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 kg/m²) 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.

Key words: Human nutrition: Milk: Protein: Calcium: Postprandial metabolism

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 (4–8h) 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
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/m²; 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 N₂ 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>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49·8</td>
<td>15·5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31·2</td>
<td>3·6</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>129</td>
<td>12</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>86</td>
<td>8</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5·58</td>
<td>0·87</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3·57</td>
<td>0·70</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1·23</td>
<td>0·27</td>
</tr>
<tr>
<td>TAG (mmol/l)</td>
<td>1·71</td>
<td>0·62</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5·66</td>
<td>0·35</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>160</td>
<td>71</td>
</tr>
</tbody>
</table>
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, the milk 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 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

**Table 2. Composition of the test meals**

<table>
<thead>
<tr>
<th>Products/components</th>
<th>Control</th>
<th>Milk</th>
<th>Protein</th>
<th>Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>864</td>
<td>948</td>
<td>948</td>
<td>864</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>3615</td>
<td>3966</td>
<td>3966</td>
<td>3615</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>8</td>
<td>29</td>
<td>29</td>
<td>8</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>109</td>
<td>109</td>
<td>109</td>
<td>109</td>
</tr>
<tr>
<td>Of which lactose</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>44</td>
<td>44</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>Ca (mg)</td>
<td>49</td>
<td>697</td>
<td>703</td>
<td>699</td>
</tr>
<tr>
<td>P (mg)</td>
<td>100</td>
<td>600</td>
<td>407</td>
<td>353</td>
</tr>
<tr>
<td>Of which lactose</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
</tbody>
</table>

**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 x time interactions.**

- P < 0.001 and meal effects
- P > 0.186
- P > 0.076
- P < 0.001.
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,
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We further provided the pro-

human studies suggest that the nature of protein does not
different from the protein used by Westphal
of cows’ milk (mainly casein and whey), and thus somewhat
we used was a total milk protein, containing the total fraction
findings of that study are difficult to interpret. The protein
six subjects showed increased postprandial lipaemia after pro-
response. Lorenzen
derived from a dairy source, did not affect the serum TAG
unknown.

increased production or to a decreased clearance remains
number of chylomicron particles in the circulation is higher
results therefore imply that after the protein and milk meal, the
apo present on chylomicrons of intestinal origin. The present
TAG concentrations after fat and casein consumption in
weight men and women. One study has reported increased
et al.
did find a decrease in TAG after casein consumption,
but only in combination with carbohydrate consumption. Cohen(18) 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 pro-
tein 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 post-
prandial 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 counter-
acted 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.(20–28) confirmed the insulinotropic potential of various
proteins, hydrolysates and free amino acids in combination
with carbohydrates in a series of studies in healthy young sub-
jects. 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 post-
prandial 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

Table 4. Fasting plasma amino acid concentrations and their changes after the test meals
(Mean values and standard deviations)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Milk</th>
<th>Protein</th>
<th>Ca</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>sd</td>
<td>Mean</td>
<td>sd</td>
<td>Mean</td>
</tr>
<tr>
<td>Total AA (μmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>2493</td>
<td>303</td>
<td>2479</td>
<td>243</td>
<td>2498</td>
</tr>
<tr>
<td>Change after 1 h</td>
<td>−126a</td>
<td>127</td>
<td>269b</td>
<td>286</td>
<td>278b</td>
</tr>
<tr>
<td>Change after 2 h</td>
<td>−248a</td>
<td>216</td>
<td>135b</td>
<td>287</td>
<td>112b</td>
</tr>
<tr>
<td>EAA (μmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>844</td>
<td>113</td>
<td>835</td>
<td>82</td>
<td>847</td>
</tr>
<tr>
<td>Change after 1 h</td>
<td>−103a</td>
<td>45</td>
<td>104b</td>
<td>122</td>
<td>107b</td>
</tr>
<tr>
<td>Change after 2 h</td>
<td>−191a</td>
<td>72</td>
<td>42b</td>
<td>102</td>
<td>26b</td>
</tr>
<tr>
<td>NEAA (μmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>1649</td>
<td>220</td>
<td>1644</td>
<td>183</td>
<td>1651</td>
</tr>
<tr>
<td>Change after 1 h</td>
<td>−24a</td>
<td>97</td>
<td>165b</td>
<td>181</td>
<td>171b</td>
</tr>
<tr>
<td>Change after 2 h</td>
<td>−57ab</td>
<td>157</td>
<td>93c</td>
<td>199</td>
<td>86bc</td>
</tr>
</tbody>
</table>

AA, amino acids; EAA, essential amino acids (methionine, threonine, valine, isoleucine, leucine, phenylalanine, lysine, tryptophan); NEAA, non-essential amino acids.

a,b Mean values within a row with unlike superscript letters were significantly different (P<0·05).
concentration, while the insulin response was not changed after the protein meal. A number of amino acids, including lysine, proline, glycine and arginine, have been found to attenuate plasma glucose levels when ingested with carbohydrates, without elevating insulin concentrations. However, other amino acids, such as leucine and phenylalanine, 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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