Dairy calcium supplementation in overweight or obese persons: its effect on markers of fat metabolism\(^1\)–\(^3\)

Murielle Bortolotti, Servane Rudelle, Philippe Schneiter, Hubert Vidal, Emmanuelle Loizon, Luc Tappy, and Kevin J Acheson

ABSTRACT

Background: Dairy calcium supplementation has been proposed to increase fat oxidation and to inhibit lipogenesis.

Objective: We aimed to investigate the effects of calcium supplementation on markers of fat metabolism.

Design: In a placebo-controlled, crossover experiment, 10 overweight or obese subjects who were low calcium consumers received 800 mg dairy Ca/d for 5 wk. After 4 wk, adipose tissue was taken for biopsy for analysis of gene expression. Respiratory exchange, glycerol turnover, and subcutaneous adipose tissue microdialysis were performed for 7 h after consumption of 400 mg Ca or placebo, and the ingestion of either randomized slow-release caffeine (SRC; 300 mg) or lactose (500 mg). One week later, the test was repeated with the SRC or lactose crossover.

Results: Calcium supplementation increased urinary calcium excretion by 16% (\(P = 0.017\)) but did not alter plasma parathyroid hormone or osteocalcin concentrations. Resting energy expenditure (59.9 ± 3.0 or 59.6 ± 3.3 kcal/h), fat oxidation (58.4 ± 2.5 or 53.8 ± 2.2 mg/min), plasma free fatty acid concentrations (0.63 ± 0.02 or 0.62 ± 0.03 mmol/L), and glycerol turnover (3.63 ± 0.41 or 3.70 ± 0.38 \(\mu\)mol · kg\(^{-1}·\)min\(^{-1}\)) were similar with or without calcium, respectively. SRC significantly increased free fatty acid concentrations, resting fat oxidation, and resting energy expenditure. During microdialysis, epinephrine increased dialysate glycerol concentrations by 250% without and 254% with calcium. Expression of 7 key metabolic genes in subcutaneous adipose tissue was not affected by treatments. As a consequence, energy accumulates as lipid in adipose tissue and other tissues, and that development leads to obesity, insulin resistance, and diseases associated with the metabolic syndrome. Although there have been many recent advances in the understanding of the mechanisms associated with obesity at the molecular level in rodents and knock-out rodent models, the medical and scientific community remains essentially at a loss with respect to ways in which to deal with this increasing pandemic in humans.

Recently, various groups of scientists working in one laboratory (Zemel’s group) proposed that dietary calcium, via its influence on plasma 1,25-dihydroxyvitamin D\(_3\) (calcitriol) concentrations, regulates the concentration of intracellular adipocyte calcium and subsequently regulates adipocyte lipid metabolism(1–8). Zemel’s group also found that high intracellular calcium concentrations inhibit lipolysis and stimulate de novo lipogenesis, whereas low intracellular concentrations increase lipolysis and inhibit de novo lipogenesis. Such results are intriguing, but they warrant further investigation, because there is considerable evidence of little de novo lipogenesis in humans (9, 10). When de novo lipogenesis does occur, much of it occurs in the liver (11), and, because of low fatty acid synthase activity, very little, if any, occurs in adipose tissue (11–18).

In studies in mice and humans, it was shown that an increase in dietary calcium in the absence of energy restriction partitions energy toward lean body mass rather than toward adipose tissue (5, 7, 19) and that this effect is accentuated during energy restriction (5, 7, 20). Furthermore, this partitioning effect of calcium is even more pronounced when the diet has a high dairy component (20–22).

INTRODUCTION

Overeating increases the energy turnover of the body; however, it is difficult in many cases for the mechanisms of energy expenditure (EE) (thermogenesis and physical activity) to match and balance the excess energy entering the body in these conditions. As a consequence, energy accumulates as lipid in adipose tissue and other tissues, and that development leads to obesity, insulin resistance, and diseases associated with the metabolic syndrome. Although there have been many recent advances in the understanding of the mechanisms associated with obesity at the molecular level in rodents and knock-out rodent models, the medical and scientific community remains essentially at a loss with respect to ways in which to deal with this increasing pandemic in humans.

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Whether dietary calcium also influences thermogenesis and fat oxidation is not known; however, Zemel et al (7) observed increases in the core temperature of mice receiving high-calcium diets, which they interpreted as a shift in efficiency from energy storage to thermogenesis, and Melanson et al (23) reported correlations between acute calcium intake and fat oxidation during 24-h measurements of energy metabolism in a room calorimeter. In contrast, it has also been established that calcium intake affects energy intake by influencing lipid digestibility (24, 25), which it does by forming indigestible calcium soaps with dietary lipids in the gastrointestinal tract; thus, fecal fat excretion is increased (24, 25). Although Zemel et al (20) accepted that some dietary energy, in the form of fats, can be lost as nonabsorbable calcium complexes in the feces—as has been shown in several human studies (24–27)—they discounted this effect as being insignificant.

In the present study, the primary objective was to investigate the effect of supplementing the diet with 800 mg dairy Ca/d on EE and markers of fat metabolism in a group of overweight or obese subjects who habitually consumed a low-calcium diet. Because caffeine ingestion has been shown to increase EE (28–30) and, to some extent, lipid oxidation (29), caffeine was given acutely at the end of the calcium supplementation period to explore possible synergistic effects between calcium and caffeine.

**SUBJECTS AND METHODS**

The study was a single-center, double-blind, placebo-controlled, randomized crossover trial, the protocol of which is shown in Figure 1. Subjects were recruited by advertisement within the university hospital and by word of mouth. The study targeted overweight and obese subjects (both male and female) with a body mass index (in kg/m²) of >27 and a habitually low daily intake of calcium. Potential volunteers were first screened by telephone, and only those who consumed little or no dairy products were subsequently interviewed by the dietitian of the Institute of Physiology to determine their habitual calcium intake. Only those with calcium intakes ≥800 mg/d were considered for the study. In general, the subjects consumed no or almost no dairy products; their calcium intake was supplied almost entirely from meat, vegetables, starchy food, fruit, bread, water, fruit juice, and beer.

Ten subjects, 3 men and 7 women, whose physical characteristics are presented in Table 1, were included in the study. After a preliminary medical examination, during which anthropometric measurements were made, the subjects were randomly assigned to receive either a placebo or calcium supplement for a period of 5 wk, during which they consumed their habitual low-calcium diet supplemented with either a placebo (sachet of 5 g...
TABLE 1
Physical characteristics of the subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>22.2 ± 1.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78.1 ± 9.1</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.65 ± 0.06</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.5 ± 1.4</td>
</tr>
<tr>
<td>Calcium intake (mg/d)</td>
<td>586 ± 137</td>
</tr>
</tbody>
</table>

All values are x ± SEM, n = 10.

maltdextrin; MD47: Roquette, Lestrem, France) taken 2 times/d or a dairy calcium supplement (sachet containing 400 mg Ca^{2+} and made up to 5 g with MD47 filler; Capocol MM-0525 BG: Arla Foods, Viby, Denmark) taken 2 times/d to provide 800 mg Ca^{2+}/d. The calcium supplement was a natural milk mineral concentrate with the following composition: 6% protein, 13% lactose, 74% ash, 6% moisture, and 24% calcium, present almost entirely as calcium phosphate. Compliance was assessed by counting the number of returned empty sachets. After 4 wk of supplementation, subjects spent a day at the Institute of Physiology; their food intake and physical activity were controlled for a period of 24 h. The next morning, a sample of abdominal subcutaneous adipose tissue was taken, and measurements of whole-body energy metabolism, lipid turnover, lipid disposal, and adipose tissue lipolysis were made for 7 h in resting, fasting conditions.

All subjects provided written informed consent. The study was carried out in accordance with the ethical standards for clinical research of the Faculty of Biology and Medicine, Lausanne University (Lausanne, Switzerland), whose ethics committee reviewed and approved the protocol.

Biopsy of adipose tissue

Under local anesthesia (0.5 mL, 1% xylocaine), abdominal percutaneous adipose tissue (≈100 mg) for biopsy was taken by aspiration through a 16-gauge needle from an abdominal spot 20 mm lateral to the umbilicus, as described previously (31). The adipose tissue sample was immediately frozen in liquid nitrogen and stored at −80 °C until mRNA extraction. The expression of mRNAs involved in adipose tissue fat metabolism, coding for hormone-sensitive lipase (HSL), lipoprotein lipase (LPL), adipose triglyceride lipase (ATGL), perilipin, peroxisome proliferator–activated receptor γ2 (PPARγ2), sterol regulatory element–binding protein 1c (SREBP1c), and phosphodiesterase 3B (PDE-3B), were measured and compared after 4 wk of calcium supplementation and 4 wk of placebo.

Metabolic measurements

After the subject was comfortably installed in a semirecumbent position in a bed, a venous catheter was inserted into the antecubital vein of each arm. One catheter was used for a primed, continuous infusion of 5-deuterated glycerol to measure glycerol turnover (32), and the other, which was kept patent with physiologic saline, was used for blood sampling. In addition, two 30-mm Cuprophane dialysis membranes (Gambro, Lund, Sweden) were placed in the abdominal subcutaneous adipose tissue on either side of the umbilicus by using microdialysis catheters (CMA 60; CMA Microdialysis, Solna, Sweden) inserted aseptically with local xylocaine anesthesia. These 2 membranes were used to measure the in vivo lipolysis of abdominal subcutaneous adipose tissue with epinephrine, one with and the other without local lipolytic stimulation. The perfusion fluid contained either physiologic saline (catheter 1) or epinephrine (1 μmol/L; Santetica SA, Mendrisio, Switzerland) to stimulate lipolysis (catheter 2), and both fluids were perfused at 0.3 μL/min by using a high-precision syringe pump (CMA Microdialysis). A fasting baseline blood sample was taken at −60 min, after which a primed (1.5 μmol/kg), continuous (0.1 μmol · kg⁻¹ · min⁻¹) infusion of 5-deuterated glycerol was begun.

At time zero (0 min), the subject ingested the contents of one of the same type of sachets he or she had been taking during the preceding 4 wk, along with a gelatin capsule containing either 500 mg lactose or 300 mg slow-release caffeine (SRC) (33), which were taken in random order. Immediately afterward, a transparent ventilated hood was placed over the subject’s head to measure respiratory exchange (Deltatrac II indirect calorimeter; Datex, Helsinki, Finland), perfusion of the microdialysis perfusates was begun, and measurements were continued for a further 6 h. Dialysates were collected every 60 min throughout the study, after calibration of each microdialysis catheter by using the no-net-flux method (34). Blood samples were taken at 60-min intervals from −60 to 360 min. Urine was collected twice during the 24 h before the acute study—from 0800 to 2300 and from 2300 to the next morning (overnight fasting sample), the time of which was recorded—and also at the end of the 7-h test. After these tests, the subject continued ingesting the same supplement for an additional week, at the end of which his or her diet and physical activity were controlled for 24 h and the measurements described above (7-h energy metabolism, whole-body glycerol turnover, and in vivo lipolysis of adipose tissue) were repeated. During the second acute study, the subject ingested a gelatin capsule containing either SRC or lactose, whichever had not been ingested in the first study. Between treatments, there was a 10-wk washout period, during which the subject consumed his or her habitual diet.

Measurements

The effects of 4–5 wk calcium supplementation on fasting whole-body lipid turnover and lipid disposal (oxidative and non-oxidative) were measured by using isotope dilution of 1,1,2,3,3-H₂ glycerol (33) and an open-circuit, ventilated-hood, indirect calorimeter (Deltatrac II). Concurrently, in vivo lipid metabolism under normal and lipolysis-stimulated conditions was measured in abdominal subcutaneous adipose tissue.

Calculations

Under the assumption that urea nitrogen contributes 90% to total nitrogen excretion, protein oxidation was calculated from urinary urea nitrogen excretion measured in the urine from the timed urine collection at the end of the test. After that step, EE and substrate utilization were calculated from the respiratory exchange data with the use of standard equations (36).

Analyses

Plasma was immediately separated from blood by centrifugation at 4 °C for 10 min at 3600 rpm and stored at −20 °C until analyzed. Fasting samples were analyzed for osteocalcin by using time-resolved amplified cryptate emission (TRACE; BRAHMS Kryptor, St- Ouen, France), for parathyroid hormone
(PTH) by using an enzymatic chemiluminescence method (Roche Diagnostics, Mannheim, Germany), and for total calcium by using an automated method (Roche Diagnostics).

All plasma samples were analyzed for glucose by using the glucose oxidase method (Beckman Glucose Analyzer II; Beckman Instruments, Fullerton, CA); for free fatty acids and triglycerides by using colorimetric methods from Wako (Freiburg, Germany) and Biomérieux (Biomérieux Vetek Inc, Switzerland), respectively; and for insulin by using a radioimmunoassay kit from Linco (St Charles, MO). We measured 1,1,2,3,3-^3H_5 glycerol enrichments on acetylated derivatives by using gas chromatography—mass spectrometry (GC 5890/MS 5971; Hewlett-Packard Instruments, Palo Alto, CA) with selective ion monitoring at 164.1 and 159 mass-to-charge ratio. Twenty-four-hour urine samples were analyzed for Ca, phosphate excretion during calcium supplementation. All of the data were analyzed by using 2-factor repeated-measures analysis of variance (ANOVA) procedures; when significant differences were observed, paired comparisons were further analyzed by using Bonferroni’s multiple-comparison test. *P ≤ 0.05* was considered significant. We used STATA software (version 9.1; Stata Corp, College Station, TX) for all statistical analyses.

**RESULTS**

**Body weight and markers of calcium intake in blood and urine**

There was no significant difference between body weight at the end of the placebo and calcium supplementation periods: 79.6 ± 3.0 and 80.0 ± 3.2 kg, respectively.

Fasting plasma calcium concentrations were 2.19 ± 0.02 mmol Ca\(^{++}\)/L after placebo treatment, and they did not change when the diet was supplemented with 800 mg Ca/d for 4–5 wk: 2.15 ± 0.03 mmol Ca\(^{++}\)/L. Similarly, neither PTH (placebo: 38.7 ± 4.1 ng/L) nor osteocalcin (placebo: 32.8 ± 2.4 μg/L) changed significantly with calcium supplementation (PTH: 39.6 ± 4.3 ng/L; osteocalcin: 33.5 ± 1.6 μg/L).

Calcium concentrations in urine collected during the day and the night after placebo treatment were 1.94 ± 0.33 and 2.36 ± 0.56 mmol/L, respectively, and they increased with calcium supplementation to 2.34 ± 0.34 and 3.86 ± 0.65 mmol/L, respectively. ANOVA indicated a significant effect of calcium on urinary calcium excretion during the night (*P = 0.028*). In consequence, total 24-h calcium excretion increased from 138.4 ± 13.1 mg/24 h with placebo to 159.9 ± 17.2 mg/24 h with calcium supplementation (*P = 0.017*). Neither the day (placebo: 10.72 ± 1.88 mmol/L; calcium: 11.97 ± 2.06 mmol/L) nor the night samples (placebo: 18.09 ± 1.78 mmol/L; calcium: 23.78 ± 2.56 mmol/L) showed significant differences in urinary phosphate excretion during calcium supplementation.

**Energy expenditure and substrate utilization**

No significant difference in resting EE was observed after 4–5 wk of dairy calcium supplementation (**Figure 2A**). Mean substrate oxidation and EE were calculated over the course of the 6-h study and are presented in **Figure 2B**. No significant differences were observed in protein oxidation or EE, and, although slight differences can be seen in carbohydrate and fat oxidation between the placebo and calcium supplementation treatments, those differences also were not significant.

At the end of the placebo supplementation period, ingestion of 300 mg SRC (**Figure 3A**) resulted in a significant (*P = 0.001*) increase of 5.4 ± 0.2 kcal/h in EE, which was accompanied by a slight (nonsignificant) decrease in carbohydrate oxidation (5.8 ± 1.8 mg/min) and a significant (*P = 0.04*) increase in fat oxidation (11.4 ± 0.7 mg/min). When SRC was given acutely after 4–5 wk of calcium supplementation (**Figure 3B**), no significant differences in EE or substrate oxidation were observed.

**Glycerol turnover**

Glycerol turnover is illustrated in **Figure 4**. It was 3.70 ± 0.38 μmol · kg\(^{-1}\) · min\(^{-1}\) at the end of the placebo supplementation period and did not change significantly after calcium supplementation (3.63 ± 0.41 μmol · kg\(^{-1}\) · min\(^{-1}\)). Acute ingestion of 300
Effect of calcium or placebo consumption on fasting resting substrate oxidation and energy expenditure, respectively; no significant differences were observed (2-factor repeated-measures ANOVA). Difference = calcium supplementation − placebo. \( n = 10 \).

**Microdialysis**

Mean glycerol concentrations analyzed in the microdialysis dialysate over the 6-h experiment without and with epinephrine-stimulated lipolysis are shown in Figure 5A and B, respectively. Neither calcium nor placebo supplementation for 4–5 wk had a significant effect on glycerol release from abdominal subcutaneous adipose tissue (241.0 ± 9.4 and 231.7 ± 11.0 μmol/L, respectively). Although glycerol concentrations tended to be higher with SRC treatment (273.4 ± 10.0 μmol/L), the difference was not significant, and it was not confirmed when SRC was taken after calcium supplementation (229.6 ± 10.7 μmol/L).

When lipolysis was stimulated by the addition of epinephrine to the microdialysis perfusate (Figure 5B), dialysate glycerol concentrations increased =3.5-fold. Nevertheless, no significant differences were observed between treatments.

**Biomarkers of fat metabolism in adipose tissue**

Changes in the expression of biomarkers of fat metabolism sampled from abdominal subcutaneous adipose tissue are presented in Figure 6. None of the changes were significant or sufficiently large to have a significant effect on lipid metabolism in adipose tissue.

**Effect of calcium supplementation and slow-release caffeine on mean 6-h blood concentrations**

Nonesterified fatty acid concentrations were, on average, 0.55 ± 0.02 mmol/L at the start of the 6-h experiment, and they rose to 0.80 ± 0.03 mmol/L by the end of the study. Calcium supplementation did not have an effect on mean 6-h nonesterified fatty acid concentrations (placebo: 0.618 ± 0.024 mmol/L; calcium: 0.633 ± 0.021 mmol/L); however, SRC in the presence and absence of calcium significantly \( (P = 0.01) \) increased nonesterified fatty acid concentrations (0.709 ± 0.025 and 0.725 ± 0.025 mmol/L, respectively).

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![Figure 2](https://academic.oup.com/ajcn/article-abstract/88/4/877/4649877)

**FIGURE 2.** A: Mean ± SEM effect of consuming 2 sachets/d of either placebo or dairy calcium (800 mg/d) for 5 wk on fasting resting energy expenditure measured over 6 h. No significant differences were observed. \( n = 10 \). B: Mean ± SEM effect of consuming 2 sachets/d of either placebo or dairy calcium (800 mg/d) for 5 wk on fasting resting substrate oxidation and energy expenditure measured over 6 h. The differences between tests (calcium supplementation − placebo) were =8.6 ± 2.1 mg/min, 4.6 ± 1.6 mg/min, −1.4 ± 0.04 mg/min, and 0.3 ± 1.6 kcal/h for carbohydrate oxidation, fat oxidation, protein oxidation, and energy expenditure, respectively; no significant differences were observed (2-factor repeated-measures ANOVA). Difference = calcium supplementation − placebo. \( n = 10 \).

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![Figure 3](https://academic.oup.com/ajcn/article-abstract/88/4/877/4649877)

**FIGURE 3.** A: Mean ± SEM acute effect of 300 mg slow-release caffeine (SRC) ingestion on fasting resting substrate oxidation and energy expenditure measured over 6 h after 4–5 wk of placebo ingestion. \( n = 10 \). The differences between tests (calcium supplementation − placebo) were =5.8 ± 1.8 mg/min, 11.4 ± 0.7 mg/min, 0.5 ± 0.0 mg/min, and 5.4 ± 0.2 kcal/h for carbohydrate oxidation, fat oxidation, protein oxidation, and energy expenditure, respectively; only fat oxidation \( (P = 0.04) \) and energy expenditure \( (P = 0.001) \) were significant (2-factor repeated-measures ANOVA with Bonferroni multiple-comparison tests). B: Mean ± SEM acute effect of ingestion of 300 mg SRC on fasting resting substrate oxidation and energy expenditure measured over 6 h after consuming 800 mg dairy Ca/d for 4–5 wk. \( n = 10 \). The differences between tests were =10.4 ± 2.0 mg/min, =4.4 ± 1.8 mg/min, 3.0 ± 0.04 mg/min, and 0.6 ± 0.07 kcal/h for carbohydrate oxidation, fat oxidation, protein oxidation, and energy expenditure, respectively; no significant differences were observed (2-factor repeated-measures ANOVA).
After placebo treatment and calcium supplementation, SRC had no significant effect on mean 6-h triacylglycerol concentrations (placebo: 1.037 ± 0.048 mmol/L; SRC: 0.934 ± 0.026 mmol/L; and calcium: 0.913 ± 0.043 mmol/L; SRC 1.065 ± 0.079 mmol/L). Insulin concentrations were 10.7 ± 0.8 μU/mL after placebo treatment and 10.8 ± 0.84 μU/mL in the presence of SRC. Although the concentrations were slightly lower after calcium (8.7 ± 0.46 μU/mL) and calcium with SRC (9.5 ± 0.5 μU/mL) treatment, these changes were not significant. Blood glucose concentrations were not influenced by treatment (placebo: 4.48 ± 0.05 mmol/L; calcium: 4.46 ± 0.06 mmol/L) or by placebo and calcium treatments with SRC (4.34 ± 0.05 and 4.35 ± 0.05 mmol/L, respectively). Similarly, plasma glycerol concentrations were not influenced by treatment (placebo: 74.8 ± 3.6 μmol/L; calcium: 73.0 ± 2.8 μmol/L) or by placebo and calcium treatments with SRC (76.5 ± 3.0 μmol/L; calcium: 78.3 ± 3.3 μmol/L, respectively).

Heart rate and blood pressure

Mean heart rate and blood pressure values are presented in Table 2. The heart rate after calcium supplementation (64 ± 2.0 beats/min) was slightly but significantly (P < 0.03) lower than that after placebo with SRC (69 ± 2 beats/min). Blood pressure was not significantly affected by treatment.

Urinary catecholamines

Norepinephrine, epinephrine, and dopamine excretions in urine collected during the day and night at the end of each supplement period (placebo or calcium) are presented in Table 3. No significant differences in urinary catecholamines were observed.

DISCUSSION

The hypothesis that dietary calcium is involved in the control of body weight and body fat stems from observations that low-calcium diets and high circulating vitamin D₃ concentrations stimulate lipid synthesis and inhibit the lipolysis of adipose tissue (2, 5–7). The inhibition of lipolysis decreases plasma free fatty acid concentrations, and that decrease in turn reduces lipid oxidation. The hypothesis is supported by animal studies on controlled calcium intakes and by the observation that vitamin D₃ inhibits lipolysis in adipocytes, apparently through the activation of a membrane receptor to vitamin D₃. In obese or overweight humans, studies so far have failed to confirm this hypothesis (26, 36), and, although lipid oxidation increased in obese subjects during 24-h energy restriction after receiving 1400 mg dairy Ca/d for 1 wk, the same dose had no effect on lipid oxidation in weight-maintenance conditions (37).

Because some reports suggest that dietary calcium supplementation may be effective only in persons with low calcium intakes, the present study was performed in subjects with a daily calcium intake of <800 mg/d. Despite their low calcium intakes, all of the participants had plasma calcium, phosphate, PTH, and osteocalcin concentrations within the normal range. This was expected, because severe calcium deprivation is required to alter these variables. Each subject was supplemented with 800 mg dairy Ca/d or a placebo for 5 consecutive weeks. Calcium supplementation increased daily urinary calcium excretion by 16% (from 138 ± 160 mg/d). Because only a portion of the ingested calcium load is absorbed from the gut, and because net enteral...
calcium absorption is low, this figure is consistent with the increase in calcium intake observed during the supplementary period.

Our observations indicate that calcium supplementation did not alter basal lipid metabolism, because plasma free fatty acid concentrations and whole-body glycerol turnover were similar in the presence and absence of calcium supplementation. By administering caffeine acutely and testing the effect of a submaximal dose of epinephrine on adipose tissue lipolysis, we also evaluated the possibility that dietary calcium may synergistically increase the effects of physiologic stimulators of lipolysis and lipid oxidation. To evaluate the response to epinephrine, we increased the local concentration of epinephrine in subcutaneous adipose tissue by infusing a solution of 1 μmol epinephrine/L through a microdialysis probe and monitoring the local release of glycerol. Epinephrine-stimulated adipose tissue glycerol concentrations were not significantly altered by calcium supplementation. The mRNA concentrations for all of the tested genes were corrected by those of hypoxanthine-guanine phosphoribosyltransferase (HPRT) taken as a reference before the calculation of the change induced by the treatment. The expression levels in the placebo condition, expressed in arbitrary units referred to HPRT concentrations, were HSL, 1406 ± 208; LPL, 5696 ± 817; ATGL, 5157 ± 1241; perilipin, 3851 ± 727; PPARγ2, 167 ± 35; SREBP1c, 11805 ± 3209; and PDE-3B, 441 ± 98. No significant differences were observed (2-factor repeated-measures ANOVA).

Our group also assessed lipid metabolism after the acute administration of SRC. Caffeine is known to increase total EE and lipid oxidation (28, 29, 33) and to enhance adipose tissue lipolysis, at least in part through the inhibition of adenosine receptors and the stimulation of the sympathetic nervous system (33). Caffeine administration enhanced total EE and lipid oxidation at the end of the placebo treatment and increased plasma free fatty acid concentrations in the presence and absence of calcium. Our group previously suggested that one potential mechanism by which caffeine stimulated thermogenesis was the ryanodine receptor (33). Caffeine is an agonist of the ryanodine receptor (38), the calcium ion–release channel of sarcoplasmic reticulum in skeletal muscle. Ryanodine receptor stimulation increases intramyocellular calcium flux, muscle contraction, heat production, glycolysis, adenosine triphosphate turnover, and mitochondrial pyruvate oxidation (39). Although this possibility is purely speculative, calcium supplementation may interfere with ryanodine receptor–stimulated heat production.

To evaluate the effect of calcium supplementation on adipose tissue lipid metabolism, transcriptomics was focused on a number of key metabolic genes; the results of this technique support the metabolic measurements. Although calcium supplementation failed to alter the expression of key genes involved in lipid metabolism, our findings suggest that calcium supplementation may influence the response to acute caffeine administration.

**TABLE 2**
Heart rate and arterial blood pressure measured during the test after 4–5 wk of either placebo or 800 mg Ca/d supplementation and after acute ingestion of 300 mg slow-release caffeine (SRC)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Placebo</th>
<th>Calcium + SRC</th>
<th>Placebo</th>
<th>Calcium + SRC</th>
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</thead>
<tbody>
<tr>
<td>Heart rate (beat/min)</td>
<td>67 ± 2</td>
<td>64 ± 2</td>
<td>69 ± 2</td>
<td>65 ± 2</td>
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<tr>
<td>Blood pressure (mm Hg)</td>
<td>114 ± 1.1</td>
<td>110 ± 1.1</td>
<td>116 ± 1.3</td>
<td>115 ± 1.8</td>
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<td>Systolic</td>
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<tr>
<td>Diastolic</td>
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<td>83 ± 0.9</td>
<td>83 ± 1.1</td>
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<td>Mean</td>
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1 All values are x ± SEM. n = 10. Statistical analysis was conducted using a 2-factor repeated-measures ANOVA with Bonferroni multiple-comparison tests.
2 The 2-factor repeated-measures ANOVA indicated a treatment effect, P = 0.02. Bonferroni multiple-comparison tests showed a significant difference in heart rate between the end of calcium supplementation and the administration of acute slow-release caffeine treatment at the end of the maltodextrin placebo supplementation period, P < 0.03.

**TABLE 3**
Urinary catecholamine excretion after 5 wk of either placebo or 800 mg Ca/d supplementation

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Calcium</th>
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<tr>
<td>Day</td>
<td>Night</td>
<td>Day</td>
</tr>
<tr>
<td>Norepinephrine (nmol/24 h)</td>
<td>182 ± 20</td>
<td>142 ± 16</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>26 ± 4</td>
<td>14 ± 3</td>
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<tr>
<td>(nmol/24 h)</td>
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</tr>
<tr>
<td>Dopamine</td>
<td>1556 ± 133</td>
<td>2120 ± 131</td>
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<tr>
<td>(nmol/24 h)</td>
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</tbody>
</table>

1 All values are x ± SEM. n = 10. None of the variables differed significantly between treatments (2-factor repeated-measures ANOVA).
storage and lipolysis, it remains possible that, in adipocytes, calcium has other effects on adipose tissue biology. Boon et al (40) recently observed a significant decrease in adipose tissue fatty acid synthase mRNA expression in a group of lean persons consuming a diet high in dairy calcium; however, the decrease occurred only at an intake of 2500 mg Ca/d, which is considerably higher than the recommended dietary intake for calcium and which is rarely attained in the regular diet. At a lower, more habitual intake of 1200 mg Ca/d, Boon et al, just as we did, observed no change in the expression of markers of adipose tissue fat metabolism and no effect of dietary calcium on EE or fat oxidation (40). In another study, in lean young men, the same group found no evidence that increasing serum calcitriol had any effect on EE, substrate metabolism, or gene expression related to fat metabolism (41).

The present observations do not support the hypothesis that increasing the calcium intake of overweight or obese low consumers of calcium increases EE and fat oxidation. It is interesting that the mechanisms proposed to support this hypothesis depend on an increase in concentrations of vitamin D₃, which exerts direct inhibitory effects on adipose tissue lipolysis through the activation of membrane-associated vitamin D₃ receptors. Although such a mechanism is supported by animal and in vitro studies (5, 6), it is contradicted by other observations made in humans (26, 36, 41). This apparent contradiction can be explained, however, by an analysis of the composition of weight loss observed in a study by Zemel et al (23) in African American adults. An energy deficit of 500 kcal/d for 24 wk represents a total deficit of 84 000 kcal, which theoretically could be due to the loss of 9.3 kg fat, 12 kg adipose tissue, or 84 kg lean body mass. At the end of the study by Zemel et al, subjects following the diet high in dairy calcium had lost approximately the amount of weight that they should have lost: 9 kg fat and 2 kg tissue that is unaccounted for, unaccounted tissue which represents 81 000–83 000 kcal or 96–99% of their target energy loss. Indeed it was the subjects in the low dairy calcium group who did not lose as much energy as they should have. Their tissue loss was equivalent to the production of calcitriol (1,25-dihydroxyvitamin D₃) in the kidney, which is normalized by vitamin D administration (44). Yanoff et al (45) observed that serum 25-hydroxyvitamin D concentrations were lower in obese American whites and blacks than in their lean peers. Calcitriol concentrations also were lower in obese American blacks than in their lean counterparts, and, although the difference was not significant between obese and lean American whites, calcitriol remained significantly lower when the 2 groups were combined. Similarly, Parikh et al (46) found that calcitriol concentrations were negatively correlated with body mass index and fat mass and were significantly lower in the obese than in the nonobese subjects. These results indicate that obesity is associated with low serum vitamin D and calcitriol concentrations, rather than with high calcitriol concentrations, as proposed by the group led by Zemel.

In summary, our present observations in a small group of overweight and mildly obese, low calcium–consuming men and women show that 5 wk of dairy calcium supplementation at 800 mg/d failed to alter a number of indexes of adipose tissue lipolysis and lipid oxidation. It also did not support the hypothesis that increasing dietary calcium will increase EE, fat oxidation, and fat loss in low calcium–consuming obese persons.

The authors’ responsibilities were as follows—all authors: the design and development of the protocol; MB, SR, PS, and LT: recruiting subjects, carrying out the clinical trial, and conducting data analysis; HV and EL: analysis of adipose tissue biopsy samples and data analysis; KJA: writing of the manuscript, which was reviewed and modified by all authors. KJA is an employee of Nestec SA; the study was instigated by KJA for Nestec SA, who requested and financed the study. None of the authors had a personal or financial conflict of interest.

REFERENCES


