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Different effects of whole milk and a fermented milk with the same fat and lactose content on gastric emptying and postprandial lipaemia, but not on glycaemic response and appetite

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Longitudinal studies indicate that milk and fermented milk products lower basal plasma cholesterol concentrations, despite their high content of saturated fat, and therefore have favourable health effects. However, there have been few studies on the postprandial effects of milk products. The present study compared the effect of whole milk with a fermented milk, A-38, on postprandial carbohydrate and lipid metabolism, gastric emptying and appetite. Eight healthy young men participated. On the two test days, they arrived fasting for collection of baseline values before consuming the meals, which for a 75 kg subject consisted of 1·4 litre milk or fermented milk, plus 165 mg [^{13}C]acetate (for later determination of gastric emptying by a [^{13}C]acetate breath test). Lactose (15 g) was added to the A-38 meal to equalize the lactose content. Postprandially the A-38 meal resulted in a slower gastric emptying rate than milk ($P<0·001$). Furthermore, the A-38 meal resulted in a greater increase and a quicker decrease of the triacylglycerol content in all lipoprotein fractions (LDL-fraction, $P<0·05$; other fractions, $P<0·001$) and of the gastrointestinal hormones (cholecystokinin and peptide YY, $P<0·05$; gastric inhibitory polypeptide and glucagon-like polypeptide-1, $P<0·001$). There were no significant differences in appetite sensations (measured by visual analogue scale) or in the glucose and insulin response ($P>0·10$). The slower emptying rate of the liquid phase after the A-38 meal is probably due to the higher viscosity of A-38. The lower and more prolonged triacylglycerol response after the milk meal might be caused by coagulation of milk in the stomach.

Milk: Fermented milk: Gastric emptying rate: Hunger: Satiety: Lipid metabolism: Insulin: Glucose

Diets high in saturated fat increase plasma cholesterol concentrations and thereby increase the risk for coronary artery disease (Keys, 1970; Rossouw *et al.* 1981; Mata *et al.* 1992). Since milk products are high in saturated fat, it is often assumed that milk increases plasma cholesterol and thereby the risk of heart disease (Jacobsen & Stensvold, 1992). However, Mann & Spoerry (1974) observed African Masais, who despite a diet high in saturated fat (mostly from milk or fermented milk and meat) had low concentrations of plasma cholesterol and a low incidence of CHD. It was suggested that milk products might contain a hypocholesterolaemic 'milk factor' (Mann & Spoerry, 1974; Rossouw *et al.* 1981). This resulted in a number of longitudinal studies in human subjects, in whom basal cholesterol concentrations were measured (Rossouw *et al.* 1981; Jacobsen & Stensvold, 1992; Agerbæk *et al.* 1995; Richelsen *et al.* 1996; Thompson *et al.* 1982), but only some studies confirmed the results of Mann & Spoerry (1974). However, animal studies were more consistent. Several milk constituents have been suggested to be the 'milk factor', but at present the evidence seems to suggest an interaction between several factors to explain the cholesterol-lowering effect of milk (for review, see Eichholzer & Stähelin, 1993).

Most studies concerning milk products have followed basal blood cholesterol concentrations over a long time. However, prolonged high postprandial triacylglycerol (TAG) levels seem to be atherogenic, since cholesteryl ester transfer protein transfers cholesteryl esters from HDL and LDL to TAG-rich lipoproteins in exchange for TAG in the opposite direction. This consequently leads to a reduction in HDL-cholesterol and more cholesterol-enriched VLDL remnants, which enhance the risk of CVD (Patsch *et al.* 1992; Frayn, 1993; Sethi *et al.* 1993; Havel, 1994; Austin, 1997; Karpe, 1997). In addition, blood glucose concentration influence the risk of CVD, since high blood glucose leads to glycation of lipoproteins, which enhances the atherogenic potential of LDL-particles (Frayn, 1993).

Abbreviations: A-38, lactic acid bacteria-fermented milk product; CCK, cholecystokinin; CRP, C-reactive protein; DOB value, delta over baseline value; GIP, gastric inhibitory polypeptide; GLP, glucagon-like peptide; PYY, peptide YY; TAG, triacylglycerol; VAS, visual analogue scale.

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Only a few studies have examined the postprandial effect of milk products. Strandhagen *et al.* (1994) found that fermented milk had a lower glycaemic response when compared with ordinary milk, which was explained by a lower rate of gastric emptying of the fermented product. The effect on lipid metabolism was not examined in the study, although differential effects would be expected from the different rates of gastric emptying and different insulin concentrations. Insulin plays an important role in postprandial lipid metabolism, since insulin, amongst other functions, stimulates the activity of lipoprotein lipase and thereby enhances the postprandial clearance of chylomicrons from the blood (Frayn, 1993). Furthermore, different sensations of satiety would be expected, as both gastric emptying and blood glucose and insulin concentrations act on the hypothalamus, which is involved in integrating satiety (Mayer & Thomas, 1967; Russek, 1970; Shimizu *et al.* 1983; Bergmann *et al.* 1992; Kaiyala *et al.* 1995; Jones *et al.* 1997). Knowledge about the physiological effect of milk products could provide beneficial information when planning diets for obese and diabetic patients for lowering the risk of CHD and diabetes.

The aims of the present study were, therefore, first to compare the effect of milk with a *Lactobacillus acidophilus*- and *Lactococcus cremoris*-fermented milk (A-38; a popular kind of yoghurt) on plasma TAG, glucose and insulin concentrations in human subjects. Second, a [^{13}C]acetate breath test was used to determine differences in gastric emptying rate between the test meals and to obtain further information regarding metabolic effects of the meals; gastrointestinal hormones were also measured. Finally, appetite sensations were recorded by using visual analogue scales (VAS).

Subjects and methods

Subjects

Eight non-smoking healthy young men (non-athletes) with a mean age 23·9 (SD 2·7) years and a mean BMI 22·8 (SD 1·2) kg/m^2 participated in the study. None had lactose intolerance, diabetes, hypertension, metabolic disorders nor infectious diseases such as hepatitis or HIV. Furthermore, on the day of the interview and during the two test days, all had normal concentrations of C-reactive protein (CRP, <5 mg/l) and Hb (8–11 mmol/l for men). None of the subjects were on daily medication. The protocol and aims of the study were fully explained to the subjects, who gave their written consent. The study was approved by the Scientific Ethics Committee of the Municipalities of Copenhagen and Frederiksberg.

Study design

The study had a randomized crossover design. The two intervention days were separated by a wash-out period of 3 months to allow the subjects to recover from their loss of blood on the first test day (350 ml per test day). Furthermore, four subjects started with milk and four started with the fermented milk (A-38) in order to eliminate seasonal variations, particularly because milk differs in fatty acid composition throughout the year (Posati *et al.* 1975).

Test meals

The test meals were prepared as individual servings and weighed in relation to body weight (milk (g) = actual body weight (kg)/75 (kg)) × 1400 (g milk)) at the experimental kitchen of the Research Department of Human Nutrition (The Royal Veterinary and Agricultural University, Frederiksberg, Denmark). For a 75 kg subject, the test meals consisted of 1400 g whole milk or 1400 g fermented whole milk, A-38, which had been fermented with a mixture of *Lactobacillus acidophilus* and *Lactococcus cremoris* (MD-foods, Viby J, Denmark). According to the Danish Food Composition tables, whole milk contains 35 g fat, 46·2 g lactose and 34 g protein/kg, while the corresponding values for A-38 are 35 g fat, 35·4 g lactose and 37 g protein/kg (Møller, 1996). Because of the different lactose contents in the two products, the test meal with A-38 was supplemented with 10·8 g lactose/kg A-38 (15·1 g/75 kg subject) to make the lactose content (and thereby the amount of easily digestible carbohydrate of the meals) equal in order to investigate specifically the effect of the physical properties of the milk fat. The lactose was dissolved in the minimum amount of water (87·5 g/75 kg subject), which the subjects drank with the A-38 test meal. To equalize the volume of the two test meals, the same amount of water (but no lactose) was given with the milk test meal. Just before the test meals were served, 165 mg sodium [^{13}C]acetate (Isotec Inc., Distributor: Campro Scientific, Veenendal, The Netherlands; catalogue no. 83-020-20-6) dissolved in 2·2 g water for a 75 kg subject (2·2 mg acetate/0·0294 g water per kg body weight) was gently mixed with the test meal, allowing subsequent determination of gastric emptying rate by a [^{13}C]acetate breath test. Table 1 shows the energy content and distribution of the test meals.

Experimental protocol

To minimize any effect of the diet eaten before the study days, the subjects were provided with food items for consumption on the day before each experimental day. For breakfast and lunch, the subjects could make their own choices, with the restriction that it should be a low-fat diet. In the afternoon they were given 100 g low-fat cake and for dinner they had 600 g of a pasta meal and bread. They were allowed to drink water *ad libitum*. The subjects were told to report the time and amount of all food items eaten and their physical activity on the day before the

Table 1. Energy content and energy distribution of the test meal for a subject weighing 75 kg*

	A-38	A-38 + lactose† test meal	Milk test meal
Fat (% energy)	48	45·2	50
Carbohydrate (% energy)	29	33·4	28
Protein (% energy)	23	21·4	22
Energy (kJ)	3864	4121	3724
Protein (g)	51·8	51·8	47·6
Fat (g)	49	49	49
Lactose (g)	49·6	64·7	64·7
Carbohydrate (g)	65·8	80·9	61·6

* Values calculated from the Danish Food composition tables (Møller, 1996).
† 15·1 g lactose (257 kJ) was added to the A-38 test meal.

test day and to repeat exactly the same pattern on the day before the second test day. They were further instructed to abstain from hard physical activity and not to drink alcohol for 2 d before each test day. On the test days the subjects arrived at 08.00 after a 12 h fast (they were allowed to drink 0·5 litres water) and were provided with a taxi to minimize physical activity.

After weighing the subjects, a Venflon catheter was inserted into an antecubital vein for blood sampling. The subjects rested in the supine position on a bed with the head slightly elevated, except during meal ingestion, at which time the subjects sat in the upright position. After 15 min rest, blood samples were taken for measurement of baseline concentrations of glucose, insulin, cholecystokinin (CCK), glucagon-like peptide (GLP)-1, gastric inhibitory polypeptide (GIP), peptide YY (PYY), CRP, Hb, NEFA, and the TAG and cholesterol contents of the lipoprotein fractions. In addition, a breath sample was taken for determination of the rate of gastric emptying. Furthermore, appetite sensations (inquiries concerning hunger, satiety, fullness and prospective consumption) were recorded by using VAS scores (100 mm VAS with words anchored at each end expressing the most extreme sensations, such as 'As hungry as I have ever felt' – 'Not at all hungry' as answer to the question: 'How hungry do you feel?' (Aitken, 1969; Hill *et al.* 1984). The subjects were told to consume the test meal within 5 min, which was achieved for the milk test meal (5·0 (SD 0·53) min), although some subjects had problems consuming the A-38 test meal in that time (6·5 (SD 2·7) min). The termination of the meal indicated *t* 0. Questionnaires to assess the palatability, taste, after-taste, smell and visual appeal of the test meals were completed by each subject immediately after the meal.

Postprandially, the subjects were observed for 8 h. Blood samples were taken for glucose and insulin every 15 min and for GIP, CCK, PYY and GLP-1 every 30 min in the first 2 h. Blood samples for GIP, CCK, PYY, GLP-1, NEFA, and the TAG and cholesterol content in the chylomicron-, VLDL-, LDL- and HDL-lipoprotein fractions were taken after 2, 3, 4, 6 and 8 h; further samples for insulin and glucose were taken 2, 4 and 8 h after the meal. At every blood sampling, appetite sensations were also recorded. Breath samples for determination of the rate of gastric emptying were obtained 5, 10, 20, 50, 80, 110, 150, 195, 255, 315, 465 min after the meal. The subjects stayed in the supine position for the first 5 h after the meal, but thereafter they were allowed to sit, walk quietly and go to the toilet. After the fifth hour, the subjects were further allowed to drink up to 1 litre water, but the total amount consumed was noted and repeated on the second test day.

Laboratory analyses

Blood was sampled without stasis through an indwelling antecubital cannula. Blood for NEFA, lipoprotein, insulin, CCK, GIP, GLP-1 and PYY analyses was collected in vacutainer tubes (Becton-Dickinson, Franklin Lakes, NJ, USA) containing EDTA; the tubes were placed on ice before and immediately after blood sampling and centrifuged at 2800 ***g*** for 15 min at 4°C. Blood for glucose was collected in sodium fluoride tubes and blood for CRP in plain tubes, which were centrifuged at 3000 rpm for 15 min at 20°C no later than 30 min after blood sampling. The plasma for NEFA determination was gassed with N_2 to avoid fatty acid oxidation and stored at − 80°C until analysis by an *in vitro* enzymic colorimetric method (Wako NEFA C-kit, code no. 994-75409E; Biofos Biosector A/S, Wako Chemicals GmbH, Neuss, Germany,) in a Cobas Mira plus analyser (Roche, Basel, Switzerland). Plasma for analysis of the TAG and cholesterol in the respective lipoprotein fractions was stored at 4°C and analysed within 48 h. First, the three fractions (chylomicrons, VLDL, and LDL + HDL) were isolated by ultracentrifugation as previously described (Tholstrup *et al.* 2001), then stored with the rest of the plasma at 4°C until their cholesterol and TAG contents were assessed by enzymic procedures on the Cobas Mira Plus analyser (Roche). For determination of cholesterol concentration, a cholesterol monotest, CHOD-PAP MPR3 kit (catalogue no. 236691; Boehringer Mannheim, Mannheim, Germany) (Trinder, 1969; Siedel *et al.* 1983; Kattermann *et al.* 1984) was used; for TAG determination, a Test-combination TAG GPO-PAP MPR2 kit (catalogue no. 701912; Boehringer Mannheim) (Trinder, 1969; Eggstein & Kuhlmann, 1974; Wahlefeld, 1974) was used. The TAG and cholesterol concentrations in the HDL-fraction were measured enzymically after precipation of plasma with PEG (Quantolip A, art. no. 8251015; Immuno AG Danmark A/S, Copenhagen, Denmark) (Kostner *et al.* 1985). Finally, the concentration in the respective ultracentrifugation lipoprotein fractions were corrected for dilution and the content in the LDL-fraction was calculated as the difference between the HDL + LDL-fraction and the precipated HDL-fraction.

EDTA-plasma for insulin analysis was stored at − 20°C until analysis by an ELISA insulin kit from Dako (code no. K6219; Dako, Glostrup, Denmark) (Gallati & Pracht, 1985; Clark & Hales, 1991; Andersen *et al.* 1993). EDTA-plasma for CCK, GIP, GLP-1 and PYY was stored at − 20°C. CCK (Rehfeld, 1998), GIP and GLP-1 (Krarup *et al.* 1983; Ørskov & Holst, 1987; Holst & Bersani, 1991; Deacon *et al.* 2000) were determined by RIA on plasma extracted with ethanol as previously described. The plasma concentration of PYY was also measured by RIA as previously described (Holst & Bersani, 1991; Näslund *et al.* 1997, 1999), but without extraction of plasma. Plasma for determination of glucose was stored at − 20°C until analysis by an enzymic colorimetric method (hexokinase–glucose-6-dehydrogenase method) using a kit (Gluco-Quant glucose, MPR3, catalogue no. 1442457) from Boehringer Mannheim and the Cobas Mira plus analyser. Serum for CRP was stored at − 20°C until analysis by an immunoturbidimetric method (Roche; T antiserum test-kit, art. no. 0721840, accelerator 1, art. no. 0721867, T standard CRP, art. no. 0737224) designed for analysis by the Cobas Mira plus analyser (Roche).

Gastric emptying

For determination of gastric emptying, a [^{13}C]acetate breath test, which is an indirect method based on the oxidation of the ^{13}C[acetate] to $^{13}CO_2$ was used. The [^{13}C]acetate was added to the test meals as mentioned earlier, and after gastric emptying, absorption and metabolism, it was recovered

in the expired air as $^{13}CO_2$. The time for absorption and metabolism of acetate after gastric emptying is rapid, thus the time of peak excretion of $^{13}CO_2$ can be used as an estimate for the rate of gastric emptying (Braden *et al.* 1995). For each sampling time, two samples of expired air were collected in 10 ml gas sample tubes from Isochem (Finchamstead, Berks., UK) and stored at room temperature, protected from daylight, until analysis. Breath samples were analysed for the $^{13}CO_2$ content by an isotope ratio MS (stable isotope detector connected to a computer; Tracermass, Europa Scientific, Cheshire, UK) with an on-line GC system (RoboPrep-G, Europa Scientific) (carrier gas He; capillary column: Pore/Pack Q, length 25 m, inner diameter 0·32 mm, flow 1 ml/min). The MS had collectors, which only measured particles with m/z ratios of 44 ($^{12}CO_2^+$) and 45 ($^{13}CO_2^+$). For calibration, a $CO_2 - N_2$ (5·6, 94·4, v/v) standard (Strandmøllen, Industrigas A/S, Klampenborg, Denmark) was used. The $^{12}CO_2/^{13}CO_2$ fraction in each breath sample was calculated as the δ value, as follows (Croset *et al.* 1995):

$$\delta\,^{13}C\ (‰) = \frac{\left(^{13}CO_2/^{12}CO_2\right)_{sample} - \left(^{13}CO_2/^{12}CO_2\right)_{standard}}{\left(^{13}CO_2/^{12}CO_2\right)_{standard}} \times 1000.$$

In order to correct for naturally occurring $^{13}CO_2$, all the sample δ values were subsequently related to the baseline (fasting) value, and expressed as delta over baseline (DOB) values.

In vitro *testing*

For visualization of how the test meals reacted in the stomach, the test meals (weighed out to correspond to 0·5 % of a test meal for a 75 kg person) were gently mixed with 5 ml gastric juice (pH 1·98, collected by a catheter from a healthy volunteer after a 12 h fast) in a 100 ml centrifugation tube, covered with parafilm and incubated for 1 h under constant shaking with twenty-nine strokes per min at 37°C. The procedure was then repeated, but without shaking. The appearance of the mixture was noted at the start and after 1 h.

Statistical analyses

All results are given as mean values and their standard errors unless otherwise stated. Results are presented as changes from the concentrations at baseline of the respective variables (glucose, insulin etc.) and analysed by a two-factor repeated-measures ANOVA with a factor for subject and a factor for meal, with subsequent Huynh–Feldt epsilon adjustment of degrees of freedom (SPSS version 7.5 for Windows; SPSS Inc., Chicago, IL, USA). The level of significance was set at $P<0·05$. Differences in baseline values and in the subjects' evaluation of the test meals by VAS scores were analysed using a paired *t* test.

Results

Gastric emptying

The rate of gastric emptying was significantly slower after the A-38 test meal, illustrated by the delayed peak excretion of $^{13}CO_2$ after the A-38 meal compared with the milk meal (time × meal interaction $P<0·001$). The excretion of $^{13}CO_2$ peaked at 110–150 min after the milk meal and at 195 min after the A-38 test meal as illustrated in Fig. 1.

In vitro *testing*

Directly after mixing the milk test meal with gastric juice, the pH of the mixture was 5·61 and the mixture turned gritty and curdled immediately. After shaking (twenty-nine strokes per min) for 1 h, a large coagulum was precipitated at the bottom of the centrifugation tube, and small particles were floating in the separated fluid. The same reaction was observed by incubating the milk test meal without shaking for 1 h, except that the coagulum was floating at the top of the fluid. When A-38 was mixed with gastric juice, pH was 3·98 and the mixture appeared slightly gritty, but no curdling was seen. After 1 h of shaking (twenty-nine strokes per min) the mixture was still coherent (no coagulum), except for a small amount of liquid separation at the bottom of the tube. The same pattern was observed after incubation without shaking.

Cholecystokinin, gastric inhibitory polypeptide, glucagon-like peptide-1 and peptide YY concentrations in plasma

There were no differences in baseline values between the test days (Table 2). However, we observed differences of interactions between meal and time for the changes from basal plasma concentration of CCK ($P<0·05$), GIP and GLP-1 ($P<0·001$) and PYY ($P<0·025$). After meal ingestion the plasma concentration of CCK increased, peaking

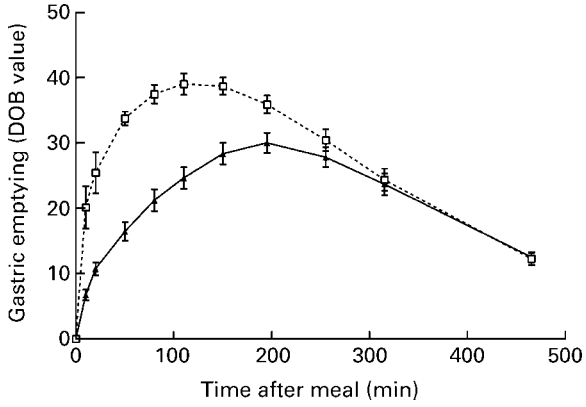

Fig. 1. Postprandial delta over baseline (DOB) values after the milk test meal and the A-38 test meal, used to estimate the rate of gastric emptying. —□—, Milk; –▲–, A-38. For details of test meals, subjects and procedures, see Table 1 and pp. 448–449. Values are means (expressed as differences from fasting values) with their standard errors shown by vertical bars. Until 195 min *n* 8, 255 min *n*7, 315 min *n* 6, 465 min *n* 5. The variability in subject number is caused by the fact that at the beginning of the study we had to localize the time for peak-excretion of $^{13}CO_2$, and only breath samples until 245, 305 and 315 min respectively on the first study day for the first three subjects were taken. As a consequence only comparative results (both test days) for five subjects for all 465 min were available. Mean values for all accessible results are shown in the figure, but the two-factor repeated measures AVOVA was performed on data from five subjects for 465 min. The meal × time effect was significant ($P<0·001$).

Table 2. Baseline values on the two test days*
(Mean values and standard deviations for eight subjects)

	Milk		A-38		Statistical significance of effect: *P*
	Mean	SD	Mean	SD	
Total TAG	1·14	0·32	1·07	0·29	0·5887
Chylomicron-TAG	0·286	0·12	0·274	0·16	0·8515
VLDL-TAG	0·533	0·19	0·504	0·13	0·6842
LDL-TAG	0·135	0·04	0·120	0·03	0·2388
HDL-TAG	0·183	0·04	0·178	0·04	0·6059
Total cholesterol	4·33	0·74	4·48	0·82	0·3751
Chylomicron-cholesterol	0·086	0·04	0·085	0·04	0·9574
VLDL-cholesterol	0·304	0·11	0·311	0·12	0·8725
LDL-cholesterol	2·900	0·58	3·024	0·63	0·3822
HDL-cholesterol	1·043	0·23	1·126	0·29	0·1191
CCK	0·450	0·33	0·738	0·42	0·1498
GIP	5·625	2·00	5·625	3·02	1·0000
GLP-1	14·380	7·76	16·380	6·74	0·3251
PYY	9·250	4·23	9·750	3·62	0·7812
Plasma glucose	5·199	0·31	5·214	0·34	0·8292
Insulin	23·750	6·70	26·500	6·91	0·1622
NEFA	0·540	0·22	0·437	0·19	0·2899
Satiety	25·00	10·4	27·13	26·3	0·8319
Hunger	72·00	15·99	66·31	16·34	0·6061
Fullness	31·25	21·78	26·13	19·28	0·6466
Prospective consumption	71·25	9·52	7·00	15·67	0·8750

* For details of subjects and procedures, see pp. 448–449.
TAG, triacylglycerol; CCK, cholecystokinin; GIP, gastric inhibitory polypeptide; GLP, glucagon-like peptide; PYY, peptide YY.

after about 60–90 min, where after it started to fall for both test meals (Fig. 2). Immediately after meal ingestion, the A-38 meal seemed to result in a slightly greater increase in CCK concentrations compared with milk, but about 240 min, there was a crossover, after which the milk meal resulted in higher concentrations than A-38. The CCK concentrations had still not reached the baseline at 480 min after the milk meal, as opposed to the A-38 meal, where the baseline was reached shortly after 360 min. After meal ingestion, GIP increased to a plateau (30–120 min), which was twice as high after the A-38 meal compared with the milk meal, but after 240 min the lines crossed, whereafter the milk test meal resulted in higher GIP concentrations than A-38. The GIP concentration reached baseline 480 min after the A-38 meal, unlike after milk, where GIP was still elevated at that time. Plasma GLP-1 and PYY concentrations both followed the same pattern as described for CCK.

Glucose, insulin and NEFA

There were no differences in baseline values between the test days (Table 2). The mean changes from basal values of postprandial concentrations of glucose, insulin and NEFA are shown in Fig. 3. There were no significant meal × time interactions for glucose, insulin or NEFA ($P > 0{\cdot}10$) and no effect of type of meal ($P > 0{\cdot}10$), but there were effects over time for glucose, insulin and NEFA. After meal ingestion, the concentration of glucose increased initially to a peak after 15 min, decreased below fasting values after 30 min and increased to baseline value at 240 min for both meals (time effect $P < 0{\cdot}001$). For both test meals, insulin concentrations increased to a peak after 30 min, and thereafter returned to baseline after 240 min (time effect $P < 0{\cdot}005$). NEFA decreased initially from 0 to 2 h for both meals and increased thereafter to baseline at 480 min (time effect $P < 0{\cdot}001$).

Triacylglycerol in the lipoprotein fractions

There were no differences in baseline values between the test days (Table 2). We observed a significant difference for meal × time interactions for the changes from baseline values of plasma concentrations of total, chylomicron-, VLDL- and HDL-TAG ($P < 0{\cdot}001$) as well as LDL-TAG ($P < 0{\cdot}05$). The concentrations for total, chylomicron- and VLDL-TAG peaked 3 h after the A-38 meal compared with a smaller increase with a peak after 6 h for the milk meal; however, the lines crossed 6 h after the meal, whereafter the milk meal had higher values than for A-38 (Fig. 4). Plasma LDL-TAG peaked 6 h after the A-38 meal compared with a lower peak value 8 h after the milk meal. Plasma HDL-TAG peaked 6 h after the A-38 meal, while the milk meal produced an initial decrease, followed by a return to baseline. Total, chylomicron-, VLDL- and HDL-TAG concentrations returned to baseline values before 8 h after the A-38 meal, as opposed to the milk meal, for which total, chylomicron- and VLDL- TAG concentrations still had not reached baseline values.

Cholesterol in the lipoprotein fractions

There were no differences in baseline values between the test days (Table 2). We observed significant meal × time interactions for the changes from baseline values of plasma concentrations of chylomicron-, VLDL-cholesterol

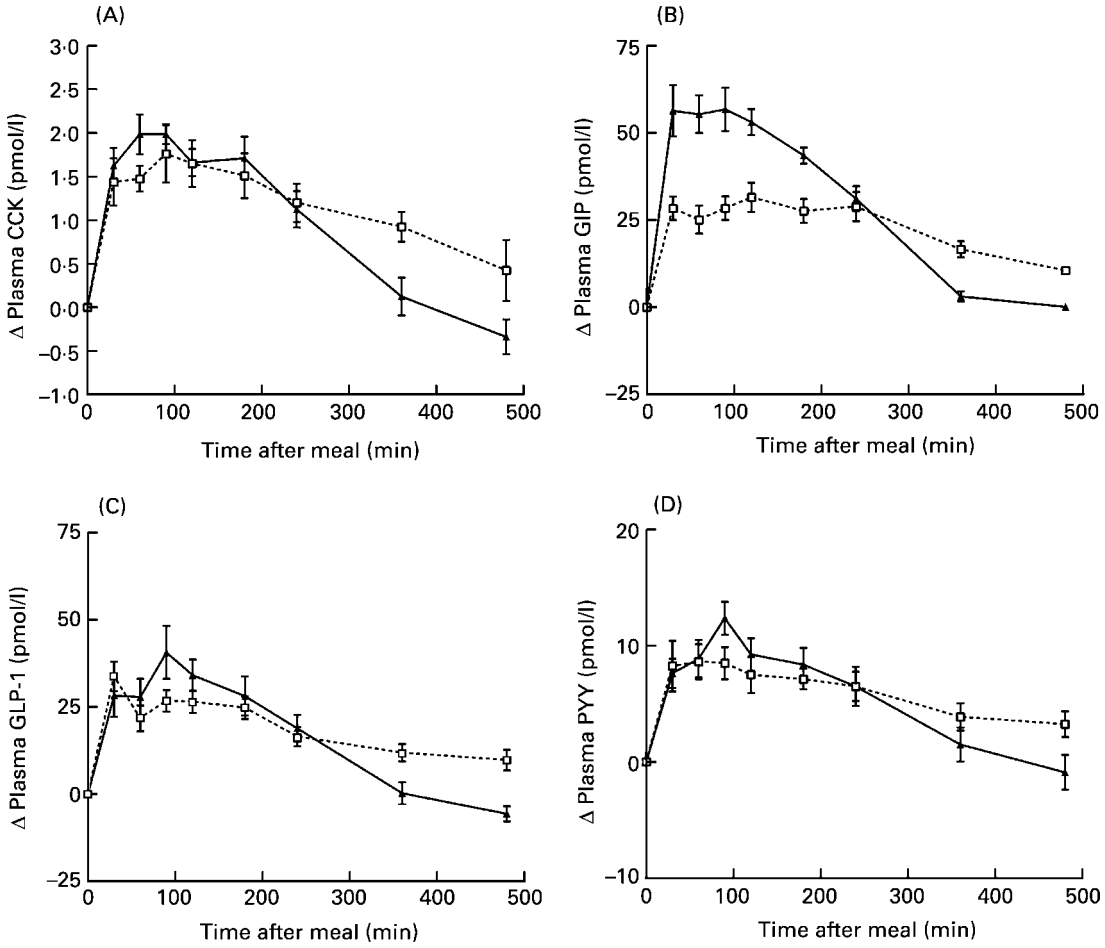

Fig. 2. Postprandial plasma concentrations of cholecystokin (CCK, (A)), gastic inhibitory peptide (GIP, (B)), glucagon-like peptide (GLP)-1 (C) and PYY (peptide YY, (D)) after the milk and A-38 test meals. –□–, Milk; –▲–, A-38. For details of test meals, subjects and procedures, see Table 1 and pp. 448–449. Values are means (expressed as differences from fasting values for eight subjects) with their standard errors shown by vertical bars. Meal × time effects were significant for CCK ($P<0·05$), GIP ($P<0·001$), GLP-1 ($P<0·001$) and PYY ($P<0·025$).

($P<0·005$) and HDL-cholesterol ($P<0·01$). Plasma chylomicron-cholesterol peaked 4 h after both test meals, with the highest peak value for the A-38 test meal; however, the lines crossed 6 h after the meal, whereafter the milk meal had higher values than A-38 (Fig. 5). Plasma VLDL-cholesterol peaked 3 h after the A-38 meal and 6 h after ingestion of milk, with the lines crossing at about 5 h. After the A-38 meal plasma HDL-cholesterol concentration decreased to a minimum at 4 h, then it began to increase; after the milk meal the concentration was initially constant for 4 h, then the concentration began to increase. For plasma total and LDL-cholesterol there were no significant meal × time effects ($P>0·10$) or effects of meal type ($P>0·10$), but both showed an effect over time ($P<0·001$). Both increased to a plateau (2–4 h) after meal ingestion, then they increased further.

Palatability and appetite ratings

Palatability ratings were not significantly different for the two meals (visual appeal $P=0·22$, smell $P=0·82$, taste $P=0·74$, aftertaste $P=0·67$, overall palatability $P=0·86$). Concerning appetite ratings (Figs. 6 and 7), there were no significant differences in meal × time interactions for the changes from baseline values of the VAS scores for satiety, hunger, prospective consumption, fullness and desire to eat some salt, 'fish or meat' (protein), sweets (carbohydrate) and fat ($P>0·10$) or in the effect of meal type ($P>0·10$) were observed, but there was an effect over time in all ratings ($P<0·001$ (salt-scores $P<0·025$)). There were no differences in baseline values between the test days (Table 2).

Discussion

The present study shows that the two milk products with the same fat content resulted in a different postprandial lipid profile. As expected, we found the whole milk resulted in a faster gastric emptying rate than A-38, but surprisingly, the milk meal resulted in a slower, lower and more long-lasting rise in TAG in the blood than A-38.

Effects on gastric emptying rate

The milk meal had a faster gastric emptying rate than the fermented milk (Fig. 1), which agrees with previous results (Strandhagen *et al.* 1994). With respect to factors that may affect the gastric emptying rate, the milk and A-38 meals

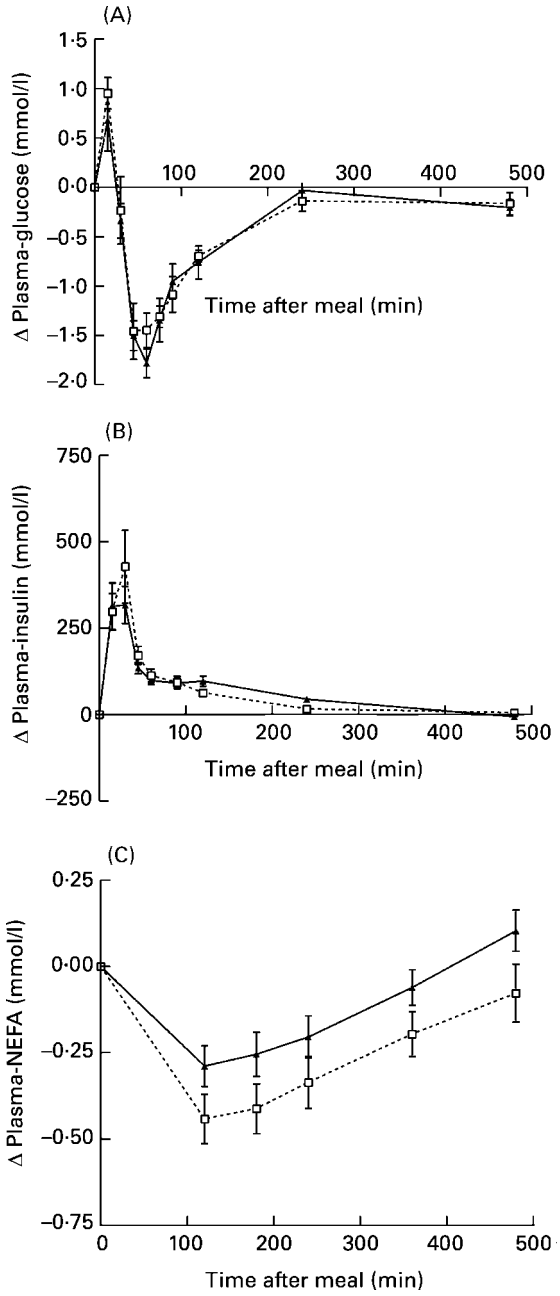

Fig. 3. Postprandial plasma concentrations of glucose (A), insulin (B) and NEFA (C) after the milk and A-38 test meals. —□—, Milk; –▲–, A-38. For details of test meals, subjects and procedures, see Table 1 and pp. 448–449. Values are means (expressed as differences from fasting values for eight subjects for all groups, except that there were seven subjects for glucose and insulin at 15 min) with their standard errors shown by vertical bars. The time effect was significant for glucose ($P<0{\cdot}001$), insulin ($P<0{\cdot}005$) and NEFA ($P<0{\cdot}001$). The effect of type of meal was not significant ($P>0{\cdot}10$). The 15 min values for insulin and glucose were missing for one subject, so only data from seven subjects were analysed by two-factor repeated measures ANOVA.

were similar in volume, chemical composition and energy content, but differed in viscosity and pH (Ehrlein & Pröve, 1982; Hunt *et al.* 1985; Ebihara *et al.* 1989; Urbain *et al.* 1989; Velchik *et al.* 1989; Ganong, 1991; Cullen & Kelly, 1993; Liljeberg & Björck, 1996). Increased viscosity (Ehrlein & Pröve, 1982) and low pH (Ebihara *et al.* 1989) both decrease gastric emptying rate, which could explain the slower emptying rate after the fermented milk meal.

A mixed meal normally consists of a mixture of liquid, solids and fat, and because of their different physical properties, an intragastric separation into three different phases occurs; liquid, fat and solid. Therefore, different gastric emptying rates for the three phases must be expected (Cortot *et al.* 1981). Several studies have shown that the solid and fat phase empty more slowly than the liquid phase (Meyer *et al.* 1986; Houghton *et al.* 1988; Siegel *et al.* 1988; Collins *et al.* 1991; Meyer, 1991; Edelbroek *et al.* 1992). Since the marker [^{13}C]acetate, according to Braden *et al.* (1995), follows the liquid phase, the rate of gastric emptying measured in our present study probably corresponds to the rate of gastric emptying of the liquid phase in the test meals.

Effects on blood glucose and insulin concentrations

We observed no difference in the plasma concentration of glucose and insulin after milk and fermented milk, A-38 (Fig. 3), which is surprising as Strandhagen *et al.* (1994), who compared whole milk and a *Lactococcus*-fermented milk, observed lower glucose and insulin levels caused by a slower gastric emptying rate after the fermented milk test meal.

The experimental designs in the study of Strandhagen *et al.* (1994) and our present study are similar. However, Strandhagen *et al.* (1994) added the lactose to the fermented milk as a powder and mixed it in the meal the day before the test day, which could have resulted in bacterial fermentation of some of the lactose in the fermented product, which could explain the lower glucose and insulin response after the fermented meal. Another explanation is that, since the lactose in our present experiment was dissolved in a glass of water and given together with the A-38 test meal and not mixed in the fermented milk, the lactose solution was emptied quicker from the stomach than the lactose in A-38, and thereby might have counteracted a possible difference in glucose and insulin concentrations that could have been caused by differences in viscosity.

Our present study was designed to investigate the effect of the physical state of the milk fat in whole milk and in the fermented milk modified with lactose to resemble whole milk with respect to carbohydrate. The results regarding postprandial glucose and insulin are, therefore, not fully representative for unmodified fermented milk. However, the different physical properties of the products did not seem to have different effects on carbohydrate metabolism in our present study.

Effect on postprandial lipid profile

Based on the results of Strandhagen *et al.* (1994), the A-38 test meal was expected to result in a slower rise in TAG due to the slower gastric emptying rate and to have a slower clearance from the blood due to the lower insulin level. However, in our present study A-38 had a slower gastric emptying, but resulted in a faster increase and higher peak concentration of TAG in the blood, compared with the

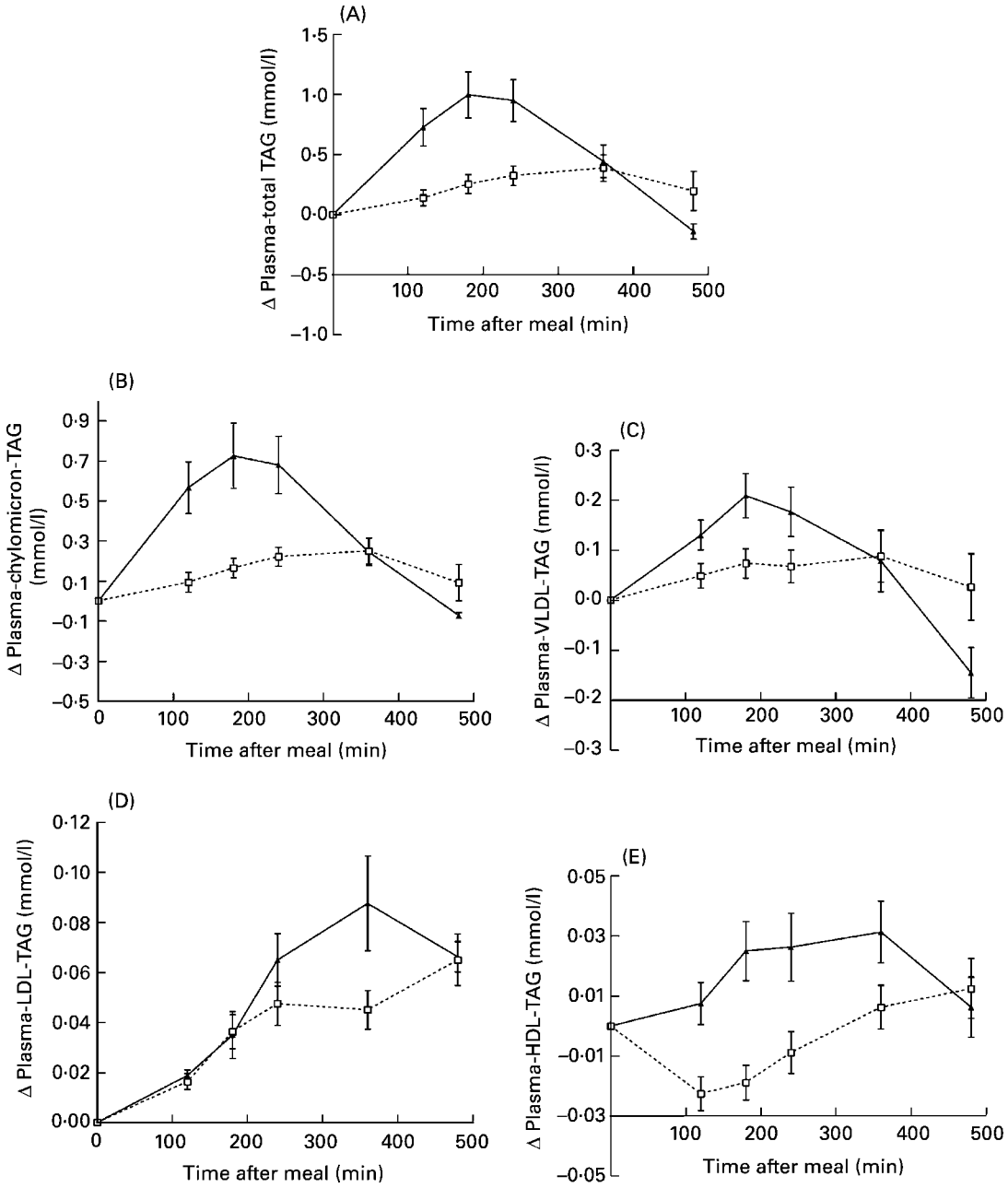

Fig. 4. Postprandial plasma concentrations of triacylglycerol (TAG) in the respective lipoprotein fractions after the milk and A-38 test meals. –-□–-, Milk; –▲–, A-38. For details of test meals, subjects and procedures, see Table 1 and pp. 448–449. Values are means (expressed as differences from fasting values for eight subjects) with their standard errors shown by vertical bars. Meal × time effects were significant for total TAG ($P<0·001$), chylomicron-TAG ($P<0·001$), VLDL-TAG ($P<0·001$), LDL-TAG ($P<0·05$) and HDL-TAG ($P<0·001$).

milk test meal (Fig. 4). The pattern of the TAG was mimicked in chylomicron- and VLDL-cholesterol (Fig. 5).

Variations in the rate of rise of the postprandial lipaemia may reflect variations in fat absorption and other gastrointestinal functions (Havel, 1997). As we determined the rate of gastric emptying of the liquid phase and not of the fat phase, the fat in the milk meal might empty more slowly from the stomach than the fat in the A-38 meal. The finding that the gastrointestinal hormones, which are all stimulated by fat in the intestine (Sykes *et al.* 1980; Douglas *et al.* 1988, 1990; Taylor & Mannon, 1991; Ørskov, 1992; Walsh, 1994; Holst, 1996; Morgan, 1996; Pedersen-Bjeregaard *et al.* 1996) remained elevated for a longer time after the milk meal (Fig. 2), further supports a more long-lasting stimulation with fat. An explanation for the restraining effect of the fat from the milk meal in the stomach could be that milk coagulates in the stomach, paralleling cheese production, where caseins in the milk are precipitated by addition of acid or rennin (enzymes from calf's stomach), consisting of chymosin or pepsin. Chymosin hydrolyses the κ-casein. In turn, the κ-casein no longer inhibits the precipitation of α̃ and β-casein, causing them to aggregate and precipitate at their isoelectric point (pH 4·6). Subsequently, both types of coagulum will gather and excrete whey. The

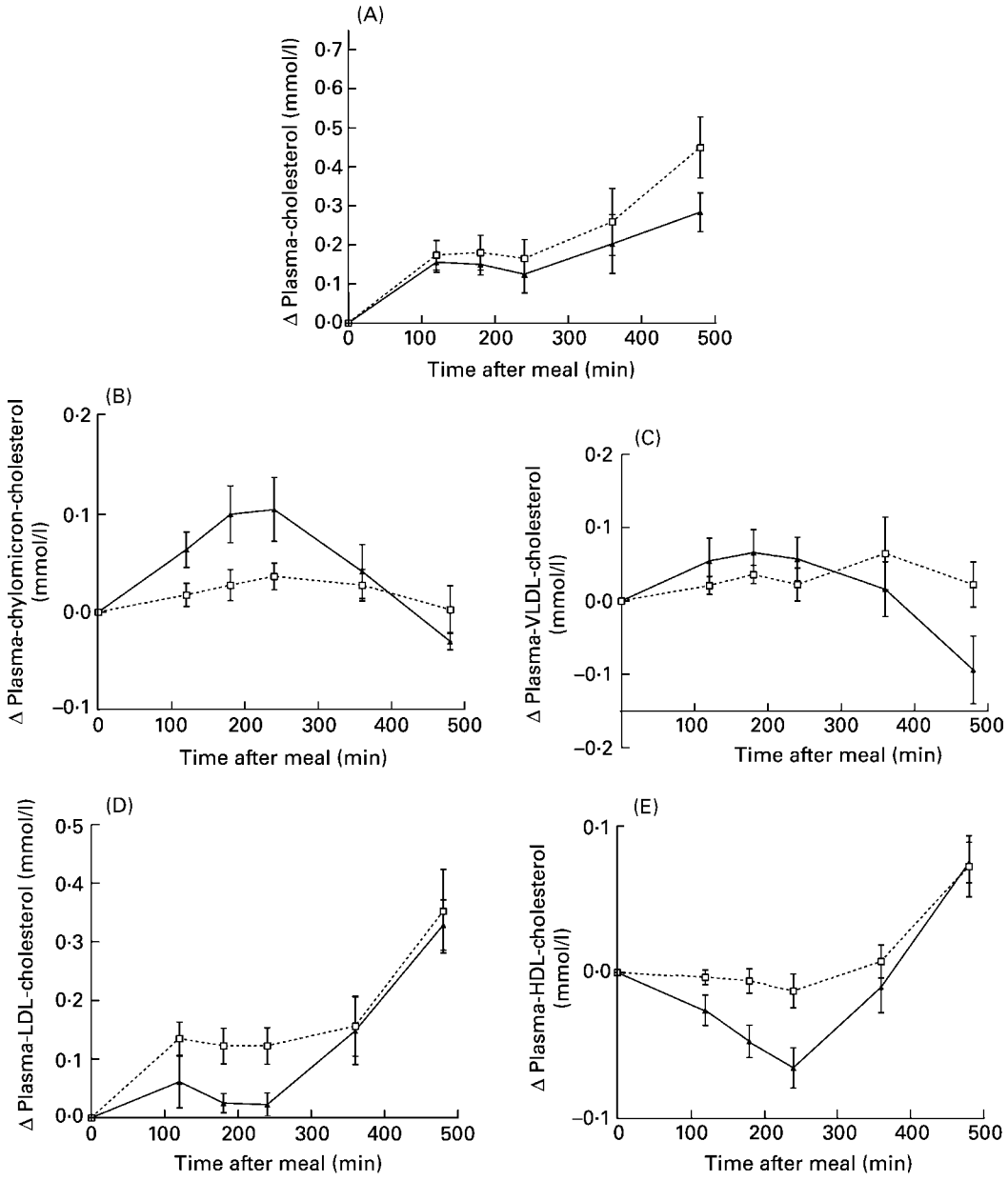

Fig. 5. Postprandial plasma concentrations of cholesterol in lipoprotein fractions after the milk and A-38 test meals. –-□–-, Milk; –▲–, A-38. For details of test meals, subjects and procedures, see Table 1 and pp. 448–449. Values are means (expressed as differences from fasting values for eight subjects) with their standard errors shown by vertical bars. Meal ×time effects were significant for chylomicron-cholesterol ($P<0{\cdot}005$), VLDL-cholesterol ($P<0{\cdot}005$) and HDL-cholesterol ($P<0{\cdot}01$). Time effects for total and LDL-cholesterol were significant ($P<0{\cdot}001$). The effects of meal type were not significant ($P>0{\cdot}10$).

milk fat particles will be confined in the coagulum, while dissolved substances (lactose, whey proteins) are emptied with the whey (Nielsen, 1983).

Human subjects are believed not to have a functional chymosin–protease in the stomach (Foltmann, 1992), but they have pepsin A and C (Foltmann, 1986), which cleave the κ-casein at the same position as chymosin (Drøhse & Foltmann, 1989). In the stomach, the pH is also low, and it is reasonable to believe that milk coagulates in the stomach, which was confirmed by our *in vitro* experiment. The milk test meal coagulated and separated fluid, where the coagulum probably consisted of precipitated casein with confined fat particles, whereas the separated fluid consisted of whey. The liquid phase with dissolved lactose and whey proteins probably has a faster emptying rate than the fat phase, and as the [^{13}C]acetate follows the liquid phase (Braden *et al.* 1995), it could explain the faster gastric emptying rate after the milk meal, even though the fat remains in the stomach in the coagulum, which only empties slowly. In contrast, the A-38 test meal did not coagulate when mixed with gastric juice and remained as a homogeneous fluid, probably indicating that it is already coagulated during production. Therefore, A-38 was probably emptied from the stomach as a mixture of fat, lactose and protein, which emptied more quickly than the coagulum consisting of fat and protein in the milk meal.

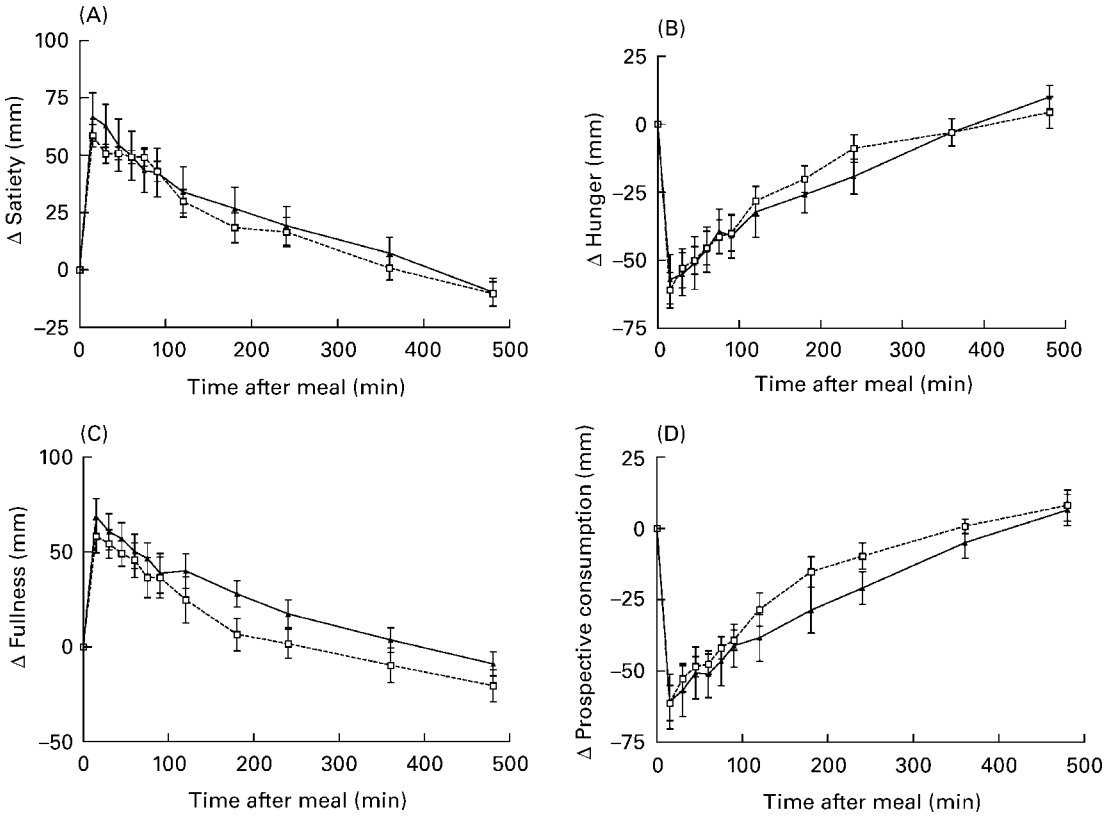

Fig. 6. Postprandial visual analogue scale scores for satiety (A), hunger (B), fullness (C) and prospective consumption (D) after the milk and A-38 test meals. –-□–-, Milk; –▲–, A-38. For details of test meals, subjects and procedures, see Table 1 and pp. 448–449. Values are means (expressed as differences from fasting values for eight subjects) with their standard errors shown by vertical bars. Time effects were significant for satiety ($P<0{\cdot}001$), hunger ($P<0{\cdot}001$), fullness ($P<0{\cdot}001$) and prospective consumption ($P<0{\cdot}001$). The effects of type of meal were not significant ($P>0{\cdot}10$).

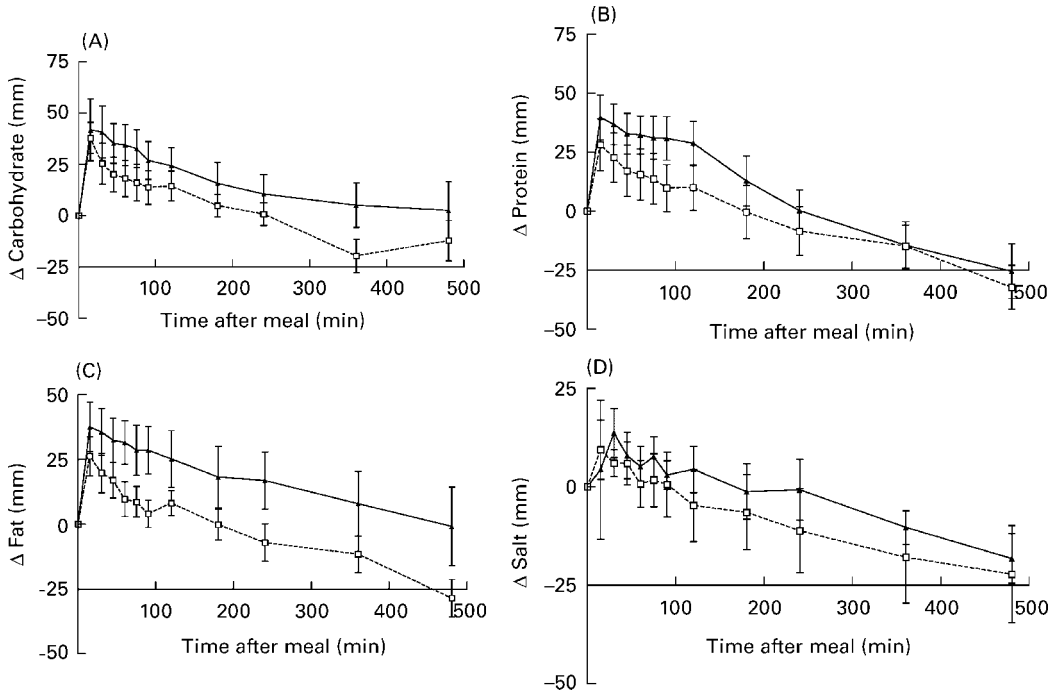

Fig. 7. Postprandial visual analogue scale scores for desire to eat carbohydrate (A), protein (B), fat (C) and salt (D) after the milk and A-38 test meals. –-□–-, Milk; –▲–, A-38. For details of test meals, subjects and procedures, see Table 1 and pp. 448–449. Values are means (expressed as differences from fasting values for eight subjects) with their standard errors shown by vertical bars. Time effects were significant for protein ($P<0{\cdot}001$), carbohydrate ($P<0{\cdot}001$), fat ($P<0{\cdot}001$) and salt ($P<0{\cdot}025$). The effects of type of meal were not significant ($P>0{\cdot}10$).

The physiological effect of coagulation of milk in the stomach could be to delay the passage of chyme from the stomach to the duodenum (Nielsen, 1983; Foltmann, 1992).

In the proximal intestine, the secretion of GIP is stimulated by fat and carbohydrate (Morgan, 1996; Sykes *et al.* 1980; Taylor & Mannon, 1991), while CCK secretion is stimulated by protein and fat (Douglas *et al.* 1988, 1990). GIP increased to a higher level after the A-38 meal, corresponding with stimulation by both fat and lactose. After the milk meal, GIP only increased half as much, corresponding to an initial stimulation with lactose only, whereas the more long-lasting stimulation could be due to the slow gastric emptying of fat. That CCK increased to the same peak level after both meals might be due to whey proteins in the fluid phase of the milk meal.

The faster clearance of TAG after the A-38 meal might be explained by a greater activation of the lipoprotein lipase. This is not caused by insulin, as the insulin concentrations were similar after the two meals. However, GIP has previously been shown to result in an increased clearance of chylomicron-TAG by stimulating the lipoprotein lipase activity (Eckel *et al.* 1979; Wasada *et al.* 1981). The 'plateau' level for GIP was twice as high after the A-38 meal, which could explain the greater clearance of TAG. However, the primary explanation for the longer presence of lipids in the blood after the milk meal is probably a prolonged release from the stomach.

Evaluation of the lipid profile

After a fat-rich meal, patients with CHD showed a greater increase in TAG, a later peak and a more long-lasting presence of lipids in the blood compared with normal subjects (Patsch *et al.* 1992; Austin, 1997; Karpe, 1997). An increased rise in TAG will, via cholesteryl ester transfer protein, cause an increased exchange of cholesteryl esters from LDL and HDL to TAG-rich particles in exchange for TAG. In this manner, high postprandial TAG levels cause a reduction in HDL-cholesterol, which is inversely correlated to the development of CHD (Patsch *et al.* 1992). Our present study indicates that after the A-38 meal, there was an increased transfer of cholesteryl esters from HDL to the chylomicron and VLDL fractions in exchange for a transfer of TAG from chylomicrons and VLDL to HDL. The A-38 meal resulted in a greater increase in HDL-TAG and a decrease in HDL-cholesterol until the TAG content in the chylomicron and VLDL-fraction peaked. Thus, the A-38 meal resulted in a decrease in HDL-cholesterol, in contrast to a constant level after the milk meal, until the TAG levels began to decrease. On the other hand, milk resulted in a more long-lasting postprandial lipaemia, which is also associated with the development of CHD (Patsch *et al.* 1992; Karpe, 1997). Thus, whether milk or A-38 are more beneficial to health cannot be concluded from our present results.

Effects on appetite

The milk and A-38 meals had similar effect on appetite sensations and on the subjects' evaluation of the test meals. Since the glucose and insulin concentrations in plasma were also similar after the two meals, this is in accordance with the glucostatic theory, according to which: (1) the sensation of satiety correlates with the glucose utilization in glucose sensitive neurons in the satiety centre in the hypothalamus (Mayer & Thomas, 1967); (2) increased glucose level in the blood mediates satiety (Shimizu *et al.* 1983; Russek, 1970).

Gastric emptying is known to affect the sensation of satiety, and as the gastric emptying rates are different after the two test meals, differences in appetite sensations would have been expected. However, Jones *et al.* (1997) found the sensations of fullness to be related to the area of the antrum, but not to the total gastric emptying rate. With respect to hunger sensations, no correlation was found with either the area of antrum or the total gastric emptying rate. In addition, the gastrointestinal hormones, GIP, GLP-1 (Lavin & Read, 1995) and CCK influence satiety (Moran & McHugh, 1988; Holt *et al.* 1992; Kaiyala *et al.* 1995). We observed different levels of CCK, GIP and GLP-1, but this did not seem to influence the appetite sensations in our present study. It could be argued that eight subjects are not enough to give valid results for VAS scores. However, according to Flint *et al.* (2000), only eight subjects are needed for valid testing of mean postprandial values in a paired design such as our present study. For comparison of fasting values eighteen subjects are needed (Flint *et al.* 2000). Thus, both test meals resulted in the same plasma glucose and insulin concentrations, as well as the same appetite sensations. When planning diets for diabetics and obese patients, for whom products resulting in low plasma glucose concentrations and high sensation of satiety are preferred, milk and A-38 seem to have equal effects.

Conclusion

In conclusion, the present study shows that milk results in a faster gastric emptying rate of the liquid phase than the fermented milk, A-38. Furthermore, the A-38 meal resulted in a higher increase in TAG in the lipoprotein fractions than the milk meal, which resulted in a lower, but more prolonged lipid response. Both a high level of TAG (A-38) and especially late postprandial lipaemia (milk) are thought to be associated with CVD, so it is difficult from our present study to conclude which product is healthier.

Acknowledgements

The skilful technical assistance of Alice Lieth at Rigshospitalet (CCK analyses) and Lene Albæk at the University of Copenhagen (GLP-1, GIP and PYY analyses) is gratefully acknowledged. Thanks also to Lene Appelt and Inge Rigstrup at the County Hospital in Glostrup for analysing the breath samples.

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