Effects of milk and milk constituents on postprandial lipid and glucose metabolism in overweight and obese men

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Abstract

Studies have suggested that two major milk constituents, casein and Ca, favourably affect postprandial responses. However, effects of milk on postprandial metabolism are unknown. We therefore investigated effects of using milk with a fat-containing meal on lipid and glucose responses in overweight men. To identify the constituent responsible for possible effects, we also studied responses to Ca and protein. A total of sixteen men (BMI $> 27 \text{ kg/m}^2$) participated in four postprandial tests. They consumed a breakfast (44 g of fat) plus a drink: a control drink, low-fat milk or a protein and Ca drink (500 ml). Blood samples were taken before the meals and at regular time points during 6 h thereafter. Compared with control, the incremental AUC (iAUC) for serum TAG was increased by 44 % after the protein meal ($P=0·015$). Although the iAUC were not different ($P=0·051$), peak glucose concentrations were reduced by 24 % after protein intake, as compared with control ($P=0·021$). The decrease of 18 % after milk intake did not reach statistical significance. Compared with the milk meal, the iAUC for insulin was 52 % lower after the control meal ($P=0·035$) and 51 % after the protein meal ($P=0·005$). The present results indicate that the intake of milk with a fat-containing meal enhances postprandial TAG and insulin responses and may blunt glucose increases. The protein fraction of milk seems to be the main determinant for the effects on TAG and glucose. Ca did not change any of the postprandial responses.

Postprandial hyperlipidaemia and hyperglycaemia are strongly associated with CVD[1,2]. In fact, postprandial changes in plasma TAG and glucose concentrations might be more strongly associated with CVD risk than their fasting concentrations[3,4]. Further, high fasting glucose concentrations are predictive for post-meal glucose excursions[5] and those of fasting TAG for postprandial hyperlipidaemia[6], which may explain why patients with the metabolic syndrome often have a disturbed postprandial metabolism[7]. As people in the Western world are in a non-fasting state for most of the day, these are important findings.

The macronutrient composition of a meal is an important determinant of the postprandial response. High-fat meals have been found to increase plasma TAG concentrations[8], to impair endothelial activity and function[9] and to increase markers of inflammation[10]. However, Westphal *et al.*[11] have shown that adding glucose to a high-fat meal resulted in a delay and reduction of postprandial lipaemia. Casein had an even stronger effect: chylomicron responses were further reduced and delayed, which could not be explained by gastric emptying time. Further, concentrations of C-peptide and insulin were elevated during the post-absorptive phase

(4–8 h) after the casein-containing meals. Whether milk and other dairy products have comparable effects on postprandial metabolism, as has milk protein alone, is not known. Furthermore, it has been reported that increased Ca intakes from dairy products reduced chylomicron TAG concentrations after a meal[12]. However, effects of Ca on other postprandial responses have not been studied before. In the present study, we therefore investigated the effects of adding low-fat milk to a high-fat breakfast on postprandial lipid and glucose metabolism in overweight and obese subjects. To evaluate which of the dairy components may be responsible for possible effects, we also studied the postprandial effects of milk protein and Ca, two major milk constituents.

Methods

Study population

Subjects were recruited in Maastricht and surroundings by advertisements in local newspapers and posters in the hospital and university buildings. People who were interested to participate were informed about the purposes

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and requirements of the study, and all gave their written informed consent before they entered the screening procedure. The study was approved by the Medical Ethical Committee of the Maastricht University Medical Centre and was registered on 8 June 2009 at ClinicalTrials.gov as NCT00917878.

Subjects were invited for the screening procedure when they met the following inclusion criteria: male; aged 18–70 years; non-smoking; BMI > 27 kg/m^2; no active CVD, familial hyperlipidaemia, inflammatory diseases or other medical conditions that might interfere with the study outcomes; no abuse of alcohol or drugs; and stable body weight during the past 3 months. During two screening visits, body weight, height and blood pressure were measured and a fasting blood sample was taken to determine serum lipid and lipoprotein concentrations. A total of sixteen men were enrolled in the study (Table 1). They were asked not to change their dietary habits, use of alcohol and level of physical exercise during the study. They were also requested to record any signs of illness, use of medication or other important details during the study period in a diary. All men completed the study.

Study design and intervention

The study consisted of four test days, which were separated by a washout period of at least 3 d. On each test day, subjects received a different test meal in randomised order, and participated in a postprandial test. Subjects were asked not to perform any strenuous physical exercise and not to consume alcohol on the day before testing. They were also asked to refrain from high-fat foods on the day prior to the test days. After an overnight fast, subjects arrived at the university by public transport or car. An intravenous cannula (Venfon®; Becton Dickinson) was inserted into an antecubital vein and blood samples were collected for analysis of fasting concentrations of metabolic risk markers. Subjects were then requested to consume one of the test meals within 10 min. Subsequent blood samples were drawn 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min after meal consumption. Subjects were allowed to drink water (250 ml) directly after the $T = 120$ and $T = 240$ blood sample collections.

The test meals consisted of 168 g butter cake and 500 ml of one of the four drinks (Table 2). In the milk intervention, subjects consumed skimmed milk (0 % fat; Campina). All drinks contained an equal amount of lactose (DMV International).

The protein drink contained the same amount of protein (REFIT Total Milk Protein; DMV International) as the milk, while the Ca content (Lactoval; DMV International) of the Ca and protein drinks was equal to that of the milk. The amino acid compositions of all meals are provided as supplementary Table S1 (available online).

Blood sampling and analyses

Venous blood was sampled using a Vacutainer system (Becton Dickinson). Blood was drawn into serum separator tubes for the analysis of lipids and lipoproteins. After clotting at room temperature for at least 30 min, serum was obtained by centrifugation at 1300 g for 15 min at room temperature and stored at -80°C. Further, blood was sampled into EDTA tubes for the analysis of insulin and NEFA concentrations and sodium fluoride (NaF) tubes were used for analysis of plasma glucose and amino acid concentrations. EDTA and NaF tubes were kept on ice until centrifugation at 1300 g for 15 min at 4°C. Plasma samples were snap-frozen in liquid N$_2$ and stored at -80°C.

Fasting serum samples were analysed for concentrations of total cholesterol (CHOD-PAP method; Roche) and HDL-cholesterol (precipitation method; Roche). LDL-cholesterol concentrations were calculated using the Friedewald equation[13]. Concentrations of TAG, with correction for free glycerol, were determined in serum samples from $T = 0$, 60, 120, 180, 240, 300 and 360 min (GPO Trinder; Sigma Diagnostics). apoB-48 concentrations were determined in plasma samples from $T = 0$, 120, 240 and 360 min by ELISA (Human Apo B-48 kit; Shibayagi). NEFA concentrations were analysed in plasma samples from $T = 0$, 60, 120, 240 and 360 min (NEFA kit; WAKO).

Glucose concentrations (Horiba ABX) and insulin concentrations (RIA; Millipore) were measured in plasma samples from $T = 0$, 15, 30, 45, 60, 90, 120, 240 and 360 min. Plasma amino acid concentrations were determined from blood samples obtained at $T = 0$, 60 and 120 min, as described elsewhere[14].

Statistics

Data are presented as mean values and standard deviations unless otherwise indicated. Fasting concentrations ($T = 0$) of the various parameters, as measured on the day of each test meal, were not statistically significant, as examined using univariate ANOVA. To evaluate the overall response of total TAG, apoB48, glucose, insulin and NEFA during the 6 h postprandial period, the incremental areas under the postprandial curve (iAUC) or decremental AUC were calculated using the trapezoidal rule. Maximal changes were calculated by subtracting fasting concentrations from the maximal or minimal value. Differences in iAUC and maximal changes between the test meals were tested for significance by univariate ANOVA followed by Bonferroni's correction for multiple comparisons. Changes in concentrations over time were analysed using linear mixed models, with diet and time as fixed factors and with diet × time as the interaction term. If the interaction term was not statistically significant, it was omitted from the

Table 1. Baseline characteristics of the sixteen men

(Mean values and standard deviations)

Characteristics	Mean	SD
Age (years)	49·8	15·5
BMI (kg/m^2)	31·2	3·6
Systolic blood pressure (mmHg)	129	12
Diastolic blood pressure (mmHg)	86	8
Total cholesterol (mmol/l)	5·58	0·87
LDL-cholesterol (mmol/l)	3·57	0·70
HDL-cholesterol (mmol/l)	1·23	0·27
TAG (mmol/l)	1·71	0·62
Glucose (mmol/l)	5·66	0·35
Insulin (pmol/l)	160	71

Table 2. Composition of the test meals

	Control	Milk	Protein	Ca
Products/components	Butter cake, 168 g; water, 500 ml; lactose, 29 g	Butter cake, 168 g; milk, 500 ml	Butter cake, 168 g; water, 500 ml; lactose, 29 g; milk protein, 23·4 g; Ca, 1·3 g	Butter cake, 168 g; water, 500 ml; lactose, 29 g; Ca, 2·3 g
Composition				
Energy (kcal)	864	948	948	864
Energy (kJ)	3615	3966	3966	3615
Protein (g)	8	29	29	8
Carbohydrates (g)	109	109	109	109
Mono- and disaccharides	74	74	74	74
Of which lactose	30	30	30	30
Fat (g)	44	44	44	44
Ca (mg)	49	697	703	699
P (mg)	100	600	407	353

model, and the factors diet and time were tested for statistical significance. If significant, *post hoc* tests with Bonferroni's correction were carried out. For time, only comparisons were made relative to those of fasting concentrations. All statistical analyses were performed using SPSS 16.0 for Macintosh OS X package (SPSS). A *P* value < 0·05 was considered as statistically significant.

Results

TAG and apoB-48

Changes in serum TAG concentrations during the postprandial phase did not differ significantly between the meals (*P* = 0·186 for meal effects; Fig. 1). Compared with fasting concentrations, all test meals increased serum TAG concentrations (*P* < 0·001 for time effect), which reached peak concentrations after 180 min and had not returned to fasting concentrations after 360 min. iAUC and maximal increases are shown in Table 3. Compared with the control meal, addition of protein to the meal significantly increased the iAUC by 44 % (*P* = 0·015). The difference between the protein and Ca meals was nearly significant (*P* = 0·062). The maximal increases in serum TAG concentrations were not different between the four meals (*P* = 0·498). Fig. 2 shows apoB-48 concentrations after the test meals. Compared with fasting values, all test meals increased apoB-48 concentrations (*P* < 0·001 for time effect). Also, a statistically significant meal effect was found (*P* = 0·016), related to differences between the milk and Ca meals (*P* = 0·030) and the protein and Ca meals (*P* = 0·042). Compared with the milk meal, the Ca meal decreased the iAUC by 48 % (*P* = 0·012; Table 3). The maximal increases from fasting values were not statistically different between the test meals (*P* = 0·076).

Glucose and insulin

Plasma concentrations of glucose and insulin are provided as supplementary Figs. S1 and S2 (available online). Compared with fasting values, all test meals increased serum glucose concentrations (*P* < 0·001 for time effect), which decreased below fasting concentrations after about 180 min. Changes between the meals, however, were not significantly different (*P* = 0·947 for meal effect). Glucose concentrations were maximally increased at 15 min after the consumption of the Ca and

protein meals, and at 30 min after consumption of the control and milk meals. The meals did not change the iAUC significantly (*P* = 0·051), but did change the maximal increase in glucose concentrations (*P* = 0·004). The difference between the protein and control meals of − 24 % reached statistical significance (*P* = 0·021). The decrease of 18 % after milk intake did not reach statistical significance (*P* = 0·111).

Insulin concentrations were maximal at 45 min after consumption of the test meals and had returned to fasting concentrations after 240 min (*P* < 0·001 for time effect). No meal effects were found (*P* = 0·585). Meal effects for the iAUC were significantly different (*P* < 0·001). Compared with the control meal, the iAUC was 52 % higher after the milk meal (*P* = 0·035). Also, the difference of 51 % between the milk and protein meals reached statistical significance (*P* = 0·005). The maximal increases were not statistically different between the four test meals (*P* = 0·227).

NEFA

Plasma NEFA concentrations significantly changed over time (*P* < 0·001), but these changes were not affected by the
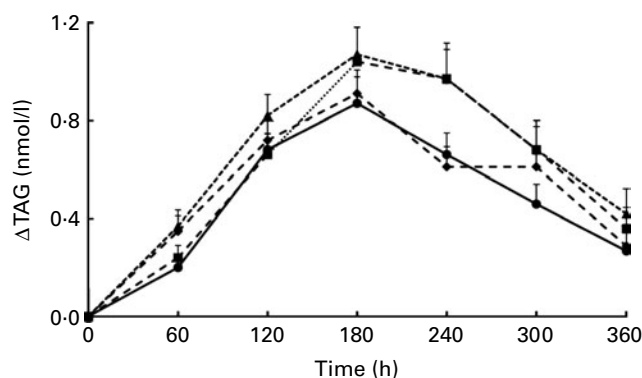

Fig. 1. Mean changes in serum TAG concentrations after consumption of the control, milk, protein, and calcium meals in a randomised crossover study with overweight and obese men (*n* 16). Values are means with their standard errors and were analysed using linear mixed models. There were no significant meal × time interactions. Time effects were *P* < 0·001 and meal effects *P* = 0·186. After Bonferroni's correction for multiple comparisons, all time points significantly differed from zero: *P* < 0·001. ●, Control; ■, milk; ▲, protein; ◆, calcium.

Table 3. Incremental AUC (iAUC) and maximal changes from fasting concentrations

(Mean values and standard deviations)

	Control		Milk		Protein		Ca		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P for meal effect
TAG									
iAUC (mmol × min/l)	181[a]	71	226[a,b]	127	249[b]	97	202[a,b]	75	0·007
Maximal change (mmol/l)	0·97	0·41	1·07	0·57	1·16	0·44	1·11	0·57	0·498
apoB-48									
iAUC (mg × min/l)	877[a,b]	432	1227[a]	419	1161[a,b]	423	887[b]	592	0·006
Maximal change (mg/l)	4·38	2·02	5·17	1·63	5·43	1·60	4·27	2·54	0·076
Glucose									
iAUC (mmol × min/l)	213	124	211	150	155	136	242	161	0·051
Maximal change (mmol/l)	3·22[a]	1·10	2·79[a,b]	1·46	2·57[b]	1·46	3·15[a,b]	1·14	0·004
Insulin									
iAUC (pmol/l × min) × 10³	131[a]	106	185[b]	129	121[a]	91	130[a,b]	78	<0·001
Maximal change (pmol/l)	1243	847	1430	806	1306	764	1278	701	0·227
NEFA									
dAUC (μmol × min/l) × 10³	69·7	54·1	11·2	36·2	54·7	47·0	59·6	35·2	0·218
Maximal change (μmol/l)	−319	180	−356	131	−274	188	−290	127	0·114

dAUC, decremental AUC.

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

meals ($P = 0.355$ for meal effect; see supplementary Fig. S3, available online). Minimal values were reached after 120 min. Since NEFA concentrations dropped below the fasting values after meal consumption, decremental AUC instead of iAUC were calculated. The decremental AUC ($P = 0.281$) and the maximal decrease in NEFA ($P = 0.114$) were not different between the interventions.

Amino acids

Plasma concentrations of total, essential and non-essential amino acids after the four meals are given in Table 4). After 60 and 120 min, amino acid concentrations were increased on the milk and protein meals, compared with the control and Ca meals. Concentrations of the individual amino acids are provided as supplemental data (Supplementary Table S2, available online).

Discussion

The aim of the present study was to compare the effects of milk and of milk protein and Ca, two major milk constituents, on postprandial lipid and glucose metabolism. Compared with control, milk increased the iAUC for apoB-48 and insulin. Further, the iAUC for TAG was increased and the maximal change in glucose was decreased when protein was added to the meal. The addition of Ca to the fat-containing meal had no effects on these parameters.

Results from other studies on postprandial effects of protein on TAG responses are not conclusive. Our finding that the addition of milk protein increased the iAUC of TAG contrasts observations of Westphal et al.[11], who found in normal-weight men and women no change in this parameter when casein was added to a high-fat, carbohydrate-containing meal. In fact, they even reported that the iAUC for the chylomicron fraction was decreased. In a second study, casein and soya reduced serum TAG concentrations at the early time

points, when added to a high-fat meal containing virtually no carbohydrates[15]. In that study, iAUC values were not reported, while TAG concentrations were not analysed in the chylomicron and VLDL fractions. The amounts of protein (about 50 g) and fat (about 70 g) given to the subjects were higher than in the present study. The authors suggested that the decreased chylomicron TAG response[11] resulted from a delayed gastric emptying and from increased insulin concentrations. Insulin activates lipoprotein lipase, which might increase chylomicron degradation[16]. The responsiveness of lipoprotein lipase, in adipose tissue, to insulin and meals may be diminished in obesity[17]. As the subjects in the present study were all overweight or obese, in contrast to those in the trials of Westphal et al.[11,15], it can be speculated that this might at least partly explain the different findings. However,
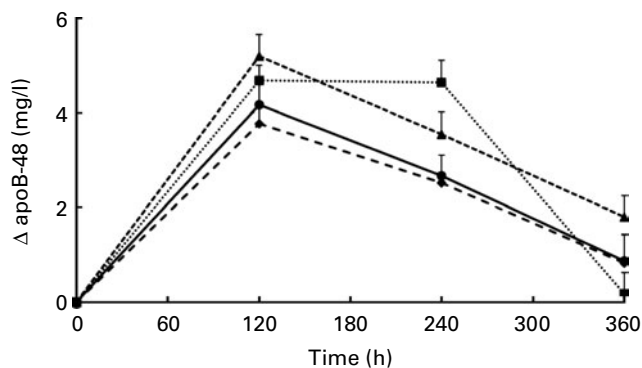

Fig. 2. Mean changes in apoB-48 concentrations after the control, milk, protein, and calcium meals in a randomised crossover study with overweight and obese men (n 16). Values are means with their standard errors and were analysed using linear mixed models. There were no significant meal × time interactions. Time effects were $P < 0.001$ and meal effects $P = 0.016$. After Bonferroni's correction for multiple comparisons, all time points significantly differed from zero: $P < 0.001$. In addition, differences between the milk and calcium meals ($P = 0.030$) and between the protein and calcium meals ($P = 0.042$) reached statistical significance. -●-, Control; -■-, milk; -▲-, protein; -◆-, calcium.

Table 4. Fasting plasma amino acid concentrations and their changes after the test meals

(Mean values and standard deviations)

	Control		Milk		Protein		Ca		P		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Time	Meal	Meal × diet
Total AA (μmol/l)									< 0·001	< 0·001	< 0·001
Fasting	2493	303	2479	243	2498	310	2453	238			
Change after 1 h	− 126[a]	127	269[b]	286	278[b]	384	− 110[a]	234			
Change after 2 h	− 248[a]	216	135[b]	287	112[b]	313	− 251[a]	219			
EAA (μmol/l)									< 0·001	< 0·001	< 0·001
Fasting	844	113	835	82	847	89	816	100			
Change after 1 h	− 103[a]	45	104[b]	122	107[b]	103	− 96[a]	81			
Change after 2 h	− 191[a]	72	42[b]	102	26[b]	92	− 177[a]	87			
NEAA (μmol/l)									< 0·001	< 0·001	< 0·001
Fasting	1649	220	1644	183	1651	251	1636	169			
Change after 1 h	− 24[a]	97	165[b]	181	171[b]	290	− 14[a]	162			
Change after 2 h	− 57[a,b]	157	93[c]	199	86[b,c]	242	− 74[a]	140			

AA, amino acids; EAA, essential amino acids (methionine, threonine, valine, isoleucine, leucine, phenylalanine, lysine, tryptophan); NEAA, non-essential amino acids.
[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$).

in a trial with overweight, type 2 diabetic subjects, Brader et al.[18] did find a decrease in TAG after casein consumption, but only in combination with carbohydrate consumption. Cohen[19] observed no effects of casein addition (23 g) on postprandial lipaemia after a high-fat (40 g) meal in normal-weight men and women. One study has reported increased TAG concentrations after fat and casein consumption in healthy male subjects[20]. However, since only four out of six subjects showed increased postprandial lipaemia after protein ingestion and results were not statistically analysed, the findings of that study are difficult to interpret. The protein we used was a total milk protein, containing the total fraction of cows' milk (mainly casein and whey), and thus somewhat different from the protein used by Westphal et al.[11]. Animal studies suggest that casein may have an unfavourable impact on lipid metabolism, as compared with soya protein[21], but human studies suggest that the nature of protein does not affect postprandial lipaemia[15]. We further provided the protein in addition to that in the meals, resulting in a higher energy intake in the protein and milk meals. However, it is not likely that a higher energy intake per se will increase postprandial TAG concentrations. For example, when extra energy is added to a fat-rich meal in the form of glucose, the TAG response may even be reduced[22,23].

We further observed that apoB-48 responses increased by the addition of milk or protein to the meal, which is in line with our observations on TAG responses. ApoB-48 is the apo present on chylomicrons of intestinal origin. The present results therefore imply that after the protein and milk meal, the number of chylomicron particles in the circulation is higher compared with the control meal. Whether this is due to an increased production or to a decreased clearance remains unknown.

The Ca supplement used in the present trial, which was derived from a dairy source, did not affect the serum TAG response. Lorenzen et al.[12] found that Ca from dairy products (350 or 800 mg), but not a calcium carbonate supplement (850 mg), reduced the iAUC for chylomicron TAG after a fat

load. The authors suggested that this decrease may be due to a reduction in fat absorption, as a number of studies have shown that Ca intake increases faecal fat excretion, probably through the binding in the intestine of Ca to fatty acids, mainly SFA[24]. However, no differences in total plasma TAG were observed, which is in line with our findings. Effects on fat absorption, however, cannot be fully excluded, as the effect on chylomicron TAG may have been too small to be detected in the total TAG fraction or may have been counteracted by an increase in VLDL.

We observed a 35 % increase in the insulin response after the milk meal, but no increase after the protein meal. Protein, especially in combination with carbohydrates, is already known for decades to induce insulin secretion[25]. Van Loon et al.[26–28] confirmed the insulinotropic potential of various proteins, hydrolysates and free amino acids in combination with carbohydrates in a series of studies in healthy young subjects. One of the most insulinotropic amino acids was leucine, which is highly present in milk protein. In addition, protein may decrease hepatic insulin extraction from the circulation. Addition of whey protein to a meal resulted in elevated postprandial insulin concentrations, while C-peptide levels were unaffected[29]. However, Westphal et al.[11] did find increased C-peptide concentrations after casein consumption, indicating an increased insulin production. Our observation that the insulin response was elevated by the milk meal, but not by the protein meal, is unexpected, as the protein content, the source of protein, the amino acid composition and the Ca and lactose content were exactly the same in both drinks. Further, changes in plasma concentrations of amino acids were comparable after the milk and protein meal. So, there may be an additional factor in milk that influences either the secretion or the clearance of insulin.

The iAUC for glucose were not different between the meals, but the peak change was decreased by the protein meal. Milk also decreased the peak change in glucose, though this change did not reach statistical significance. Ca had no effect. Thus, we observed a reduction in the maximal glucose

concentration, while the insulin response was not changed after the protein meal. A number of amino acids, including lysine[30], proline[31], glycine[32] and arginine[33], have been found to attenuate plasma glucose levels when ingested with carbohydrates, without elevating insulin concentrations. However, other amino acids, such as leucine[34] and phenyl-alanine[35], have been shown to cause a reduction in plasma glucose and a concurrent increase in insulin secretion. The increased insulin levels after the milk diet did not affect NEFA concentrations. This is in contrast to the reduced NEFA concentrations observed by Westphal et al.[11,15], who contributed this effect to the increased insulin response. However, Brader et al.[18] did not find significant differences in NEFA AUC, despite an increase in insulin after casein consumption.

In conclusion, the present results indicate that the intake of milk with a fat-containing meal enhances postprandial TAG and insulin responses and may blunt glucose increases. The protein fraction of milk seems to be the main determinant for the effects on TAG and glucose. Ca did not change any of the postprandial responses.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0007114512005314

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