Effect of dairy calcium from cheese and milk on fecal fat excretion, blood lipids, and appetite in young men

Karina V Soerensen, Tanja K Thorning, Arne Astrup, Mette Kristensen, and Janne K Lorenzen

ABSTRACT

Background: Calcium from different dairy sources might affect blood lipids and fecal fat excretion differently because of differences in the food matrix and nutritional composition.

Objective: We investigated whether milk- and cheese-based diets with similar calcium contents affect a saturated fatty acid–induced increase in blood lipids differently.

Design: Fifteen healthy, young men participated in a randomized 3 × 2-wk crossover study in which the following 3 isocaloric diets that were similar in fat contents and compositions were compared: control diet (nondairy diet (≈500 mg Ca/d)), milk diet (semiskimmed milk–based diet (1700 mg Ca/d)), and cheese diet (semihard cow-cheese–based diet (1700 mg Ca/d)). Blood was drawn before and after each period, and feces were collected for 5 d during each period.

Results: Saturated fatty acid–induced increases in total and low-density lipoprotein (LDL) cholesterol were lower with the milk diet (mean ± SD: 0.57 ± 0.13 and 0.53 ± 0.11 mmol/L, respectively) (P < 0.01) and cheese diet (0.41 ± 0.15 and 0.47 ± 0.12 mmol/L, respectively) (P < 0.05) than with the control diet (0.89 ± 0.12 and 0.84 ± 0.11 mmol/L, respectively). Fecal fat excretion increased more with the consumption of both the milk (5.2 ± 0.4 g/d) and cheese (5.7 ± 0.4 g/d) diets than with the control diet (3.9 ± 0.3 g/d) (P < 0.001). Changes in blood pressure, high-density lipoprotein cholesterol, triglycerides, and lipid ratios did not differ.

Conclusions: Compared with the control diet, milk- and cheese-based diets attenuated saturated fatty acid–induced increases in total and LDL cholesterol and resulted in increased fecal fat excretion; however, effects of milk and cheese did not differ. Because the diets contained similar amounts of saturated fat, similar increases in total and LDL cholesterol could be expected; however, both milk and cheese attenuated these responses, which seem to be explained by their calcium contents. This trial was registered at clinicaltrials.gov as NCT01317251.

INTRODUCTION

Cardiovascular disease (CVD) is the main cause of mortality and morbidity worldwide causing ~17 million deaths every year (1). Generally, a reduction or substitution of energy from saturated fat is perceived as an important dietary approach to prevent and reduce CVD, and consequently, a reduction in saturated fat rich foods has been recommended. However, recent evidence suggested that this picture may be more complex because SFAs interact with other nutrients depending on food matrixes, which exert different physiologic effects (2, 3). Dairy foods contain substantial amounts of SFAs and have, therefore, been linked to increased CVD risk. However, recent meta-analyses of mainly prospective cohort studies that investigated the association between the consumption of dairy foods and CVD risk and mortality failed to support this assumption (4, 5), which suggested that dairy products have characteristics that might counteract the negative effect of SFAs on CVD. A wide range of different dairy products exists, and the processing of, eg, milk into cheese, yogurt, and sour cream results in different nutritional compositions and structures of these products, which consequently may exert different physiologic effects. Calcium has been suggested as a nutrient of importance for the prevention of CVD, and several studies have shown that high calcium intake improves the blood lipid profile (6–12). However, the consistency and magnitude of effects observed have varied substantially, and not all studies were able to confirm these findings (13–16).

One proposed mechanism of action for the lipid-lowering properties of dairy or calcium is via increased fecal fat excretion (6, 17). The increase in fecal fat loss has been suggested to be caused by either the formation of insoluble calcium soaps or the formation of insoluble conjugates between calcium-phosphate complexes and bile acids (18). As a consequence, the lipid metabolism may be altered. However, results have not been consistent across studies, and thus, other factors may come into play as well. One factor could be that calcium from different dairy products behave differently depending on the food matrix. Different structural forms of calcium in different dairy products lead to differences in intestinal calcium-binding mechanisms and, in turn, differences in physiologic effects and magnitude. To our knowledge, no previous studies have investigated whether similar amounts of calcium coming from different dairy matrixes could explain conflicting results on blood lipids. However, 2 of our previous studies suggested that such differences might exist because dairy fat from cheese appeared less hypercholesterolemic than did an equal amount of dairy fat from butter (2, 19); however, calcium contents were not matched. Still, the studies suggested that different dairy products exert different blood lipid

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periods were separated by a washout period. Dietary intervention was as follows: a diet that was high in semiskimmed milk (1.5% fat), a diet that was high in semihard cow cheese, or a low-calcium control diet that contained butter but no other dairy products. Dietary intervention periods were separated by a washout period ≥14 d duration. During each dietary intervention period, feces was collected for 5 d (days 10–14), and urine was collected for 24 h (day 14) in preweighed plastic containers. Fasting body weight was measured to the nearest 0.5 kg with subjects wearing light clothing (Lindeltronic 8000 scale; Lindells, Sweden), and height was measured to the nearest 0.5 cm with a wall-mounted stadiometer (Seca). Two blood pressure measurements were performed in the supine position after 10 min rest by using an automatically inflated cuff (UA-787; A&D Co Ltd), and the mean was calculated. A fasting blood sample was drawn before and after each period. Subjects were asked to fast ≥12 h before these measurements were taken (maximum of 0.5 L H₂O allowed) and were instructed to abstain from hard physical activity and alcohol consumption for 24 h. The subjective appetite sensation in response to the 3 diets was investigated after standardized breakfast test meals on days 1, 8 and 14 during each period. Because of the nature of the diets, it was not possible to blind the study (subjects and staff). The study was carried out at the Department of Nutrition, Exercise and Sports, Faculty of Sciences, University of Copenhagen, Frederiksberg, Denmark, from April 2011 to March 2012 and was approved by the Municipal Ethical Committee of Copenhagen (H-1-2011-004) (registered at www.clinicaltrials.gov; NCT01317251).

**Subjects and Methods**

**Study design**

The study was designed as an open-label, crossover, intervention study in which subjects were randomly assigned to the sequence of 3 different 14-d dietary intervention periods by simple randomization by using web-based software (Randomization.com). Intervention diets were as follows: a diet that was high in semiskimmed milk (1.5% fat), a diet that was high in semihard cow cheese, or a low-calcium control diet that contained butter but no other dairy products. Dietary intervention periods were separated by a washout period ≥14 d duration. During each dietary intervention period, feces was collected for 5 d (days 10–14), and urine was collected for 24 h (day 14) in preweighed plastic containers. Fasting body weight was measured to the nearest 0.05 kg with subjects wearing light clothing (Lindeltronic 8000 scale; Lindells, Sweden), and height was measured to the nearest 0.5 cm with a wall-mounted stadiometer (Seca). Two blood pressure measurements were performed in the supine position after 10 min rest by using an automatically inflated cuff (UA-787; A&D Co Ltd), and the mean was calculated. A fasting blood sample was drawn before and after each period. Subjects were asked to fast ≥12 h before these measurements were taken (maximum of 0.5 L H₂O allowed) and were instructed to abstain from hard physical activity and alcohol consumption for 24 h. The subjective appetite sensation in response to the 3 diets was investigated after standardized breakfast test meals on days 1, 8 and 14 during each period. Because of the nature of the diets, it was not possible to blind the study (subjects and staff). The study was carried out at the Department of Nutrition, Exercise and Sports, Faculty of Sciences, University of Copenhagen, Frederiksberg, Denmark, from April 2011 to March 2012 and was approved by the Municipal Ethical Committee of Copenhagen (H-1-2011-004) (registered at www.clinicaltrials.gov; NCT01317251).

**Subjects**

Fifteen young, healthy men were recruited through advertising at university campuses in the Copenhagen area and Internet postings. To be included in the study, subjects had to be 18–50 y of age, have a BMI (in kg/m²) of 20–28, and be weight stable (weight change <3 kg ≤3 mo before the study). Exclusion criteria were as follows: any known chronic illnesses, use of supplements or medications that could affect study outcomes, smoking, excessive physical activity (>10 h/wk), allergies toward milk, lactose intolerance, participation in other scientific studies, or inability to follow the protocol. All subjects gave written consent after having received verbal and written information about the study. At enrollment, all subjects filled in a food-frequency questionnaire (20) to assess their habitual calcium intakes. Furthermore, their daily physical activity levels were estimated by use of a 3-d physical activity registration. Hereafter, the energy-requirement (ER) level was calculated as follows (21):  

\[
ER (MJ) = 0.064 \times \text{body weight (kg)} + 2.84 \times \text{PAL (J)}
\]

where PAL is the physical activity level. According to the estimated ER, subjects were allocated to an energy level of either 13 or 15 MJ. After the first dietary intervention period, 3 subjects shifted from 13- to 15-MJ diets because of increased feelings of hunger and a small weight loss.

**Experimental diets**

Estimated compositions of the 3 intervention diets were similar in terms of total energy and macronutrient and fatty acid composition with ~31.5 percentage of energy from fat, 52.9 percentage of energy from carbohydrates, and ~15.5 percentage of energy from protein (Table 1) but differed with respect to the amount and type of dairy foods consumed. The amount of milk and cheese per 10 MJ corresponded to an intake ~800 mg dairy calcium from either ~670 mL semiskimmed milk (1.5% fat) (Letmaelck; Arla Foods) or 120 g semihard cow cheese (45% fat/dry weight) (Klovborg; Arla Foods). The amount of calcium from nondairy sources was kept similar across the 3 diet periods. Mineral water was provided ad libitum, but consumption was registered by subjects, and calcium intake from this source was calculated (mineral water: 117 mg Ca/L). Subjects were allowed to drink nonsweetened coffee and tea and artificially sweetened beverages as long as these drinks were based on the water provided. Four daily menus were prepared to vary the diet, and when possible, one daily meal was served at the Department of Nutrition, Exercise and Sports, whereas remaining meals were provided for home consumption. On Fridays, subjects were provided all foods for consumption at home during the weekend. The diets provided consisted of breakfast, lunch, and evening meals as well as fruits and snacks. The diets were based on typical Danish food items and dishes and were most often delivered to

| TABLE 1 Nutrient composition of the 3 diets normalized per 10 MJ¹ |
|---------------------------------|-----------------|-----------------|
| Diet                           | Control         | Milk            |
| Energy (kJ)²                   | 10,007 (9266)   | 10,012 (10,603) |
| Energy density (kJ/g)          | 5.5             | 5.7             |
| Weight (g)                     | 1838            | 1742            |
| Fat (% of energy)²             | 31.7 (28.9)     | 31.6 (28.3)     |
| SFA (g)                        | 45.1            | 46.5            |
| MUFA (g)                       | 25.1            | 23              |
| PUFA (g)                       | 6.6             | 5.7             |
| Carbohydrate (% of energy)     | 52.9            | 52.9            |
| Protein (% of energy)          | 15.4            | 15.5            |
| Dietary fiber (g)              | 19.2            | 20.3            |
| Total calcium (mg)             | 362             | 1143            |
| Dairy calcium (mg)             | 0               | 781             |

¹ Nutrient contents were estimated by using Dankost 3000 dietary assessment software (Danish Catering Center).  
² Energy and fat contents were measured, and numbers based on measured fat and energy values are shown in parentheses.
subjects as preportioned and precooked meals. Subjects were instructed to carefully adhere to the diet, consume all foods provided, and not consume anything else as well as to report any deviations from the diet plan. A moderate seasoning of the foods was allowed. Independent of the experimental diet, breakfast meals used for appetite assessment were similar in energy density and macronutrient and fatty acid composition but were adjusted to each subject’s individual energy requirement. Breakfast meals consisted of wheat buns with cheese, meat, jam, and chocolate depending on the diet type (control, milk, and cheese) in combination with either orange juice or milk (see Supplemental Table 1 under “Supplemental data” in the online issue). The macronutrient and micronutrient compositions were calculated with Dankost 3000 dietary assessment software (Dankost 3000, version 2.5; Danish Catering Center).

Analytic procedures

Diet

Samples of the 3 diets were homogenized and freeze dried. The total fat content was determined by using the method of Weibull-Stoldtts with the use of ANKOM equipment (ANKOM Technology). The total energy content was determined by using bomb calorimetry (IKA-calorimeter system 4000; IKA). Because it was not possible to sufficiently homogenize butter, this was not included in any of the diet samples, but the content of fat and energy from butter was added to measured values.

Feces

Before analysis, fecal samples were freeze dried and homogenized. For each subject, all samples from the same diet period were pooled. Fecal energy excretion was determined by using bomb calorimetry (IKA-calorimeter system 4000). Samples were hydrolyzed with 3N HCl before the total fat content was measured by acidic Bligh and Dyer extraction (22).

The fecal calcium content was measured by using atomic absorption spectrophotometry with a PYE UNICAM SP9 atomic absorption spectrophotometer (Philips Electron Optics). Before analysis, samples were subjected to dry ashing at 525°C for 6 h, and the ashes were dissolved in acid (6.5% HNO₃). The solution was diluted with a lanthanum chloride solution before the calcium concentration was measured.

Urine

The urinary calcium content was analyzed with an ABX Pentra 400 analyzer (HORIBA ABX SAS) by photometric analysis by using an ABX Pentra Calcium CP ready-to-use kit (HORIBA ABX SAS).

Blood

Plasma glucose was measured by using an enzymatic endpoint method (hexokinase) (Gluco-quant Glucose/HK; Roche Diagnostics); the intraassay CV was 1.4%. Concentrations of triacylglycerols and total cholesterol were assessed by using colorimetric test kits (Roche TG; Roche Diagnostics GmbH); intraassay variations were 0.6% and 0.9%, respectively. HDL cholesterol was measured by using homogeneous enzymatic colorimetric test kits (Roche HDL cholesterol plus second generation; Roche Diagnostics GmbH); intraassay precisions were 1.8%. All analyses were performed by using an ABX Pentra 400 chemistry analyzer (ABX Pentra; Horiba ABX). LDL cholesterol was calculated by using Friedewald’s equation (23). Insulin was measured by using a solid-phase, 2-site chemiluminescent immunometric assay (Immulite/immuliter 1000 insulin; Diagnostic Products Corp) with an Immulite 1000 analyzer (Diagnostic Products Corp). Intraassay and interassay CVs were 2.5% and 4.9%, respectively. For insulin concentrations under the detection limit (14.4 pmol/L), one-half of the detection limit was applied (7.2 pmol/L).

Appetite registration

Subjective appetite was tested 3 times during each diet period on days 1, 8, and 14. Subjects met fasting, as described previously, at the Department to perform an appetite sensation test by using visual analog scales. Subjects consumed their breakfast test meals and were asked to fill in a small booklet with questions related to appetite by indicating their subjective feelings on a 100-mm horizontal line that showed extreme answers to questions at each end (24). This method was done at time point 0 (before the meal) and 60, 120, and 180 min postprandially. In this period of time, subjects were instructed to consume no other foods and only drink 0.5 L H₂O. After the consumption of the breakfast meal, subjects were allowed to leave the Department and completed appetite registrations, eg, at home, at work, or in class.

Statistical analysis and calculations

The sample size was based on 2 previous studies (25, 26) in which differences in fecal fat excretion between mixed dairy products and the control was 6 g/d and between milk and the control was 3 g/d. Thus, with an expected difference of 4 g/d and an SD of 5 g/d, a total of 14 subjects had to be included (α = 0.05, β = 1–0.80). With the inclusion of 16 subjects, a small dropout was allowed.

The AUC (total increase >0) from 0 to 180 min was calculated for all appetite variables by using the trapezoidal method. Fat and energy digestibility were calculated as follows:

\[
\text{Digestibility} = \frac{100 \times |\text{consumed} \text{ (analyzed)} - \text{excreted}|}{\text{consumed} \text{ (analyzed)}}
\]  

All statistical analyses were performed with the Statistical Analysis System software package (version 9.3; SAS Institute Inc). All dependent variables were controlled for the homogeneity of variance and normal distribution by investigation of residual plots and normal probability plots. Potential outlying observations were identified by using Cooks distances (Cook’s D ≥1). An ANOVA was performed by using the PROC MIXED procedure (SAS Institute Inc) to compare baseline values (fecal outcome variables excepted) between the 3 dietary intervention periods, in which the period and diet were modeled as fixed variables, and the subject was included as a random variable. An ANOVA was applied to investigate the effect of the diet on dependent variables (values obtained after the intervention), in which the subject was modeled as a random variable, the diet, period, and period × diet interaction were included as fixed variables, and the corresponding baseline value was included as a covariate when available. For the AUC for hunger, fullness,
satiety, prospective consumption, well-being, and thirst, an ANCOVA was applied on days 1, 8, and 15 separately, in which the subject was modeled as a random variable, and the diet, period, and period × diet were included as fixed variables. For all analyses, the period × diet interaction was only omitted from the model when \( P > 0.1 \). Unadjusted post hoc pairwise comparisons between diets were made when the effect of a diet was significant. Pearson’s correlations between fecal fat and changes in total and LDL cholesterol were made, and a multiple linear regression analysis was applied to identify explanatory variables of changes in total and LDL cholesterol. Included in the analysis were fecal fat excretion, fat intake, body weight, and the corresponding baseline concentration as well as the subject as a random variable. Results are presented as means ± SD, the statistical significance was defined as \( P < 0.05 \), and trends are reported for \( 0.05 \leq P < 0.10 \).

### RESULTS

All 15 subjects recruited for the study completed all 3 dietary intervention periods. Subjects were young, healthy, normal-weight men with relatively high habitual calcium intakes (Table 2). None of the outcomes assessed differed at baseline between diets (\( P > 0.10 \)), and no differences in body weight change were shown between the 3 intervention periods (Table 3). Also no

### TABLE 2

Subject baseline characteristics (n = 15)

<table>
<thead>
<tr>
<th>Values</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>27.7 ± 4.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.9 ± 6.2</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.83 ± 0.1</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>23.1 ± 2.3</td>
</tr>
<tr>
<td>Energy requirement (MJ/d)</td>
<td>14.2 ± 1.8</td>
</tr>
<tr>
<td>Habitual calcium intake (mg/d)</td>
<td>1,120 ± 223</td>
</tr>
</tbody>
</table>

\(^1\) All values are means ± SDs.

### TABLE 3

Body weight, BP, and blood lipid, insulin, and glucose concentrations before and after each dietary intervention period and fecal variables during each 2-wk dietary intervention period

<table>
<thead>
<tr>
<th>Body weight (kg)</th>
<th>Control</th>
<th>Milk</th>
<th>Cheese</th>
<th>P-diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>77.2 ± 6.1</td>
<td>76.9 ± 6.3</td>
<td>77.5 ± 6.6</td>
<td>NS</td>
</tr>
<tr>
<td>After</td>
<td>77.1 ± 6.1</td>
<td>77.2 ± 6.5</td>
<td>77.5 ± 6.5</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>124.0 ± 5.4</td>
<td>127.1 ± 6.7</td>
<td>122.3 ± 7.2</td>
<td>NS</td>
</tr>
<tr>
<td>After</td>
<td>123.6 ± 7.6</td>
<td>122.8 ± 9.2</td>
<td>121.6 ± 5.7</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>71.3 ± 5.1</td>
<td>72.2 ± 7.0</td>
<td>68.9 ± 6.9</td>
<td>NS</td>
</tr>
<tr>
<td>After</td>
<td>72.2 ± 7.3</td>
<td>70.8 ± 7.0</td>
<td>71.0 ± 6.2</td>
<td>NS</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0.78 ± 0.23</td>
<td>0.76 ± 0.31</td>
<td>0.85 ± 0.31</td>
<td>0.08</td>
</tr>
<tr>
<td>After</td>
<td>0.82 ± 0.23</td>
<td>0.91 ± 0.31</td>
<td>0.83 ± 0.27</td>
<td>0.013</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>4.23 ± 0.81</td>
<td>4.26 ± 0.93</td>
<td>4.43 ± 1.12</td>
<td>0.012</td>
</tr>
<tr>
<td>After</td>
<td>5.12 ± 0.93(^a)</td>
<td>4.83 ± 1.12(^b)</td>
<td>4.84 ± 1.08(^b)</td>
<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>2.57 ± 0.62</td>
<td>2.56 ± 0.74</td>
<td>2.71 ± 0.74</td>
<td>NS</td>
</tr>
<tr>
<td>After</td>
<td>3.42 ± 0.97(^a)</td>
<td>3.09 ± 0.97(^b)</td>
<td>3.18 ± 0.89(^b)</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>1.30 ± 0.27</td>
<td>1.35 ± 0.23</td>
<td>1.33 ± 0.19</td>
<td>0.02</td>
</tr>
<tr>
<td>After</td>
<td>1.34 ± 0.27</td>
<td>1.33 ± 0.19</td>
<td>1.28 ± 0.19</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin (pmol/L)(^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>27.5 ± 16.7</td>
<td>24.1 ± 17.7</td>
<td>38.0 ± 21.0</td>
<td>NS</td>
</tr>
<tr>
<td>After</td>
<td>32.5 ± 16.8(^a)</td>
<td>33.1 ± 23.0(^a)</td>
<td>23.0 ± 41.3(^b)</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>5.41 ± 0.24</td>
<td>5.35 ± 0.35</td>
<td>5.37 ± 0.26</td>
<td>NS</td>
</tr>
<tr>
<td>After</td>
<td>5.38 ± 0.24</td>
<td>5.33 ± 0.38</td>
<td>5.30 ± 0.33</td>
<td>NS</td>
</tr>
<tr>
<td>Fecal variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excretion of DM (g/d)</td>
<td>34.2 ± 1.8(^a)</td>
<td>44.6 ± 3.1(^b)</td>
<td>40.5 ± 3.0(^b)</td>
<td>0.002</td>
</tr>
<tr>
<td>Energy excretion (kJ/d)</td>
<td>712 ± 34(^a)</td>
<td>844 ± 53(^b)</td>
<td>808 ± 60(^b)</td>
<td>0.032</td>
</tr>
<tr>
<td>Percentage of energy excreted</td>
<td>5.3 ± 0.3</td>
<td>5.6 ± 0.3</td>
<td>5.4 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Fat excreted (g/d)</td>
<td>3.9 ± 0.3(^a)</td>
<td>5.2 ± 0.4(^b)</td>
<td>5.7 ± 0.4(^b)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Percentage of fat excreted</td>
<td>3.8 ± 0.3(^a)</td>
<td>4.5 ± 0.3(^b)</td>
<td>5.1 ± 0.3(^b)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

\(^1\) All values are means ± SDs. \( n = 15 \). P-diet refers to the overall effect of diet in an ANOVA model in which the value obtained after each period was modeled as the dependent variable, and diet were modeled as fixed variables, the corresponding baseline value was included as a covariate when available, and the subject was modeled as a random variable. \( P > 0.10 \) for the period × diet interaction for all dependent variables. Different superscript letters in the same row indicate significantly different values in post hoc pairwise comparisons that were based on an ANOVA model (\( P < 0.05 \)), BP, blood pressure; DM, dry matter.

\(^2\) \( n = 14 \) because 5 of 6 samples were under the detection limit for insulin for one subject.
period × diet interaction was observed for any of the outcomes. Also, subjects did not report any changes in physical activity levels, deviations from diets, or adverse events that could have been related to interventions during the study.

**Diet composition**

The chemical measurement of total energy and fat in the diet differed from table values (Table 1). The measured energy content was higher than that calculated for the milk and cheese diet but lower for the control diet; thus, for diets estimated to provide 10 MJ/d, the diets differed by up to ~1.5 MJ/d. Diet-composition tables often differ from values obtained from analyses of specific food, and the 3 diets differed slightly with respect to individual foods. The fat content was ~3% of energy lower than calculated for all 3 diets. Calcium intake from ad libitum water consumption did not differ between diets (data not shown).

**Study compliance**

Because of high habitual calcium intake, subjects were considered to be in calcium balance before the study. Thus, the excretion of calcium via urine and feces was expected to be high when calcium in the diet was high if subjects were compliant to the diets and collected all urine and feces as requested. The total urinary and fecal calcium excretion was measured, and to assess compliance with the protocol, the relative excretion (compared with calcium consumed) was calculated. Compared with the control diet (60.9 ± 11.8%), the relative calcium excretion (feces plus urine) tended to be higher during the milk (71.2 ± 13.1%; P = 0.059) and cheese (74.9 ± 18.2%; P = 0.020) diets (data not shown).

**Blood lipids and blood pressure**

All 3 diets increased total and LDL cholesterol compared with at baseline as expected because of the high content of SFA (Table 3, Figure 1). Compared with the control diet, the milk diet attenuated the increase in both total (0.57 ± 0.49 compared with 0.89 ± 0.48 mmol/L, respectively) and LDL cholesterol (0.53 ± 0.41 compared with 0.85 ± 0.43 mmol/L, respectively) (P < 0.05). Compared with the control diet, the cheese diet also attenuated both total cholesterol (0.41 ± 0.60 compared with 0.89 ± 0.48 mmol/L, respectively) and LDL cholesterol (0.47 ± 0.46 compared with 0.85 ± 0.43 mmol/L, respectively) (P < 0.01). The magnitude of effect for milk tended to be smaller than for cheese but did not differ (P > 0.1). Overall, there tended to be an effect of the diet on changes in triacylglycerol (P = 0.08), but pairwise comparisons did not reveal differences between diets. HDL cholesterol and blood pressure did not differ between the 3 diets (P > 0.1).

**Fecal fat excretion, energy excretion, and dry weight**

Fecal fat excretion was increased with cheese (5.7 ± 0.4 g/d) and milk (5.2 ± 0.4 g/d) diets in comparison with the control diet (3.9 ± 0.3 g/d) (P < 0.001) (Table 3), but no difference was shown between the milk and cheese diets (P > 0.10). Similarly, fecal energy excretion increased during both the cheese (808 ± 60 kJ/d) and milk (844 ± 53 kJ/d) diets in comparison with during the control diet (712 ± 34 kJ/d) (P < 0.05), but no difference was observed between cheese and milk diets (P > 0.10). Concurrently, increases in fecal dry matter were shown with both milk (44.6 ± 3 g/d) and cheese (40.5 ± 3 g/d) diets in comparison with the control diet (34.2 ± 1.8 g/d) (P < 0.01); again, no differences were seen between milk and cheese diets (P > 0.10).

**Correlation between changes in lipids and fecal fat excretion**

Fecal fat excretion correlated with the change in LDL concentration for all 3 diets (R² = 0.163, P = 0.002) (Figure 2) and the change in total cholesterol (R² = 0.137, P = 0.007). In a multiple linear regression analysis which included fecal fat excretion, fat intake, body weight, and baseline total or LDL cholesterol as explanatory variables and the subject as a random variable, only fat excretion explained the variation in total (P = 0.023) and LDL (P = 0.028) cholesterol.

**Insulin and glucose changes**

Insulin differed between diets (P = 0.02), whereby the decrease seen after cheese consumption differed significantly from small increases observed after the control (P = 0.02) and milk (P = 0.006) periods (Table 3), whereas no effect of diets on fasting glucose concentrations were observed (P > 0.10).

**Appetite sensation and palatability**

Subjects rated the 3 different breakfast meals equally palatable (data not shown). To investigate the acute effect of the diet on

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**FIGURE 1.** Mean ± SD changes in total and LDL cholesterol after 2-wk consumption of control, milk, and cheese diets. *Total and LDL cholesterol after both milk and cheese diets differed from after the control diet in an ANOVA adjusted for the corresponding baseline variable, diet, and period (P < 0.05) (n = 15).

**FIGURE 2.** Correlations (95% CIs) between changes in LDL-C and fecal fat excretion during the control (black), milk (open), and cheese (gray) periods (R² = 0.163, P = 0.002) (n = 15). LDL-C, LDL cholesterol.
DISCUSSION

The association between milk and dairy consumption and risk of CVD has recently been addressed in 2 meta-analyses, both of which showed a neutral or even a small beneficial effect of consuming milk and dairy foods despite their high SFA contents (4, 5). It has been hypothesized that the high content of calcium in dairy products may be responsible for this protective effect. In the current study, we showed that both milk- and cheese-rich diets attenuated an increase in total and LDL cholesterol and resulted in increased fecal fat excretion compared with consumption of a fat-matched control diet, which was in accordance to the results of previous studies (17, 26). However, although results from previous studies have indicated that magnitude of effect may depend on the dairy-product type, differences between milk- and cheese-based diets were not shown in the current study (17, 25, 27).

The following 2 possible mechanisms have been put forward on the effects of dairy foods on lipid metabolism: 1) the formation of calcium fatty acid soaps and 2) the formation of amorphous calcium phosphates, both of which take place in the duodenum (18). It can be speculated that both of these mechanisms may be affected differently depending on the food matrix. In milk, fat is present as small globules enclosed by a membrane (28), whereas in cheese, fat is encapsulated by a protein structure including casein (the fat structure depends somewhat on the cheese type). In cheese, part of the calcium is bound to casein and may, therefore, facilitate a higher degree of interaction between fat and calcium when cheese is consumed compared with when milk is consumed (19). Moreover, if calcium and fat do not enter the duodenum at the same time, the interaction between calcium and fat may be limited. In the case of a milk-rich diet, calcium is contained in the liquid phase, characterized by a faster gastric emptying rate than in solid phases (29, 30). Thus the liquid phase containing the calcium may enter the duodenum before the majority of the fat from the solid phase of the meal. This and potentially other matrix differences may, in turn, affect the formation of calcium–fatty acid soaps and amorphous calcium phosphates in the intestine differently and, in turn, also fat and bile acid metabolism. However, we were not able to confirm a matrix-dependent difference in the current study although both changes in total and LDL cholesterol and fat excretion pointed in this direction.

In the current study, changes in fecal fat excretion correlated with changes in total and LDL cholesterol despite the fact that the increase in fecal fat excretion was lower than usually seen with dairy-rich diets according to a recent meta-analysis (17). The link between fecal fat excretion and changes in blood lipids is based on the assumption that fecal fat excretion reflects the effect calcium has on bile acid reabsorption, which leads to the de novo synthesis of bile acids with plasma cholesterol as a substrate. Thus, our results suggested only modest effects on bile acid metabolism. Nonetheless, the results are in line with those of 2 previous studies in which improved blood lipid concentrations on high calcium intakes were shown without very large increases in fecal fat excretion (9, 31), which suggested that calcium may affect blood lipids via other mechanisms that are not linked to bile acid metabolism. In contrast, a study by Ditscheid et al (10) showed a decrease in total cholesterol with high calcium phosphate supplementation without differences in fecal fat excretion. Still, Ditscheid et al (10) detected increased fecal bile acid excretion and proposed that this was caused by the formation of amorphous calcium phosphate; thus, bile acid metabolism may be affected without major changes in fecal fat–excretion patterns. This effect may also have been the case in our study; however, we did not measure fecal bile acid excretion, and fecal fat excretion remained the most important determinant of changes in blood lipids.

The difference in fecal fat excretion between high and low dairy calcium diets in the current study was small (1.3 ± 0.4 and 1.8 ± 0.4 g/d for milk and cheese, respectively, compared with the fat control), which could be speculated to have resulted from the relatively low dose of calcium in the current study (117 mg Ca/MJ) compared with in other studies (25, 27). In a meta-analysis, the mean difference in fecal fat excretion between high and low dairy calcium diets was 5.2 g/d (95% CI: 1.6, 8.8 g/d) (17), and although total dairy calcium intakes varied across studies, they showed no clear dose-response relation, and thus, this variance does not appear to explain the small differences observed in the current study. An incomplete fecal collection would result in an underestimation of fecal fat excretion and, thus, explain these small differences. The percentage of relative calcium excretion (in urine and feces) was lower than expected (≤80%) with milk and cheese diets on the basis of previous studies, which suggested that fecal collections may have been incomplete. However, fecal wet- and dry-matter excretions in the current study were similar to what we have seen before (25, 27), and there seemed to be no other plausible explanation for this relatively low relative calcium excretion compared with what we have previously seen. Thus, we do not believe this has affected the results because milk and cheese affected calcium excretion to the same extent.

In contrast to previous intervention studies (2, 19), we showed that insulin decreased in response to a cheese-based diet compared with control and milk-based diets. However, Struijk et al (32) recently showed cheese intake to be inversely associated with 2-h fasting plasma glucose concentrations in ~6000 Danish adults after 5 y follow-up, although the incidence of type 2 diabetes was not affected. The lower glycemic index of cheese than milk has been proposed as responsible for the differential effects on glucose metabolism of different dairy foods. Cheese also contains fermentation products such as lactic acids and short-chain fatty acids, which may affect hepatic insulin metabolism; however, additional studies are needed to elucidate and confirm our findings.

We also investigated whether calcium could have an effect on appetite because this has been suggested as a possible explanation for the inverse associations between calcium consumption and body weight gain (33). The mechanisms by which dietary intake of calcium may regulate appetite are unknown, but calcium has
been proposed to be an indicator of food availability, which also is
correlated with the finding that an appetite-suppressing effect of
milk was predominantly seen in individuals with low habitual
calcium intake (34). Also, calcium may participate in eliciting
hormonal responses that affect physiologic functions involved in
the regulation of food intake. We showed that prospective food
consumption on day 8 was increased with the milk compared
cheese diets, which indicated that subjects who consumed the milk
diet had a greater desire to eat than did subjects who consumed the
cheese diet; however, no other differences were observed. This
observation is in agreement with the finding that macronutrients in
drinks exhibit weaker effects on appetite than in solid foods.

The evidence concerning the effects of calcium on appetite is
scarcely based on studies that varied substantially in designs
(8, 34-36). In a 6-mo weight-loss study, Gilbert et al (34) showed
that 1000 mg Ca/d from milk resulted in a smaller increase in the
desire to eat and hunger assessed at baseline and after 1 and 6 mo
with the milk supplement than with placebo, and similar results
were recently obtained by Jones et al (36) during a 12-wk trial in
which an increase in peptide YY was also seen in the supple-
mented group. In another weight-loss study, Major et al (8)
showed that supplementation with calcium carbonate (1200 mg/
d) and vitamin D supplementation resulted in suppressed appetite
in habitual low-calcium consumers (<600 mg/d) assessed at
standardized breakfast meals before and after the intervention.
In contrast, we previously showed no effect of the calcium con-
tent or source in an acute study in which the effect on appetite
and gut peptides was assessed after standardized breakfast meals
of low (15 mg/MJ), medium (84 mg/MJ), and high (172 mg/MJ)
dairy calcium contents and calcium carbonate (183 mg/MJ) (36).
Thus, little evidence exists to substantiate an effect of dairy or
calcium on appetite regulation. However, note that the effect of
calcium on appetite regulation and body weight in most studies
has been difficult to distinguish from effects of other dairy com-
ponents such as protein.

A major strength of the current study was its highly controlled
dietary intervention, whereby total fat, SFA, and calcium were
matched, which made it possible to conclude differences between
dairy foods. However, calculated dietary values differed sub-
stantially from measured values, and thus, it can be speculated
that, if the diets contained similar amounts of energy (and fat), it
would have been likely that the effect size of the attenuation of
total and LDL cholesterol as well as the difference in fecal fat
excretion would have been greater than what was observed. Fur-
thermore, the study was carried out as a crossover design, which
increased the power substantially by eliminating interindividual
variations. However, it can be speculated that a population with
a more atherogenic lipid profile would be more susceptible to SFA-
induced increases in blood lipids and, therefore, have resulted in
more-pronounced differences between diets. Finally, the calcium
content of the control diet was low compared with in the 2 dairy-
based diets, and thus, we could not conclude whether dairy com-
pared with nondairy calcium exert different effects when given with
similar amounts and types of fat.

In conclusion, compared with the control diet, both milk- and
cheese-based diets attenuated SFA-induced increases in total and
LDL cholesterol and resulted in increased fecal fat excretion;
however, effects of milk and cheese did not differ. Fecal fat
excretion was correlated to changes in LDL cholesterol and
explained a significant proportion of the changes in total and LDL
cholesterol in a multiple linear regression. These results do not
support that the effect on blood lipids differ substantially between
milk and cheese consumption with similar calcium contents but
do support previous findings that calcium-rich dairy foods may be
less atherogenic than their calcium-poor counterparts.

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The authors’ responsibilities were as follows—AA, JKL, and MK: de-
signed the study; KVS and TKT: carried out experimental work; KVS and MK:
analyzed data and prepared the first draft of the manuscript; and all authors:
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