

Original Research

Does Fat in Milk, Butter and Cheese Affect Blood Lipids and Cholesterol Differently?

Tine Tholstrup, PhD, Carl-Erik Høy, PhD, Lene Normann Andersen, MS, Robin D.K. Christensen, MS, Brittmarie Sandström, PhD

Research Department of Human Nutrition, Center of Advanced Food Research, the Royal Veterinary and Agricultural University, Copenhagen (T.T., L.N.A., R.D.K.C., B.S.), Section of Biochemistry and Nutrition, Bio Centrum-DTU, Technical University of Denmark, Lyngby (C.-E.H), DENMARK

Key words: milk fat, cheese, butter, plasma cholesterol, glucose, insulin

Objective: To compare the effects of isoenergetic amounts of milk, cheese and butter (adjusted to the same content of lactose and casein) on fasting and postprandial blood lipids and lipoproteins, and on postprandial glucose and insulin response.

Design: The experiments were designed to provide 20% of total energy from dairy fat, as either whole milk, mean (\pm SD) 2164 (\pm 97) g, butter 93 (\pm 4) g, and hard cheese 305 (\pm 45) g, which were served to 14 healthy young men for three periods of three weeks each, separated by washout periods, in a randomized, cross-over study with strictly controlled dietary intake. Fasting blood samples were taken at the end of the study periods. Measurements of the postprandial effect of the three different dairy test products (0.7 g of milk fat/kg body weight) were carried out on day 4 of each intervention period. Blood samples were taken before and at 2, 4, 6 and 8 hours following intake of the meals.

Results: Fasting LDL cholesterol concentration was significantly higher after butter than cheese diet ($p = 0.037$), with a borderline significant difference in total cholesterol ($p = 0.054$) after the experimental periods of three weeks. Postprandial glucose showed a higher response after cheese diet than after milk diet ($p = 0.010$, diet \times time interaction).

Conclusions: A different effect of fat in milk and butter could not be confirmed in this study. The moderately lower LDL cholesterol after cheese diet compared to butter diet should be investigated further.

INTRODUCTION

Milk and milk products play an important part in a healthy diet as they contribute to intakes of essential nutrients and protein of a nutritionally high quality. In Denmark dairy products contribute more than 50% of total calcium intake [1]. However, due to the high content of cholesterol raising saturated fatty acids (SFA) in milk fat, a decrease in the intake of fat-rich dairy products is recommended. Although there is no doubt that mixtures of long-chain saturated fatty acids (SFA) in milk fat increase plasma cholesterol [2,3], there is some controversy regarding to the specific effect of milk products. Thus, a neutral or a hypocholesterolemic effect of milk products shown in some studies [4–10] has led to the hypothesis that

milk contains a beneficial “milk factor”, which somehow modulates the cholesterol raising effect of the milk fat [11,12]. This hypothesis is in line with a finding that whole milk reduces plasma and hepatic triacylglycerol (TAG) in rats in a manner that cannot simply be predicted by the fat content of whole milk [13]. A beneficial effect of milk was described in an internal report from an epidemiological study (The Caerphilly and Speedwell Prospective Heart Disease Studies) in which a high intake of whole milk was associated with a low risk of CHD in middle-aged men [14]. Although heavily debated [15,16] these controversial results were never published. Others have found a hypercholesterolemic effect of milk [17,18], as could be predicted from the fatty acid composition [17].

The inconsistency of the effect of different milk products on

Address reprint requests to: Tine Tholstrup, PhD, Research Department of Human Nutrition, The Royal Veterinary and Agricultural University, 30 Rolighedsvej, DK-1958 Frederiksberg, DENMARK. E-mail: Tine.Tholstrup@fhe.kvl.dk

Supported by The Danish Dairy Research Foundation and The Danish Research Development Program for Food Technology (FOETEK).

Journal of the American College of Nutrition, Vol. 23, No. 2, 169–176 (2004)

Published by the American College of Nutrition

serum cholesterol does not include butter. Several human metabolic studies show that butter is hypercholesterolemic when compared with other fat sources [2,3,19]. A single study reported the same effect of whole milk and butter [20]. The effect of cheese on plasma cholesterol concentration has, to our knowledge, not been investigated.

In milk the fat and the majority of the milk phospholipids are present in the microscopic globules emulsified in water. Cream used for butter production is an oil/water emulsion, which is mechanically agitated (churned) to cause a partial breakdown of the emulsion. By this process the membrane of the fat globules, as well as the globules, are damaged [21]. It may be hypothesized that if a more hypocholesterolemic factor associated with some milk products exists, it may be removed during the butter production process. Cheese is prepared from the curd precipitated by rennin or lactic acid. However, further chemical changes occur during fermentation of the lactose and protein during aging. Contrary to milk and butter the fat in cheese is encapsulated by the casein structure [21]. Thus, the physical state of dairy fats in milk, cream for butter, and cheese is different. Whether this may affect the rate of absorption of the products, following the synthesis and clearance of chylomicrons and thereby concentration of lipoproteins remains to be examined.

In this study we compared the effect of high intakes of dairy fat as whole milk, butter and hard cheese on fasting and postprandial blood lipids and lipoproteins, and on postprandial glucose and insulin responses in healthy young men. The butter and the hard cheese diets were adjusted for the casein and lactose content of milk in order to specifically investigate the impact of differences in the physical form of the dietary milk fat.

MATERIALS AND METHODS

Subjects

Fourteen young men were recruited for the study. Their ages ranged from 20 to 31 years (mean 23 years), body weight from 67 to 83 kg (mean 73 kg), body mass index from 20 to 27 kg/m² (mean 22 kg/m²), total plasma cholesterol: 2.9–5.0 mmol/L (mean 4.1 mmol/L), and plasma TAG: 0.6–2.0 mmol/L (mean 0.9 mmol/L). The subjects had no history of atherosclerotic disease, and all were apparently healthy as assessed by a medical questionnaire. They were non-smokers (except for one, who smoked less than 10 cigarettes daily throughout all three study periods) and were not taking any medication. The subjects had a moderate physical activity level (training max. 1–2 hours twice a week and/or cycling daily to work). They continued with the same physical activity throughout the trial. Three different diets were served in randomized order for three weeks each, in a cross-over design. The three intervention periods were separated by a period of at least one-month on habitual diet.

The participants' habitual diets were assessed from 7-day weighed food records. We calculated energy intake and nutrient composition using a national database (Dankost, National Food Agency, Denmark). Habitual energy intake ranged from 9 to 19 MJ (mean 14 MJ) with 26–42% of energy from fat (mean 32 E%). Eleven E% was derived from saturated fatty acids and the ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) was 0.2–0.8 (mean 0.4). Intake of cholesterol was 175–665 mg/10 MJ (mean 371 mg/10 MJ), and dietary fiber intake ranged from 1.4 to 3.2 g/MJ (mean 2.4 g/MJ).

Test Diets

We served three diets, with a high content of whole milk (MI), butter (BU), or cheese (CH), respectively. Energy intake was individualized based on the food records of the subjects, taking body weight, gender, age, and level of physical activity into account (World Health Organization, 1985). The subjects were given a strictly controlled isocaloric diet with 35% of energy from fat, of which 20 E% derived from milk fat (total contribution of SFA was 16 E%), 17 E% from protein, and 48 E% from carbohydrates. All food items other than milk, cheese and butter were constant and identical in the three test diets. The MI diet contained 1.5 L of whole milk/10 MJ (54 g of fat and 1779 mg calcium per 10 MJ), the BU diet contained 64 g of butter/10 MJ (54 g of fat and 10 mg calcium per 10 MJ), and the CH diet contained 205 g of hard cheese, "Samsø", 45% fat of dry weight, i.e. 26% fat/10 MJ (1989 mg calcium/10 MJ). (E.g. for a subject with a daily intake of 14 MJ the diet contained 2.1 L whole milk, 287 g of hard cheese or 90 g of butter). In order to specifically investigate the effect of the physical condition of the milk fats we balanced the dairy products with regard to protein and lactose. Thus 72 g of lactose/10 MJ were added to diet CH and 72 g of lactose/10 MJ and 52 g of milk protein (80% casein and 20% whey protein) were added to diet BU, incorporated in bread and cakes.

The experimental meals consisted of ordinary and common food items, which were prepared and cooked in customary ways. All foods were prepared and weighed in individual servings in the experimental kitchen at the Research Department of Human Nutrition of the Royal Veterinary and Agricultural University. On weekdays lunch was served at the Department. All other meals were provided daily as packages with instructions for preparation. Meals for the weekend were provided on Fridays. Body weight without heavy clothing was recorded before lunch three times a week. Physical activity was kept constant and daily records of tea and coffee intake were kept. Physical activity, illness and deviation from the protocol were monitored as described earlier [22]. Duplicate portions of each of the three test diets were collected in each period of the study. The portions were pooled and the Danish Institute of Agricultural Sciences, Research Center Foulum, determined the fatty acid compositions and cholesterol contents. Fatty acid compositions and cholesterol contents are presented in Table 1.

Table 1. Nutrient Contents and (Major) Fatty Acids of the Experimental Diets per 10 MJ

	MI	CH	BU
Protein (g)	105	105	97.2
Total fat (g)	82.5	89.1	87.3
Fatty acids (mass %)			
SFA			
8:0	0.70	0.72	0.71
10:0	1.63	1.75	1.79
12:0	2.26	2.41	2.41
14:0	6.82	7.39	7.27
15:0	0.63	0.74	0.68
16:0	25.16	24.90	24.93
17:0	0.39	0.45	0.43
18:0	9.00	9.10	9.32
20:0	0.16	0.16	0.19
22:0	0.23	0.23	0.19
MUFA			
14:1	0.63	0.72	0.69
16:1	1.40	1.58	1.55
17:1	0.23	0.28	0.18
18:1	28.66	29.23	29.09
20:1	0.31	0.39	0.44
22:1	0.16	0.11	0.13
PUFA			
18:2	20.80	18.81	18.98
18:3	0.54	0.61	0.69
18:4	0.31	0.45	0.33
Cholesterol (mg)	266	249	257

Abbreviations: MI = diet high in milk, BU = diet high in butter, CH = diet high in cheese, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids.

Postprandial Test Meals

The effect of a meal high in milk, butter or cheese was investigated on day 4 of each 3-week experimental period. The test diet for the postprandial studies consisted of 0.7 g of milk fat/kg body weight, i.e. a subject weighing 75 kg had a meal containing 1.5 L of milk, 200 g of cheese, or 64 g of butter, respectively, together with a measured quantity of bread. The meals constituted 4.7 MJ. The cheese test meal was supplemented with bread containing lactose (33.8 g/4.7 MJ). The butter test meal was supplemented with bread containing lactose (33.8 g/4.7 MJ) and milk protein (80% casein and 20% whey protein) (24.4 g/4.7 MJ). Forty-five % of the energy was derived from fat (98% from milk fat), 35% from carbohydrates, and 20% from protein. The energy content of the test meal ranged from 4.2 MJ to 5.2 MJ (mean 4.6 MJ), and the content of milk fat in each test meal ranged from 47 g to 58 g (mean 51 g).

Blood Analysis

Blood samples were taken by venipuncture with minimum stasis and collected into siliconized vacutainers. Subjects refrained from heavy physical activity or alcohol intake for at least 24 hours, fasted for at least 12 hours, and rested supine for

15 minutes before sampling. Fasting samples: Morning blood samples were taken before each of the three study periods (2 samples before the first and one sample before the second and third dietary period) and on day 14, 20, and 21 of each dietary period. Sampling was performed after at least 12 hours of fasting and after 15 minutes of supine rest. Postprandial samples were taken on day 4 of the study periods before and at 2, 4, 6 and 8 hours after intake of the dairy products. The plasma concentration of C-reactive protein (CRP) was assessed to rule out infectious diseases in the subjects at the time of blood collection. Values were in the normal range (<5 mg/L) except for one measurement for one subject. Data from this occasion were excluded from analysis of plasma lipids and lipoproteins. Blood for lipid and lipoprotein analysis was collected in tubes containing EDTA, which were immediately placed on ice and centrifuged at $3000 \times g$ for 15 min at 4°C. Plasma for apolipoprotein and fatty acid analysis was stored at -80°C. Plasma for lipoprotein analysis was stored at 4°C and analyzed within 48 hours. Chylomicrons, VLDL + chylomicron remnants, and LDL + HDL fractions were separated by ultra-centrifugation. Chylomicrons were isolated by careful over-layering 3 mL of plasma with 2.5 mL of salt solution of density 1.006 kg/L. The UC tubes (13 × 64 mm) were centrifuged for 23 minutes at 20°C at 30,000 rpm in an ultra-centrifuge (L7-55, Beckmann Instruments, Palo Alto, CA) using a fixed angle rotor (= 96.1 mm) (50.4 Ti, Beckmann Instruments). The tubes were sliced 45 mm from the bottom, and the top fraction ($S_f > 400$) was transferred and adjusted to a total volume of 5 mL with a salt solution of density 1.006 kg/L. The bottom fraction ($S_f < 400$) was transferred to another UC tube, adjusted to 5.5 mL with salt solution of density 1.006 kg/L and centrifuged at 50,000 rpm for 16 h at 4°C. After tube slicing 30 mm from the bottom, the top fraction containing VLDL and chylomicron remnants, and the bottom fraction containing LDL + HDL, were transferred to separate tubes and adjusted to a final volume of 5 mL.

Cholesterol and TAG concentrations were assessed by enzymatic procedures (Boehringer Mannheim GmbH, Mannheim, FRG) on a Cobas Mira analyzer (Roche, Basel, Switzerland). Total high-density lipoprotein (HDL) and HDL₃ cholesterol, respectively were separated as supernatants, after precipitation with polyethylene glycol (Quantolip, Immuno AG, Vienna, Austria) [23] and the cholesterol concentrations were measured enzymatically. HDL₂ cholesterol was calculated by subtracting HDL₃ cholesterol from the total HDL cholesterol. LDL cholesterol was calculated from the difference of cholesterol in the infranatant. The concentrations of plasma apolipoproteins apo B and A-1 were determined by immunoturbidimetry on a Cobas Mira analyzer (Roche, Basel, Switzerland) using monospecific polyclonal antibodies against apo B and A-1 (kits from Roche, Basel, Switzerland). Precision was determined by the control solution from the manufacturer. Fatty acid profile of chylomicrons was determined before and during the postprandial period by a method previously described [24].

Ethics

The protocol and the aim of the study were fully explained to the subjects, who gave their written consent. The Scientific Ethics Committee of the municipalities of Copenhagen and Frederiksberg (01-197/96) approved the research protocol.

Statistical Methods

There were no significant differences between the three samplings in blood lipids and lipoproteins between day 14, 20, and 21, so those values were pooled. The mean value was calculated and used in the statistical analysis. We used paired *t*-tests to compare the effect of the three diets, and to compare each of the interventions with the level at baseline. To analyze, and present the pair wise differences in lipid and lipoprotein values, we used a one-way analysis of covariance with a factor for diet and the baseline value as covariate, which is presented as difference between means (95% confidence interval); analyzed using the general linear model procedure: SAS package (Version 8.2; SAS Institute Inc., Cary, NC, USA). All statistical tests were 2-sided, and a cutoff level of $p < 0.05$ was used for assessing statistical significance. To analyze postprandial data repeated-measures analysis of variance (R-MANOVA) with Huynh-Feldt adjustment of degrees of freedom were used to assess effect of time, difference in effect of the experimental fats, and interaction between effects of time and type of fat during the 0 to 8 hours period of the day. The graphs of the time course illustrate how the means differ. A paired *t* test was used to compare the effect on plasma glucose at specific time points. Statistical analyses of R-MANOVA were conducted using the SPSS computer program (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Effect of Experimental Diet after Three Weeks (Fasting Samples)

The body weights of the volunteers did not differ significantly at the end of the three experimental periods; 73.4 (± 4.9) kg mean (\pm SD) on the MI diet, 73.9 (± 5.4) kg on the BU diet, and 73.4 (± 5.2) kg on the CH diet. The fasting mean plasma concentrations of total cholesterol, VLDL cholesterol, LDL cholesterol, HDL cholesterol, HDL₂-, HDL₃-, ratio of LDL/HDL cholesterol, TAG, Apo A-1, and Apo B for baseline values and values after the three test diets are given in Table 2. The differences between means after three weeks' intervention are presented in Table 3. When the difference between the means after the three different dietary interventions was analyzed, BU diet resulted in 0.21 mmol/L higher LDL cholesterol compared to CH ($p = 0.037$), adjusted for the baseline value. A difference that only appeared borderline significant when evaluating total cholesterol ($p = 0.054$) the same way. When analyzing the difference between the mean LDL/HDL cholesterol ratio after the three weeks' intervention, MI tended to be higher than CH ($p = 0.069$) when the baseline value was used as covariate.

No significant differences were observed between diet MI, BU and CH in plasma HDL, HDL₂, HDL₃, VLDL cholesterol, apo A-1 and apo B concentrations.

Effect of a Single Meal Given on Day Four of the Study Periods

There was an overall increase in plasma total TAG, chylomicron and VLDL TAG with a measured maximum at 4 hours

Table 2. Fasting Values of Plasma Lipids, Lipoproteins and Glucose Concentrations, before and after Three Weeks' Dietary Intervention

Variable	Before	MI	CH	BU
Total cholesterol	4.11 \pm 0.16	4.18 \pm 0.17	4.05 \pm 0.15	4.26 \pm 0.18
VLDL-cholesterol	0.21 \pm 0.03	0.19 \pm 0.03	0.19 \pm 0.03	0.17 \pm 0.03
LDL-cholesterol	2.64 \pm 0.16 ^{ab}	2.81 \pm 0.18	2.67 \pm 0.15	2.87 \pm 0.17
HDL-cholesterol	1.23 \pm 0.06 ^{ac}	1.15 \pm 0.07	1.16 \pm 0.06	1.19 \pm 0.05
HDL ₂ -cholesterol	0.37 \pm 0.03 ^{bc}	0.34 \pm 0.03	0.32 \pm 0.02	0.34 \pm 0.03
HDL ₃ -cholesterol	0.86 \pm 0.04	0.81 \pm 0.05	0.85 \pm 0.04	0.85 \pm 0.03
Total triacylglycerols	0.89 \pm 0.09 ^{abc}	0.73 \pm 0.08	0.75 \pm 0.08	0.71 \pm 0.08
LDL/HDL cholesterol	2.26 \pm 0.20 ^{abc}	2.62 \pm 0.26 ^c	2.41 \pm 0.20	2.50 \pm 0.19
Apo A-1 [g/L]	1.39 \pm 0.04 ^{abc}	1.31 \pm 0.04	1.27 \pm 0.05	1.31 \pm 0.04
Apo B [g/L]	0.86 \pm 0.05	0.89 \pm 0.05	0.87 \pm 0.06	0.89 \pm 0.05
Glucose	4.84 \pm 0.09	4.89 \pm 0.08	4.94 \pm 0.09	4.91 \pm 0.07

Values are mean \pm SEM; 3 samplings before and in the end of the study periods. Blood lipids and glucose given as mmol/L. To convert values for total, LDL, VLDL, HDL, HDL₂, HDL₃-cholesterol to milligrams per deciliter, multiply by 38.67. To convert values for triacylglycerols to milligrams per deciliter multiply by 88.54.

Abbreviations: MI = diet rich in milk, CH = diet rich in cheese, BU = diet rich in butter.

^a Different from MI ($p < 0.05$).

^b Different from BU ($p < 0.05$).

^c Different from CH ($p < 0.05$).

Table 3. Difference between Means from Fasting Values of Plasma Lipids and Lipoproteins after Three Weeks of Dietary Intervention

	Butter vs. Cheese	Butter vs. Milk	Cheese vs. Milk	<i>p</i> -value*
Total Cholesterol (mmol/L)	0.20 [†] (−0.00; 0.41)	0.07 (−0.13; 0.28)	−0.13 (−0.33; 0.08)	0.145
VLDL-cholesterol (mmol/L)	−0.02 (−0.07; 0.02)	−0.02 (−0.07; 0.02)	−0.00 (−0.05; 0.04)	0.523
LDL-cholesterol (mmol/L)	0.21 [‡] (0.01; 0.40)	0.06 (−0.13; 0.26)	−0.14 (−0.34; 0.05)	0.098
HDL-cholesterol (mmol/L)	0.03 (−0.06; 0.12)	0.04 (−0.05; 0.13)	0.01 (−0.08; 0.10)	0.691
HDL ₂ -cholesterol (mmol/L)	0.02 (−0.01; 0.06)	0.00 (−0.03; 0.04)	−0.02 (−0.05; 0.02)	0.417
HDL ₃ -cholesterol (mmol/L)	0.00 (−0.06; 0.07)	0.04 (−0.03; 0.10)	0.03 (−0.04; 0.10)	0.497
LDL/HDL-ratio	0.08 (−0.15; 0.31)	−0.13 (−0.37; 0.10)	−0.22 [§] (−0.45; 0.02)	0.182
Total Triacylglycerols (mmol/L)	−0.03 (−0.13; 0.06)	−0.02 (−0.12; 0.07)	0.01 (−0.08; 0.10)	0.792

Values are the difference between means (95% confidence interval), *n* = 14.

* Analyzed using a one-way analysis of covariance, with a factor for diet and adjusting to the baseline value using it as a covariate.

[†] *p* = 0.054, [‡] *p* = 0.037, [§] *p* = 0.069.

and a decrease to fasting values after 8 hours. There was no significant difference between the effect of milk, butter and cheese meals on these variables, when diet × time interaction, delta values between baseline and values at the different time points were analyzed.

There was an overall increase in chylomicron cholesterol and VLDL cholesterol with a measured maximum at 4 hours and decrease to fasting values after 8 hours similar to the pattern of TAG in the same fractions. Plasma cholesterol concentrations of total HDL and HDL₃ decreased with the lowest measured values at 4 hours and increased to fasting after 8 hours. MI, CH and BU meals resulted in a different response in HDL₂ cholesterol (diet × time interaction) (delta values between baseline and values at the different time points were analyzed) (*p* < 0.05) with the graph showing a slightly higher HDL₂ cholesterol concentration after CH than MI and BU at 8 hours. No other differences were observed.

There was an overall increase in plasma glucose with a measured maximum at 30 minutes after intake of the meals, followed by a decrease between 30 and 60 minutes (Fig. 1a). There was a different response to MI compared to CH (*p* = 0.010, diet × time interaction). At 30 and 60 minutes CH meals resulted in a higher glucose response than MI (*p* < 0.05). There was an overall increase in plasma insulin, with a measured maximum at 30 minutes after intake of the meals, followed by a decrease with a minimum between 30 and 60 minutes (Fig. 1b). The responses after the three diets did not differ. The fatty acid composition (myristic, palmitic, stearic and oleic acid) in chylomicron TAG, before and postprandially at 2, 4, 6, and 8 hours after intake of the test diets, were not different when analyzed by paired *t* test (data not shown).

DISCUSSION

This study was designed to compare the effects of the physical state of milk fat present in whole milk, butter, and hard cheese. The main finding of this study was the lack of difference in cholesterolemic effect of the diets containing whole milk and butter.

Reports of hypocholesterolemic effect of milk [7–9] led us to expect MI to be less cholesterolemic than BU diet, which has generally been confirmed to be cholesterol raising [2,3,19]. However, the observation that MI diet increased LDL cholesterol to the same extent as BU diet [20] agrees with findings by Steinmetz *et al.*, who showed that the effect on cholesterol was as could be predicted from the FA composition of milk [17]. There may be several reasons for the lack of agreement in previous findings on the effects of milk. Overall there is a lack of standardization in the studies. The amount and composition of dairy products have differed, as have the study populations' composition and the duration of the interventions. In addition, in several of the studies the total dietary intake was not controlled [6,7,9], or a control diet for comparison has either been lacking or has not been fully described [4,5,10]. However, results from this strictly controlled dietary study with cross-over design do not support the hypothesis of a hypocholesterolemic milk factor.

CH diet caused a slightly lower increase in plasma LDL cholesterol concentration compared to BU diet (≈7%). In contrast to CH both BU and MI resulted in an increase in LDL cholesterol (8.7% and 6.4%, respectively). Nobody has previously compared the effects of either fat in milk and butter with fat in neither cheese nor the three specific dairy products. Calcium content may affect plasma cholesterol by decreasing

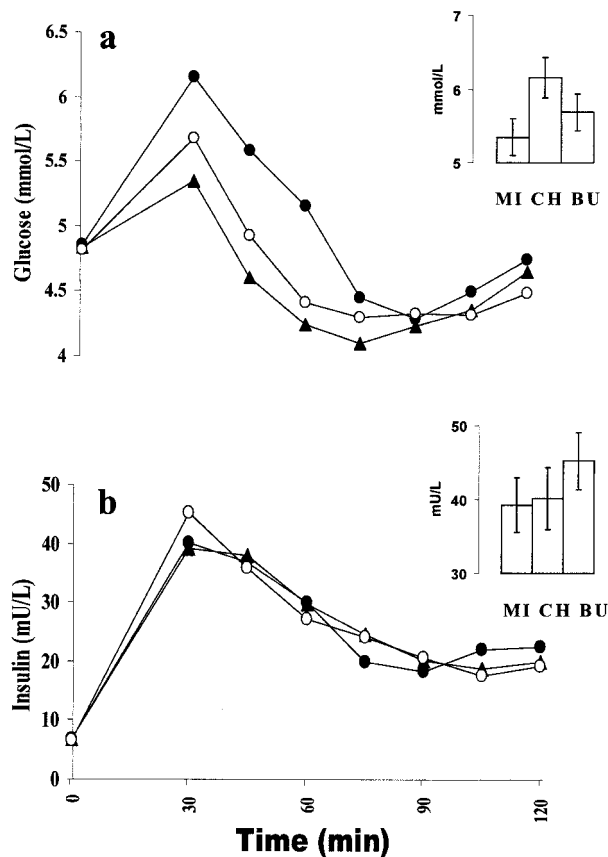


Fig. 1. Graphs show mean values of plasma glucose (a) and insulin (b) before and after intake of the test meals (0.7 g milk fat/kg body weight) of MI (black triangle), CH (black circle), and BU (open circle). Samplings at hours 0 are fasting values, other values are postprandial. Bars show mean values \pm SEM 30 minutes after intake of test meals, $n = 14$. See Table 1 for details of fatty acid composition of the test diets. Abbreviations: MI = milk diet, CH = cheese diet, BU = butter diet.

the absorption of exogenous cholesterol [25,26]. Total difference in daily calcium intake between CH and BU was 1979 mg per 10 MJ, which is high, compared to average intake of calcium in Danish men, which is 941 mg/10 MJ [27]. Thus, it is possible that the content of calcium in CH resulted in a lack of increase in plasma LDL cholesterol compared to BU due to a moderate cholesterol decreasing effect of calcium, as suggested [28]. However, if the high calcium content of CH diet should have prevented a LDL cholesterol increasing effect of the high intake of milk fat in cheese, MI with a rather similar content of calcium should have affected LDL cholesterol in the same way, which was not the case. Another reason that CH did not cause an increase in LDL cholesterol may be due to the fact that cheese is fermented. Thus studies have shown a moderate cholesterol lowering effect of fermented dairy products [29,30].

The study was designed to compare the effects of the three fats with each other. However, although habitual diet was not

controlled (but apparently rather similar before each test periods due to the cross-over design), we found the comparisons of values after intervention with baseline values relevant to report (Table 2). The results of this comparison underline the tendency found in the comparison of the three fats (Table 3). The cholesterol raising effect of the diets high in dairy products was not surprising as saturated fatty acids were 16 E% in the experimental diet *versus* 11 E% in the habitual diet. The pronounced decrease in plasma TAG after all three experimental diets compared to baseline values could be explained by the lower content of carbohydrates in the experimental diet compared to baseline diet [31].

The overall postprandial response after meals high in common dairy products, and thereby relatively high in fat, is in accordance with our previous findings [32]. The similar pattern in increase of total chylomicron, VLDL TAG and of cholesterol in chylomicron and VLDL is generally observed [33]. A decrease in HDL cholesterol is due to an increased CETP activity in the postprandial state, as we have shown recently [32]. There was no significant difference in total or chylomicron TAG, in accordance with the lack of differences in postprandial concentration of the major fatty acids in chylomicron TAG after intake of the test diets. The higher glycemic response after cheese diet compared to milk diet may be explained by a higher accessibility of the added lactose in the cheese diet. The texture of foods is known to affect gastric emptying, which is more rapid after intake of fluid foods than after solid foods [34]. The texture of foods is also known to affect gastric inhibitory peptide (GIP) and glucagon-like peptide (GLP)-I differently, and thereby possibly affect glucose (and insulin) response [35]. However, the consistency of milk and cheese, although different when consumed, may be more similar in the stomach, where milk coagulates causing casein to precipitate. This may be the reason why the milk diet did not cause the expected more rapid response in plasma glucose.

This study was designed to investigate the effect of the physical state of fat in whole milk, butter and hard cheese, and the products were therefore modified to adjust for differences in content of lactose and casein. The results of the study are therefore not fully applicable to the effect of the products, whole milk, butter and hard cheese. However, the adjustment for lactose content could not have affected the lipid values [10]. That the naturally higher casein content in whole milk than in butter could have resulted in a more favorable blood profile cannot be ruled out. However, animal studies have not shown any consistent effect of casein on cholesterol concentration [36–38] and a lack of an effect of casein on plasma cholesterol has been repeatedly been shown in humans [39,40].

In conclusion, the hypothesis that milk may be less cholesterolemic than butter was not confirmed by this study. The moderately lower LDL cholesterol after cheese diet compared to butter diet is interesting and should be investigated further.

ACKNOWLEDGMENTS

We thank our technicians, especially Karen Rasmussen, dietician Hanne Jensen, Berit Christensen and the other staff of the metabolic kitchen for their excellent contribution.

REFERENCES

- Levnedsmiddelstyrelsen, Danskernes kostvaner 1995. Hovedresultater. Sundhedsministeriet: LST, 1996.
- Wood R, Kubena K, O'Brien B, Tseng S, Martin G: Effect of butter, mono- and polyunsaturated fatty acid-enriched butter, trans fatty acid margarine, and zero trans fatty acid margarine on serum lipids and lipoproteins in healthy men. *J Lipid Res* 34:1–11, 1993.
- Cox C, Mann J, Sutherland W, Chisholm A, Skeaff M: Effects of coconut oil, butter, and safflower oil on lipids and lipoproteins in persons with moderately elevated cholesterol levels. *J Lipid Res* 36:1787–1795, 1995.
- Mann GV, Spoerry A: Studies of a surfactant and cholesteremia in the Maasai. *Am J Clin Nutr* 27:464–469, 1974.
- Antila M, Ali-Yrkkö S, Antila V, Antila P, Rönnemaa T, Järveläinen H, Viikari J: Is fat globule membrane essential for cholesterol-lowering effect of milk? [Letter]. *Lancet* 1(8168 Pt 1):602, 1980.
- Thompson LU, Jenkins DJA, Amer MAV, Reichert R, Jenkins A, Kamulsky J: The effect of fermented and unfermented milks on serum cholesterol. *Am J Clin Nutr* 36:1106–1111, 1982.
- Rossouw JE, Burger E-M, van der Vyver P, Ferreira JJ: The effect of skim milk, yogurt, and full cream milk on human serum lipids. *Am J Clin Nutr* 34:351–356, 1981.
- Marks J, Howard AN: Hypocholesterolaemic effect of milk [Letter]. *Lancet* 2(8041):763, 1977.
- Hepner G, Fried R, St Jeor S, Fusetti L, Morin R: Hypocholesterolemic effect of yogurt and milk. *Am J Clin Nutr* 32:19–24, 1979.
- Howard AN, Marks J: Effect of milk products on serum-cholesterol [Letter]. *Lancet* 1(8149):957, 1979.
- Bernstein BA, Richardson T, Amundson CH: Inhibition of cholesterol biosynthesis by bovine milk, cultured buttermilk, and orotic acid. *J Dairy Sci* 59:539–543, 1975.
- Nair CR, Mann GV: A factor in milk which influences cholesteremia in rats. *Atherosclerosis* 26:363–367, 1977.
- Schneeman BO, Rice R, Richter BD: Reduction of plasma and hepatic triacylglycerides with whole milk-containing diets in rats. *J Nutr* 119:965–970, 1989.
- Elwood PC, Burr ML, Bainton D, Yarnell JWG, Fehily AM, Baker IA: Progress Report VII 1991 MRC Epidemiology Unit, 1991.
- Anonymous: Milk, butter, and heart disease. *The Lancet* 337:607–608, 1991.
- Shaper AG, Wannamethee G, Walker M: Milk, Butter and Heart Disease [Letter]. *BMJ* 302:785–786, 1991.
- Steinmetz KA, Childs MT, Stimson C, Kushi LH, McGovern PG, Potter JD, Yamanaka WK: Effect of consumption of whole milk and skim milk on blood lipid profiles in healthy men. *Am J Clin Nutr* 59:612–618, 1994.
- Keim NL, Marlett JA, Amundson CH: The cholesterolemic effect of skim milk in young men consuming controlled diets. *Nutr Res* 1:429–442, 1981.
- Berner LA: Roundtable discussion on milkfat, dairy foods, and coronary heart disease risk. *J Nutr* 123:1175–1184, 1993.
- Roberts DCK, Truswell AS, Sullivan DR, Gorrie J, Darnton-Hill I, Norton H, Thomas MA, Allen JK: Milk, plasma cholesterol and controls in nutritional experiments [Letter]. *Atherosclerosis* 42:323–325, 1982.
- Fellows P: "Food Processing Technology. Principles and Practice," 2 ed. Cambridge: Woodhead Publishing Ltd, 2000.
- Tholstrup T, Marckmann P, Jespersen J, Sandström B: Fat high in stearic acid favorably affects blood lipids and factor VII coagulant activity in comparison with fats high in palmitic acid or high in myristic and lauric acids. *Am J Clin Nutr* 59:371–377, 1994.
- Ng TKW, Hassan K, Lim JB, Lye MS, Ishak R: Nonhypercholesterolemic effects of a palm-oil diet in Malaysian volunteers. *Am J Clin Nutr* 53:1015S–1120S, 1991.
- Kleman LP, Finley JW, Leveille GA: Estimation of the absorption coefficient of stearic acid in SALATRIM. *J Agricul Food Chem* 42:484–488, 1994.
- Yacowitz H, Fleischman AI, Bierenbaum ML: Effects of oral calcium upon serum lipids in man. *Br Med J* 5446:1352–1354, 1965.
- Bierenbaum ML, Fleischman AI, Raichelson RI: Long term human studies on the lipid effects of oral calcium. *Lipids* 7:202–206, 1972.
- Andersen NL, Fagt S, Groth MV, Hartkopp HB, Møller A, Ovesen L, Warming DL: Danish Dietary Habits 1995. Publ. No. 235 ed. Copenhagen: Danish Veterinary and Food Administration, 1996.
- Bostick RM, Fosdick L, Grandits GA, Grambsch P, Gross M, Louis TA: Effect of calcium supplementation on serum cholesterol and blood pressure. A randomized, double-blind, placebo-controlled, clinical trial. *Arch Fam Med* 9:31–38, 2000.
- Pereira DI, Gibson GR: Effects of consumption of probiotics and prebiotics on serum lipid levels in humans. *Crit Rev Biochem Mol Biol* 37:259–281, 2002.
- Agerholm-Larsen L, Bell ML, Grunwald GK, Astrup A: The effect of a probiotic milk product on plasma cholesterol: a meta-analysis of short-term intervention studies. *Eur J Clin Nutr* 54:856–860, 2000.
- Parks EJ, Hellerstein MK: Carbohydrate-induced hypertriglycerolemia: historical perspective and review of biological mechanisms. *Am J Clin Nutr* 71:412–433, 2000.
- Tholstrup T, Sandström B, Bysted A, Hølmer G: Effect of six dietary fatty acids on postprandial lipid profile, plasma free fatty acids, lipoprotein lipase and cholesterol ester transfer activities in healthy young men. *Am J Clin Nutr* 73:198–208, 2001.
- Karpe F: Postprandial lipid metabolism in relation to coronary heart disease. *Proc Nutr Soc* 56:671–678, 1997.
- Hunt JN, Knox MT: Regulation of gastric emptying. Heidel W (ed): "Handbook of Physiology." Washington DC: American Physiological Society, pp 1917–1936, 1968.
- Murphy MC, Isherwood SG, Sethi S, Gould BJ, Wright JW, Knapper JA, Williams CM: Postprandial lipid and hormone responses to meals of varying fat contents: modulatory role of lipoprotein lipase? *Eur J Clin Nutr* 49:579–588, 1995.

36. Carroll KK, Hamilton MG: Effects of dietary protein and carbohydrate on plasma cholesterol levels in relation to atherosclerosis. *J Food Sci* 40:18–23, 1975.
37. West CE, Deuring K, Schutte JB, Terpstra AHM: The effect of age on the development of hypercholesterolemia in rabbits fed semi-purified diets containing casein. *J Nutr* 112:1287–1295, 1982.
38. DiFrancesco L, Allen OB, Mercer NH: Long-term feeding of casein or soy protein with or without cholesterol in Mongolian gerbils. II. Plasma lipid and liver cholesterol responses. *Acta Cardiol.* 45:273–290, 1990.
39. Teixeira SR, Potter SM, Weigel R, Hannum S, Erdman Jr JW, Hasler CM: Effects of feeding 4 levels of soy protein for 3 and 6 wk on blood lipids and apolipoproteins in moderately hypercholesterolemic men. *Am J Clin Nutr* 71:1077–1084, 2000.
40. Sacks FM, Breslow JL, Wood PG, Kass EH: Lack of an effect of dairy protein (casein) and soy protein on plasma cholesterol of strict vegetarians. An experiment and a critical review. *J Lipid Res* 24:1012–1020, 1983.

Received September 9, 2002; revision accepted June 4, 2003.