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Comparison of the effects of cow's milk, fortified soy milk, and calcium supplement on plasma adipocytokines in overweight or obese women

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ABSTRACT

Background: Obesity is an escalating public health problem. Adipose tissue synthesizes and secretes a variety of biological molecules, termed adipcytokines, that may contribute to obesity-linked metabolic abnormalities including cardiovascular diseases.
Objectives: We compared the effects of cow's milk, calcium-fortified soy milk, and a calcium supplement on adipo cytokines in premenopausal overweight and obese women.
Materials and Methods: In this clinical trial, 100 healthy, overweight or obese premenopausal women were randomly assigned to one of the following dietary regimens for 8 *weeks:* (a) a control diet (b), a calcium-supplemented diet containing 800 mg/day calcium carbonate, (c) a high-milk diet containing three servings of low-fat milk, and (d) a soy-milk diet containing three servings of calcium-fortified soy milk. All diets required a 500-kcal/day reduction in energy. At baseline and after 8 weeks, anthropometric indices and plasma leptin, adiponectin, TNFα, CRP, and IL-6 were measured.
Results: Plasma CRP and leptin were significantly correlated with all anthropometric indices except for WHR, and plasma adiponectin had a significant negative correlation with WHR at baseline. Plasma leptin, CRP, and IL-6 decreased significantly in all groups ($P < 0.01$; except for CRP in the control group), but there were no significant differences among the four groups for these three measures.
Conclusions: We conclude that a dietary reduction of 500-kcal/day has beneficial effects on plasma adipocytokines, but calcium intake either as calcium carbonate or as milk leads to no differences. These results merit further research.

▸ *Implication for health policy/practice/research/medical education:*
Obesity and its complications are the major concerns of health care practitioners, and finding proper diet to reduce this problem is critical. Results of this article are useful for nutritionists, epidemiologists and physicians.

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1. Background

Obesity is an escalating public health problem (1-4) Defined as the accumulation of excess body fat (5, 6). obesity is a principle causative factor in the development of metabolic disorders such as insulin resistance, hypertension, hyperglycemia, dyslipidemia, and atherosclerosis (6, 7). Adipose tissue is currently considered to be a hormonally active system in the control of metabolism and not only as a store of excess energy (5, 8, 9). It synthesizes and secretes a variety of biological molecules, including

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adiponectin, leptin, tumor necrosis factor alpha (TNFα), and interlukin-6 (IL-6) (5, 9-11). Recent research has revealed that substances such as C-reactive protein (CRP), IL-6, and TNFα mirror oxidative stress and may play a role in promoting adverse vascular outcomes in metabolic syndrome and type 2 diabetes (12, 13). TNFα is overexpressed in white adipose tissue in obesity and decreases with weight loss and improvement of insulin sensitivity (14). Subsequent studies have demonstrated that CRP concentrations are significantly related to various measures of body fat, and weight loss may lead to a decrease in CRP concentration (15, 16).

Leptin is a hormone primarily secreted by the adipose tissue and represents several physiological functions (17). Although the principal effects of leptin in the central nervous system are the control of food intake and energy expenditure, there is a significant relationship between leptinaemia and the chronic subinflammatory state that accompanies obesity which suggests that other possible peripheral biological effects are associated with its cytokine-like structure (18).

In sharp contrast to most adipokines, adiponectin expression and serum concentrations do not increase and actually decrease in a variety of obese and insulin-resistant states (14). Adiponectin is produced in fat cells (19), and in addition to its effects on insulin sensitivity, it has anti atherogenic properties (14, 18).

2.Objectives

Various studies have demonstrated that weight loss results in significant increases in serum levels of adiponectin and significant reduction in plasma CRP (20, 21). Sun and Zemel's findings indicated that dietary calcium suppresses obesity-associated inflammatory status by modulating proinflammatory and anti-inflammatory factor expression in mice (22). A few studies have specifically focused on the effects of calcium or dairy consumption on plasma adipocytokines (16, 22-25). One of these studies was conducted on mice (22), and 2 of them evaluated the effect of calcium on CRP (16, 23). In the present study, we examined the effects of cow's milk, fortified soy milk, and calcium supplement on serum adipocytokines in overweight or obese women.

3. Material and Methods

3.1 Study sample

Using an effect size of 0.55 for our sample size equations, 20 people for each group were estimated. Then we added 25% to this number, so 25 people for each group were included in the analyses. One hundred healthy, premenopausal, overweight or obese women ranging in age from 20 to 50 years volunteered to participate in this randomized clinical trial, and 85 completed the study. Fifteen subjects dropped out for various reasons, including thyroid disease, not wanting or being able to consume soy milk or a calcium supplement, and choosing not to continue participation. There were no significant differences between those who completed the study and those who did not for any of the main variables. Inclusion criteria were as follows: body mass index (BMI) more than 25 kg/m^2; taking no medications or supplements that might affect metabolism of calcium, vitamin D, or weight loss; absence of menopause; stable body weight (body weight changed less than 3kg for the last 2 months); and absence of diabetes, hypertension, coronary-artery, thyroid, and kidney diseases. The subjects were nonpregnant and nonlactating with no allergy to milk or soy milk and lactose intolerance. We also made sure that the subjects had not participated in any other studies in the 6 months preceding the screening. The study was approved by the ethics committee of the National Nutrition and Food Technology Research Institute. Each potential participant was informed of the possible risks and benefits associated with this study and provided written signed consent.

3.2 Study interventions

For the first 2 weeks of the study, we collected the subjects' baseline dietary data and physical activity assessments. Then, we randomized the subjects to one of the following dietary regimens for 8 weeks:

1. A control diet that required a 500-kcal/day reduction in total intake (based on the Harris-Benedict equation), with 500 to 600 mg/day of dietary calcium (about 1.5 to 2 servings of dairy products);
2. A calcium-supplemented diet identical to the control diet except for the addition of 800 mg/day of calcium (as calcium carbonate);
3. A milk diet that required a 500 kcal/day reduction in total intake and containing three servings (220 ml each) of low fat milk (1.5%); and
4. A soy-milk diet that required a 500-kcal/day reduction in total intake and three servings of calcium fortified soy milk per day. The total calcium intake for the milk diet and the soy-milk diet were between 1,200 and 1,300 mg/day.

Daily caloric requirements for all diets were calculated by using the Harris-Benedict equation (15). After adjusting the caloric intakes for each participant's physical activity level, individualized meal plans were given to each participant based on a 500 kcal/day reduction from their estimated caloric requirements (26). The diets for all groups were designed to provide comparable levels of macro nutrients as follows: 55% carbohydrate, 18% protein, and 27% fat. At baseline and at 2-week intervals, weight, waist circumference, and hip circumference were measured, and 24-hr dietary records (27) as well as physical-activity records (2 weekdays and 1 weekend day) were taken. Body fat was measured at the beginning of the study and at Week 8 using a Bodystat bioelectric impedance analysis (Quadscan4000 model).

At baseline and after 8 weeks, fasting blood samples (10 ml) were obtained in the morning. The plasma was separated and frozen at -80°C for later analy-

sis. Adiponectin (Mercodia, Uppsala, Sweden), leptin (Mercodia,Uppsala,Sweden), TNFα (Diaclon, France), IL-6 (Diaclon, Besancon, France), and CRP (Diagnostics Biochem Canada) were measured using a sandwich Elisa method.

3.3 Statistical analysis

Dietary records were analyzed using Nutritionist 4 (N IV). Statistical analyses were performed with SPSS software, version 15. One-way ANOVAs were used to compare the characteristics of the subjects in the four groups at baseline and also to compare the mean differences of plasma adipocytokines among the four groups. The effect of the intervention in each group was tested using paired-samples t-tests, and the relationships between plasma adipocytokines and anthropometric indices were estimated using Pearson correlations. $P < 0.05$ was considered significant.

4. Results

Of the 100 women meeting the general eligibility criteria, 15 dropped out before completing the weight-loss period (5, 3, 3, and 4 persons in control, calcium supplement, high milk, and soy milk groups respectively). Plasma CRP, IL-6, and TNFα were not normally distributed, so we used logs of them in the analysis.

The baseline characteristics of subjects are shown in *Table 1*. It is apparent that there were no significant differences between the age, weight, BMI, or energy and calcium intake of subjects across the four groups.

The relationship among plasma adipocytokines and anthropometric indices at baseline are shown in *Table 2*. It is clear that plasma CRP and leptin were significantly correlated with all anthropometric indices except for waist to hip ratio WHR, that plasma adiponectin had a significant negative correlation with WHR, and that there were no significant correlations among TNFα and IL-6 and the anthropometric indices.

Table 3 shows that after 8 weeks, the correlations among plasma adipocytokines and anthropometric indices were the same as they were at baseline, except for plasma leptin, which showed no significant correlation. The anthropometric indices of all groups decreased significantly after 8 weeks of weight-loss intervention ($P < 0.001$for all), but the amount of these reductions was significantly different among the four groups for only waist circumference ($P = 0.029$) and WHR ($P = 0.015$). After adjusting for baseline values with analyses of covariance, however, changes in weight and BMI were significantly different among the four groups ($P = 0.017$ and $P = 0.019$, respectively). Post hoc tests showed that the changes in waist circumference and WHR were significantly higher in the high-milk (6.32 ± 2.50 cm and 0.048 ± 0.019) and soy-milk (5.84 ± 1.47 cm and 0.044 ± 0.019) groups than in the control group (3.98 ± 2.77 cm and 0.021 ± 0.016; $P < 0.01$ and $P < 0.05$, respectively). Also, the reductions in weight and BMI were significantly greater in the high-milk group (4.43 ± 1.93 kg and 1.74 ± 0.73 kg/m²) than in the control group (2.87 ± 1.55 kg and 1.15 ± 0.62 kg/m²; $P < 0.01$for all).

Table 1. Baseline characteristics of subjects under study

Variables	Control (n = 20)	Ca supplement (n = 22)	High milk (n = 22)	Soy milk (n = 21)	P [b]
Age, y	38.25 ± 9.49 [a]	35.77 ± 8.70	38.27 ± 10.43	37.54 ± 9.27	0.78
Weight, kg	76.78 ± 9.6	78.16 ± 11.43	76.24 ± 10.57	80.05 ± 13.32	0.69
BMI, kg/m²	30.78 ± 3.13	31.54 ± 4.12	30.01 ± 3.55	31.09 ± 4.13	0.58
Energy, kcal/d	1839 ± 169	1870 ± 201	1937 ± 177	1901 ± 148	0.32
Calcium, mg/d	512 ± 172	532 ± 149	484 ± 131	509± 101	0.73

[a] mean ± SD
[b] One way ANOVA

Table 2. Correlation of plasma adipocytokines and anthropometric indices at baseline

Anthropometric indices adipocytokines	Weight, kg	Waist circumference, cm	BMI, kg/m²	Body fat mass (%)	WHR
CRP, ng/ml	r = 0.34 p = 0. 001	r = 0.41 p = 0. 0001	r = 0.42 p = 0. 0001	r = 0.41 p = 0. 0001	NS
TNFα, pg/ml	NS [a]	NS	NS	NS	NS
IL-6, pg/ml	NS	NS	NS	NS	NS
Leptin, ng/ml	r = 0.49 p = 0. 0001	r = 0.52 p = 0. 0001	r = 0.56 p = 0. 0001	r = 0.47 p = 0. 0001	NS
Adiponectin, ng/ml	NS	NS	NS	NS	r = - 0.27 p = 0. 013

[a] NS = not significant

Table 3. Correlation of plasma adipocytokines and anthropometric indices after intervention

Anthropometric indices Adipocytokines	**Weight**, kg	**Waist circumference**, cm	**BMI**, kg/m²	**Body fat mass** (%)	**WHR**
CRP, ng/ml	r = 0.31 *p* = 0.003	r = 0.37 *p* = 0.0001	r = 0.41 *p* = 0.0001	r = 0.40 *p* = 0.0001	r = 0.24 0.026
TNFα, pg/ml	NS [a]	NS	NS	NS	NS
IL-6, pg/ml					
	NS	NS	NS	NS	NS
Leptin, ng/ml	NS	NS	NS	NS	NS
Adiponectin, ng/ml	NS	NS	NS	NS	r = - 0.23 *p* = 0.03

[a] NS = not significant

The data in *Table 4* indicate that plasma IL-6 and leptin decreased significantly in all four groups, but there were no significant reduction in plasma TNFα or adiponectin. The reduction of plasma CRP was significant in the three experimental groups but not the control group. Mean differences of plasma adipocytokines among the four groups were not significant.

5. Discussion

Results of this study showed that plasma CRP and leptin were significantly correlated with body weight, waist circumference, body fat, and BMI. Inflammatory markers, such as CRP and IL-6, are higher in obese individuals than in lean subjects (12). The insulin resistance and atherosclerosis study showed that CRP values correlated with BMI, waist circumference, and fasting hyperinsulinemia (13). There are several studies that indicate that serum leptin concentration is proportional to body-fat mass (17, 28, 29).

Additionally, in this study there was a significant correlation between plasma adiponectin and WHR. It seems

Table 4. Within and between groups comparison of the effects of 8-week weight-loss intervention on plasma adipocytokines

Variables [a]	**Groups** [b]	**Time**		**Changes**
		Before	**After**	
TNFα, pg/ml	Control (19)	6.60 ± 4.57	6.26 ± 4.17	-0.34 ± 0.44
	Ca supplement (21)	6.48 ± 1.94	5.91 ± 1.78	-0.82 ±0.26
	low fat milk (21)	8.30 ± 6.72	7.91 ± 5.91	-0.38 ± 0.50
	Soy milk (20)	4.96 ± 2.51	4.94 ± 2.84	-0.12 ± 0.40
CRP, ng/ml	Control (19)	2.92 ± 1.99	2.60 ± 2.63	-0.31 ± .043
	Ca supplement (21)	2.60 ± 2.24	2.05 ± 1.88	0.54 ± 0.26 [e]
	low fat milk (21)	1.92 ± 2.06	1.17 ± 1.32	0.75 ± 0.19 [c]
	Soy milk (20)	3.29 ± 2.35	2.27 ± 2.11	0.97 ± 0.38 [e]
IL-6, pg/ml	Control (19)	1.56 ± 1.20	1.15 ± 0.86	-0.40 ± 0.11 [d]
	Ca supplement (21)	1.54 ± 0.64	1.03 ± 0.51	-0.55 ± 0.11 [d]
	low fat milk (21)	1.18 ± 0.57	0.89 ± 0.37	-0.29 ± 0.08 [c]
	Soy milk (20)	1.02 ± 0.48	0.76 ± 0.42	-0.24 ± 0.10 [d]
leptin, ng/ml	Control (19)	21.54 ± 9.42	14.88 ± 7.56	-6.65 ± 1.40 [c]
	Ca supplement (21)	21.96 ± 8.65	18.56 ± 9.31	-5.10 ± 1.22 [e]
	low fat milk (21)	20.37 ± 12.3	11.75 ± 9.05	-8.61 ± 1.27 [c]
	Soy milk (20)	20.75 ± 9.85	14.29 ± 5.85	-5.78 ± 1.68 [d]
Adiponektin, ng/ml	Control (19)	8.37 ± 1.99	7.96 ± 2.55	-0.40 ± 0.61
	Ca supplement (21)	8.61 ± 2.69	8.36 ± 2.04	-0.55 ± 0.46
	low fat milk (21)	10.32 ± 3.65	10.08 ± 3.41	-0.24 ± 0.56
	Soy milk (20)	8.33 ± 2.62	7. ± 2.1063	-0.53 ± 0.31

[a] All values are Mean ± SD
[b] No significant differences were seen among groups (One way ANOVA)
[c] Significant difference between before and after $P < 0.001$
[d] Significant difference between before and after $P < 0.01$
[e] Significant difference between before and after $P < 0.05$

that visceral obesity is predominantly associated with reduced levels of adiponectin; obese women who have normal upper-body fat have normal adiponectin levels as well (13).

In this study, a dietary reduction of 500 kcal/day for 8 weeks led to significant decreases in plasma IL-6 and leptin and no significant changes in plasma TNFα in any group. Plasma CRP decreased significantly in all three experimental groups but not the control group. Leptin is related to the risk of diabetes (30) and cardiovascular disease (31) in the elderly. It has been reported that an imposed energy deficit causes decreases in circulating leptin (29, 32). Copolla *et al*. reported that after 12 months of a multidisciplinary program of weight reduction, obese women lost at least 10% of their original weight, and their CRP levels decreased significantly ($P < 0.01$) (20). Additionally, Madsen *et al*. showed that weight loss was associated with a significant decrease in CRP (21). Sun and Zemel found that a high-calcium diet significantly inhibited the expression of TNFα and IL-6 and stimulated the expression of adiponectin in visceral fat ($P < 0.001$) in male aP2-agoti transgenic mice (6). They also found similar effects in a retrospective analysis of archival clinical samples from obese subjects, with high dairy intake reducing CRP and increasing adiponectin under both eucaloric and hypocaloric conditions. Sun and Zemel suggested that although the reduction of CRP levels was independent of changes in body weight, it is not possible to fully ignore the effect of reduced adiposity, as subjects exhibited reduced body fat. Circulating 1α, 25-dihydroxycholecalciferol was reduced with increased Ca intake, concurrent with reductions in pro-inflammatory indices, suggesting a role for dietary Ca in attenuating the cytokine dysregulation related to diet-induced obesity (25). Also, research has suggested that parathyroid hormone regulates the circulating levels of the inflammatory cytokines interleukin-6 and tumor necrosis factor-α, which in turn stimulate production of CRP and calcium supplementation, by suppressing parathyroid hormone production, might also decrease serum CRP (23).

In contrast, Pittas *et al*. found that daily consumption of either 500 mg of calcium citrate and 700 IU of vitamin D or placebo for 3 years had no significant effects on plasma IL-6 and CRP (26). Also Grey *et al*. reported that after 1 year of calcium supplementation (1g calcium citrate per day), there was no difference between the CRP levels of the supplement and placebo groups (25).

Despite the 5-cm reduction in waist circumference and approximately 5% reduction in body weight, there was no significant change in plasma adiponectin in this study. Maestu *et al*. reported that after a 10-week intervention, no change in adiponectin concentration was observed, despite significant weight and fat mass loss (6.5% reduction) in male competitive bodybuilders (17). Xydakis *et al*. observed no change in plasma adiponectin after 4 to 6 weeks of weight loss (11). On the other hand, Madsen *et al*. found that a mean weight loss of about 12% after 8 weeks of VLCD resulted in a significant 22% increase in serum levels of adiponectin. They also found that relatively large weight losses (more that 10-11%) were necessary to obtain a significant increase in plasma adiponectin levels (21).

Despite the reductions in waist circumference, WHR, body weight, and BMI were significantly different across all four groups, but plasma adipocytokine changes were not significantly different in any group. We assume that, although the reductions in anthropometric indices were significantly different among the four groups, they were not high enough to exert different effects on the subjects' adipocytokines expressions.

For this study, we conclude that calcium intake as calcium carbonate, calcium-fortified soy milk, or low-fat milk leads to no differences in plasma adipocytokines. Still, given the short duration of this study, future studies with longer experimental periods are warranted.

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Conflict of interest

There is no conflict of interest.

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