Effect of dairy calcium or supplementary calcium intake on postprandial fat metabolism, appetite, and subsequent energy intake[1–3]

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ABSTRACT

Background: High calcium intake has been shown to increase fecal fat excretion.

Objective: Our aim was to examine whether a high calcium intake from dairy products or from supplements affects postprandial fat metabolism and appetite through fat malabsorption.

Design: Four different isocaloric meals were tested in 18 subjects according to a randomized crossover design. The test meals contained high (HC meal: 172 mg/MJ), medium (MC meal: 84 mg/MJ), or low (LC meal: 15 mg/MJ) amounts of calcium from dairy products or a high amount of calcium given as a calcium carbonate supplement (Suppl meal: 183 mg/MJ). Concentrations of plasma total triacylglycerol, chylomicron triacylglycerol, serum total cholesterol, HDL cholesterol, cholecystokinin, glucagon-like peptide 1, ghrelin, peptide YY, glucose, and insulin and appetite sensation were measured before and at regular intervals until 420 min postprandially.

Results: Dairy calcium significantly diminished the postprandial lipid response. The baseline adjusted area under the curve for chylomicron triacylglycerol was ≈17% lower after the MC meal ($P = 0.02$) and ≈19% lower after the HC meal ($P = 0.007$) than after the LC meal and ≈15% lower after the MC meal ($P = 0.0495$) and ≈17% lower after the HC meal ($P = 0.02$) than after the Suppl meal. No consistent effects of calcium on appetite sensation, or on energy intake at the subsequent meal, or on the postprandial responses of cholecystokinin, glucagon-like peptide 1, ghrelin, peptide YY, insulin, or glucose were observed.

Conclusions: Increased calcium intakes from dairy products attenuate postprandial lipidemia, most probably because of reduced fat absorption, whereas supplementary calcium carbonate does not exert such an effect. This may be due to differences in the chemical form of calcium or to cofactors in dairy products. Calcium did not affect appetite sensation, glucose metabolism, or gut hormone secretion. *Am J Clin Nutr* 2007;85:678–87.

KEY WORDS Dietary calcium, calcium supplementation, postprandial lipidemia, appetite, men, cholecystokinin, glucagon-like peptide 1, ghrelin, peptide YY

INTRODUCTION

The possibility that a high calcium intake may affect energy balance has attracted considerable attention. Several observational studies have found inverse associations between calcium intake or intake of dairy products and body weight, composition, or both (1–6). On the basis of a reanalysis of data from 4 observational studies, Davies et al (1) concluded that differences in calcium intake could explain ≈3% of the variation in body weight. However, other observational studies have failed to find this association (7–9). Relatively few intervention studies have been reported, and those that have been reported are ambiguous with respect to the effect of calcium intakes on body weight and body composition (1, 10–16). In addition, in both animal models and in human studies it has been found that calcium from dairy products has a more profound effect on body weight and composition than does supplementary calcium (6, 14). Many different mechanisms have been suggested to be responsible for the positive effect of a high calcium intake on energy balance. Zemel et al (6) suggested that calcium affects adipocyte metabolism and fat oxidation. Animal studies have found that dairy calcium increases fecal fat excretion (17). In a short-tem intervention study, we recently showed that increases in daily calcium intake increase fecal fat and energy excretion, whereas we failed to find any effect on energy expenditure (18). An increase in calcium intake of ≈1300 mg/d, mainly from low-fat dairy products, caused a daily increase in fecal fat excretion of 8.2 g in subjects consuming a diet with 30% of energy derived from fat. Others have shown a similar effect, though quantitatively smaller (19–21). The difference in effects might have been due to the source of calcium or to the protein source (18). The subjects in our study

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[2] Supported by the Danish Dairy Research Foundation, FOOD Graduate School, Arla Foods Ingredients amba, Pharma Vinci A/S, The Dutch Dairy Association, Programme Commission on Food and Health (Danish Research Agency), and the Danish Medical Research Council. The cacao butter was a gift from Aarhus United Denmark.

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were given calcium from dairy products, whereas the subjects in the other studies were given calcium supplements or fortified products. The increased fat excretion was presumably due to formation of insoluble calcium fatty acid soaps or to the binding of bile acids, which impairs the formation of micelles (22–25). When fat is absorbed, it enters blood circulation in the form of intestinally derived triacylglycerol-rich lipoproteins, ie, chylomicrons. Thus, if calcium partly inhibits fat absorption, a decrease in the postprandial increase in chylomicron triacylglycerol would be expected. As far as we know, the effect of calcium on postprandial fat absorption has not been examined previously. Dairy calcium may produce weight loss by a mechanism other than fat malabsorption. Although we, and others, have failed to find any effect of calcium on 24-h energy expenditure during energy balance (18, 26), the effects of calcium on appetite regulation have been less well studied. It is also possible that the increased fecal energy loss produced by dairy calcium might be recognized by the body and result in a compensatory increase in appetite and calorie intake. The aim of the present study was to examine whether a high calcium intake from dairy products or from supplements affects postprandial fat metabolism and appetite.

SUBJECTS AND METHODS

Subjects

Eighteen male subjects were recruited through advertising at universities in Copenhagen and on the internet. The subjects were healthy, between 18 and 50 y old, and moderately overweight [body mass index (in kg/m^2): 24–31]. The exclusion criteria were as follows: lactose intolerance, milk allergy, diabetes, hypertension, hyperlipidemia, smoking, elite athletes, regular use of medication, use of dietary supplements, blood donation within the 6 mo preceding the study, and a hemoglobin concentration <130 g/L. The subjects were instructed to not donate blood during the study period. All subjects gave written consent after having received verbal and written information about the study. The study was carried out at the Department of Human Nutrition, Faculty of Life Sciences, University of Copenhagen, Frederiksberg, Denmark, and was approved by the Municipal Ethical Committee of Copenhagen and Frederiksberg in accordance with the Helsinki-II declaration (KF 01-144/02). The subjects received 3000 Danish crowns (≈US$500) on completion of all tests.

Experimental design

Four different isocaloric meals were tested in 18 subjects according to a randomized crossover design The subjects were randomly assigned to the sequence of the test meals. There was a washout period of ≥3 wk between the test meals. Each test meal lasted 8.5 h. The subjects were instructed to not eat or drink dairy products or food items rich in fat, such as potato crisps or chocolate, for 2 d immediately before each test meal or to drink alcohol or perform hard physical activities on the day before each test meal. The subjects consumed a standardized meal supplied by the study, which consisted of a pasta dish, orange juice, and biscuits (4 MJ; 17.6% of energy from protein, 22.1% of energy from fat, and 60.3% of energy from carbohydrate) on the evening before the test. The subjects fasted after consuming this meal at no later than 2000. They were supplied with 0.5 L water and were instructed to consume half of it before they went to bed and the remaining half in the morning before leaving the house. On the morning of the tests, the subjects arrived at the Department of Human Nutrition by car, bus, train, or slowly walking (ie, the least strenuous means of transportation). On arrival they were weighed while wearing only underwear and after emptying their bladder. Weight was measured in kilograms with one decimal by a Lindetronic 8000 scale (Copenhagen, Denmark). A Venflon catheter (BD Infusion Therapy AB, Helsingborg, Sweden) was then inserted in an antecubital arm vein. After 20 min of rest, a fasting blood sample was taken and the subject's appetite sensation was assessed with a visual analogue scale (VAS). The test meal was then served. The subjects were instructed to consume the meal within 15 min, after which they completed a questionnaire on the palatability of the meal. Blood samples were collected 15, 30, 60, 90, 120, 180, 300, and 420 min after the start of the meal, and appetite sensation was assessed every 30 min throughout the 7 h after the meal. During the day the subjects were allowed to read, walk around at the Department of Human Nutrition, listen to the radio, or watch television or videos. One to 3 subjects were tested on each test day. The subjects were allowed to talk with each other as long as the conversation did not involve food, appetite, or related issues. The subjects were not allowed to eat or drink during the day except for 2 glasses of water (250 mL each), which were served 180 and 300 min after the meal (after the blood samples were drawn). At the end of day, after the last blood sample was taken, an ad libitum meal consisting of a pasta salad (15% of energy from protein, 30% of energy from fat, and 55% of energy from carbohydrate) and water was served, food intake was registered, and energy intake was calculated. The subjects were instructed to eat until "comfortable satisfaction." Afterward, they completed a questionnaire on the palatability of the meal, and appetite sensation was assessed. The subjects' water intake was registered on the first test day, and this intake was repeated on the following test days.

Test meals

Four isocaloric test meals were provided to the subjects in a randomized order on 4 different days (**Table 1**). The test meals contained dairy proteins as the main protein source and a high amount of calcium from dairy products (HC meal), a medium amount of calcium from dairy products (MC meal), a low amount of calcium from dairy products (LC meal), or a low amount of calcium from dairy products and a calcium supplement served as a drink containing calcium carbonate (Suppl meal).

The test meals consisted of a bread roll, dairy products, chocolate, and water (Table 1). All of the test meals consisted of the same servings of bread rolls, yogurt, and liquid. The content of macronutrients and micronutrients in each meal was estimated by using Dankost 3000 dietary assessment software (Danish Catering Center, Herlev, Denmark) (27) and information from Arla Foods Ingredients amba (Viby, Denmark). The calcium and energy contents were analyzed. The test meals had the same macronutrient composition: 15% of energy from protein, 39% of energy from fat, and 46% of energy from carbohydrate. In addition, all test meals contained the same amounts of lactose, the content of which was adjusted by adding lactose powder to the bread roll or the drink, and the same amounts of whey and casein, which were achieved with the addition of whey powder (Lacprodan DI-9213; Arla Foods Ingredients amba) and casein powder (Miprodan 30; Arla Foods Ingredients amba).

TABLE 1
The content and nutrient composition of the 4 test meals

	Test meals[1]			
	LC	MC	HC	Suppl
Bread roll				
Milk (g)	0	0	75	0
Water (g)	75	75	0	100
Flour (g)	122.5	122.5	122.5	122.5
Cacao butter (g)	49.25	48	47.25	49.25
Casein powder (g)[2]	14.25	5	0	27.75
Whey powder (g)[3]	1.25	1.25	0	4.75
Lactose (g)	15	5.5	0	31.5
Sugar (g)	0	1.5	5.5	0
Chocolate (g)	2.5	2.5	2.5	2.5
Yogurt				
Yogurt (g)	21	75	75	21
Water (g)	54	0	0	54
Gelatin (g)	0.5	0	0	0.6
Lactose (g)	2	2	2	2
Sugar (g)	2.3	2.3	2.3	7.8
Drink				
Milk (g)	0	266	554	0
Water (g)	488	255.5	0	463
Casein powder (g)[2]	13.5	7	0	0
Whey powder (g)[3]	3.5	1.5	0	0
Lactose (g)	16.5	12	0	0
Sugar (g)	5.5	4	0	0
Calcium supplement (g)[4]				1000
Nutrient composition[5]				
Energy (kJ)	4464	4146	4619	4649
Protein (% of energy)	14.9	14.9	14.9	14.9
Fat (% of energy)	39.3	39.1	39.3	39.3
Carbohydrate (% of energy)	45.9	45.9	45.8	45.9
Calcium (mg)	68	350	793	850
Phosphate (mg)	521	1253	1913	521
Casein (g)	27.2	27.8	27.4	27.2
Whey (g)	4.0	4.2	4.3	4.0
Lactose (g)	34.2	34.9	34.8	34.2

[1] LC, MC, and HC, low, medium, and high amounts of calcium from dairy products; Suppl, high amounts of calcium as supplementary calcium carbonate.
[2] Miprodan 30; Arla Foods Ingredients amba, Viby, Denmark.
[3] Lacprodan DI-9213; Arla Foods Ingredients amba.
[4] Calcium carbonate; Pharma-Vinci A/S, Frederiksværk, Denmark.
[5] The content of protein, fat, carbohydrate, and phosphate were estimated by using the Dankost 3000 dietary assessment software (Danish Catering Center, Herlev, Denmark) and information from Arla Foods Ingredients amba. The contents of calcium and energy were measured.

The test meals were prepared at the Department of Human Nutrition from normal Danish food items. Portions were matched to each subject's individual energy requirement and adjusted to the nearest 1 MJ. Each test meal contained 50% of the subject's daily energy requirement. The subjects' energy requirements were determined by using the following formula, which is based on a meta-analysis of our 24-h calorimetry studies (B Buemann, unpublished observations, 2003):

$$\text{24-h EE(kJ/d)} = 1780.6 + 101.2 \times \text{FFM} + 32.2 \times \text{FM} + 212.4 \times \text{SPA} + 4.03 \times \text{DE} - 4.17 \times \text{age} \quad (1)$$

Spontaneous physical activity was 5.8% for men and duration of exercise (DE) was set to 30 min (28). Body composition was measured at baseline with the bioelectrical impedance method, and fat-free mass and fat mass were calculated.

The subjects' habitual diet was assessed with the use of a 3-d weighed food record (2 weekdays and 1 Saturday). The subjects were given both verbal and written instructions on how to complete the food record, and digital kitchen scales were supplied. Dankost 3000 dietary assessment software (Danish Catering Center) (27) was used to calculate the energy and nutrient composition of the diets.

Visual analogue scale

Visual analogue scales, 100 mm in length with words expressing the most positive and the most negative rating anchored at each end, were used to measure subjective appetite sensation (hunger, satiation, fullness, prospective food consumption, thirst, well-being, and desire to eat meat or fish or something sweet, salty, or fatty) and palatability of the test and ad libitum meals (**Table 2**). The questions were provided in small booklets, one question at a time. The subjects were instructed to not compare their ratings with each other and could not refer to their previous ratings when filling in the visual analogue scale.

Analyses

Venous blood samples were drawn at baseline and 15, 30, 60, 90, 120, 180, 300, and 420 min after the test meal started. Blood samples for plasma analyses were collected in tubes containing EDTA and placed on ice immediately. Blood samples for serum analyses were collected in serum tubes and kept at room temperature for 30 min to coagulate. All samples were centrifuged at 2800 × *g* for 15 min at 4 °C and stored at −20 °C until analyzed (exceptions are described below).

Triacylglycerol

Triacylglycerol was measured in blood samples drawn at baseline and 60, 120, 300, and 420 min after the start of the test meal. The chylomicrons were isolated within 24 h by ultracentrifugation as described elsewhere (29). Plasma total triacylglycerol, chylomicron triacylglycerol, and triacylglycerol concentrations in the bottom fraction were measured with an enzymatic endpoint method (Test-Combination Triacylglycerol GPO-PAP kit; Roche, Basel, Switzerland) by using a Cobras Mira Plus (Roche). The intraassay and interassay CVs were 0.5% and 1.4%, respectively. The percentage of recovery was calculated, and the chylomicron triacylglycerol concentration was adjusted to the concentration corresponding to 100% recovery.

Plasma concentrations of glucose, cholecystokinin, ghrelin, glucagon-like peptide 1, and peptide YY

Plasma concentrations of glucose, cholecystokinin (CCK), ghrelin, glucagon-like peptide 1 (GLP-1), and peptide YY (PYY) were measured in blood samples drawn at baseline and 15, 30, 60, 90 (only glucose), 120, 180, 300 (except glucose), and 420 min after the start of the test meal. Glucose was measured with the use of an enzymatic endpoint method (Hexokinase) (Gluco-quant Glucose/HK, Roche Diagnostics, Basel, Switzerland) with a Cobras Mira Plus (Roche). The intraassay and interassay CVs were 1.1% and 1.3%. CCK concentrations in plasma were measured by radioimmunoassay with an antiserum (code no. 92128) that

TABLE 2
Questions on appetite, desire to eat specific foods, and palatability of the test meals

Answer (0 mm)	Question	Answer (100 mm)
I am not hungry at all	How hungry do you feel?	I have never been more hungry
I am completely empty	How satisfied do you feel?	I cannot eat another bite
Not at all full	How full do you feel?	Totally full
Nothing at all	How much do you think you can eat?	A lot
Yes, very much	Would you like to eat something sweet?	No, not at all
Yes, very much	Would you like to eat something salty?	No, not at all
Yes, very much	Would you like to eat something fatty?	No, not at all
Yes, very much	Would you like to eat meat or fish?	No, not at all
Not at all	How thirsty do you feel?	Very much
Not comfortable at all	How comfortable do you feel?	Very comfortable
Good	Visual appeal	Bad
Good	Smell	Bad
Good	Taste	Bad
Much	Aftertaste	None
Good	Overall palatability	Bad

binds the circulating CCK with equimolar potency without cross-reactivity with any gastrin. Details of the assay and its reliability parameters are described elsewhere (30).

Plasma ghrelin concentrations were determined by using a radioimmunoassay kit (catalog no. GHRT-89k; Linco Research, St Charles, MO) that measures total ghrelin (intact and desoctanoylated ghrelin). The sensitivity was 100 pg/mL, and the intraassay CV was <10%. Quality controls were always within acceptable limits.

Radioimmunoassay of PYY in plasma was performed by using antiserum code no. 8412-2II (31), which reacts equally with PYY1–36 and PYY3–36. Synthetic human PYY 1–36 or PYY 3–36 (Peninsula Laboratories, St Helens, United Kingdom) were used for standards. [^{125}I]-PYY1–36 (code no. IM259) was from Amersham Biosciences, Buckinghamshire, United Kingdom. The assay buffer consisted of 0.05 mol sodium phosphate/L (pH 7.5), 400 KIE Trasylol-aprotinin/mL, 0.1 mol NaCl/L, 10 mmol EDTA/L, and 0.6 mmol merthiolate/L. Unknown plasma samples (150 μL) + 150 μL assay buffer or 150 μL charcoal-treated plasma + 150 μL standards were preincubated with antiserum (100 μL) and diluted 1:20.000 (final concentration) for 48 h at 4 °C. Then, 100 μL tracer (5 fmol; specific activity: 70 MBq/nmol) was added, and the mixture was incubated for 24 h before bound and free peptide moieties were separated by plasma-coated charcoal (32). The detection limit of the assay was <2.5 pmol/L, and 50% inhibition was obtained with 23 pmol PYY/L. Recovery of PYY added to plasma in concentrations between 5 and 50 pmol/L deviated <15% from expected values. The intraassay CV was <5%. The antiserum showed no cross-reaction with human neuropeptide Y or human pancreatic peptide in concentrations up to 500 pmol/L.

GLP-1 concentrations in plasma were measured by radioimmunoassay after extraction of plasma with 70% ethanol (by vol, final concentration). Carboxy-terminal GLP-1 immunoreactivity was determined by using antiserum code no. 89390, which has an absolute requirement for the intact amidated carboxy-terminus of GLP-1 7–36 amide and cross-reacts <0.01% with carboxy-terminally truncated fragments, and 89% with GLP-1 9–36 amide, the primary metabolite of dipeptidyl-peptidase IV–mediated degradation (33). The sum of the 2 components (total GLP-1 concentration) reflects the rate of secretion of the L cell. Sensitivity was <5 pmol/L, and the intraassay CV was <10%.

Serum concentrations of insulin, total cholesterol, and HDL cholesterol

Serum concentrations of insulin, total cholesterol, and HDL cholesterol were measured in blood samples drawn at baseline and 30, 60, 90, 120, 180, 300 (except insulin), and 420 min after the start of the test meal. Insulin was measured by solid-phase, 2-site chemiluminescent immunometric assay (Immulite/immuliter 1000 insulin; Diagnostic Products Corporation, Los Angeles, CA) with the use of an Immulite 1000 analyzer (Diagnostic Products Corporation). The intraassay and interassay CVs were 2.5% and 4.9%, respectively. Total cholesterol was measured with an enzymatic endpoint method (CHOD-PAP) (cholesterol kit; Roche) by using a Cobras Mira plus (Roche, Basel, Switzerland). The intraassay CV was 0.9%, and the interassay CV was 1.5%. HDL cholesterol was measured with an enzymatic colorimetric test (HDL cholesterol plus second generation; Roche) by using a Cobras Mira Plus (Roche). The intraassay and interassay CVs were 1.9% and 3.5%, respectively.

Calcium and energy contents of the test meals

A portion of each meal was freeze-dried and homogenized. Gross energy content was obtained by using a bomb calorimeter (Ika-calorimeter system C4000; Ika, Heitersheim, Germany). The analyses were performed as duplicates. Before the calcium content was measured, the samples were lyophilized and microwave digested (MES-1000; CEM Corporation, Matthews, NC) with 65% HNO_3 (suprapur; Merck, Darmstadt, Germany) and 30% H_2O_2 (suprapur; Merck). Calcium was measured by atomic absorption spectroscopy (SpectraAA-200 VARIAN; Varian Techtron Pty, Limited, Victoria, Australia) after dilution with a lanthanium oxide solution (Merck). Standards were prepared from a 1000-mg Ca/L standard (Tritisol; Merck) by dilution with lanthanium oxide solution. A reference diet (Standard Reference Material 1548a, Typical Diet; National Institute of Standards and Technology, Gaithersburg, MD) was analyzed in the same run. The percentage of recovery of calcium in the reference diet was

89%. The calcium content in the samples was adjusted to concentrations corresponding to 100% recovery.

Calculations and statistical methods

Data were analyzed with the use of SAS software (version 8; SAS Institute, Cary, NC). Data are reported as means ± 95% confidence limits (CLs) unless otherwise indicated. A P value <0.05 is considered significant.

Repeated-measures analysis of variance (ANOVA) was used to assess the effect of time, meal, and interaction of meal and time. To adjust for differences in baseline values, these factors were included as covariate in all analyses. The analysis was performed in PROC MIXED. Based on the structure of the covariance matrix, the Gaussian model of spatial correlation was chosen for covariance structure. The study had a crossover design, so "subject" was included as a random effect. We tested for a significant effect of sequence of the test meals, and sequence of the test meals was included as a covariate when this was the case.

Area under the curve (AUC) was calculated as the total increase above zero. Analysis of covariance (ANCOVA) was used to examine the effect of meal on AUC. ANCOVA was performed in PROC MIXED. "Subject" was included as a random effect and baseline values as a covariate in all analyses. The sequence of the test meals was included as a covariate when it was found to have a significant effect. ANOVA was used to examine the effect of meal on the palatability of the meals and ad libitum intake. ANOVA was performed in PROC MIXED, with subject included as a random effect. Model control for all analyses was performed in PROC MIXED, and data were transformed before analysis if necessary. Least-squares means were used to estimate the adjusted means. Pairwise comparisons of the least-squares means were performed by paired t test.

The recording of appetite sensation measured by VAS after intake of the ad libitum meal was not included in the calculation of AUC or in any of the analyses.

RESULTS

All 18 subjects completed the 4 test meals. The characteristics of the subjects and their habitual intake are presented in **Table 3**. Blood variables from one subject were excluded from the data analyses because of abnormal triacylglycerol values. The record of appetite sensation after the HC meal was missing for one subject.

Plasma triacylglycerol response

The postprandial responses in plasma total and chylomicron triacylglycerol are shown in **Figure 1**. Plasma total and chylomicron triacylglycerol concentrations increased after intake of each of the 4 test meals. The concentrations peaked 180 min after intake of the MC and the HC meals and 300 min after intake of the LC meal and the Suppl meal. At the end of the study, the concentrations had decreased to values similar to the baseline concentration after intake of the LC, MC, and HC meals but not after intake of the Suppl meal. Repeated-measures ANOVA showed a significant meal-by-time interaction on the plasma total triacylglycerol concentration ($P = 0.04$) when adjusted for baseline concentration. However, pairwise comparisons of the adjusted means showed no persistent effects. There was a significant effect of time on chylomicron triacylglycerol concentrations ($P < 0.0001$) when adjusted for baseline concentration.

TABLE 3
Baseline characteristics and habitual intake[1]

	Subjects ($n = 18$)
Age (y)	25.7 ± 1.2 (25.9 ± 1.3)
Height (m)	184 ± 2 (184 ± 2)
Weight (kg)[2]	91.4 ± 1.2 (91.3 ± 1.3)
BMI (kg/m^2)[3]	27.1 ± 0.4 (27.1 ± 0.5)
Habitual intake[4]	
Energy (kJ/d)	12985 ± 624 (13138 ± 652)
Protein (% of energy)	15.4 ± 0.5 (15.2 ± 0.5)
Carbohydrate (% of energy)	51.2 ± 1.4 (51.3 ± 1.5)
Fat (% of energy)	26.7 ± 1.1 (26.4 ± 1.2)
Alcohol (% of energy)	6.6 ± 1.5 (7.0 ± 1.6)
Calcium (mg/d)	1380 ± 104 (1395 ± 110)

[1] All values are $\bar{x}$ ± SE. Eighteen subjects were included in the study and all completed the tests; however; one subject was excluded from part of the data analyses because of abnormal triacylglycerol values. The values in parentheses are for the 17 subjects included in these analyses.

[2] The mean (±SE) weight at the end of the study was 91.5 ± 1.5 (91.4 ± 1.5) kg.

[3] The mean (±SE) BMI at the end of the study was 27.1 ± 0.5 (27.1 ± 0.5).

[4] Assessed with a 3-d weighed food record.

There was no significant meal-by-time interaction and no significant effect of meal.

The AUC for plasma total and chylomicron triacylglycerol are shown in Figure 1. ANCOVA showed that meal had a significant effect on AUC for chylomicron triacylglycerol ($P = 0.01$) when adjusted for baseline concentration. The adjusted AUC for chylomicron triacylglycerol was lower after intake of the MC (≈17%; $P = 0.02$) and HC (≈19%; $P = 0.007$) meals than after the LC meals. Similarly, the AUC was lower after intake of the MC (≈15%; $P = 0.0495$) and the HC (≈17%; $P = 0.02$) meals than after the Suppl meal. There were no significant differences between the other meals. Meal had no significant effect on AUC for plasma total triacylglycerol adjusted for baseline concentration.

Serum cholesterol

Repeated-measures ANOVA of serum total cholesterol and of HDL cholesterol, both adjusted for baseline concentrations, showed no significant meal-by-time interaction and no significant effect of meal (data not shown). However, a significant effect of time on both serum total cholesterol ($P = 0.01$) and HDL cholesterol ($P < 0.0001$) was observed.

ANCOVA showed that meal had no significant effect on the AUC for total cholesterol when adjusted for the baseline concentration of total cholesterol or on the AUC for HDL cholesterol when adjusted for the baseline concentration of HDL cholesterol and the sequence of the test meals (data not shown).

Plasma concentrations of glucose, cholecystokinin, ghrelin, glucagon-like peptide 1, and peptide YY

The postprandial responses of glucose, CCK, ghrelin, GLP-1, PYY, and insulin are shown in **Figure 2**. Repeated-measures ANOVA showed a significant meal-by-time interaction on GLP-1 ($P = 0.007$) when adjusted for baseline concentration. Comparisons of adjusted least-squares means showed that the GLP-1 concentration was higher 60 min after intake of the Suppl

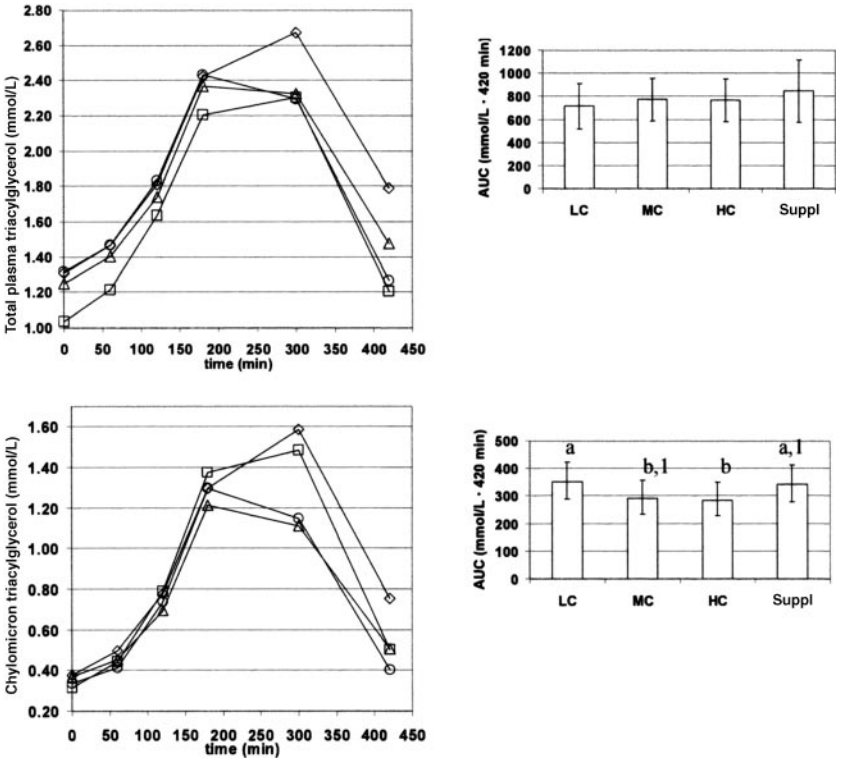

FIGURE 1. Mean plasma total triacylglycerol and chylomicron triacylglycerol and area under the curve (AUC) in response to the 4 test meals: low (LC; □), high (HC; △), and medium (MC; ○) amounts of calcium from dairy products or high amounts of calcium as supplementary calcium carbonate (Suppl; ◇). $n = 17$. The mean AUCs are adjusted for the baseline concentration, and the bars represent 95% confidence limits. Repeated-measures ANOVA showed a significant interaction of meal and time for plasma total triacylglycerol concentration ($P = 0.04$) and a significant effect of time on chylomicron triacylglycerol concentration ($P < 0.0001$) when adjusted for the baseline concentration. ANCOVA showed a significant effect of meal on the AUC for chylomicron triacylglycerol ($P = 0.01$) when adjusted for the baseline concentration. Values with different lowercase letters are significantly different, $P < 0.05$. [1]$n = 16$ because of missing values due to analytical problems.

meal than after the LC ($P = 0.03$), MC ($P = 0.01$), and HC ($P = 0.005$) meals. No significant differences were observed at the other time points, and no significant meal-by-time interaction or significant effect of meal was observed on CCK, ghrelin, PYY, glucose, or insulin when adjusted for baseline concentrations. However, a strong significant effect of time on CCK, ghrelin, PYY, and insulin ($P < 0.0001$ for all) was observed when adjusted for baseline concentration and on glucose ($P < 0.0001$) when adjusted for baseline concentration and the sequence of the test meals.

The AUCs for CCK, GLP-1, ghrelin, PYY, glucose, and insulin are shown in Figure 2. ANCOVA showed that meal had no significant effect on the AUC for CCK, GLP-1, ghrelin, PYY, or insulin when adjusted for baseline concentrations or on the AUC for glucose when adjusted for the baseline concentration and the sequence of the test meals.

Palatability of the test meal

ANOVA showed that meal had a significant effect on the palatability of the test meal with regard to taste ($P = 0.02$), visual appeal ($P < 0.0001$), and overall palatability ($P < 0.0001$) (data not shown). The subjects found that the taste of the HC meal was significantly better than the taste of the LC ($P = 0.0008$) and the supplement ($P = 0.007$) meals when adjusted for the sequence of the test meals. The subjects found that the HC meal was significantly more visually appealing than were the LC ($P < 0.0001$) and Suppl ($P = 0.02$) meals, and the LC meal was significantly less appealing than were the MC ($P = 0.0003$) and Suppl ($P = 0.02$) meals. The subjects found that the overall palatability of the HC meal was significantly better than that of the LC ($P < 0.0001$) and the Suppl ($P = 0.0002$) meals and that of the MC meal was significantly better than that of the LC ($P = 0.0003$) and the Suppl ($P = 0.01$) meals.

Appetite sensation

The postprandial responses in subjective appetite sensation measured by VAS are shown in **Figure 3** (data on well-being and desire to eat meat or fish or something salty or sweet are not shown). Repeated-measures ANOVA showed no significant meal-by-time interaction and no significant effect of meal on hunger, satiation, fullness, prospective food consumption, thirst, well-being, or desire to eat meat or fish or something sweet, salty, or fatty after adjustment for sensation measured at baseline. However, a strong significant effect of time on all measures, except well-being, was observed ($P < 0.0001$ for all). ANCOVA showed that meal had no significant effect on AUC for any of the measures after adjustment for sensation measured at baseline (Figure 3).

At the end of the day, the subjects were served an ad libitum meal. ANOVA showed that meal did not significantly affect energy intake at this meal (data not shown). Meal had no significant effect on the palatability of the ad libitum meal, except on the smell and the visual appeal of the meal. The subjects found the smell of the ad libitum meal to be significantly better after intake

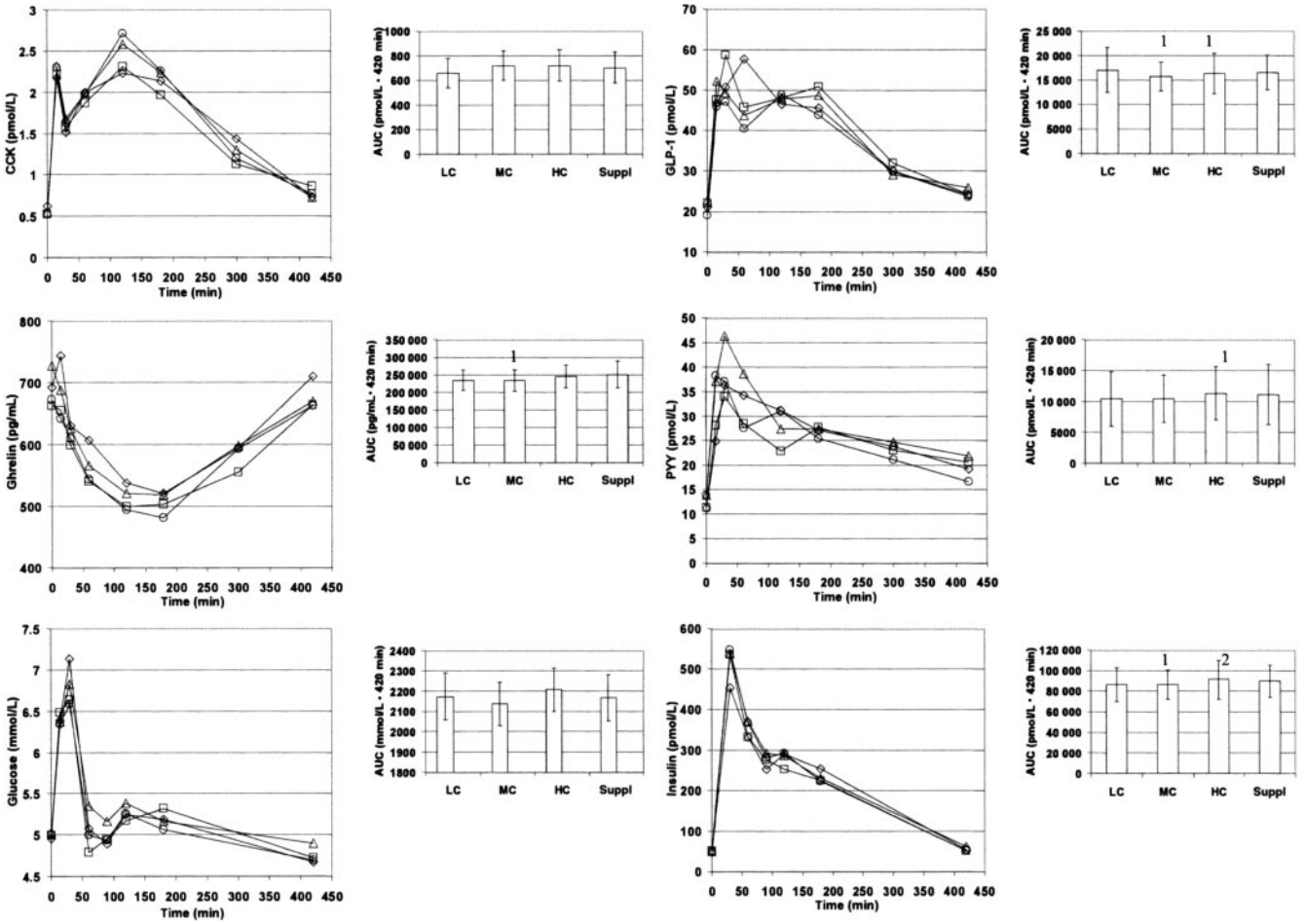

FIGURE 2. Mean cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), ghrelin, peptide YY (PYY), glucose, and insulin concentrations and area under the curve (AUC) in response to the 4 test meals: low (LC; □), high (HC; △), and medium (MC; ○) amounts of calcium from dairy products or high amounts of calcium as supplementary calcium carbonate (Suppl; ◇). $n = 17$. The bars for the AUCs represent 95% confidence limits. Repeated-measures ANOVA showed a significant interaction of meal and time for GLP-1 ($P = 0.007$) when adjusted for the baseline concentration. Comparisons of adjusted least-squares means showed that, at 60 min, the GLP-1 concentration was higher after intake of Suppl than after the LC ($P = 0.03$), MC ($P = 0.01$), and HC ($P = 0.005$) meals. There was a significant effect of time on CCK, ghrelin, PYY, and insulin ($P < 0.0001$ for all) when adjusted for the baseline concentration and on glucose ($P < 0.0001$) when adjusted for the baseline concentration and sequence of the test meals. ANCOVA showed that meal had no significant effect on the AUC for CCK, GLP-1, ghrelin, PYY, or insulin when adjusted for the baseline concentration or on the AUC for glucose when adjusted for the baseline concentration and sequence of the test meals. [1]$n = 16$ because of missing values due to analytical problems. [2]$n = 15$ because of missing values due to analytical problems.

of the HC ($P = 0.0008$) and the Suppl ($P = 0.04$) meals than after the intake of the LC meal. The subjects found the ad libitum meal to be significantly more visually appealing after intake of the HC ($P = 0.009$) and MC ($P = 0.01$) meals than after intake of the LC meal.

DISCUSSION

The present study evaluated the effects of calcium intake on postprandial fat metabolism as a proxy for fat absorption. The major finding was that a high calcium intake from dairy products, milk, and low-fat yogurt**,** but not from a calcium supplement, decreased postprandial lipidemia. No significant effect of a high calcium intake from either dairy products or the supplement on appetite sensations, appetite hormones, or calorie intake at the subsequent meal was found.

Postprandial fat metabolism

Consumption of the high-fat meals resulted, as expected, in pronounced postprandial lipidemia. Compared with the LC meal, the lipid response in chylomicron triacylglycerol was reduced by ≈17% by the MC meal and by ≈19% by the HC meal, which indicated that a high calcium intake from dairy products reduces the lipid response but also that a plateau value exists above which an increased calcium intake does not seem to have any additional effect. The total lipid response in plasma total triacylglycerol was not affected by calcium intake.

The decrease in the lipid response in chylomicron triacylglycerols may reflect either a decrease in fat absorption or an increase in chylomicron clearance. However, no evidence in the literature suggests that calcium intake interferes with chylomicron clearance, and the unaffected concentrations of total cholesterol and HDL cholesterol suggest that this is unlikely. It is therefore most likely that the decreased postprandial lipid response was due to decreases in fat absorption. It was previously shown that the lipid response in chylomicron triacylglycerols increases with the amount of fat ingested (0-50 g per meal) (34). As far as we know, the present study is the first to show that an increased calcium intake from dairy products exerts a lowering effect on

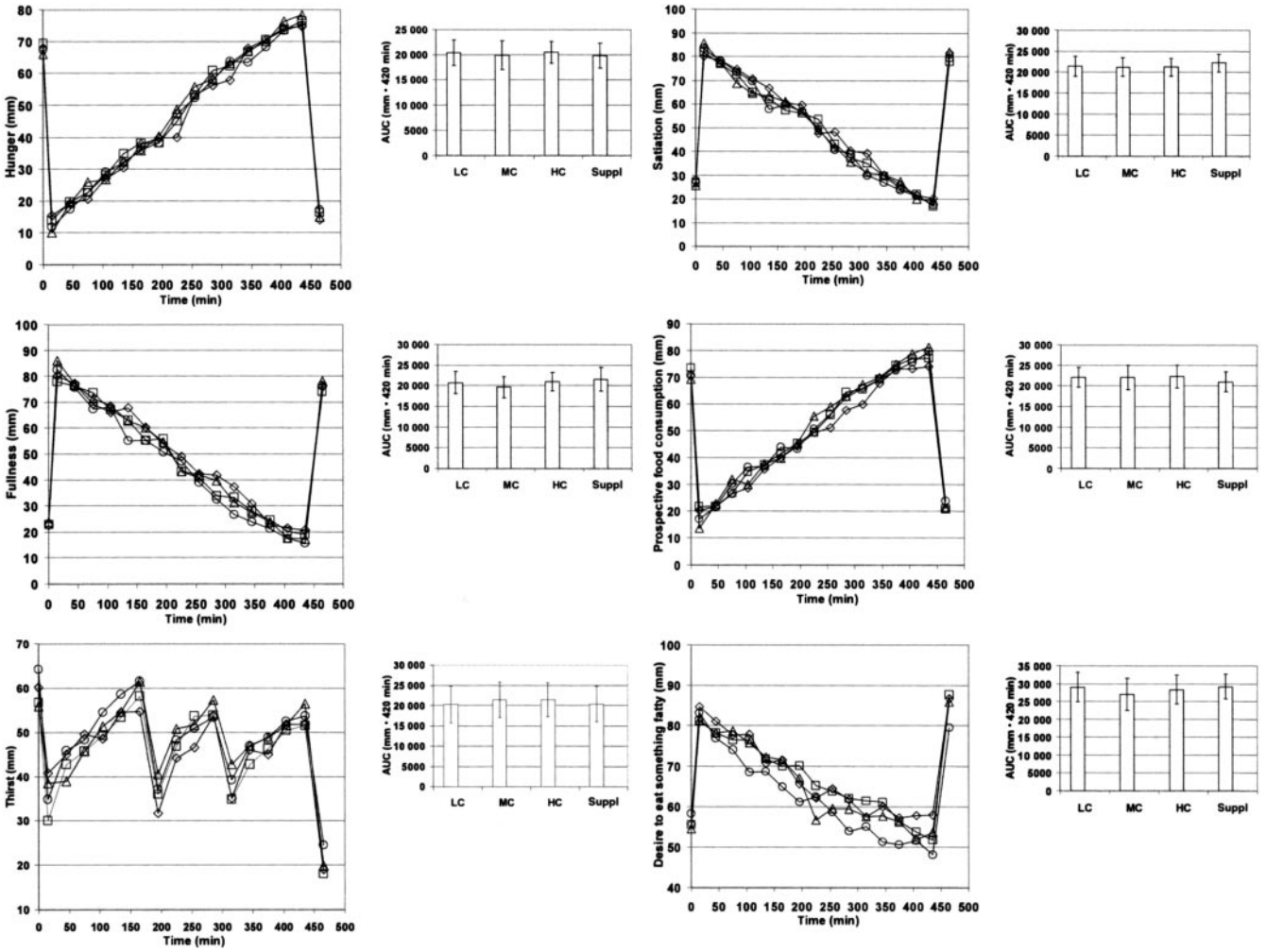

FIGURE 3. Mean appetite sensation and desire to eat something fatty and area under the curve (AUC) in response to the 4 test meals: low (LC; □), high (HC; △), and medium (MC; ○) amounts of calcium from dairy products or high amounts of calcium as supplementary calcium carbonate (Suppl; ◇) ($n = 18$, except for the HC meal, for which $n = 17$). The bars for the AUCs represent 95% confidence limits. Repeated-measures ANOVA showed a strong significant effect of time on all measures ($P < 0.0001$ for all) when adjusted for the sensation measured at baseline. ANCOVA showed that meal had no significant effect on AUC for any of the measures when adjusted for sensation measured at baseline.

postprandial fat absorption. However, several studies, in both animals and humans, have shown that calcium intake increases the fecal excretion of fat, presumably via the formation of insoluble calcium fatty acid soaps in the gut or by binding of bile acids, which impairs the formation of micelles (17–21, 24, 25, 35, 36).

Our main aim in studying postprandial fat metabolism was to examine whether the previously reported increased fecal fat excretion induced by a diet high in dairy calcium could be reflected in a diminished postprandial plasma excursion of triacylglycerol and chylomicron triacylglycerol, but we are aware that the findings might also have implications for the role of a diet high in dairy products in the risk of cardiovascular diseases (CVD). Observational studies have previously shown an inverse association between dairy and calcium intakes and CVD and dyslipidemia (2, 37–39). Orlistat, a gastrointestinal lipase inhibitor that reduces dietary fat absorption by ≈30%, has been shown to have a positive effect on fasting lipid profile by decreasing fasting total cholesterol and LDL (40, 41) and reducing postprandial lipids (42). Although the effect of calcium in the present study is not as pronounced as the effect of Orlistat, it is likely that a long-term high intake of calcium from dairy products may have beneficial effects on the lipid profile and, thereby, on the risk of CVD. A beneficial effect on the lipid profile was previously shown in intervention studies using calcium from supplements or fortified foods (43, 44).

It was previously shown that calcium from dairy products has a more profound effect on body weight than does calcium from supplements (6, 14). The mechanism behind this difference is unknown. We previously suggested that this may be due in part to the time at which the supplement is consumed. If calcium is to inhibit the absorption of fat it, is a condition that fat and calcium are present in the gut at the same time (5). We therefore gave the supplement as a part of the meal and in a solution that mimicked the milk given with the other meals. However, the lipid response in chylomicron triacylglycerols was not significantly different after the intake of the LC and Suppl meals, which indicated that there must be another property of milk calcium that we did not take into account, eg, the chemical form of calcium, other bioactive components in dairy products, differences in the solubility

of calcium from milk and calcium carbonate, or differences in pH. In dairy products, calcium is largely found as calcium phosphate and it is possible that phosphate contributes to the effect of calcium. It was previously shown that supplemental calcium increases the fecal excretion of phosphate and inhibits its absorption, probably because of the formation of insoluble calcium phosphate, which has been suggested to bind bile acids and thereby partly impairs the formation of micelles (25).

Appetite sensation and regulation

One of our considerations was that a partial inhibition of fat absorption by increased calcium intake could have a compensatory stimulatory effect on food intake, which might lead to increased food intake. However, we found no effect of a high calcium intake, from either dairy products or the supplement, on the sensation of appetite, on the secretion of the appetite-regulating hormones, or on energy intake at the subsequent ad libitum meal. A significant interaction was found between meal and time for GLP-1 secretion, but this was due to an inexplicably high concentration of GLP-1 60 min after intake of the supplement meal and it was not a persistent difference between the meals. In addition, there was no significant difference in the total GLP-1 response; therefore, we do not attach much importance to the significant interaction between meal and time. Our results indicate that the apparent inhibition of fat absorption does not have a stimulating effect on energy intake and that the previously observed effect of calcium on body weight and composition is not due to a reduced energy intake. However, we cannot exclude a subchronic effect of calcium on appetite.

We used a VAS to measure appetite sensation. The reproducibility and validity of the VAS was previously examined and found to be reliable (45). Flint et al found that, to detect a difference of 10% in the mean value, a study with a paired design should include ≥18 subjects (20 subjects for the variable "desire to eat something salty") to obtain a power of 0.9 (45). In the present study we used the AUC and not mean values in the data analyses. However, because the study included 18 subjects, it seems unlikely that the negative outcome found in the present study was due to type 2 errors. If possible, a gap should be entered because the study limitations apply to the entire study and not just the part dealing with appetite sensation and regulation.

The study had some limitations. Although all efforts were exerted to make the 4 meals as similar as possible, except for the calcium intakes, there were some differences in the compositions of the meals, and we could not rule out that these differences may have confounded the outcome. Moreover, we cannot exclude the possibility that an adaptation to a long-term high calcium intake may occur, which could reduce the effect. However, because our subjects had a relatively high habitual calcium intake, it seems unlikely that a total adaptation takes place. Further studies are necessary to examine this aspect further.

Consistent with our previous finding that a diet high in dairy calcium induces fat malabsorption in humans, the present findings indicate that the effect is also reflected in an attenuated postprandial lipid response after intake of dairy calcium from milk and low-fat yogurt, but not after intake of supplementary calcium. This may have been due to differences in the chemical form or to cofactors in the dairy products. The findings of the present study do not suggest any effect on appetite regulation as assessed by appetite sensations, appetite hormones, or ad libitum intake of a subsequent meal.

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JKL, IT, and AA designed the study. JKL and SN collected the data. JJH and JFR analyzed most of the hormones. JKL analyzed the data. All authors participated in the discussion of the results and commented on the manuscript. AA is a member of the Arla Nutrition Advisory Board and receives an honorarium for attending each board meeting.

REFERENCES

1. Davies KM, Heaney RP, Recker RR, et al. Calcium intake and body weight. J Clin Endocrinol Metab 2000;85:4635–8.
2. Jacqmain M, Doucet E, Despres JP, Bouchard C, Tremblay A. Calcium intake, body composition, and lipoprotein-lipid concentrations in adults. Am J Clin Nutr 2003;77:1448–52.
3. Lin YC, Lyle RM, McCabe LD, McCabe GP, Weaver CM, Teegarden D. Dairy calcium is related to changes in body composition during a two-year exercise intervention in young women. J Am Coll Nutr 2000;19:754–60.
4. Loos RJ, Rankinen T, Leon AS, et al. Calcium intake is associated with adiposity in black and white men and white women of the HERITAGE Family Study. J Nutr 2004;134:1772–8.
5. Lorenzen JK, Mølgaard C, Michaelsen KF, Astrup A. Calcium supplementation for 1 y does not reduce body weight or fat mass in young girls. Am J Clin Nutr 2006;83:18–23.
6. Zemel MB, Shi H, Greer B, Dirienzo D, Zemel PC. Regulation of adiposity by dietary calcium. FASEB J 2000;14:1132–8.
7. Kamycheva E, Joakimsen RM, Jorde R. Intakes of calcium and vitamin D predict body mass index in the population of Northern Norway. J Nutr 2003;133:102–6.
8. Phillips SM, Bandini LG, Cyr H, Colclough-Douglas S, Naumova E, Must A. Dairy food consumption and body weight and fatness studied longitudinally over the adolescent period. Int J Obes Relat Metab Disord 2003;27:1106–13.
9. Rajpathak SN, Rimm EB, Rosner B, Willett WC, Hu FB. Calcium and dairy intakes in relation to long-term weight gain in US men. Am J Clin Nutr 2006;83:559–66.
10. Haub MD, Simons TR, Cook CM, Remig VM, Al-Tamimi EK, Holcomb CA. Calcium-fortified beverage supplementation on body composition in postmenopausal women. Nutr J 2005;4:21.
11. Reid IR, Horne A, Mason B, Ames R, Bava U, Gamble GD. Effects of calcium supplementation on body weight and blood pressure in normal older women: a randomized controlled trial. J Clin Endocrinol Metab 2005;90:3824–9.
12. Shapses SA, Heshka S, Heymsfield SB. Effect of calcium supplementation on weight and fat loss in women. J Clin Endocrinol Metab 2004;89:632–7.
13. Thompson WG, Rostad HN, Janzow DJ, Slezak JM, Morris KL, Zemel MB. Effect of energy-reduced diets high in dairy products and fiber on weight loss in obese adults. Obes Res 2005;13:1344–53.
14. Zemel MB, Thompson W, Milstead A, Morris K, Campbell P. Calcium and dairy acceleration of weight and fat loss during energy restriction in obese adults. Obes Res 2004;12:582–90.
15. Zemel MB, Richards J, Mathis S, Milstead A, Gebhardt L, Silva E. Dairy augmentation of total and central fat loss in obese subjects. Int J Obes Relat Metab Disord 2005;29:391–7.
16. Zemel MB, Richards J, Milstead A, Campbell P. Effects of calcium and dairy on body composition and weight loss in African-American adults. Obes Res 2005;13:1218–25.
17. Papakonstantinou E, Flatt WP, Huth PJ, Harris RB. High dietary calcium reduces body fat content, digestibility of fat, and serum vitamin D in rats. Obes Res 2003;11:387–94.
18. Jacobsen R, Lorenzen JK, Toubro S, Krog-Mikkelsen I, Astrup A. Effect of short-term high dietary calcium intake on 24-h energy expenditure, fat oxidation, and fecal fat excretion. Int J Obes Relat Metab Disord 2005;29:292–301.
19. Denke MA, Fox MM, Schulte MC. Short-term dietary calcium fortification increases fecal saturated fat content and reduces serum lipids in men. J Nutr 1993;123:1047–53.
20. Shahkhalili Y, Murset C, Meirim I, et al. Calcium supplementation of chocolate: effect on cocoa butter digestibility and blood lipids in humans. Am J Clin Nutr 2001;73:246–52.

21. Welberg JW, Monkelbaan JF, de Vries EG, et al. Effects of supplemental dietary calcium on quantitative and qualitative fecal fat excretion in man. Ann Nutr Metab 1994;38:185–91.
22. Gacs G, Barltrop D. Significance of Ca-soap formation for calcium absorption in the rat. Gut 1977;18:64–8.
23. Govers MJ, Termont DS, Van Aken GA, van der MR. Characterization of the adsorption of conjugated and unconjugated bile acids to insoluble, amorphous calcium phosphate. J Lipid Res 1994;35:741–8.
24. Govers MJ, Van der MR. Effects of dietary calcium and phosphate on the intestinal interactions between calcium, phosphate, fatty acids, and bile acids. Gut 1993;34:365–70.
25. van der MR, Welberg JW, Kuipers F, et al. Effects of supplemental dietary calcium on the intestinal association of calcium, phosphate, and bile acids. Gastroenterology 1990;99:1653–9.
26. Boon N, Hul GB, Viguerie N, Sicard A, Langin D, Saris WH. Effects of 3 diets with various calcium contents on 24-h energy expenditure, fat oxidation, and adipose tissue message RNA expression of lipid metabolism-related proteins. Am J Clin Nutr 2005;82:1244–52.
27. Møller A, Saxholt E, Christensen AT, Hartkopp HB, Hess Ygil K. Danish Food Composition Databank, revision 6.0. Food Informatics, Institute of Food Safety and Nutrition, Danish Institute for Food and Veterinary Research. June 2005. Internet: http://www.foodcomp.dk/ (accessed 12 December 2006).
28. Klausen B, Toubro S, Astrup A. Age and sex effects on energy expenditure. Am J Clin Nutr 1997;65:895–907.
29. Tholstrup T, Sandstrom B, Bysted A, Holmer G. Effect of 6 dietary fatty acids on the postprandial lipid profile, plasma fatty acids, lipoprotein lipase, and cholesterol ester transfer activities in healthy young men. Am J Clin Nutr 2001;73:198–208.
30. Rehfeld JF. Accurate measurement of cholecystokinin in plasma. Clin Chem 1998;44:991–1001.
31. Ekman R, Wahlestedt C, Bottcher G, Sundler F, Hakanson R, anula P. Peptide YY-like immunoreactivity in the central nervous system of the rat. Regul Pept 1986;16:157–68.
32. Holst JJ, Bersani M. Assays for peptide products of somatostatin gene. In: Conn MP, ed. Methods in neurosciences. San Diego, CA: Academic Press, 1991:3–22.
33. Orskov C, Rabenhoj L, Wettergren A, Kofod H, Holst JJ. Tissue and plasma concentrations of amidated and glycine- extended glucagon-like peptide I in humans. Diabetes 1994;43:535–9.
34. Dubois C, Beaumier G, Juhel C, et al. Effects of graded amounts (0–50 g) of dietary fat on postprandial lipemia and lipoproteins in normolipidemic adults. Am J Clin Nutr 1998;67:31–8.
35. Behling AR, Kaup SM, Choquette LL, Greger JL. Lipid absorption and intestinal tumour incidence in rats fed on varying levels of calcium and butterfat. Br J Nutr 1990;64:505–13.
36. Xu C, Wensing T, Beynen AC. Effects of high calcium intake on fat digestion and bile acid excretion in feces of veal calves. J Dairy Sci 1998;81:2173–7.
37. Iso H, Stampfer MJ, Manson JE, et al. Prospective study of calcium, potassium, and magnesium intake and risk of stroke in women. Stroke 1999;30:1772–9.
38. Patton JS, Carey MC. Watching fat digestion. Science 1979;204:145–8.
39. Pereira MA, Jacobs DR Jr, Van Horn L, Slattery ML, Kartashov AI, Ludwig DS. Dairy consumption, obesity, and the insulin resistance syndrome in young adults: the CARDIA Study. JAMA 2002;287:2081–9.
40. Davidson MH, Hauptman J, DiGirolamo M, et al. Weight control and risk factor reduction in obese subjects treated for 2 years with orlistat: a randomized controlled trial. JAMA 1999;281:235–42.
41. Rossner S, Sjostrom L, Noack R, Meinders AE, Noseda G. Weight loss, weight maintenance, and improved cardiovascular risk factors after 2 years treatment with orlistat for obesity. European Orlistat Obesity Study Group. Obes Res 2000;8:49–61.
42. Suter PM, Marmier G, Veya-Linder C, et al. Effect of orlistat on postprandial lipemia, NMR lipoprotein subclass profiles and particle size. Atherosclerosis 2005;180:127–35.
43. Reid IR. Effects of calcium supplementation on circulating lipids: potential pharmacoeconomic implications. Drugs Aging 2004;21:7–17.
44. Vaskonen T. Dietary minerals and modification of cardiovascular risk factors. J Nutr Biochem 2003;14:492–506.
45. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Int J Obes Relat Metab Disord 2000;24:38–48.