported. Blood pressure and heart rate were measured weekly.

Reactive oxygen species/oxidative stress

Plasma malondialdehyde and plasma oxidized LDL were measured at baseline and on day 7, day 28, and day 84. Malondialdehyde was measured with the use of a fluorometric assay based on the method of Yagi (20), and plasma oxidized LDL was measured by enzyme-linked immunosorbent assay (Assay Designs, Ann Arbor, MI; Linco Research, St Charles, MO; and Bioscience, San Diego, CA).

Inflammatory markers and cytokines

Plasma samples were collected at baseline and on day 7, day 28, and day 84. Interleukin-6, monocyte chemoattractant protein 1, adiponectin, TNF-α, and CRP concentrations in plasma were determined by enzyme-linked immunosorbent assay (Assay Designs, Linco Research, and Bioscience).

Additional biochemical variables

Plasma glucose, insulin, and fasting lipid profiles (cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride) were measured at baseline and on day 7, day 28, and day 84. They were assessed by standard clinical techniques in the clinical laboratory.

Homeostasis model assessment of insulin resistance

The homeostasis model assessment of insulin resistance (HOMA-IR) was used as a screening index of changes in insulin sensitivity. HOMA-IR was calculated via a standard formula from fasting plasma insulin and glucose as follows (21):

$$\text{HOMA-IR} = [\text{insulin}\ (\mu\text{U/mL}) \times \text{glucose}\ (\text{mmol/L})]/22.5 \quad (1)$$

Biomarker assay variability

Intra-assay variation for the oxidative stress biomarkers and inflammatory stress biomarkers ranged from 4.23% to 7.81%. Interassay variation ranged from 6.84% to 9.22%.

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 a 48-h abstention from exercise at baseline and on day 84. 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 (SensorMedics, Anaheim, CA).

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 was $\geq$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. The metabolic rate was calculated with the use of the Weir equations (22). The respiratory quotient was calculated as carbon dioxide production/oxygen consumption.

A 24-h urine sample was collected for nitrogen analysis for each assessment to calculate the nonprotein respiratory quotient

TABLE 1
Baseline subject characteristics[1]

	All subjects	Low dairy	Adequate dairy
Age (y)	37.0 ± 9.9	39.5 ± 10.2	34.4 ± 9.4
Weight (kg)	94.3 ± 12.1	95.6 ± 12.5	93.0 ± 13.7
Height (cm)	175.4 ± 9.0	175.0 ± 11.9	175.8 ± 5.2
BMI (kg/m^2)	30.7 ± 4.1	31.2 ± 5.4	30.1 ± 4.4
Waist circumference (cm)	103.0 ± 5.8	104.7 ± 9.6	101.3 ± 7.6
Systolic pressure (mm Hg)	127.7 ± 8.3	129.8 ± 11.5	125.5 ± 17.9
Diastolic pressure (mm Hg)	85.9 ± 4.0	83.2 ± 6.6	82.5 ± 10.4
LDL-C (mg/dL)	120.2 ± 11.3	132.1 ± 24.4	108.3 ± 37.5
HDL-C (mg/dL)	47.0 ± 6.7	44.6 ± 7.3	49.4 ± 12.9
Triglycerides (mg/dL)	139.9 ± 36.2	142.5 ± 83.9	137.2 ± 53.0
Sex (M/F)	19/21	9/11	10/10
Ethnicity (*n*)			
African American	7	3	4
Hispanic	9	5	4
White	22	11	11
Other	2	1	1

[1] All values are means ± SDs; n = 20/group. LDL-C, LDL cholesterol; HDL-C, HDL cholesterol. There were no significant group differences.

TABLE 2
Effects of low-dairy (LD) compared with adequate-dairy (AD) diets on body weight, body composition, and waist circumference in overweight and obese subjects with metabolic syndrome[1]

Variable and obesity status	Diet	Change from baseline			*P* value
		Baseline	Day 28	Day 84	
Weight (kg)					
Overweight	LD	87.8 ± 12.7	2.7 ± 2.3	2.4 ± 1.2	NS
	AD	89.4 ± 5.5	−1.4 ± 0.3	−0.1 ± 0.1	
Obese	LD	103.4 ± 16.7	−0.7 ± 1.0	0.0 ± 2.6	
	AD	98.9 ± 11.1	−0.6 ± 1.3	−0.6 ± 2.1	
All	LD	95.6 ± 12.5	1.0 ± 1.3	1.1 ± 1.2	
	AD	93.0 ± 13.7	−1.0 ± 0.7	−0.4 ± 0.4	
BMI (kg/m^2)					
Overweight	LD	28.4 ± 1.3	0.9 ± 0.8	0.8 ± 0.5	Obesity status, $P < 0.001$; diet, NS; day, NS
	AD	26.5 ± 1.4	−0.4 ± 0.1	0.0 ± 0.3	
Obese	LD	34.0 ± 2.3	0.2 ± 0.4	0.0 ± 0.7	
	AD	33.7 ± 3.2	−0.2 ± 0.4	−0.2 ± 0.6	
All	LD	31.2 ± 5.4	0.3 ± 0.4	0.4 ± 0.9	
	AD	30.1 ± 4.4	−0.3 ± 0.2	0.1 ± 0.3	
Waist (cm)					
Overweight	LD	103.9 ± 9.9	−0.3 ± 2.4	−0.1 ± 3.6	Diet, $P < 0.03$; diet × day, $P < 0.03$; obesity status, NS; day, NS
	AD	97.5 ± 4.1	−1.2 ± 2.3	−2.9 ± 2.3	
Obese	LD	106.9 ± 9.9	0.3 ± 0.2	0.5 ± 0.5	
	AD	103.4 ± 8.4	−1.8 ± 0.3	−2.6 ± 0.8	
All	LD	104.7 ± 9.6	−0.3 ± 0.3	−0.2 ± 0.	
	AD	101.3 ± 7.6	−1.5 ± 0.6	−2.8 ± 0.8	
Fat mass (kg)					
Overweight	LD	37.8 ± 9.1	−0.3 ± 0.8	1.0 ± 1.9	Diet, $P < 0.05$; diet × obesity status, $P < 0.05$; day, NS; obesity status, NS
	AD	37.0 ± 5.8	−1.9 ± 1.0	−1.7 ± 1.4	
Obese	LD	42.4 ± 7.7	−0.2 ± 1.4	−0.3 ± 2.2	
	AD	41.5 ± 7.5	−0.4 ± 0.4	−0.8 ± 0.3	
All	LD	40.8 ± 8.6	0.0 ± 1.1	0.4 ± 1.6	
	AD	39.1 ± 4.2	−1.1 ± 0.6	−1.3 ± 0.9	
Trunk fat mass (kg)					
Overweight	LD	21.7 ± 3.8	0.4 ± 0.3	0.1 ± 0.1	Diet, $P < 0.02$; obesity status, NS; day, NS
	AD	20.4 ± 2.7	−1.4 ± 0.9	−1.2 ± 0.6	
Obese	LD	24.2 ± 4.8	0.5 ± 0.9	1.1 ± 1.5	
	AD	22.3 ± 3.9	−0.3 ± 0.3	−1.5 ± 0.3	
All	LD	23.8 ± 5.9	0.4 ± 0.7	0.6 ± 1.0	
	AD	21.0 ± 3.9	−0.8 ± 0.4	−1.4 ± 0.7	
Lean mass (kg)					
Overweight	LD	55.2 ± 8.3	−0.3 ± 0.2	−0.2 ± 1.0	NS
	AD	47.8 ± 6.6	1.6 ± 2.5	1.8 ± 2.4	
Obese	LD	57.6 ± 16.5	−0.9 ± 1.3	0.0 ± 1.1	
	AD	52.6 ± 9.3	0.5 ± 1.1	0.3 ± 1.3	
All	LD	56.1 ± 5.3	−0.5 ± 1.8	−0.1 ± 1.2	
	AD	49.3 ± 10.0	1.1 ± 0.6	0.8 ± 0.8	

[1] All values are means ± SDs; $n = 40$ [20/group (10 overweight and 10 obese)].

and, subsequently, substrate oxidation. Quality control included calibration of the gas analyzers before each measurement with the use of known gases of 95% oxygen and 5% carbon dioxide.

Statistical analysis

Changes from baseline values were computed for all outcome variables. Our analysis evaluated differences between treatments (adequate dairy or low dairy) measured over multiple time points (days 7, 28, and 84). These data were analyzed with the use of a repeated-measures approach; data were analyzed with the use of the PROC MIXED procedure for repeated measures in SAS (version 9.1; SAS Inc, Cary, NC). The effects of baseline, gender, compliance, and obesity status were included in the models and, where significant, are shown in the data tables. When these factors were not significant, they were removed from the final model.

TABLE 3
Effects of low-dairy (LD) compared with adequate-dairy (AD) diets on glucose, insulin, and insulin resistance in overweight and obese subjects with metabolic syndrome[1]

Variable and obesity status	Diet	Baseline	Change from baseline			*P* value
			Day 7	Day 28	Day 84	
Glucose (mmol/L)						
Overweight	LD	4.84 ± 0.39	−0.32 ± 0.95	0.31 ± 0.91	0.38 ± 0.61	NS
	AD	4.83 ± 0.59	−0.77 ± 0.71	−0.63 ± 0.46	−0.04 ± 0.22	
Obese	LD	5.78 ± 2.03	−0.98 ± 0.26	−1.0 ± 0.52	−0.17 ± 0.75	
	AD	4.40 ± 1.00	0.14 ± 0.16	−0.52 ± 0.43	0.03 ± 0.17	
All	LD	5.62 ± 1.86	−0.65 ± 0.24	−0.35 ± 0.45	0.11±-0.51	
	AD	4.57 ± 0.84	−0.22 ± 0.28	−0.57 ± −0.32	0.01 ± 0.22	
Insulin (μU/mL)						
Overweight	LD	9.47 ± 5.89	0.39 ± 0.68	2.55 ± 2.31	1.65 ± 2.46	Diet, $P < 0.05$; obesity status,
	AD	15.58 ± 2.86	−4.75 ± 1.86	−4.692.79	−3.32 ± 2.22	NS; day, NS
Obese	LD	17.80 ± 7.24	4.64 ± 4.91	2.85 ± 2.42	1.74 ± 3.23	
	AD	13.23 ± 2.8	−2.29 ± 1.80	−2.19 ± 2.01	−3.43 ± 2.41	
All	LD	16.42 ± 7.52	2.52 ± 4.55	2.64 ± 2.87	1.72 ± 3.14	
	AD	14.01 ± 5.17	−3.51 ± 1.18	−3.43 ± 1.48	−3.38 ± 1.27	
HOMA-IR						
Overweight	LD	2.94 ± 0.24	−0.83 ± 1.19	−0.08 ± 1.28	−0.28 ± 1.05	Diet, $P < 0.05$; obesity status,
	AD	3.45 ± 2.18	−1.45 ± 0.67	0.08 ± 0.26	−0.60 ± 0.43	NS; day, NS
Obese	LD	4.14 ± 3.25	1.41 ± 1.32	0.51 ± 0.42	2.86 ± 2.79	
	AD	2.78 ± 0.94	−0.33 ± 0.44	−0.40 ± 0.93	−0.58 ± 0.66	
All	LD	3.94 ± 2.97	0.92 ± 0.46	0.21 ± 0.34	1.19 ± 0.68	
	AD	3.05 ± 1.48	−0.71 ± 0.35	−0.28 ± 0.26	−0.59 ± 0.34	

[1] All values are means ± SDs; $n = 40$ [20/group (10 overweight and 10 obese)]. HOMA-IR, homeostasis model assessment of insulin resistance.

RESULTS

Subject baseline characteristics are shown in **Table 1**. All enrolled subjects completed the trial, and 28 met the a priori compliance criteria described in Subjects and Methods (full compliance for 9 of the 12 wk of the study); an additional 5 subjects were fully compliant for 8 of the 12 wk of the study. There was no significant change in body weight for either treatment group, but the adequate-dairy group exhibited a significant decrease in fat mass (≈1 kg, $P < 0.05$). Most of this loss of body fat was accounted for by a decrease in trunk fat

TABLE 4
Effects of low-dairy (LD) compared with adequate-dairy (AD) diets on blood pressure (BP) and heart rate in overweight and obese subjects with metabolic syndrome[1]

Variable and obesity status	Diet	Baseline	Change from baseline			*P* value
			Day 7	Day 28	Day 84	
Systolic BP (mm Hg)						
Overweight	LD	133.3 ± 8.1	1.5 ± 1.0	0.7 ± 1.6	0.6 ± 1.2	Diet, $P < 0.01$; diet × day, $P < 0.01$;
	AD	122.4 ± 14.1	−1.9 ± 2.6	−2.4 ± 2.8	−5.1 ± 2.6	obesity status, NS; day, NS
Obese	LD	127.3 ± 12.2	−1.4 ± 2.6	1.5 ± 1.7	2.1 ± 1.5	
	AD	127.1 ± 18.2	−1.7 ± 1.2	−1.4 ± 1.0	−9.0 ± 2.1	
All	LD	129.8 ± 11.5	0.1 ± 1.6	1.3 ± 1.4	1.4 ± 1.8	
	AD	125.5 ± 17.9	−1.8 ± 2.2	−1.9 ± 1.7	−7.1 ± 3.1	
Diastolic BP (mm Hg)						
Overweight	LD	84.5 ± 10.6	3.0 ± 2.9	2.0 ± 0.9	1.5 ± 0.5	Diet × day × obesity status, $P < 0.05$;
	AD	82.1 ± 9.3	−1.6 ± 0.5	3.4 ± 3.2	−2.9 ± 1.0	diet, NS; obesity status, NS; day, NS
Obese	LD	82.9 ± 6.4	−0.9 ± 2.7	0.5 ± 1.9	2.2 ± 2.9	
	AD	82.6 ± 11.5	−1.3 ± 3.8	−1.4 ± 1.2	−5.2 ± 2.4	
All	LD	83.2 ± 6.6	1.3 ± 1.9	0.8 ± 1.4	1.9 ± 2.2	
	AD	82.5 ± 10.4	−1.5 ± 0.7	1.0 ± 2.7	−4.1 ± 1.9	
Heart rate (beats/min)						
Overweight	LD	76.8 ± 6.2	−1.3 ± 2.1	0.7 ± 2.1	2.4 ± 2.2	NS
	AD	84.0 ± 6.6	−0.1 ± 1.1	−5.5 ± 2.1	−9.7 ± 3.6	
Obese	LD	76.4 ± 12.9	−0.7 ± 1.1	0 ± 1.6	2.0 ± 1.9	
	AD	76.6 ± 8.7	4.6 ± 1.8	1.5 ± 1.7	−5.6 ± 2.5	
All	LD	76.4 ± 11.7	−1.0 ± 1.1	0.5 ± 1.8	2.2 ± 3.3	
	AD	79.3 ± 8.8	2.3 ± 1.6	−2.0 ± 1.9	−7.7 ± 2.3	

[1] All values are means ± SDs; $n = 40$ [20/group (10 overweight and 10 obese)].

TABLE 5
Effects of low-dairy (LD) compared with adequate-dairy (AD) diets on oxidative biomarkers in overweight and obese subjects with metabolic syndrome[1]

Biomarker and obesity status	Diet	Baseline	Change from baseline			P value
			Day 7	Day 28	Day 84	
Malondialdehyde (nmol/L)						
Overweight	LD	1.95 ± 0.47	0.43 ± 0.47	0.59 ± 0.37	0.80 ± 0.11	Diet, $P < 0.01$; obesity status,
	AD	2.80 ± 0.53	−1.34 ± 0.40	−0.86 ± 0.46	−0.81 ± 0.44	$P < 0.01$; day, NS
Obese	LD	5.14 ± 1.50	−0.42 ± 1.22	1.13 ± 1.47	0.70 ± 1.14	
	AD	4.88 ± 1.63	−1.51 ± 0.83	−1.79 ± 0.91	−1.96 ± 0.89	
All	LD	4.61 ± 1.40	−0.05 ± 0.36	0.86 ± 0.74	0.74 ± 0.45	
	AD	4.31 ± 1.15	−1.42 ± 0.51	−1.34 ± 0.71	−1.39 ± 0.89	
Oxidized LDL (ng/mL)						
Overweight	LD	389 ± 62	110 ± 85	38 ± 96	72 ± 83	Diet, $P < 0.02$; obesity status,
	AD	249.2 ± 72	−38 ± 33	−55 ± 19	−70 ± 28	$P < 0.02$; diet × day, $P < 0.02$
Obese	LD	495 ± 74	34 ± 86	64 ± 78	38 ± 70	
	AD	370 ± 89	−51 ± 38	−105 ± 36	−95 ± 22	
All	LD	442 ± 67	72 ± 15	51 ± 67	55 ± 65	
	AD	315 ± 90	−45 ± 23	−78 ± 34	−88 ± 36	

[1] All values are means ± SDs; $n = 40$ [20/group (10 overweight and 10 obese)].

($P < 0.02$), which was also reflected in the significant decrease in waist circumference, which decreased (3 cm; $P < 0.03$) as shown in **Table 2**. The low-dairy diet exerted no significant effect on body composition, weight, or waist circumference (Table 2).

The effects of dietary treatment on insulin resistance and blood pressure are summarized in **Tables 3** and **4**, respectively. The diets exerted no significant effect on plasma glucose, but the adequate-dairy diet resulted in a significant reduction in plasma insulin that was manifested at day 7 and maintained through the end of the study (day 84; $P < 0.05$). Similarly, the HOMA-IR data showed a significant improvement in insulin sensitivity by day 7, maintained through the end of the study ($P < 0.05$) (Table 3). The adequate-dairy diet also resulted in a significant decrease in systolic blood pressure ($P < 0.01$), and this effect increased progressively from day 7 to day 84 ($P < 0.01$) (Table 4). The adequate-dairy diet also exerted a significant effect on diastolic blood pressure in the obese subgroup on day 84 ($P < 0.05$), but was without effect in the low-dairy group. In general, diet exerted little effect on plasma lipids, although the adequate-dairy diet resulted in decreases in plasma cholesterol ($P < 0.02$) and triglycerides ($P < 0.05$) in the obese subjects only (data not shown).

The effect of dietary treatments on oxidative stress biomarkers is summarized in **Table 5**. The adequate-dairy diet resulted in significant attenuation of oxidative stress, as shown by significant decreases in both malondialdehyde and oxidized LDL. By day 7 there was a 35% decrease in malondialdehyde, which was maintained through the 12 wk of the study ($P < 0.01$). Similarly, oxidized LDL had decreased by 11% by day 7 and progressed to a 25% reduction by the end of the study ($P < 0.02$). The low-dairy diet exerted no significant effect on oxidative stress biomarkers, but there was a significant effect of obesity status on both malondialdehyde and oxidized LDL ($P < 0.01$ and $P < 0.02$, respectively).

The effects of dietary treatments on inflammatory markers are summarized in **Table 6**. The adequate-dairy diet significantly reduced each of the inflammatory stress biomarkers studied. A significant reduction in TNF-α was observed by day 7 in the adequate-dairy group, with a progressively greater effect seen on days 28 and 84 ($P < 0.01$); this effect was more pronounced in the obese than in the overweight subgroup. Monocyte chemoattractant protein 1 was similarly decreased by day 7 for the adequate-dairy diet, with progressively greater effects shown on days 28 and 84 ($P < 0.02$ for the diet and time effects). Interleukin-6 was decreased by day 7 on the adequate-dairy diet, with progressively greater effects shown over time, particularly in the obese subgroup ($P < 0.02$). CRP followed a similar trend, although the effect was initially smaller in magnitude for the obese subgroup but became significantly greater among obese subjects by the end of the study ($P < 0.02$ for both the diet effect and interaction). Overall, the adequate-dairy diet resulted in a 47% decrease in circulating CRP levels by day 84. Adiponectin was significantly increased by the adequate-dairy diet, with a 25% increase noted by day 7 that progressed to a 53% increase by day 84 ($P < 0.005$). This effect was significantly more pronounced in the obese subgroup ($P < 0.01$).

DISCUSSION

Data from this study show that an increase in dairy intake from suboptimal to adequate levels (≈3.5 servings/d) significantly attenuates both oxidative and inflammatory stress in metabolic syndrome. Notably, although these effects may result, in part, from reductions in adiposity on higher dairy diets (17, 18), the rapid onset (within the first 7 d of dietary change) suggest that there is an adiposity-independent effect as well. This is further supported by our previous evidence that showed direct effects of dairy components on adipocyte cytokine expression and secretion (23–25).

Our results are at variance with the recent report by Wennersberg et al (15), because they showed no effect of a 6-mo dairy intervention on body composition or oxidative or inflammatory biomarkers in Scandinavian subjects with metabolic syndrome, although they did find an improvement in the HOMA index of comparable magnitude to that reported here. However, there are some key design differences between the 2 trials. The Wennersberg (15) study did not control macronutrient and energy intake, and the dairy intervention group exhibited increases in

TABLE 6
Effects of low-dairy (LD) compared with adequate-dairy (AD) diets on inflammatory biomarkers in overweight and obese subjects with metabolic syndrome[1]

Biomarker and obesity status	Diet	Baseline	Change from baseline			P value
			Day 7	Day 28	Day 84	
TNF-α (nmol/L)						
Overweight	LD	14.47 ± 4.03	0.45 ± 0.24	0.36 ± 1.54	0.29 ± 1.99	Diet, $P < 0.01$; diet × obesity,
	AD	12.47 ± 1.61	−2.34 ± 1.28	−2.86 ± 1.13	−2.62 ± 1.18	$P < 0.01$; diet × day, $P < 0.01$; day, NS;
Obese	LD	15.09 ± 2.42	1.45 ± 1.25	1.58 ± 0.70	0.68 ± 1.30	obesity status, NS
	AD	18.55 ± 1.16	−2.29 ± 1.58	−3.60 ± 1.25	−7.41 ± 0.71	
All	LD	14.22 ± 2.87	0.79 ± 0.66	0.98 ± 1.69	0.49 ± 1.40	
	AD	17.03 ± 3.18	−2.31 ± 1.44	−3.16 ± 1.36	−5.99 ± 2.07	
MCP-1 (pg/mL)						
Overweight	LD	195.2 ± 36.2	23.7 ± 19.7	−10.9 ± 11.0	−22.1 ± 22.7	Diet, $P < 0.02$; diet × day,
	AD	134.8 ± 26.6	−12.9 ± 18.8	−32.1 ± 15.9	−45.6 ± 9.3	$P < 0.02$; obesity status, NS; day, NS
Obese	LD	160.0 ± 47.7	7.0 ± 12.1	20.2 ± 10.9	28.8 ± 22.6	
	AD	182.5 ± 66.8	−24.5 ± 18.0	−39.5 ± 24.7	−45.5 ± 22.7	
All	LD	165.9 ± 45.3	15.3 ± 3.0	15.6 ± 12.8	25.4 ± 23.1	
	AD	176.6 ± 59.8	−18.7 ± 11.5	−35.7 ± 19.9	−45.3 ± 29.9	
IL-6 (pg/mL)						
Overweight	LD	25.3 ± 4.3	−0.7 ± 0.9	−2.8 ± 2.5	1.8 ± 2.2	Diet, $P < 0.02$; diet × obesity
	AD	31.2 ± 3.4	−3.7 ± 0.9	−11.0 ± 1.9	−9.3 ± 1.1	status × day, $P < 0.01$; day, NS;
Obese	LD	44.2 ± 4.1	2.7 ± 2.6	0.4 ± 1.8	−1.3 ± 1.5	obesity status, NS
	AD	41.6 ± 2.7	−11.0 ± 2.7	−13.4 ± 2.3	−18.1 ± 3.3	
All	LD	31.8 ± 1.6	1.0 ± 2.6	−1.5 ± 2.9	0.3 ± 3.8	
	AD	37.4 ± 2.9	−8.0 ± 2.2	−12.2 ± 3.2	−13.7 ± 3.8	
CRP (ng/mL)						
Overweight	LD	29.8 ± 19.5	8.6 ± 11.6	2.3 ± 4.2	4.5 ± 9.3	Diet, $P < 0.02$; diet × obesity
	AD	15.0 ± 8.9	−6.9 ± 4.7	−7.6 ± 4.1	−6.4 ± 4.4	status × day, $P < 0.02$; obesity status,
Obese	LD	27.0 ± 15.6	7.6 ± 9.7	2.5 ± 3.1	−6.7 ± 11.2	NS; day, NS
	AD	24.5 ± 16.6	−3.0 ± 2.4	−3.3 ± 2.8	−11.7 ± 6.6	
All	LD	24.8 ± 15.3	8.1 ± 4.9	2.4 ± 3.2	−1.1 ± 5.5	
	AD	21.9 ± 14.5	−5.0 ± 2.8	−5.5 ± 3.7	−9.1 ± 5.3	
Adiponectin (ng/mL)						
Overweight	LD	15.73 ± 4.55	2.62 ± 2.63	−4.02 ± 3.72	−2.31 ± 2.64	Diet, $P < 0.01$; diet × day, $P < 0.021$;
	AD	21.08 ± 6.62	3.18 ± 2.80	3.19 ± 2.62	5.27 ± 2.16	diet × obesity status × day, $P < 0.01$
Obese	LD	22.24 ± 7.93	−1.62 ± 3.37	0.89 ± 5.31	−3.22 ± 4.8	
	AD	16.85 ± 6.09	5.17 ± 3.35	6.56 ± 2.03	11.82 ± 3.06	
All	LD	21.16 ± 7.65	−0.55 ± 1.82	−1.56 ± 5.38	−2.98 ± 1.59	
	AD	18.26 ± 6.32	4.17 ± 2.44	4.89 ± 2.33	9.01 ± 5.64	

[1] All values are means ± SDs; $n = 40$ [20/group (10 overweight and 10 obese)]. TNF-α, tumor necrosis factor-α; MCP-1, monocyte chemoattractant protein 1; IL-6, interleukin-6; CRP, C-reactive protein.

energy and fat intakes that were not shown in the present study. In addition, subjects recruited for the present study were not habitual consumers of dairy products, and those in the control (low-dairy) group maintained their dairy intake at <0.5 serving/d and their calcium intake at <600 mg/d. In contrast, the baseline calcium intake of the milk intervention group in the Wennersberg (15) study was 815 mg/d, possibly too high to observe an effect of additional dairy foods. Similarly, van Meijl et al (26) observed only a reduction of TNF-α and an increase in soluble TNF-α receptor, but no effects on other inflammatory biomarkers in overweight and obese subjects fed a low-fat dairy diet for 8 wk. However, the estimated calcium intake of their control subjects was 931 mg/d, which suggests that they were already consuming sufficient levels of calcium, leaving little opportunity for dairy to exert significant effects. Notably, a post hoc analysis of subjects below and above a habitual calcium intake of 700 mg/d showed a significant effect of the dairy intervention on waist circumference and sagittal

abdominal diameter only in those with habitually low calcium intakes, but no effect on oxidative or inflammatory markers. It is possible that a lower threshold exists for dairy effects on these biomarkers, but data to evaluate this concept are not available.

We showed previously that calcitriol stimulates inflammatory cytokine production in adipocytes (23) and that dietary calcium–induced suppression of calcitriol attenuates adiposity-induced inflammatory cytokines in a mouse model of obesity (16, 26). We have also shown comparable effects of dairy foods in humans, because dairy significantly reduced circulating biomarkers of oxidative and inflammatory stress in otherwise healthy overweight and obese adults (16, 18).

We observed few significant differences between overweight and obese subjects. Although increased adiposity is generally associated with increased oxidative and inflammatory stress, the effects of BMI have been studied previously in the absence of complicating factors. Thus, the additional inflammatory stress that results from metabolic syndrome appears to exert a greater effect than the adiposity differences between overweight and obese subjects. However, the oxidative biomarkers (malondialdehyde and oxidized LDL) were both higher in the obese than in the overweight individuals, and the adequate-dairy diet exerted significantly greater effects on both oxidative biomarkers in the obese subjects compared with the overweight subjects.

These data also indicate that other components of metabolic syndrome are significantly improved by an increase in dairy food intake to an adequate level. Numerous studies have shown an inverse relation between dietary calcium and blood pressure (27–29), and an increase in dairy intake to adequate levels is a key component of the DASH diet for blood pressure control (30). Whereas these effects are attributable, in part, to dietary calcium, food sources of calcium have consistently exerted greater effects (30). In addition to calcium, dairy contains bioactive components, including angiotensin-converting enzyme inhibitory peptides (31), that also appear to contribute to these effects. We showed the adequate-dairy diet to significantly reduce systolic pressure in both overweight and obese participants and to reduce diastolic pressure in obese, but not overweight, subjects. These data confirm that the achievement of adequate levels of dairy consumption attenuates the elevated blood pressure that is typically characteristic of metabolic syndrome.

Previous studies have suggested that milk proteins, particularly the whey fraction, possess insulinotropic effects in healthy individuals (32), whereas data from the CARDIA study show a strong inverse association between dairy consumption and abnormal glucose homeostasis, as well as the development of the insulin resistance syndrome (33). Furthermore, a higher intake of low-fat dairy appears to reduce the risk of type 2 diabetes (34–37). However, the underlying mechanism of this protective effect is not well understood. Numerous studies have indicated a relation between calcium and vitamin D insufficiency and type 2 diabetes (35), and calcium has been proposed to exert a direct role (34). However, other dairy components, such as angiotensin-converting enzyme inhibitory peptides, may play a significant role (33, 34). Consistent with these observations, data from the present study suggest that an increase in dairy food intake from suboptimal to adequate levels results in an improvement in insulin sensitivity, because circulating insulin and HOMA-IR were markedly improved by the adequate-dairy diet.

Key strengths of this study include the randomized controlled design, the maintenance of the macronutrient composition in the diets, and the assessment of early and sustained time points to isolate the effects of dairy-induced changes in adiposity. Our findings are limited by the relatively short duration of the intervention (12 wk), the use of self-report diet records to assess compliance, and the inability to blind subjects to the use of test (dairy) compared with control foods. In addition, the design of this study may have suffered additional confounding because of the use of readily available foods as control products, some of which may have contributed constituents that exerted potential positive (soy, fruit) or negative (luncheon meats, *trans* fatty acids in peanut butter crackers) independent effects that were not controlled for. Finally, although our selection criteria of subjects with very low habitual dairy and calcium intakes strengthens our design by increasing the likelihood that all subjects started below anticipated calcium and dairy thresholds for treatment effects, it also limits the external validity of our findings, because we cannot extrapolate these findings to individuals with more moderate levels of habitual dairy intake.

Nonetheless, the data from this study support our previous cellular and mouse data, as well as clinical trial data from overweight and obese individuals without metabolic syndrome. Notably, the effects in metabolic syndrome, which is characterized by a higher level of oxidative and inflammatory stress, are greater in magnitude than we recently reported in otherwise healthy overweight and obese individuals (18). The rapid onset of these improvements indicates that they are likely independent of any changes in adiposity that may result from dairy-rich diets. Moreover, these data also suggest that other key components of metabolic syndrome are significantly improved by an increase of dairy food intake to an adequate level.

The authors' responsibilities were as follows—MBZ and RAS: planned and directed the study, performed statistical assessments, and undertook primary manuscript writing; RAS: conducted the clinical study; TT: conducted the biomarker analyses; and RAS, TT, and MBZ: participated in study interpretation. The authors reported no conflicts of interest.

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