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Applied nutritional investigation

Effects of weight loss from a high-calcium energy-reduced diet on biomarkers of inflammatory stress, fibrinolysis, and endothelial function in obese subjects

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ABSTRACT

Objective: Obesity is characterized by chronic subclinical inflammation, which is critical to endothelial dysfunction. Weight loss, induced by lifestyle interventions, is associated with a decline in biomarkers of inflammation and endothelial dysfunction. There is little evidence that high dietary calcium intake may reduce inflammation and improve endothelial function. The purpose of this study was to evaluate the effects of weight loss from a high-calcium energy-reduced diet on biomarkers of inflammation, fibrinolysis, and endothelial function in obese individuals.
Methods: In this randomized clinical trial, we analyzed the data from 35 obese adults who lost at least 3% of initial body weight, during a period of 16 wk of energy restriction (−800 Kcal/d). Individuals were randomized into the following dietary regimens: (1) a high calcium diet (HCD; 1200–1300 mg/d) or (2) a low-calcium diet (LCD; <500 mg/d).
Results: After 16 wk of intervention subjects on HCD compared with those on LCD exhibited greater reduction in waist circumference and waist-to-hip ratio. Participants on HCD presented a significant reduction in all biomarkers of endothelial dysfunction evaluated in the study (intracellular adhesion molecule-1, vascular cell adhesion molecule 1, and E-Selectin), whereas subjects on LCD showed a significant decrease in intracellular adhesion molecule-1 and E-Selectin. Biomarkers of inflammation and fibrinolysis were reduced in both diets, although without reaching statistical significance. The reduction in all markers of inflammation, fibrinolysis, and endothelial dysfunction was similar in both diets.
Conclusion: The findings of this study suggest that increased calcium intake during weight loss has no benefits with respect to biomarkers of inflammation, fibrinolysis, and endothelial function.

Introduction

Obesity is becoming a global epidemic in both children and adults [1]. Overwhelming evidence supports the role of obesity in the pathogenesis and progression of cardiovascular disease [2], a leading cause of mortality in Westernized societies [3].

Adipose tissue, once considered a simple energy warehouse, is now regarded as an active endocrine and paracrine organ that releases a large number of bioactive mediators (adipokines) that influence not only body weight homeostasis but also inflammation, coagulation, fibrinolysis, insulin resistance, and atherosclerosis [3–5]. Most adipokines with proinflammatory properties are overproduced with increasing adiposity, whereas some adipokines with antiinflammatory or insulin-sensitizing properties, like adiponectin, are decreased. This dysregulation of adipokines production may promote obesity-linked metabolic disorders and cardiovascular disease [5].

Obesity represents a disease state characterized by chronic subclinical inflammation. In fact, inflammatory markers, such as C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), monocyte-chemoattractant protein-1 (MCP-1), and plasminogen activated inhibitor (PAI-1), are increased in obese individuals compared with lean subjects [6,7]. Inflammatory mechanisms are critical to all stages of cardiovascular disease progression and play a causal role in vascular endothelial dysfunction that represents a crucial early event in atherosclerosis and subsequent coronary heart disease events [7]. Weight loss, induced by lifestyle interventions, is associated with

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a decline in biomarkers of inflammation and endothelial dysfunction [8–10].

Recent evidence indicates that a calcium-rich diet may affect energy balance, with an antiobesity effect [11,12]. Some studies have suggested that during energy restriction, a high dietary calcium intake improves weight loss and/or abdominal adiposity reduction in obese individual [13–16], which probably enhances the effect of energy restriction on biomarkers of inflammation and endothelial dysfunction.

A recent observational study [17] identified an inverse association between dairy products consumption and levels of various inflammatory markers among healthy adults. Few interventional studies investigated the effect of dietary calcium on inflammation and endothelial function. The majority of these studies were conducted without concomitant energy restriction (and/or weight loss) and their results are conflicting [18–22].

Therefore, the purpose of this study was to evaluate the effects of weight loss from a high-calcium energy-reduced diet on biomarkers of inflammatory stress, fibrinolysis, and endothelial function in obese individuals.

Materials and methods

Study design

This randomized, controlled clinical trial was conducted at the Laboratory of Clinical and Experimental Pathophysiology, CLINEX, located at Pedro Ernesto University Hospital, Rio de Janeiro State University.

Potential participants were recruited at the Department of Plastic Surgery, among candidates for lipoplasty, and underwent initial eligibility screening by registered dieticians. Subjects were assessed during a 2-wk run-in period to evaluate their baseline diet and clinical status, after which a 16-wk intervention period followed. The weight and physical activity of the eligible individuals were stable, with no more than a 3-kg weight variation over the preceding 12 wk and no recent change in exercise frequency or intensity. Using a computer-generated randomization with the software STATA version 10.0 (StataCorp, College Station, TX, USA), we randomized participants into a high-calcium diet (HCD) or a low-calcium diet (LCD). We used block randomization to balance the size of HCD and LCD groups.

Participants in both groups received individual instruction from the study dietician, who provided nutritional assessment at baseline (week 0) and weeks 4, 8, 12, and 16. The development and reinforcement of strategies for continued success were made at the same time points. Anthropometric parameters and blood pressure levels were measured at baseline and at weeks 4, 8, 12, and 16. Fasting plasma levels of circulating glucose, insulin, leptin, calcium, parathormone (PTH), 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], lipid profile, highly sensitive CRP (hs-CRP), TNF-α, intracellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), E-Selectin, and PAI-1 were evaluated at baseline and at week 16. Blood samples were collect between 8 and 10 A.M. after an overnight fast (12 h). Participants were asked to collect 24-h urine at baseline and at week 16. Urinalysis was performed for calcium and creatinine.

Approval for the study was obtained from the Ethics Committee of Pedro Ernesto University Hospital. All participants gave written informed consent. The procedures followed in this study were in accordance with institutional guidelines.

Subjects

Subjects were initially screened according to the following criteria: age between 20 and 60 y, body mass index (BMI) between 30.0 and 34.9 kg/m^2, and a low habitual dietary calcium intake (<500 mg/d). The exclusion criteria included the following: following special diets (i.e., vegetarian); intolerance to lactose; use of medications affecting the metabolism, such as metformin, insulin, diuretics, statins, and β-blockers; current calcium supplementation or use of any medication known to interfere with calcium metabolism and body weight. Individuals with eating disorders, major depression, or a medical history of drug addiction were also excluded. In addition, those with any metabolic disease, such as diabetes mellitus or hypothyroidism, or chronic diseases severely affecting the cardiovascular, gastrointestinal, and renal systems were also excluded. Pregnant or lactating women were not allowed into the study.

Of the 198 individuals initially screened, 67 entered the run-in period. Seventeen individuals did not complete this phase and were not enrolled in the study. During the run-in period, potential participants were submitted to dietary, anthropometric, and biochemical evaluation. Fourteen subjects (21%) failed to complete the run-in period because (1) their calcium intake was more than 500 mg/d ($n = 6$); (2) the changes in body weight during the period resulted in BMI values less than 30 or more than 35 kg/m^2 ($n = 7$); and (3) the plasma glucose levels were maintained at values more than 126 mg/dL ($n = 1$). After completing the run-in period, participants were then randomized to the following outpatient dietary regimens: (1) a HCD [1200–1300 mg/d, supplemented with non-fat powdered milk (60 g/d)] or (2) a LCD (<500 mg/d) (Fig. 1). Both groups were instructed to follow a calorie-restricted regimen. All calcium in both diets was derived from food sources. Thirty-nine subjects (78%) completed the study, 19 in the HCD group and 20 in the LCD group. The non-completers left the study because of scheduling conflicts, moving to a distant area, illness or death of a relative, or loss of interest. In the present analysis of data, to ensure that all subjects really experienced weight loss during the study period, we included only the participants that lost at least 3% of their initial body weight. Therefore, included in the final analysis were 18 participants in HCD and 17 in LCD.

A modest weight loss of 5% to 15% of initial body weight over a period of 6 mo is considered realistic and of proven health benefit [23], so we adopted a cutoff point of 3% of weight loss, because the duration of the present study was 16 wk (~4 mo).

Diets

The dietary assessments during the run-in period used a 3-d food record, covering two weekdays and one weekend day, to estimate current calcium consumption. During the intervention phase, the food records were reviewed and clarified in an interview with a registered dietician every 4 wk to assess dietary adherence. Nutrient analysis of the 3-d food record was performed using the software NutWin (São Paulo Federal University, UNIFESP, São Paulo, Brazil).

In both intervention arms, low- and high-calcium diets, energy intake was formulated to correspond to an 800 kcal/d reduction in the baseline total daily energy expenditure, calculated using the World Health Organization equations [24]. Both diets were structured to provide comparable levels of macronutrients and fibers: ~22% of calories from protein, ~28% from fat, ~50% from carbohydrate, and ~19 g of fibers per day. Participants in the HCD received 60 g/d of non-fat powdered milk. Reported total energy, percentage of energy from protein, fat, and carbohydrate, and fiber content were similar in both diets (Table 1). Calcium intake in HCD and LCD was 1245.6 ± 20.3 and 456.7 ± 15.3 mg/d, respectively ($P < 0.001$) (Table 1).

Subjects were encouraged to use a meal plan based on their calorie intake and an exchange list of nutrients. Participants were supplied with an analog kitchen scale to assist in accurately measuring their food portions. Neither subjects nor dieticians were blinded to the assigned diet.

Anthropometric measurements

Height, weight, and waist and hip circumferences were measured between 8 and 10 A.M. after a 12-h fast. Height was obtained using a stadiometer and weight was measured with a Fillizola calibrated scale, accurate to ±0.1 kg, after participants, wearing a hospital gown, attempted to empty their bladder. BMI was calculated using the standard equation (kg/m^2). Waist circumference was measured in the standing position, midway between the lower margin of the last rib and the iliac crest. The measurements were taken at mid exhalation. Hip circumference was measured at the widest point of the hip/buttocks area with the measuring tape parallel to the floor. Waist-to-hip ratio was determined by dividing waist circumference by hip circumference.

Body fat was estimated using an equation, based on skinfold thickness, which was validated using a four-compartment model as the method of reference [25]. This equation includes the sum of the triceps, subscapular, suprailiac, and mid-thigh skinfold thicknesses. These skinfold thicknesses were measured on the left side of the body, using a Lange skinfold caliper (Beta Technology, Santa Cruz, CA,USA), according to standard techniques [26].

The anthropometric measurements were taken twice and mean values were used in all analyses.

Laboratorial parameters

Plasma samples were stored at −80°C. Total cholesterol, high-density lipoprotein cholesterol, and triglyceride concentrations were measured at the hospital clinical laboratories using an automated analyzer. Low-density lipoprotein cholesterol was calculated using the Friedewald formula [27] when triglycerides did not exceed 400 mg/dL. Radioimmunoassay was used to determine plasma leptin and insulin levels (Linco Research, St Charles, MO, USA). Fasting plasma glucose was determined by the use of the glucose oxidase method. The insulin resistance status was assessed by the use of homeostasis model assessment of insulin resistance (HOMA-IR) index [28]. The value of

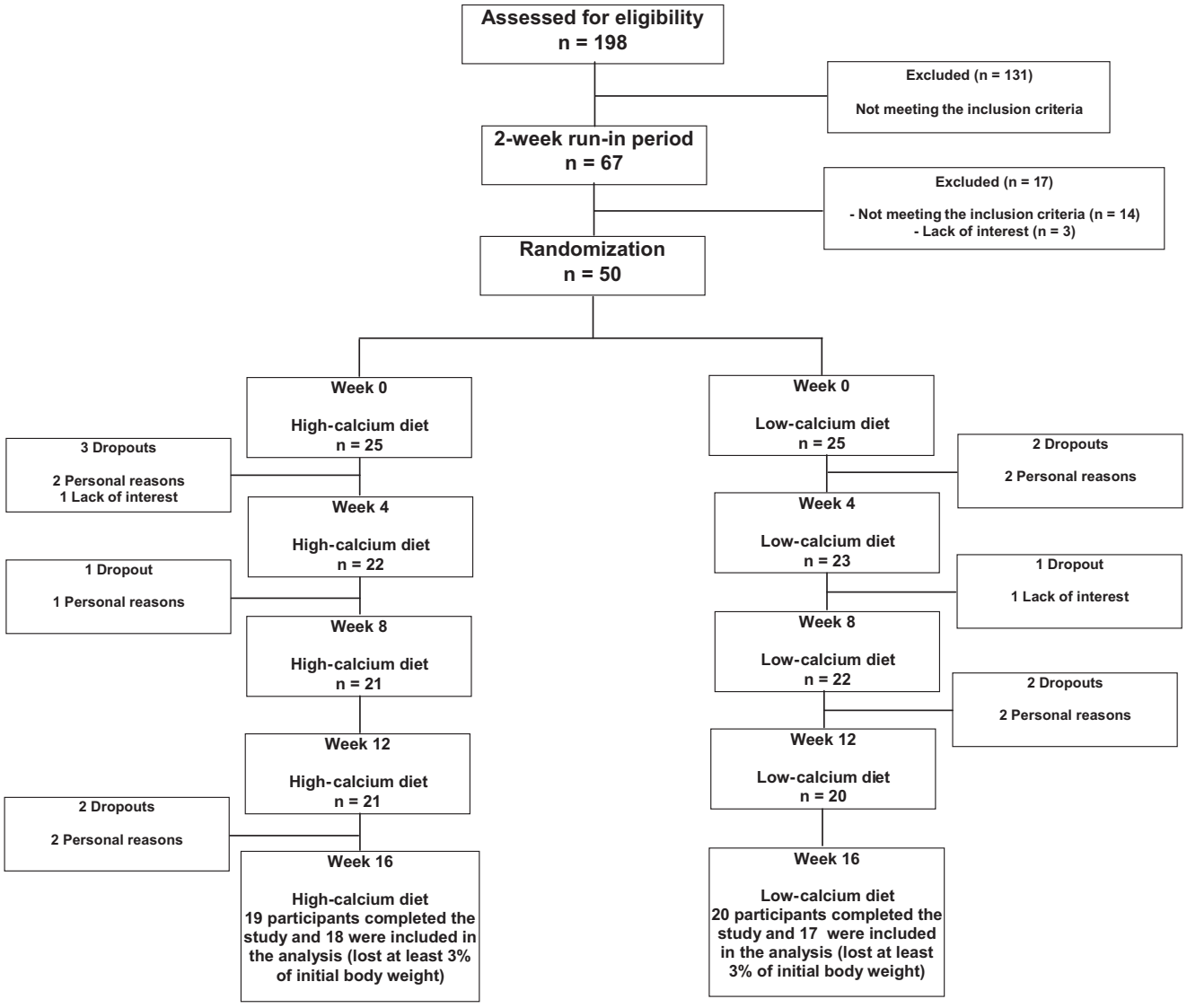

Fig. 1. Flow diagram of the study.

1,25$(OH)_2$vitD was determined by radioimmunoassay using the commercial kit of Diasorin SpA (Saluggia, Vercelli, Italy). Plasma PTH intact molecule was assessed by electrochemiluminescence (Roche Diagnostics GmbH, Mannheim, Germany). Serum and urinary calcium were determined by colorimetry. Urinary creatinine was assessed by kinetic method. Highly sensitive CRP was determined by turbidimetry using a commercial kit (Biosystems, Barcelona, Spain). Plasma levels of TNF-α were determined by enzyme immunometric method using commercial kits (Assay Designs, Ann Arbor, MI, USA). The values of ICAM-1, VCAM-1, E-Selectin, and PAI-1 were determined by luminex xMAP method using a commercial kit of multiple dosing (EMD Millipore Corporation, Billerica, MA, USA).

Table 1
Nutrient composition of reported intake during the study

Nutrient	High-calcium diet ($n = 18$)	Low-calcium diet ($n = 17$)	P
Energy (Kcal/d)	1719.6 ± 45.4	1698.1 ± 51.3	0.63
Protein (% energy)	21.7 ± 0.5	21.2 ± 0.6	0.30
Fat (% energy)	28.4 ± 0.6	28.2 ± 0.7	0.54
Carbohydrate (% energy)	49.9 ± 0.5	50.7 ± 0.6	0.15
Fiber (g/d)	15.6 ± 0.4	17.2 ± 0.5	0.33
Calcium (mg/d)	1245.6 ± 20.3	456.7 ± 15.3	<0.001

Data are mean ± SEM.

Blood pressure

Blood pressure was recorded using a calibrated Dinamap 1846 Critikon automated sphygmomanometer (Critikon, Tampa, FL, USA), after a resting period of at least 10 min in the sitting position. An appropriate arm cuff was used. Arm position was adjusted so that the cuff was at the level of the right atrium. Blood pressure was measured on the dominant arm, every 3 min for 15 min, at a constant temperature of 22–24°C. The first value was discarded, and the mean of the last four readings was used in the analysis.

Statistical methods

Mean values and standard errors were used to summarize quantitative variables. Normality was tested by using the Shapiro-Wilk normality test. Skewed data (triglycerides, insulin, leptin, PTH, HOMA-IR, hs-CRP, PAI-1, ICAM-1, and E-selectin) were log transformed to improve normality. A t test for independent samples was used to assess the differences of variables between the two groups at baseline. For biochemical variables (evaluated at week 0 and week 16), intra- and intergroup differences were assessed by a t test. For anthropometric parameters and blood pressure levels (that were evaluated at weeks 0, 4, 8, 12, and 16), we used repeated measures ANOVA to evaluate intra- and intergroup differences. Multiple linear regression analysis was used to adjust for confounding factors (age, gender, and baseline values of each variable). Categorical variables were compared by χ^2 test. A $P < 0.05$ was considered significant. All

Table 2
Baseline characteristics of study subjects

Characteristic	High-calcium diet ($n = 18$)	Low-calcium diet ($n = 17$)	P
Age (y)	40.5 ± 2.4	44.1 ± 2.6	0.24
Sex (female/male)	17/1	15/2	0.51
Body weight (kg)	83.3 ± 2.6	81.6 ± 2.6	0.62
Body mass index (kg/m^2)	32.2 ± 0.3	32.1 ± 0.4	0.83
Body fat (%)	43.2 ± 0.8	42.6 ± 1.2	0.67
Body fat (kg)	35.9 ± 1.0	34.5 ± 1.1	0.35
Waist circumference (cm)	98.9 ± 1.2	98.7 ± 1.5	0.94
Hip circumference (cm)	112.9 ± 1.5	111.1 ± 1.8	0.47
Waist-to-hip ratio	0.88 ± 0.01	0.89 ± 0.02	0.52
Glucose (mg/dL)	99.5 ± 2.1	97.6 ± 3.5	0.65
Insulin (μU/mL)	21.2 ± 1.5	23.1 ± 2.8	0.55
HOMA-IR	5.2 ± 0.3	5.8 ± 0.8	0.50
Total cholesterol (mg/dL)	208.0 ± 9.2	208.1 ± 12.3	0.99
HDL-cholesterol (mg/dL)	53.2 ± 3.4	49.2 ± 1.6	0.29
LDL-cholesterol (mg/dL)	124.6 ± 7.7	128.9 ± 9.8	0.73
Triglycerides (mg/dL)	157.6 ± 21.7	147.6 ± 15.8	0.71
Leptin (ng/mL)	30.0 ± 3.9	27.3 ± 3.1	0.59
Total serum calcium (mg/dL)	9.4 ± 0.1	9.4 ± 0.1	0.58
Ionized serum calcium (mg/dL)	5.3 ± 0.1	5.4 ± 0.1	0.09
24-h urine calcium/creatinine (mg/mg)	0.13 ± 0.02	0.12 ± 0.02	0.33
Parathormone (pg/mL)	34.1 ± 3.3	30.0 ± 2.5	0.34
1.25(OH)$_2$vit D (pg/mL)	79.7 ± 3.5	68.9 ± 3.9	0.06
hs-CRP (mg/L)	0.26 ± 0.02	0.36 ± 0.05	0.11
Tumor necrosis factor-α (pg/mL)	3.1 ± 0.6	4.3 ± 0.9	0.26
PAI- 1 (pg/dL)	106.2 ± 9.3	104.8 ± 16.3	0.94
ICAM-1 (ng/mL)	146.0 ± 25.1	193.8 ± 44.0	0.33
VCAM-1 (ng/mL)	930.3 ± 43.7	1016.4 ± 97.3	0.42
E-Selectin (ng/mL)	31.2 ± 3.1	34.1 ± 3.4	0.57
Systolic blood pressure (mmHg)	118.1 ± 2.0	114.1 ± 2.0	0.20
Diastolic blood pressure (mmHg)	76.6 ± 2.1	72.1 ± 1.9	0.11
Mean blood pressure (mmHg)	90.5 ± 2.1	86.1 ± 1.8	0.12

HDL, high-density lipoprotein; hs-CRP, high sensible C-reactive protein; ICAM-1, intracellular adhesion molecule 1; LDL, low-density lipoprotein; PAI-1, plasminogen activator inhibitor 1; VCAM-1, vascular cell adhesion molecule 1.
Data are mean ± SEM.

statistical analyses were performed using the software STATA 10.0 (STATACorp., College Station, TX, USA).

Results

Both treatment groups were comparable in all characteristics at baseline (Table 2). As expected from the experimental design, there was a significant reduction in all anthropometric parameters of the participants allocated in both HCD and LCD, because of the energy deficit of 800 kcal/d (Table 3). Subjects in HCD demonstrated a significantly greater reduction in waist circumference and waist-to-hip ratio when compared with those in the LCD (Table 3 and Fig. 2). Regarding body weight and body fat, the differences between groups were not statistically significant (Table 3 and Fig. 2). The reduction in anthropometric measures presented a continuous trend during all the study period (Fig. 2). Approximately 85% of weight loss corresponded to estimated fat loss in both groups.

Serum concentration of glucose, insulin, HOMA-IR, total cholesterol, triglycerides, and leptin decreased significantly in both groups (Table 4). There was no significant difference between groups in relation to the modifications in metabolic profile during the study period (Table 4). Serum levels of PTH and 1,25(OH)$_2$vitD were significantly reduced after 16 wk of intervention only in the HCD. Comparisons of changes in PTH values between groups showed a significantly greater reduction in this variable in the HCD (Table 4). Systolic, diastolic, and mean blood pressure presented a significant reduction in both groups. Participants in HCD compared to subjects in LCD presented a significantly higher reduction in blood pressure (Table 3 and Fig. 2).

Serum levels of hs-CRP and TNF-α decreased from baseline to the end of the study (week 16) in both diets. However, the reduction in these biomarkers of inflammation was not statistically significant in both groups (Fig. 3). The comparative analysis of the changes in these parameters between HCD and LCD showed no statistical significance for hs-CRP ($P = 0.20$) and for TNF-α ($P = 0.61$), even after adjusting for confounding factors (age, gender, and baseline values of each variable) ($P > 0.05$).

PAI-1 was also reduced during the study period without reaching statistical significance in HCD and LCD, although there was a trend to be statistically significant in both groups (Fig. 3). Participants in HCD and in LCD showed a similar reduction in this biomarker of fibrinolysis ($P = 0.97$), even after adjusting for confounding factors ($P > 0.05$).

HCD subjects presented a significant reduction in serum levels of all the studied adhesion molecules (ICAM-1, VCAM-1, and E-Selectin), whereas participants in LCD presented a significant decrease only in ICAM-1 and E-Selectin (Fig. 3). The comparative analysis between HCD and LCD showed that the reduction in these biomarkers of endothelial dysfunction was similar in both groups: ICAM-1 ($P = 0.65$), VCAM-1 ($P = 0.47$), and E-Selectin ($P = 0.99$). The decrease in these biomarkers remained similar in both diets even after controlling for confounders ($P > 0.05$).

Table 3
Anthropometric parameters and blood pressure levels at baseline (week 0) and at the end of the study (week 16) in the different groups of calcium intake

	High-calcium diet (HCD) ($n = 18$)				Low-calcium diet (LCD) ($n = 17$)				$P^{\ddagger}$	$P^{\S}$
	Week 0	Week 16	Δ	P^{*}	Week 0	Week 16	Δ	$P^{\dagger}$		
Body weight (kg)	83.3 ± 2.6	78.0 ± 2.1	−5.3 ± 0.9	<0.001	81.6 ± 2.6	77.2 ± 2.8	−4.4 ± 0.6	<0.001	0.42	0.60
Body mass index (kg/m^2)	32.2 ± 0.3	30.1 ± 0.4	−2.0 ± 0.3	<0.001	32.1 ± 0.4	30.3 ± 0.5	−1.8 ± 0.3	<0.001	0.54	0.78
Body fat (%)	43.2 ± 0.8	40.4 ± 0.8	−2.9 ± 0.4	<0.001	42.6 ± 1.2	40.1 ± 1.3	−2.5 ± 0.4	<0.001	0.60	0.66
Body fat (kg)	35.9 ± 1.0	31.4 ± 1.0	−4.5 ± 0.7	<0.001	34.5 ± 1.1	30.8 ± 1.4	−3.7 ± 0.5	<0.001	0.38	0.74
Waist circumference (cm)	98.9 ± 1.2	90.8 ± 1.3	−8.1 ± 1.3	<0.001	98.7 ± 1.5	92.9 ± 1.6	−5.8 ± 0.6	<0.001	0.04	0.04
Waist-to-hip ratio	0.88 ± 0.01	0.84 ± 0.01	−0.04 ± 0.0	<0.001	0.89 ± 0.02	0.86 ± 0.02	−0.03 ± 0.01	<0.001	0.04	0.04
Systolic blood pressure (mmHg)	118.4 ± 2.6	109.4 ± 2.6	−9.0 ± 1.6	<0.001	114.1 ± 2.0	110.0 ± 2.1	−4.1 ± 1.2	0.005	0.02	0.03
Diastolic blood pressure (mmHg)	76.2 ± 2.0	69.3 ± 2.2	−7.3 ± 1.2	<0.001	72.1 ± 1.9	68.2 ± 1.4	−3.9 ± 1.1	0.003	0.04	0.04
Mean blood pressure (mmHg)	90.5 ± 2.1	82.7 ± 2.3	−7.8 ± 1.1	<0.001	86.1 ± 1.8	82.2 ± 1.6	−3.9 ± 0.9	<0.001	0.01	0.02

Data are mean ± SEM. Δ = week 16 − week 0.
* P value refers to changes during the study, considering the values at weeks 0, 4, 8, 12, and 16 in HCD, and was estimated by repeated measures ANOVA.
† P value refers to changes during the study, considering the values at weeks 0, 4, 8, 12, and 16 in LCD, and was estimated by repeated measures ANOVA.
‡ HCD versus LCD by repeated measures ANOVA.
§ HCD versus LCD diet by repeated measures ANOVA adjusted for age, gender, and baseline values of each variable.

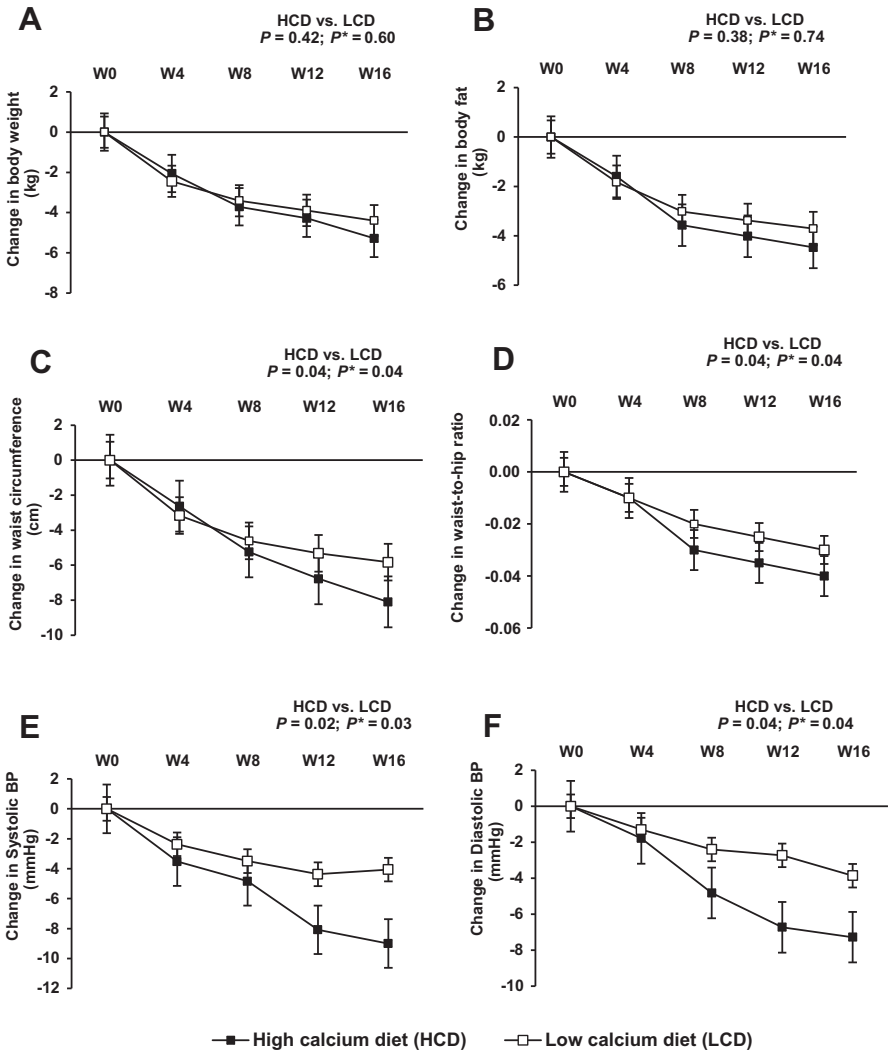

Fig. 2. Mean changes from baseline (week 0) in (A) body weight, (B) body fat, (C) waist circumference, (D) waist-to-hip ratio, (E) systolic blood pressure, and (F) diastolic blood pressure, according to dietary intervention ($n = 35$). *P* value was estimated by repeated measures ANOVA.

Discussion

In the present clinical trial, based on a sample of obese individuals who lost at least 3% of initial body weight from an energy-reduced diet (randomized to a HCD or a LCD), the main findings were as follows: (1) a significant reduction in all biomarkers of endothelial dysfunction (ICAM-1, VCAM-1, and E-Selectin) in HCD; (2) a significant decrease in ICAM-1 and E-Selectin in LCD; (3) biomarkers of inflammation and fibrinolysis reduced in both diets, although without reaching statistical significance; (4) the reduction in all biomarkers of inflammation, fibrinolysis, and endothelial dysfunction was similar in both diets.

In the present study, we selected only patients who really lost weight, because our objective was to evaluate the effects of a high-calcium intake, during weight loss, on inflammation, fibrinolysis, and endothelial function. Although it was not the aim of the present study, participants in HCD compared to subjects in LCD presented a significantly higher decrease in abdominal obesity and blood pressure.

CRP is a hepatically derived inflammatory response marker that increases up to 1000-fold in response to infection and tissue destruction. Standard tests for PCR determine these highly elevated CRP levels, but cannot adequately assess the normal ranges of this protein. Highly sensitive CRP detects levels of CRP within the normal range as well as higher levels. Observational and epidemiological studies have suggested the potential predictive role of hs-CRP in the prediction of cardiovascular events [29,30].

Cross-sectional and interventional studies have shown that hs-PCR is positively associated with adiposity parameters such as BMI [31] and that weight loss in obese individuals reduces significantly the levels of this protein [8,9,32,33]. In a systematic review [8] of lifestyle intervention studies, the mean weight change was –6.2 kg during a mean period of 7.5 mo and the mean change in hs-CRP levels was –0.9 mg/L (range, –2.3 to 0.5 mg/L). Heilbronn et al. [9] investigated the effects of weight loss in 83 obese women (mean BMI of 33.8 kg/m^2 and mean CRP of 5.6 mg/L). Subjects were placed on very-low-fat, energy-restricted

Table 4
Metabolic variables at baseline (week 0) and at the end of the study (week 16) in the different groups of calcium intake

	High-calcium diet ($n = 18$)				Low-calcium diet ($n = 17$)				$P^{\ddagger}$	$P^{\S}$
	Week 0	Week 16	Δ	P^{*}	Week 0	Week 16	Δ	$P^{\dagger}$		
Glucose (mg/dL)	99.5 ± 2.1	93.7 ± 2.3	−5.8 ± 1.9	0.006	97.6 ± 3.5	94.0 ± 2.8	−3.6 ± 1.6	0.03	0.64	0.52
Insulin (μU/mL)	21.2 ± 1.5	17.1 ± 1.56	−4.1 ± 1.23	0.004	23.1 ± 2.8	18.6 ± 2.6	−4.5 ± 1.9	0.03	0.91	0.82
HOMA-IR	5.2 ± 0.3	3.9 ± 0.4	−1.3 ± 0.3	0.001	5.8 ± 0.8	4.4 ± 0.6	−1.4 ± 0.5	0.02	0.69	0.68
Total cholesterol (mg/dL)	208.0 ± 9.2	191.2 ± 9.6	−16.8 ± 5.0	0.004	208.1 ± 12.3	192.5 ± 9.9	−15.6 ± 5.2	0.008	0.87	0.71
HDL-cholesterol (mg/dL)	53.2 ± 3.4	51.9 ± 3.2	−1.3 ± 1.4	0.38	49.2 ± 1.6	48.6 ± 2.1	−0.6 ± 1.5	0.70	0.74	0.84
LDL-cholesterol (mg/dL)	124.6 ± 7.7	117.1 ± 8.4	−7.4 ± 4.7	0.13	128.9 ± 9.8	120.3 ± 8.0	−8.6 ± 4.6	0.08	0.87	0.89
Triglycerides (mg/dL)	157.6 ± 21.7	114.1 ± 0.2	−43.4 ± 12.2	0.003	147.6 ± 15.8	117.6 ± 11.6	−29.9 ± 7.6	0.001	0.54	0.46
Leptin (ng/mL)	30.0 ± 3.9	23.0 ± 3.5	−7.0 ± 2.2	0.006	27.3 ± 3.1	22.8 ± 4.0	−4.5 ± 2.3	0.006	0.45	0.57
Total serum Ca^{++} (mg/dL)	9.4 ± 0.1	9.5 ± 0.1	0.1 ± 0.1	0.34	9.4 ± 0.1	9.4 ± 0.1	−0.04 ± 0.1	0.78[†]	0.26	0.32
Ionized serum Ca^{++} (mg/dL)	5.3 ± 0.1	5.3 ± 0.1	0.03 ± 0.01	0.70	5.4 ± 0.1	5.4 ± 0.1	−0.04 ± 0.1	0.64[†]	0.54	0.58
24-h urine Ca^{++}/Creatinine (mg/mg)	0.13 ± 0.02	0.16 ± 0.02	0.03 ± 0.02	0.12	0.12 ± 0.02	0.12 ± 0.02	−0.00 ± 0.01	0.92[†]	0.18	0.16
Parathormone (pg/mL)	34.1 ± 3.3	29.7 ± 2.9	−4.4 ± 1.3	0.004	30.0 ± 2.5	31.6 ± 2.4	1.6 ± 2.4	0.50[†]	0.04	0.03
$1.25(OH)_2$vit D (pg/mL)	79.7 ± 3.5	68.9 ± 3.7	−10.8 ± 5.3	0.04	68.9 ± 4.0	68.4 ± 4.1	−0.5 ± 5.1	0.49[†]	0.15	0.12

HDL, high-density lipoprotein; LDL, low-density lipoprotein; Δ = week 16 − week 0.
Data are mean ± SEM.
[*] *P* value refers to differences between baseline and end of study (week 0 versus week 16) in HCD and was estimated by *t* test.
[†] *P* value refers to differences between baseline and end of study (week 0 versus week 16) in LCD and was estimated by *t* test.
[‡] HCD versus LCD diet by *t* test.
[§] HCD versus LCD diet by *t* test adjusted for age, gender, and baseline values of each variable.

diets (5700 kJ/d, 15% fat) for 12 wk. Weight change was −7.9 kg and CRP was significantly decreased by 26% ($P < 0.001$).

In the present study we did not observe a significant reduction in hs-PCR levels in both groups. The possible explanations for this lack of agreement between our results and the findings of the literature are that, in comparison with the studies cited in the above paragraph [8,9,32], (1) our participants in HCD and in LCD presented lower baseline levels of BMI (32.2 and 32.1 kg/m^2, respectively) and hs-CPR (0.26 and 0.36 mg/L, respectively); and (2) the magnitude of weight loss in HCD and LCD was also lower (−5.3 and −4.4 kg, respectively).

Participants in HCD and in LCD did not present a significant reduction in serum levels of TNF-α. This finding is also divergent from what was observed in other studies, in which weight loss was associated with a significant decrease in TNF-α [10,34]. The possible mechanisms responsible for this disagreement with other studies are the same as were mentioned for hs-PCR.

PAI-1 is the most important endogenous inhibitor of tissue plasminogen activator and is a main determinant of fibrinolytic activity. There is now compelling evidence that obesity is associated with elevated PAI-1 levels [35]. Weight loss is associated with a significant reduction in PAI-1, which is proportional to the degree of weight loss [33,36]. We observed a decrease in PAI-1 levels with a trend to be statistically significant in both groups.

The present study showed a significant decrease in ICAM-1 and E-Selectin in both groups. However VCAM-1 was significantly reduced only in HCD. These findings are in agreement with other studies that evaluated the effect of weight loss on adhesion molecules and observed a significant reduction in its serum levels [10,33,36,37].

Our results demonstrated that the reduction in biomarkers of inflammation (hs-CRP and TNF-α), fibrinolysis (PAI-1), and endothelial dysfunction (ICAM-1, VCAM-1, and E-Selectin) was similar in HCD and LCD. Participants in HCD, compared to subjects in LCD, although they presented a similar weight loss during the study, had a greater decrease in abdominal obesity and even so the reduction in these biomarkers was similar.

The comparison of our results (in relation to the effect of a high calcium intake) with other studies is difficult, because of the small number of published studies evaluating the effect of dietary calcium on these biomarkers, and to the best of our knowledge only one study was conducted during weight loss [21]. In the former study, Zemel and Sun [21] performed an evaluation of hs-CRP in archival samples from two previous clinical trials conducted in obese individuals. Twenty-four weeks of feeding a high-dairy eucaloric and hypocaloric diet resulted in an 11% ($P < 0.03$) and 29% ($P < 0.01$) decrease in hs-CPR, respectively (post- versus pretest), whereas there was no significant change in the low-dairy groups.

Although our study was conducted during weight loss, we will compare our findings with studies during a eucaloric diet. Recently, Stancliffe et al. [22] determined the early (7 d) and sustained (4 and 12 wk) effects of adequate-dairy (3.5 servings/d) compared with low-dairy (0.5 servings/d) weight-maintenance diets in subjects with metabolic syndrome. Inflammatory markers were suppressed with adequate-dairy intake: at 12 wk TNF-α showed a 35% reduction ($P = 0.05$), IL-6 21% reduction ($P = 0.02$), and MCP-1 24% decrease ($P = 0.05$). Low-dairy diet exerted no effect on inflammatory markers. Although diet had no effect on body weight, adequate-dairy diet reduced waist-circumference and trunk fat ($P < 0.01$ for both).

Zemel et al. [18] evaluated the acute effects of a dairy-rich diet on inflammatory stress in overweight and obese subjects in the absence of adiposity changes. Twenty subjects (10 obese, 10 overweight) participated in a blinded, randomized, cross-over study of dairy- compared with soy-supplemented eucaloric diets. Inflammatory stress biomarkers were measured on days 0, 7, and 28 of each dietary period. The dairy-supplemented diet resulted in lower inflammatory markers (TNF-α: 15%, $P = 0.002$; IL-6: 13%, $P = 0.01$; MCP-1: 10%, $P = 0.0006$) and increased adiponectin (20%, $P = 0.002$), whereas the soy-supplemented diet 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.

On the other hand, two other interventional studies [19,20] did not observe significant changes in biomarkers of inflammation and of endothelial dysfunction. Wennersberg et al. [20] evaluated 121 overweight subjects ($n = 121$), with traits of the metabolic syndrome, that were randomly assigned into milk or control groups. The milk group was instructed to consume three to five portions of dairy per day. The control group maintained

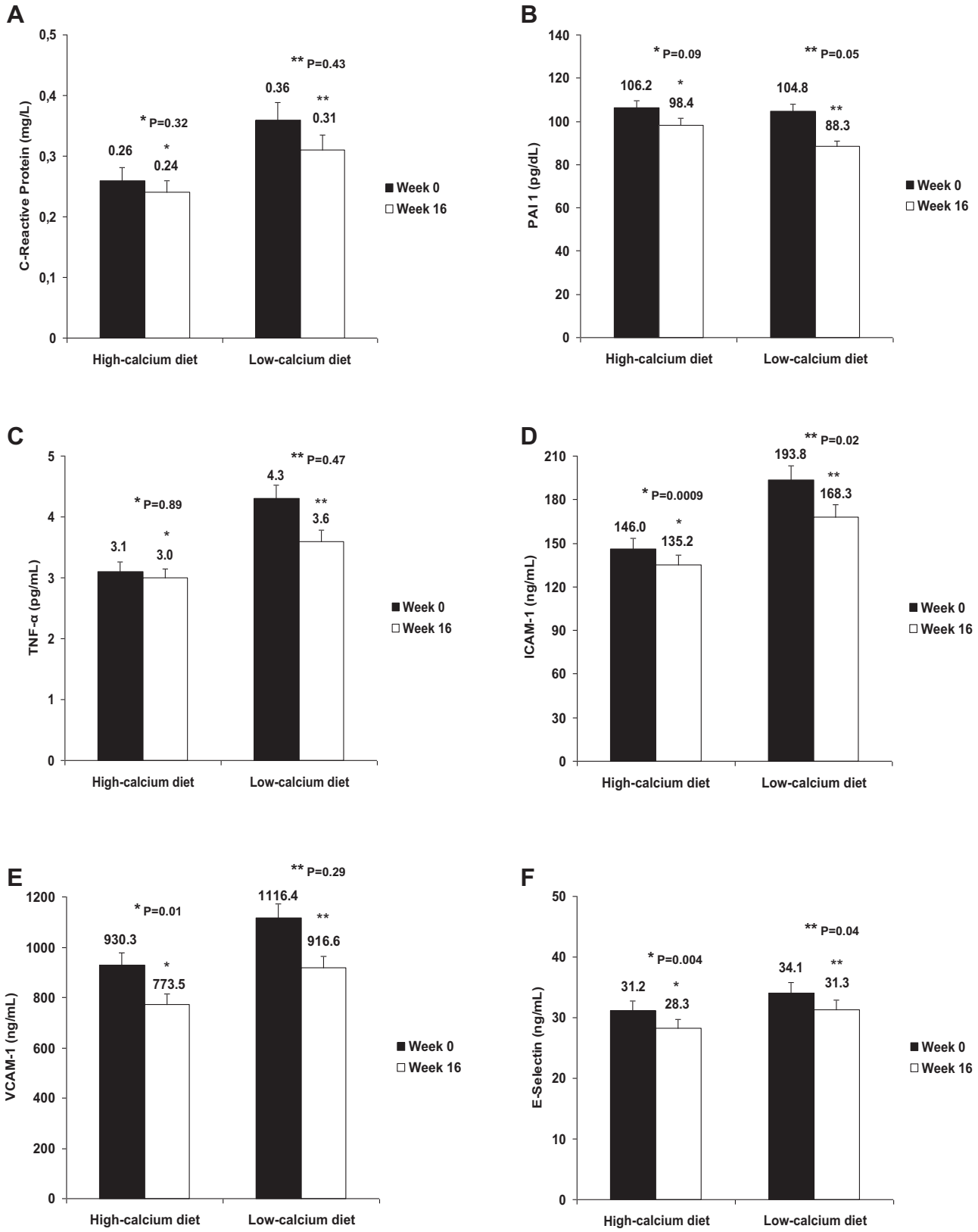

Fig. 3. Mean levels of (A) highly sensible C-reactive protein (hs-CRP), (B) tumor necrosis factor-α (TNF-α), (C) plasminogen activated inhibitor (PAI-1), (D) intracellular adhesion molecule 1 (ICAM-1), (E) vascular cell adhesion molecule 1 (VCAM-1), and (F) E-Selectin at baseline (week 0) and at the end of the study (week 16), according to dietary intervention ($n = 35$). *P* value was estimated by *t* test.

their habitual diet. Clinical investigations were conducted on admission and after 6 mo. There were no significant differences between changes in markers of inflammation and endothelial function in the milk and the control groups. When the sexes were analyzed separately, VCAM-1 decreased ($P = 0.001$) in women in the milk group. Van Meijl and Mensink [19] investigated the effects of low-fat dairy consumption on inflammatory markers and adhesion molecules in overweight and obese subjects. Thirty-five subjects consumed, in a random order, low-fat dairy products (500 mL low-fat milk and 150 g low-fat yogurt) or carbohydrate-rich control products (600 mL fruit juice and three fruit biscuits) daily for 8 wk. Plasma concentration of TNF-α was decreased ($P = 0.07$) after the dairy diet consumption. Low-fat dairy consumption did not affect other markers of low-grade systemic inflammation (IL-6 and MCP-1) and endothelial dysfunction (ICAM-1 and VCAM-1).

At present, there is no consensus about the effect of dietary calcium on inflammation and endothelial function. However, the lack of a positive effect of calcium in the present study may have been influenced by the higher baseline levels (without statistical significance) of hs-CRP, TNF-α, ICAM-1, VCAM-1, and E-Selectin in LCD.

Although in the present study we did not find any effect of higher dietary calcium on inflammatory biomarkers, the proposed mechanism by which dietary calcium intake could modulate inflammatory stress is probably mediated, in part, by the reduction in body fat. However, it has been shown that calcium may have additional effects via suppression of $1,25(OH)_2$vitD. Elevated serum levels of calcitriol, besides increasing intracellular calcium concentration, appear to increase the production of reactive oxygen species, through modulation of mitochondrial uncoupling. These two mechanisms modulate the production and release of cytokines [18,38].

There are several limitations to our study. First, although this study was a randomized clinical trial, it was open-labeled. Second, we consider this study exploratory, and additional research with a longer period of intervention is therefore indicated. Third, we cannot exclude that an additional bioactivity of dairy products (i.e., non-calcium-mediated), such as that resulting from the high concentration of leucine and of angiotensin-converting enzyme inhibitors, contributed to the proposed benefits [39].

In conclusion, the findings of this study suggest that increased calcium intake during weight loss has no benefits beyond those achieved with energy restriction on biomarkers of inflammation, fibrinolysis, and endothelial function. However, more studies are necessary to evaluate this important issue.

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