Diets with high-fat cheese, high-fat meat, or carbohydrate on cardiovascular risk markers in overweight postmenopausal women: a randomized crossover trial[1,2]

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ABSTRACT

Background: Heart associations recommend limited intake of saturated fat. However, effects of saturated fat on low-density lipoprotein (LDL)-cholesterol concentrations and cardiovascular disease risk might depend on nutrients and specific saturated fatty acids (SFAs) in food.

Objective: We explored the effects of cheese and meat as sources of SFAs or isocaloric replacement with carbohydrates on blood lipids, lipoproteins, and fecal excretion of fat and bile acids.

Design: The study was a randomized, crossover, open-label intervention in 14 overweight postmenopausal women. Three full-diet periods of 2-wk duration were provided separated by 2-wk washout periods. The isocaloric diets were as follows: *1*) a high-cheese (96–120-g) intervention [i.e., intervention containing cheese (CHEESE)], *2*) a macronutrient-matched nondairy, high-meat control [i.e., nondairy control with a high content of high-fat processed and unprocessed meat in amounts matching the saturated fat content from cheese in the intervention containing cheese (MEAT)], and *3*) a nondairy, low-fat, high-carbohydrate control (i.e., nondairy low-fat control in which the energy from cheese fat and protein was isocalorically replaced by carbohydrates and lean meat (CARB).

Results: The CHEESE diet caused a 5% higher high-density lipoprotein (HDL)-cholesterol concentration ($P = 0.012$), an 8% higher apo A-I concentration ($P < 0.001$), and a 5% lower apoB:apo A-I ratio ($P = 0.008$) than with the CARB diet. Also, the MEAT diet caused an 8% higher HDL-cholesterol concentration ($P < 0.001$) and a 4% higher apo A-I concentration ($P = 0.033$) than with the CARB diet. Total cholesterol, LDL cholesterol, apoB, and triacylglycerol were similar with the 3 diets. Fecal fat excretion was 1.8 and 0.9 g higher with the CHEESE diet than with CARB and MEAT diets ($P < 0.001$ and $P = 0.004$, respectively) and 0.9 g higher with the MEAT diet than with the CARB diet ($P = 0.005$). CHEESE and MEAT diets caused higher fecal bile acid excretion than did the CARB diet ($P < 0.05$ and $P = 0.006$, respectively). The dominant type of bile acids excreted differed between CHEESE and MEAT diets.

Conclusions: Diets with cheese and meat as primary sources of SFAs cause higher HDL cholesterol and apo A-I and, therefore, appear to be less atherogenic than is a low-fat, high-carbohydrate diet. Also, our findings confirm that cheese increases fecal fat excretion. This trial was registered at clinicaltrials.gov as NCT01739153. *Am J Clin Nutr* doi: 10.3945/ajcn.115.109116.

INTRODUCTION

Cardiovascular disease (CVD)[4] is still one of the main causes of death in developed countries (1). Health authorities in several countries recommend a limited intake of regular-fat cheese, and the American and Danish Heart Associations recommend the replacement of high-fat dairy products with low-fat alternatives (2, 3). However, observational studies showed a modest inverse association between intake of dairy products and CVD risk (4, 5). In addition, observational data on specific dairy products implied that it is questionable whether cheese consumption is detrimental to heart health, and some studies even suggested a cardioprotective effect (6–8). A distinct effect of cheese compared to other dairy products may have multiple explanations. Cheese has a high content of calcium, which has been associated with reduced fat digestibility as measured by increased fecal fat excretion in humans (9, 10). We previously showed that a high-fat, dairy-calcium rich diet significantly decreased the LDL-cholesterol concentration as well as the ratio of LDL cholesterol to HDL cholesterol in humans compared with the effects of a low-calcium macronutrient-matched diet (11). The effect on

blood lipids may not solely be a result of increased fecal fat excretion (12), but other mechanisms responsible for this effect are still unclear. An increased bile acid excretion (13) leading to hepatic de novo synthesis of bile acids and increased liver LDL-receptor expression and clearance of LDL particles from the systemic circulation could be potential mechanisms.

Most human-intervention studies on cheese used butter as a control to ensure a similar fat content and composition (12, 14, 15). However, during free-living conditions, a person who reduces intake of cheese in an attempt to improve her or his own health is unlikely to compensate by increasing the intake of butter. More likely, the person would replace the cheese with other nondairy foods (e.g., charcuterie and cold cuts on bread) or limit the amount of cheese while increasing the amount of bread. Similar to dairy products, meat is a major contributor to saturated fat intake (16). A recent study observed that saturated fat from dairy products was inversely associated with CVD, whereas saturated fat from meat was positively associated with CVD (17). It was suggested that the association between saturated fat and risk of CVD could depend on specific SFAs or other concomitant nutrients in foods containing saturated fat.

Therefore, the aim of the current study was to explore the effect of cheese and meat as sources of SFAs on blood lipids, apolipoproteins, and the fecal excretion of fat and bile acids. An isocaloric replacement with carbohydrates was also explored. We chose to study postmenopausal overweight women because these women have increased CVD risk but have been less investigated within cardiovascular research than have men.

METHODS

Subjects

A total of 19 postmenopausal women were recruited through announcements in local newspapers and queries to potentially eligible persons who signed up on a list of volunteers for diet-studies at the Department of Nutrition, Exercise and Sports, University of Copenhagen. Inclusion criteria were as follows: female sex, postmenopausal >1 y, 45–68 y of age, BMI (in kg/m^2) from 25 to 32, total cholesterol concentration >4.5 and <7 mmol/L, and systolic blood pressure <160 mm Hg or diastolic blood pressure <100 mm Hg. Reasons for exclusions were as follows: chronic diseases, milk or nut allergy, use of dietary supplements <2 mo before and during the entire study period, smoking, >10 h vigorous physical activity per week, use of prescription medicine that could have affected the results of the study, hormone replacement therapy, blood donation <1 mo before study commencement and during the entire study period, simultaneous participation in other clinical studies, and an inability to comply with procedures required by the study protocol. All subjects gave their written consent after having received verbal and written information about the study. Before study commencement, subjects filled in a food-frequency questionnaire that was used to assess their habitual calcium intakes (18). Energy requirements of subjects were estimated on the basis of basal metabolic rate (height, weight, and age) and included the physical activity level estimated by using a physical activity scale (19). On the basis of individual energy requirements, subjects were allocated to a weight-maintenance diet.

Design

The study was a randomized, crossover, open-label intervention with 3 full-diet periods of 2-wk duration separated by washout periods ≥2 wk. A 2-wk intervention period was chosen because a new steady state for blood lipids was shown to be reached after ∼2 wk on a new diet regimen (20–22). Subjects were randomly assigned to the sequence of diets by using website random-sequence software (http://www.randomization.com).

Conditions before and on sampling days were standardized. In addition to fasting for a minimum of 12 h (0.5 L H_2O allowed), subjects were asked to maintain their habitual diets and to refrain from physical exercise and avoid alcohol intake 24 h before blood sampling. All blood samples were drawn by a trained laboratory technician. In each of the 3 intervention periods, pre-intervention and postintervention fasting blood samples were drawn. A nonfasting blood sample was also drawn on the last day of each intervention period 3 h after ingestion of a standardized breakfast meal to investigate postprandial triacylglycerol concentrations. In addition to blood samples, blood pressure and anthropometric measures (waist and hip circumferences, weight, and height) were measured pre-intervention and postintervention. Furthermore, subjects collected all feces during the last 5 d of each intervention period. Moreover, during each intervention period, subjects filled in diaries of water intake, defecation frequency, and physical activity. The study was carried out at the Department of Nutrition, Exercise and Sports, Faculty of Sciences, University of Copenhagen, Frederiksberg, Denmark, from January 2013 to August 2013 and was approved by the Ethical Committee of Copenhagen (H-4-2012-156) and registered at clinicaltrials.gov as NCT01739153.

Diets

Three experimental isocaloric, weight-maintenance diets were designed and planned with Dankost3000 dietary assessment software (Danish Catering Center). Two diets had a high content of saturated fat in the food matrix of either cheese or meat [i.e., an intervention containing cheese (CHEESE) and a nondairy control with a high content of high-fat processed and unprocessed meat in amounts matching the saturated fat content from cheese in the intervention containing cheese (MEAT).

The third diet had a high-carbohydrate content [i.e., a nondairy low-fat control in which the energy from cheese fat and protein was isocalorically replaced by carbohydrates and lean meat (CARB)]. All 3 diets had similar protein contents. The CHEESE diet contained cheese [one-half Danbo (27% fat weight/weight) and one-half cheddar (33% fat weight/weight)] in daily amounts from 96 g for an 8-MJ diet to 120 g for a 10-MJ diet. Danbo cheese was chosen because it is the most commonly eaten cheese in Denmark, whereas cheddar was chosen on the basis of palatability and high sale counts worldwide and, thus, of international interest No dairy products except for cheese were included in this diet. The daily amount of high-fat meat used to replace cheese in the MEAT diet was ∼164 g for a 10-MJ diet. The main carbohydrate-rich foods used to replace cheese in the CARB diet were fruit (84 g), white bread, pasta and rice (58 g), marmalade (20g), ands cake, sweetened biscuits, and chocolate (13 g) (10-MJ diet). The CHEESE and MEAT diets were designed to match the macronutrient composition of the average Danish diet (15% of energy from protein, 35% of energy from

fat, 15% of energy from saturated fat, and 50% of energy from carbohydrates) (16), whereas the CARB diet had a lower fat content (∼25% of energy) and a higher carbohydrate content (∼60% of energy) but the same protein content (15% of energy) and protein bioavailability as in the MEAT and CHEESE diets. The fat composition of the MEAT diet was equalized to that of the CHEESE diet. Hence, focus was on the 2 primary sources of saturated fat within their food matrices and not on providing a direct comparison between meat and cheese as whole foods. The contribution of different foods to the total content of SFAs, MUFAs, and PUFAs in the CHEESE, MEAT, and CARB diets are shown in **Table 1**. Cheese and meat contributed almost similarly to the total content of SFAs of the CHEESE diet and MEAT diet, respectively. Moreover, cheese provided 38% of total fat and lean meat provided 11% of total fat (used to match the protein content) in the CHEESE diet, whereas high-fat meat provided 52% of total fat in the MEAT diet. Despite the lower fat content of the CARB diet, it was planned to have the same distribution of SFAs, MUFAs, and PUFAs as in the CHEESE diet. Also, all diets had a comparable content of dietary fiber. Nutrient compositions of the 3 diets are shown in **Table 2**, and fatty acid compositions are shown in **Table 3**.

The standardized breakfast meal given after the 2-wk diet periods provided 2.5 MJ and included similar foods and macronutrient compositions as in the just-completed diet period (**Supplemental Table 1**).

All foods were provided to the subjects for the entire 3 intervention periods. Subjects picked up their foods at the Department to be consumed at home. Full diets were composed of breakfast, lunch, dinner, and snacks. Foods on the 3 experimental diet days that were repeated every third day are shown in **Supplemental Table 2**.

Analytic procedures

Diets

The dietary energy content was measured in duplicates by using indirect calorimetry with a bomb calorimeter (Ika-calorimeter system C4000; IKA). Total lipids were extracted from the homogenized meals as described previously (23). Lipids were *trans* methylated and analyzed by using gas chromatography-flame ionization detector (24).

Blood

Serum LDL cholesterol and HDL cholesterol were assessed by using an enzymatic colorimetric procedure (ABX Pentra LDL Direct CP and ABX Pentra HDL Direct 100 CP, respectively; Horiba). Total cholesterol and triacylglycerol concentrations were assessed in serum by using enzymatic procedures (cholesterol oxidase-phenol + aminophenazone and glycerol-3-phosphate oxidase-phenol + aminophenazone, respectively). Serum apolipoprotein (apo)A-I and apoB concentrations were measured by using an immunoturbidometric assay (ABX Pentra apo A-I and ABX Pentra apoB, respectively; Horiba). All analyses were carried out with an ABX Pentra 400 Analyzer (Horiba). HOMA-IR was calculated as follows (25):

$$HOMAIR = [fasting\ serum\ insulin\,(\mu IU/mL) \times \\ fasting\ plasma\ glucose\,(mmol/L)] \div 22.5 \quad (1)$$

Feces

All collected feces were weighed and frozen at −20°C, freeze dried and homogenized, and combined for each subject from each period. Feces samples were acid hydrolyzed with 3 M HCl at 80°C for 1 h, after which the fat content was measured by using the method of Bligh and Dyer (26) with modification (27). A stepwise application of chloroform, methanol, and water was used to extract lipids into the chloroform phase, and after evaporation, the fat content was determined gravimetrically. The fecal energy content was measured in duplicates by using indirect calorimetry with a bomb calorimeter (Ika-calorimeter system C4000). Bile acids were determined by using HPLC as described by Dekker et al. (28).

TABLE 1
Contribution of different foods in percentage of the total content of SFAs, MUFAs, and PUFAs in three 2-wk intervention diets[1]

	SFAs			MUFAs			PUFAs		
	CHEESE diet	MEAT diet	CARB diet	CHEESE diet	MEAT diet	CARB diet	CHEESE diet	MEAT diet	CARB diet
Cheese	53	—	—	30	—	—	7	—	—
Chocolate	14	17	24	11	12	19	3	3	4
Meat	11	48	28	15	71	41	5	24	11
Coconut milk	7	11	20	0	1	1	0	1	1
Coconut fat	—	11	6	—	1	1	—	1	0
Sweetened biscuits	6	6	10	6	6	11	3	4	5
Almonds, peanuts	2	—	0	12	—	3	10	—	2
Canola oil	1	—	1	20	—	10	27	—	13
Sunflower oil	—	1	—	—	3	—	—	22	—
Bread, pasta, rice	4	5	7	5	6	12	36	38	49
Other[2]	2	1	4	1	0	2	8	7	15
Sum	100	100	100	100	100	100	100	100	100

[1]CARB, nondairy low-fat control in which the energy from cheese fat and protein was isocalorically replaced by carbohydrates and lean meat; CHEESE, intervention containing cheese; MEAT, a nondairy control with a high content of high-fat processed and unprocessed meat in amounts matching the saturated fat content from cheese in the intervention containing cheese.

[2]Fruit, dried fruit, vegetables, eggs, corn-flour starch, and saccharose.

TABLE 2
Nutrient composition of three 2-wk intervention diets[1]

	CHEESE diet	CARB diet	MEAT diet
Energy,[2] kJ	10,008 (11,070)	10,002 (10,469)	10,004 (10,516)
Protein, % of energy	15.3	15.0	15.1
Carbohydrate, % of energy	48.7	61.9	48.5
Sugar, % of energy	7.5	8.9	6.9
Dietary fiber,[3] g/d	35.8	37.0	36.6
Fat, % of energy	36.0	23.0	36.4
SFAs, % of fat	49.9 (52.1)	49.8 (50.8)	49.8 (51.6)
MUFAs, % of fat	35.0 (36.6)	34.2 (35.2)	35.8 (35.9)
PUFAs, % of fat	15.1 (11.3)	16.0 (14.1)	14.4 (12.5)
Calcium,[3] mg/d	1278	434	401
Phosphorus,[3] mg/d	1679	1278	1375
Sodium,[3] mg/d	2997	2887	2814
Cholesterol,[3] mg/d	199	142	185

[1]Nutrient contents were estimated with Dankost 3000 dietary assessment software (Danish Catering Center). CARB, nondairy low-fat control in which the energy from cheese fat and protein was isocalorically replaced by carbohydrates and lean meat; CHEESE, intervention containing cheese; MEAT, a nondairy control with a high content of high-fat processed and unprocessed meat in amounts matching the saturated fat content from cheese in the intervention containing cheese.

[2]Content assessed by using a duplicate analysis is shown in parentheses.

[3]Content per 100-MJ diet.

Anthropometric measures

Height without shoes was measured to the nearest 0.5 cm by using a wall-mounted stadiometer (Seca). Fasting body weight to the nearest 0.1 kg was measured pre-intervention and post-intervention by using a Lindeltronic 800 scale (Lindells) with subjects wearing underwear and having emptied their bladder in advance. Waist and hip circumferences were measured by using a flexible nonelastic tape during slow exhalation and in a standing upright position with a 25–30-cm distance between the feet of subjects. Waist circumference was measured horizontally at the midpoint between the lower rim of the ribs and top of the iliac crest. The hip circumference was measured at the level of the trochanter major and pelvic symphysis. An average of 2 consecutive measurements rounded to the nearest 0.5 cm was used.

Blood pressure

Fasting blood pressure was measured by using an automatic sphygmomanometer pre-intervention and postintervention. Fasting blood pressure was measured 2 cm above the elbow bend on the right arm after 15 min of rest in a reclined position and with an empty bladder. A cuff with an appropriate size was placed around the naked arm, and the average of 3 consecutive measurements was used. With intention to minimize the white-coat effect, the project worker remained in the room in the resting period. There was no conversation during blood pressure measurements.

Statistical analyses and data presentation

All statistical analyses were performed with SAS 9.4 software (SAS Institute Inc.). The power calculation was based on a previous study from our research group with a similar experimental design (10). In that study, a difference ± SD of 0.38 ± 0.46 mmol/L in LDL-cholesterol change (pre-intervention

to postintervention) was observed between diets rich in butter or cheese providing similar amounts of saturated fat. A total of 14 completers would have ensured 80% power to detect a similar difference at a $P < 0.05$ significance level.

An ANCOVA was applied by using the PROC MIXED procedure (SAS Institute Inc.) to investigate the effect of the diet on dependent variables (postintervention values) in which the subject number was modeled as a random variable, and the diet, period, and period $\times$ diet interaction were included as fixed variables. The pre-intervention concentration was included as a covariate in the analysis of postintervention blood analyses, and in postprandial blood analyses, the fasting postintervention concentration was included as a covariate. In addition, the influence of weight change was tested for in the statistical analyses. If the global F test was significant, pairwise comparisons between diets were subsequently performed by using post hoc t tests. All dependent variables were controlled for the homogeneity of variance and normal distribution by using plots of residuals and normal probability. Potential outlying observations were identified by using Cook's distances. No carryover effect

TABLE 3
Fatty acid as a percentage of total fatty acids in three 2-wk intervention diets[1]

	CHEESE diet	CARB diet	MEAT diet
6:0	1.30	0.13	0.13
8:0	1.03	1.49	1.40
10:0	1.61	1.04	1.01
12:0	3.18	6.99	6.61
14:0	5.39	3.33	3.83
14:1	0.45	0.15	0.26
15:0	0.49	0.04	0.18
16:0	25.20	23.34	23.74
16:1	1.15	1.03	1.75
17:0	0.40	0.26	0.43
18:0	12.64	13.42	13.80
cis 18:1n–9	33.43	32.45	32.10
18:1n–7	1.20	1.21	1.48
cis 18:2n–6	9.27	12.21	11.04
18:3n–6	0.00	0.00	0.00
20:0	0.47	0.47	0.33
18:3n–3	1.64	1.52	0.85
20:1n–9	0.33	0.30	0.32
20:2n–6	0.05	0.05	0.11
20:3n–6	0.06	0.04	0.06
22:0	0.24	0.14	0.12
20:3n–3	0.01	0.00	0.02
20:4n–6	0.15	0.18	0.25
22:1n–9	0.04	0.02	0.00
22:2n–6	0.00	0.00	0.01
20:5n–3	0.03	0.02	0.01
24:0	0.13	0.10	0.07
22:4n–6	0.01	0.02	0.05
24:1	0.03	0.02	0.00
22:5n–3	0.05	0.05	0.07
22:6n–3	0.01	0.01	0.00

[1]Measured by using a duplicate analysis. CARB, nondairy low-fat control in which the energy from cheese fat and protein was isocalorically replaced by carbohydrates and lean meat; CHEESE, intervention containing cheese; MEAT, a nondairy control with a high content of high-fat processed and unprocessed meat in amounts matching the saturated fat content from cheese in the intervention containing cheese.

was observed for any variable. All values in results, tables, and graphs are expressed as means ± SEMs. One subject was excluded from all feces analyses because of a very low feces dry weight that indicated constipation or incomplete collection of feces. The remaining analyses included all completing subjects.

RESULTS

Subjects

Of 19 subjects enrolled in the study, 14 individuals completed all 3 diet periods. Two subjects dropped out before study commencement, 2 subjects dropped out because they got influenza, and one subject dropped out because of a hip injury not related to the study. At baseline, the age of the 14 completing subjects was 59.0 ± 7.4 y, body weight was 79.6 ± 8.7 kg, BMI was 28.8 ± 1.9, systolic blood pressure was135.4 ± 11.7 mm Hg, and diastolic blood pressure was 87.6 ± 6.5 mm Hg. Baseline total cholesterol, HDL-cholesterol, and LDL-cholesterol concentrations were 6.1 ± 0.8, 1.6 ± 0.3, and 3.7 ± 0.6 mmol/L, respectively. Habitual calcium intakes of subjects were 963 ± 369 mg/d.

Blood lipids and apolipoproteins

Pre- and postintervention fasting concentrations for total cholesterol, LDL cholesterol, HDL cholesterol, triacylglycerol, apoB, and apo A-I and the ratios for total cholesterol:HDL cholesterol, LDL cholesterol:HDL cholesterol, and apoB:apo A-I as well as 3-h postprandial concentrations are shown in **Table 4**.

The CHEESE diet caused a 5% higher HDL-cholesterol concentration (0.07 ± 0.02 mmol/L; $P = 0.012$), an 8% higher apo A-I concentration (0.09 ± 0.02 g/L; $P < 0.001$), and a 5% lower apoB:apo A-I ratio ($P = 0.008$) than with the CARB diet. Also, the MEAT diet caused an 8% higher HDL-cholesterol concentration (0.11 ± 0.02 mmol/L; $P < 0.001$) and a 4% higher apo A-I concentration (0.05 ± 0.02 g/L; $P = 0.033$) than with the CARB diet. There were no differences between CHEESE and MEAT diets in HDL-cholesterol and apo A-I concentrations. There were no significant differences between diets in total cholesterol, LDL cholesterol, triacylglycerol, apoB, and total cholesterol:HDL-cholesterol or LDL-cholesterol:HDL-cholesterol ratio. There was a tendency for a difference in 3-h postprandial triacylglycerol between diets.

Glucose and insulin

Pre- and postintervention fasting concentrations and 3-h postprandial concentrations of glucose and insulin are shown in Table 4 as is HOMA-IR. There were no differences in fasting glucose, insulin concentrations, or HOMA-IR between the 3 diets. However, there was a tendency for a difference between diets in 3-h postprandial glucose and insulin concentrations.

Fecal fat, energy, and bile acid excretion

Fecal excretions of fat, energy, and bile acids are shown in **Table 5**. Fecal fat excretion was 40% higher with the CHEESE diet (1.8 ± 0.3 g/d; $P < 0.001$) and 21% higher with the MEAT diet (0.9 ± 0.3 g/d; $P = 0.005$) than with the CARB diet. Likewise, fecal fat excretion was 16% higher with the CHEESE

diet (0.9 ± 0.3 g/d; $P = 0.004$) than with the MEAT diet. There was no difference between diets in fecal energy excretion.

Fecal bile acid excretion was 28% higher with the CHEESE diet (44.8 ± 21.3 μmol/d; $P = 0.046$) and 39% higher with the MEAT diet (64.0 ± 21.4 μmol/d; $P = 0.006$) than with the CARB diet. There was no significant difference in total fecal bile acid excretion between CHEESE and MEAT diets. However, the excretion of taurine-conjugated bile acids was significantly higher with the CHEESE diet than with the MEAT diet (26.6 ± 11.1 μmol/d; $P = 0.013$) and CARB diet (27.0 ± 11.1 μmol/d; $P = 0.015$). In contrast, the excretion of deconjugated bile acids was significantly lower with the CHEESE diet (−32.4 ± 10.7 μmol/d; $P = 0.004$) and CARB diet (−54.1 ± 10.7 μmol/d; $P < 0.001$) than with the MEAT diet. Furthermore, there was a trend toward a higher fecal excretion of deconjugated bile acids with the CHEESE diet than with the CARB diet (21.7 ± 10.6 μmol/d; $P = 0.053$).

Body weight, waist and hip circumferences, and blood pressure

Body weight, waist and hip circumferences, and blood pressure did not differ significantly between the 3 diets (data not shown).

DISCUSSION

The most-important findings of the current study were that SFAs in the food matrices of cheese and meat caused higher HDL-cholesterol and apo A-I concentrations than did carbohydrates. Also, cheese caused a lower apoB:apo A-I ratio than did carbohydrates. Hence, cheese consumption of 2–3 times (96–120 g/d) the average intake in Danish adults in a diet with a total fat content similar to the average Danish intake (36% of energy) did not cause detrimental changes in blood lipid concentrations.

A similar effect of CHEESE and MEAT diets on blood lipids could have been expected if only the fat content and ratio between SFAs, MUFAs, and PUFAs were depended on. Diets enriched with SFAs were previously shown to increase apo A-I gene expression, whereas the expression decreased with glucose-enriched diets (29). Studies also showed reductions in HDL cholesterol and apo A-I when saturated fat was replaced with carbohydrates (30, 31). Consistent with our findings, a few studies observed significant positive associations between cheese intake and HDL-cholesterol and apo A-I concentrations (32, 33). Triacylglycerol concentrations did not differ between the 3 diets in our study. This result may be explained by the types of carbohydrates used to replace cheese in the CARB diet, which were primarily fruit and bread, because studies that showed increased triacylglycerol by carbohydrates mainly used pure glucose or fructose (34). Also, the short diet-period durations, isocaloric design, and similar body weight could explain the similar triacylglycerol concentrations with the 3 diets.

Danish dietary guidelines recommend reducing the intake of saturated fat to reduce CVD risk. However, our trial and others studies suggested that the choice of nutrients or foods as a replacement for SFAs is highly important with respect to CVD risk (35). Recent meta-analyses on dietary fatty acids and risk of coronary outcomes did not suggest MUFAs or PUFAs to be preferable replacements for SFAs (36, 37). A meta-analysis by

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TABLE 4
Pre-intervention and postintervention blood variables with three 2-wk intervention diets[1]

	CHEESE diet	CARB diet	MEAT diet	P-diet
Fasting concentrations				
Total cholesterol, mmol/L				NS
Pre-intervention	5.98 ± 0.21	6.21 ± 0.23	6.23 ± 0.23	
Postintervention	5.79 ± 0.29	5.74 ± 0.24	5.96 ± 0.21	
LDL cholesterol, mmol/L				NS
Pre-intervention	3.64 ± 0.18	3.86 ± 0.21	3.84 ± 0.19	
Postintervention	3.58 ± 0.23	3.68 ± 0.22	3.82 ± 0.18	
HDL cholesterol, mmol/L				0.002
Pre-intervention	1.56 ± 0.08	1.53 ± 0.07	1.55 ± 0.07	
Postintervention	1.39 ± 0.07[a]	1.30 ± 0.06[b]	1.42 ± 0.07[a]	
Triacylglycerol, mmol/L				NS
Pre-intervention	1.18 ± 0.14	1.08 ± 0.06	1.26 ± 0.11	
Postintervention	1.21 ± 0.12	1.13 ± 0.10	1.10 ± 0.09	
LDL cholesterol:HDL cholesterol ratio				NS
Pre-intervention	2.43 ± 0.17	2.62 ± 0.20	2.56 ± 0.18	
Postintervention	2.66 ± 0.21	2.93 ± 0.22	2.79 ± 0.19	
Total cholesterol:HDL cholesterol ratio				NS
Pre-intervention	3.94 ± 0.20	4.16 ± 0.23	4.11 ± 0.20	
Postintervention	4.25 ± 0.25	4.52 ± 0.24	4.31 ± 0.22	
apoB, g/L				NS
Pre-intervention	0.98 ± 0.04	1.03 ± 0.05	1.03 ± 0.05	
Postintervention	0.97 ± 0.05	1.00 ± 0.05	1.01 ± 0.05	
apo A-I, g/L				0.002
Pre-intervention	1.37 ± 0.05	1.38 ± 0.05	1.40 ± 0.04	
Postintervention	1.27 ± 0.05[a]	1.19 ± 0.04[b]	1.26 ± 0.04[a]	
apoB:apo A-I ratio				0.028
Pre-intervention	0.73 ± 0.04	0.77 ± 0.05	0.75 ± 0.04	
Postintervention	0.78 ± 0.05[a]	0.85 ± 0.05[b]	0.81 ± 0.05[b]	
Insulin, pmol/L				NS
Pre-intervention	73.6 ± 8.3	76.1 ± 7.3	77.0 ± 7.4	
Postintervention	77.8 ± 10.4	70.0 ± 7.0	72.3 ± 8.7	
Glucose, mmol/L				NS
Pre-intervention	5.71 ± 0.12	5.77 ± 0.12	5.64 ± 0.11	
Postintervention	5.18 ± 0.36	5.38 ± 0.09	5.53 ± 0.08	
HOMA-IR				NS
Pre-intervention	2.72 ± 0.34	2.81 ± 0.30	2.82 ± 0.27	
Postintervention	2.65 ± 0.41	2.58 ± 0.32	2.42 ± 0.24	
Postprandial concentrations[2]				
Total cholesterol postintervention, mmol/L	5.69 ± 0.25	5.96 ± 0.26	6.02 ± 0.20	NS
LDL cholesterol postintervention, mmol/L	3.45 ± 0.21	3.74 ± 0.22	3.76 ± 0.17	NS
HDL cholesterol postintervention, mmol/L	1.39 ± 0.06	1.39 ± 0.07	1.41 ± 0.06	NS
Triacylglycerol postintervention, mmol/L	1.90 ± 0.21	1.59 ± 0.15	1.52 ± 0.14	0.089
Insulin postintervention, pmol/L	161.8 ± 20.3	202.5 ± 26.5	179.2 ± 27.7	0.073
Glucose postintervention, mmol/L	5.29 ± 0.11	4.97 ± 0.20	5.31 ± 0.12	0.067

[1]All values are means ± SEMs. $n = 14$. P-diet reports the overall effect by using an ANCOVA whereby postintervention was modeled as the dependent variable, whereas diet, pre-intervention (when available), period, and diet × period were modeled as fixed variables, and the subject number was modeled as a random variable. For pre-intervention differences and for diet × period interactions for all dependent variables, $P > 0.05$. NS was defined as $P > 0.10$. Means that do not share a common superscript letter are significantly different at $P < 0.05$ on the basis of post hoc pairwise comparisons by using t tests. apo A-I, apolipoprotein A-I; apoB, apolipoprotein B; CARB, nondairy low-fat control in which the energy from cheese fat and protein was isocalorically replaced by carbohydrates and lean meat; CHEESE, intervention containing cheese; MEAT, a nondairy control with a high content of high-fat processed and unprocessed meat in amounts matching the saturated fat content from cheese in the intervention containing cheese.

[2]Measured 3 h after intake of a breakfast meal with a macronutrient and food composition similar to that in the intervention period just completed.

Mensink et al. (38) on fatty acid intake and blood lipids predicted significant increases in HDL cholesterol when 1% of energy from carbohydrate was replaced by SFAs but also predicted simultaneous increases in total cholesterol and LDL cholesterol. An increase in HDL cholesterol by SFAs in the food matrices of cheese and meat in the current study was consistent with this meta-analysis. The fact that total cholesterol and LDL cholesterol were similar with the CHEESE, MEAT, and CARB diets may be explained by the fact that the cheese fat replaced by carbohydrates consisted of 69% SFAs, 28% MUFAs, and 3% PUFAs.

TABLE 5
Fecal excretion of fat, energy, and bile acids with three 2-wk intervention diets

	CHEESE diet	CARB diet	MEAT diet	P-diet
Fecal fat excretion, g/d	5.8 ± 0.4[a]	3.9 ± 0.2[b]	4.9 ± 0.4[c]	0.001
Fecal energy excretion, kJ/d	679.6 ± 53.4	631.7 ± 36.3	736.1 ± 46.7	NS
Fecal total bile acid excretion, μmol/d	206.1 ± 24.8[a]	155.3 ± 16.2[b]	225.9 ± 30.7[a]	0.018
Taurine-conjugated bile acids	84.2 ± 15.4[a]	57.2 ± 5.7[b]	56.6 ± 5.8[b]	0.025
Glycine-conjugated	52.2 ± 10.5	50.8 ± 9.0	71.4 ± 12.8	NS
Total conjugated bile acids	133.0 ± 19.0	107.0 ± 11.2	120.1 ± 17.0	NS
Total deconjugated bile acids	73.1 ± 9.7[a]	48.3 ± 6.7[b]	105.8 ± 16.6[c]	<0.001

[1] All values are means ± SEMs. $n = 13$. P-diet reports the overall effect of diet by using an ANCOVA whereby diet, period, and diet × period were modeled as fixed variables, and the subject number was modeled as a random variable. For pre-intervention differences and for diet × period interactions for all dependent variables, $P > 0.05$. NS was defined as $P > 0.10$. Means that do not share a common superscript letter are significantly different at $P < 0.05$ on the basis of post hoc pairwise comparisons by using t tests. CARB, nondairy low-fat control in which the energy from cheese fat and protein was isocalorically replaced by carbohydrates and lean meat; CHEESE, intervention containing cheese; MEAT, a nondairy control with a high content of high-fat processed and unprocessed meat in amounts matching the saturated fat content from cheese in the intervention containing cheese.

MUFAs have been suggested to reduce LDL cholesterol and increase HDL cholesterol significantly. Therefore, the MUFA contents of CHEESE and MEAT diets in our study may have attenuated the effect of SFAs on LDL cholesterol. However, because calcium in dairy products has been suggested to possibly counteract the effect of SFAs on LDL cholesterol (11), we expected lower LDL cholesterol with the CHEESE diet than with the low-calcium MEAT diet, which was not supported by our results.

The higher fecal excretion of fat with CHEESE and MEAT diets than with the CARB diet were expected because of differences in the fat content. Differences in fecal fat excretion were rather small with the CHEESE diet, causing 1.8 and 0.9-g/d higher fecal fat excretion than with the MEAT and CARB diets, respectively. Fecal fat excretion has been suggested as possible a mechanism behind the lower LDL-cholesterol concentration by intake of calcium-rich dairy products such as cheese (10, 11, 39). Our results could not support this suggestion because LDL cholesterol was similar in the CHEESE and MEAT diets. It is possible that the 0.9-g/d difference in fecal fat excretion between CHEESE and MEAT diets was too small to result in differences in LDL cholesterol. It could also be speculated that SFAs in meat may have less effect on LDL cholesterol than do SFAs in cheese, which would counteract the effect of an increased fecal fat excretion by cheese on LDL cholesterol.

Energy excretion did not differ between diets, which questions the importance of the higher fecal fat excretion by cheese on energy balance and body weight in a mixed diet, especially because calcium has been shown to cause a higher fecal fat excretion when consumed as part of a high-fat diet than with a low-fat diet (11).

Like others, we observed no significant difference in total bile acid excretion between macronutrient-matched diets with a high-calcium content (CHEESE diet) than with a low-calcium content (MEAT diet) (9, 40). Increased fecal bile acid excretion by bile acid sequestrants has been shown to reduce the stimulation of the bile acid sensing nuclear receptor farnesoid X receptor, which is highly expressed in the intestine and liver (41, 42). Consequently, the gene expression of the first rate-limiting enzyme in hepatic cholesterol synthesis, cholesterol 7α-hydroxylase, gene expression is suppressed, which increases bile acid synthesis and the clearance of systemic circulating LDL cholesterol. In the current study, higher fecal bile acid excretion with CHEESE and MEAT diets than with the CARB diet may, therefore, potentially explain the similar LDL-cholesterol concentrations with the 3 diets despite differences in fat contents. Also, animal studies suggested that less farnesoid X receptor stimulation (e.g., because of increased fecal bile acid excretion and reduced bile acid recirculation) independently augments apo A-I gene expression (43) and HDL-particle remodeling (44). This effect is consistent with our finding of higher apo A-I and HDL cholesterol with the CHEESE and MEAT diets than with the CARB diet.

Three-hour postprandial triacylglycerol concentrations tended to differ between diets with a higher concentration after the CHEESE breakfast than after the CARB breakfast. However, postprandial triacylglycerol was similar after MEAT and CARB breakfasts. Future meal studies should determine whether this effect is transitory or present during the entire postprandial period.

The current study had a strong crossover design and thoroughly planned, isocaloric, highly controlled intervention diets, which were provided to subjects during intervention periods. The matching of macronutrients, the SFA:MUFA:PUFA ratio, and dietary fiber, sugar, and sodium contents in CHEESE and MEAT diets made it possible to compare food-specific SFAs and micronutrients in cheese and meat. However, some of the adjustments could also be considered limitations of the study that might prevent a direct comparison between cheese and meat as whole foods. The protein sources of the CHEESE diet differed from those of the MEAT and CARB diets, but to our knowledge, no human studies have investigated effects of casein compared with meat proteins on blood lipids. In our study, the different HDL-cholesterol responses could, however, not be explained by the different protein sources. The fat in the beef and pork meat in the MEAT diet contained 45% SFAs, 49% MUFAs, and 6% PUFAs, and if unbalanced, it may have altered our results. However, a direct comparison of SFAs from cheese and meat would require the isolation of these. This isolation would neglect the potential impact of other concomitant micronutrients and their interactions in the food matrix, which may be of importance. Other limitations of the study were the rather short study duration and the narrow study population. The latter makes it

difficult to extrapolate the results to the more-heterogeneous population.

In conclusion, diets with cheese and meat as primary sources of SFAs cause higher HDL-cholesterol and apo A-I concentrations and, therefore, appear to be less atherogenic than a low-fat, high-carbohydrate diet. Our findings confirm that cheese increases fecal fat excretion.

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