INTRODUCTION

Single meals usually contain all 3 macronutrients: fat, carbohydrate, and protein. The digestion and metabolism of these 3 main constituents are known to interact directly, eg, during the absorption stage, and indirectly, eg, via the activation of enzymes or the secretion of hormones. Fat metabolism has been studied repeatedly after the consumption both of fat alone and of fat combined with carbohydrates. It has been shown by others (1–3) and by ourselves (4) that carbohydrates added to a fatty meal cause pronounced alterations in gastric emptying and in lipoprotein metabolism. Postprandial lipemia is both delayed and reduced. The lowering of the postprandial lipemia is most probably caused by insulin acting via several mechanisms. One mechanism concerns the release of free fatty acids (FFAs) from adipose tissue, which is markedly suppressed by insulin through its inhibitory activity on the intracellular hormone-sensitive lipase. Because the rate of hepatic VLDL production is strongly dependent on the FFA supply (5), hepatic triacylglycerol synthesis and VLDL secretion are consequently slowed down (6–8). Finally, insulin might stimulate lipoprotein lipase (9), resulting in an accelerated clearance of triacylglycerol-rich lipoproteins (10).

Much less work has been done on the interactions caused by the protein component in a meal. The results are conflicting, in that either an increase in postprandial lipemia or no effect at all is reported (11, 12). However, it could be postulated that proteins such as casein interact with lipid metabolism as a result of their insulinotropic activity (13, 14). We therefore aimed to investigate the effect of casein added to various fat-rich meals to see whether effects could be observed in the presence or absence of oligosaccharides in the meal. Furthermore, both the postprandial phase and the postabsorption phase were studied for up to 8 h after the meal.

SUBJECTS AND METHODS

Subjects

Twenty-four healthy students (12 males, 12 females) took part in the study. Inclusion criteria were as follows: normal weight (body mass index, in kg/m², >18.5 but <25.0); nonsmoking; normal alcohol habits; no history of obesity, diabetes, or liver and kidney diseases; normal blood pressure; and no regular use of medications (Table 1). The subjects were instructed to refrain from any unusual changes in their habits concerning physical activity and nutritional behavior 4 wk before and throughout the study. Their habitual dietary intake was recorded by self-report; average energy intakes from carbohydrates, fat, and protein were 49.2%, 37.4%, and 13.2% of energy, respectively.

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TABLE 1
Characteristics of the subjects†

<table>
<thead>
<tr>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Age (y)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>Height (cm)</td>
</tr>
<tr>
<td>Weight (kg)</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/L)</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
</tr>
<tr>
<td>Free fatty acids (mmol/L)</td>
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</tbody>
</table>

† All values are x ± SEM; n = 12 men, 12 women.

Design

Each individual was studied 4 times, with intervals of ≥1 wk between the 4 oral fat loads. The liquid test meals consisted of 30% whipping cream, 3 mL (1 g fat) being given per kg body weight. The cream (per 100 mL) contained 30 g fat (18.2 g saturated and 9.04 g monounsaturated fatty acids), 3.5 g carbohydrates, and 2.5 g protein. The cream was mixed, in randomized order, with 300 mL water containing or not containing 75 g of a monosaccharide-oligosaccharide mixture (Dextro O.G-T; Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany). The protein-rich meal consisted of 30% whipping cream and 50 g sodium caseinate (Numico Research, Wageningen, Netherlands; Table 2) dissolved in water, with or without the oligosaccharide mixture. The meals were drunk within 15 min between 0730 and 0800. All of the test meals were well tolerated, and no gastrointestinal symptoms were reported. An ethics committee approved the protocol, and all subjects gave their written informed consent.

The first blood sample was withdrawn after a fasting period of 12 h. Further blood samples were then taken immediately before and 1, 2, 3, 4, 5, 6, 7, and 8 h after the oral fat tolerance test. No other source of energy was provided, but water was allowed ad libitum. The participants did not engage in any physical activity during the test and had avoided exercising during the 24 h before the tests. Venous blood samples were collected under standardized conditions, and serum was separated from the blood cells by centrifugation for 10 min at 3000 × g. Analyses of the lipoproteins and the metabolic variables were carried out within 24 h.

Determination of lipids and conventional lipoproteins

The separation of chylomicrons by ultracentrifugation and reproducibility issues are described in detail elsewhere (15). To isolate chylomicrons, 1 mL plasma was layered under 2 mL saline (9 g NaCl/L, d = 1.006 g/mL) and ultracentrifuged in polycarbonate tubes (Beckman Instruments, Krefeld, Germany) at 20,000 rpm in a 50.3 Ti rotor for 20 min at 10 °C. Chylomicrons were carefully isolated from the supernatant fluid. To determine triacylglycerol in VLDL, the serum was ultracentrifuged for 18 h under the same conditions, and the supernatant fluid containing VLDL plus chylomicrons was aspirated off. The triacylglycerol in VLDL was calculated by subtracting chylomicron triacylglycerol from total triacylglycerol in this fraction. All values were corrected for different yields by weighing the tubes before ultracentrifugation and after removal of the supernatant fluid.

Triacylglycerol concentrations were measured by use of commercial enzymatic methods in a random-access analyzer (Hitachi 911; Roche Diagnostics, Mannheim, Germany). All reagents and calibrators were from Roche Diagnostics. Plasma glucose was measured by use of a commercial enzymatic method (GOD; Roche Diagnostics), insulin by a commercial radioimmunoassay (BI-Insulin IRMA; BIO-RAD, Munich, Germany), and FFAs by a commercial enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany). C-peptide was analyzed by use of the Immulite system (DPC Diagnostic Products Corporation, Los Angeles, distributed in Germany by DPC Biermann GmbH, Bad Nauheim, Germany), which is a fully automatic random-access chemiluminescence-enhanced enzyme immunoassay system (16). Arginine was measured by HPLC.

Gastric emptying

All gastric emptying tests were done in combination with the test meals, 150 mg [13C]sodium acetate being dissolved in the fatty meal (17). Breath samples were collected before and then every 15 min for 240 min after the test meal and were analyzed for isotopic enrichment by using an isotope ratio mass spectrometer with an online gas-chromatographic purification system. The half-time of gastric emptying was calculated after curve fitting of the 13C exhalation to a modified power exponential function.

Statistics

Data are presented as means ± SEMs. The gastric emptying data are presented as medians and 25th and 75th percentiles. To evaluate the overall response of total triacylglycerol, triacylglycerol in VLDL, triacylglycerol in chylomicrons, FFAs, glucose, insulin, and C-peptide during the 8-h postprandial period, the areas under the postprandial curve (AUCs) were calculated by the trapezoidal rule. Statistical analysis of the data was performed by using a two-factor repeated-measures analysis of variance. Differences between the test meals were tested for significance by using Tukey’s post hoc test. Differences in the gastric emptying between the different test meals were checked for significance by

TABLE 2
Amino acid composition of the intact casein protein

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Value (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Alanine</td>
<td>2.77</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>3.52</td>
</tr>
<tr>
<td>L-Aspartate</td>
<td>6.11</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>0.45</td>
</tr>
<tr>
<td>L-Glutamate</td>
<td>20.56</td>
</tr>
<tr>
<td>L-Glycine</td>
<td>1.74</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>3.45</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>5.42</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>8.70</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>7.36</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>2.60</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>4.72</td>
</tr>
<tr>
<td>L-Proline</td>
<td>10.26</td>
</tr>
<tr>
<td>L-Serine</td>
<td>5.44</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>4.46</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>1.16</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>5.02</td>
</tr>
<tr>
<td>L-Valine</td>
<td>6.24</td>
</tr>
</tbody>
</table>
The use of Wilcoxon’s signed-rank test with Bonferroni correction. SPSS for WINDOWS (version 7.5; SPSS Inc, Chicago) was used for the analyses. Significance was set at \( P < 0.05 \).

RESULTS

Gastric emptying

The effects on gastric emptying of the addition of oligosaccharides or casein to fat, separately or in combination, are shown in Figure 1. The half-time of gastric emptying after fat alone was 127 min and was substantially prolonged to 193 min after the addition of oligosaccharides (\( P < 0.01 \)). When casein was added to fat alone or in combination with oligosaccharides, no further significant effect was detectable by use of the \( ^{13}C \) breath test.

Figure 1 shows the half-time of gastric emptying in 24 normolipidemic subjects after 4 different meals: 1) 1 g fat/kg body wt, 2) fat combined with 50 g sodium caseinate, 3) fat combined with 75 g oligosaccharides, and 4) fat combined with oligosaccharides and caseinate. Results are shown as box plots, in which the parts of the plot are the median, the box (which indicates the 25th and 75th percentiles), and the error bars (which indicate the 5th and 95th percentiles). Significantly different from fat alone, \( P < 0.05 \) (Wilcoxon’s signed-rank test with Bonferroni correction).

Postprandial lipemia

The kinetics of total serum triacylglycerol after the ingestion of the 4 different meals is shown in Figure 2A. Compared with fat alone, oligosaccharides exerted 2 pronounced effects: they delayed the triacylglycerol peak from 3 to 5 h and lowered the triacylglycerol concentrations, particularly at early time points. Casein had a similar but considerably weaker action; the triacylglycerol peak was delayed by \( \approx 1 \) h.

The triacylglycerol concentrations in chylomicrons and in VLDL are shown in Figure 2, B and C. As in serum, a delay and a reduction were observed for each density fraction. A strong effect was exerted by oligosaccharides, a somewhat weaker one by casein, and the greatest by the combination of the 2. The reductions were more pronounced in chylomicrons than in VLDL, in which delay was the predominant effect.

The AUCs are shown in Table 3. When fat alone was compared with fat plus oligosaccharides, the reductions in triacylglycerol

FIGURE 1. Half-time of gastric emptying in 24 normolipidemic subjects after 4 different meals: 1) 1 g fat/kg body wt, 2) fat combined with 50 g sodium caseinate, 3) fat combined with 75 g oligosaccharides, and 4) fat combined with oligosaccharides and caseinate. Results are shown as box plots, in which the parts of the plot are the median, the box (which indicates the 25th and 75th percentiles), and the error bars (which indicate the 5th and 95th percentiles). Significantly different from fat alone, \( P < 0.05 \) (Wilcoxon’s signed-rank test with Bonferroni correction).

FIGURE 2. Mean (± SEM) postprandial triacylglycerol concentrations in serum (A), chylomicrons (B), and VLDL (C) in 24 normolipidemic subjects after 4 different meals in each case: 1) 1 g fat/kg body wt (continuous line), 2) fat combined with 75 g oligosaccharides (broken line), 3) fat combined with 50 g sodium caseinate (dotted line), and 4) fat combined with oligosaccharides and caseinate (dot-dash line). For all panels, there were significant effects of time (\( P < 0.001 \)) and meal (\( P < 0.001 \)) and a significant meal \( \times \) time interaction (\( P < 0.001 \)), ANOVA for repeated measures.
Postprandial responses to a casein meal

AUCs in serum, chylomicrons, and VLDL were 10%, 21%, and 6%, respectively. The effects of casein on the triacylglycerol AUCs were significant only when casein was added together with oligosaccharides. This additional lowering caused by casein was 8%, 17%, and 6% for triacylglycerol in serum, chylomicrons, and VLDL, respectively.

Carbohydrate metabolism

As was to be expected, fat alone did not significantly alter concentrations of C-peptide, insulin, or glucose (Figure 3). When oligosaccharides and oligosaccharides plus casein were added, however, these variables were affected in 2 phases: in an early phase up to 3 h, which was dominated by glucose regulation, and in a second phase, between 4 and 8 h, when the specific effect of casein was observed. In the early phase, C-peptide and insulin increased sharply and glucose returned to baseline.

In the second phase, concentrations of C-peptide and insulin were elevated by between 30% and 92% after the casein-insulin increased sharply and glucose returned to baseline. No effect of casein was observed. In the early phase, C-peptide and insulin, expressed as percentages, were more pronounced during the postabsorption phase (4–8 h) than during the early phase (0 and 3 h), particularly when casein had been given together with oligosaccharides. Because the FFA AUC was markedly lowered by oligosaccharides and by casein, there was a pronounced lowering by combination of the 2, by 46% compared with the AUC of FFAs after fat alone.

DISCUSSION

The effects of additions of oligosaccharides to a fatty meal have been investigated repeatedly (2–4). The effect of protein added to fat has been studied much less and with conflicting results. Sullivan (11) studied 6 subjects over 3 h and reported that casein caused an increase in postprandial lipemia. Cohen (12) added sodium caseinate to fat and did not observe any change in postprandial lipemia over a period of 7 h. Neither investigator studied the effect of casein plus oligosaccharides on postprandial lipemia and, consequently, neither looked at the variables of carbohydrate metabolism. Our study considered for the first time the interaction of all 3 nutritional components. We found that casein 1) moderately reduces and delays the postprandial lipemia and 2) markedly lowers postprandial and postabsorptive concentrations of FFAs. We postulate that the insulinotropic action of casein is responsible for both of these effects.

Figure 4 shows that the addition of leucine to the incubation medium stimulates FFAs for 3 h, after which there was an increase similar to that after fat alone but delayed by 3 h. Casein added to fat alone caused significant reductions in FFAs at almost all time points, which resulted in a curve parallel to that given by fat alone but with the concentrations being lower by 13–36%. When both oligosaccharides and casein were added to the fat, the effects on FFA were additive. Compared with fat alone, the suppression reached maximum values of 18–72% at time points of 0.5–6 h.

The AUCs of C-peptide, insulin, and FFAs reflect these changes (Table 3). Note that the casein-induced changes in C-peptide and insulin, expressed as percentages, were more pronounced during the postabsorption phase (4–8 h) than during the early phase (0 and 3 h), particularly when casein had been given together with oligosaccharides. Because the FFA AUC was markedly lowered by oligosaccharides and by casein, there was a pronounced lowering by combination of the 2, by 46% compared with the AUC of FFAs after fat alone.

TABLE 3

Areas under the curves

<table>
<thead>
<tr>
<th></th>
<th>Fat</th>
<th>Fat + casein</th>
<th>Percentage change</th>
<th>Fat + oligosaccharides</th>
<th>Fat + oligosaccharides + casein</th>
<th>Percentage change</th>
<th>P for meal effect (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triacylglycerol (mmol · h/L)</td>
<td>13.3 ± 2.1 †</td>
<td>13.5 ± 1.8</td>
<td>1</td>
<td>12.0 ± 1.4 ‡</td>
<td>11.1 ± 2.3 ‡</td>
<td>−8</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum</td>
<td>3.8 ± 0.8</td>
<td>3.9 ± 0.9</td>
<td>3</td>
<td>3.0 ± 0.7 ‡</td>
<td>2.5 ± 0.8 ‡</td>
<td>−17</td>
<td>0.001</td>
</tr>
<tr>
<td>Chylomicron</td>
<td>5.5 ± 0.5</td>
<td>5.8 ± 0.4</td>
<td>5</td>
<td>5.1 ± 0.7 ‡</td>
<td>4.8 ± 0.6 ‡</td>
<td>−6</td>
<td>0.001</td>
</tr>
<tr>
<td>VLDL</td>
<td>6.3 ± 0.5</td>
<td>4.9 ± 0.3</td>
<td>−20</td>
<td>4.5 ± 0.7 ‡</td>
<td>3.4 ± 0.2 ‡</td>
<td>−24</td>
<td>0.001</td>
</tr>
<tr>
<td>Glucose (mmol · h/L)</td>
<td>49.6 ± 9.7</td>
<td>50.5 ± 8.5</td>
<td>2</td>
<td>53.9 ± 7.6 ‡</td>
<td>52.5 ± 7.8 ‡</td>
<td>−2</td>
<td>0.001</td>
</tr>
<tr>
<td>Insulin (pmol · h/L)</td>
<td>176 ± 54</td>
<td>227 ± 0.42</td>
<td>29</td>
<td>844 ± 49 ‡</td>
<td>931 ± 51 ‡</td>
<td>10</td>
<td>0.001</td>
</tr>
<tr>
<td>0–3 h</td>
<td>91 ± 74</td>
<td>118 ± 0.42</td>
<td>30</td>
<td>126 ± 40 ‡</td>
<td>182 ± 20 ‡</td>
<td>44</td>
<td>0.001</td>
</tr>
<tr>
<td>C-peptide (pmol · h/L)</td>
<td>3166 ± 100</td>
<td>3314 ± 101</td>
<td>5</td>
<td>8099 ± 277 ‡</td>
<td>8717 ± 326 ‡</td>
<td>8</td>
<td>0.001</td>
</tr>
<tr>
<td>0–3 h</td>
<td>2270 ± 70</td>
<td>2711 ± 65</td>
<td>19</td>
<td>2735 ± 277 ‡</td>
<td>3758 ± 326 a ‡</td>
<td>37</td>
<td>0.001</td>
</tr>
</tbody>
</table>

† n = 24. Values within a row with different superscript letters are significantly different, P < 0.05 (post hoc tests with Tukey’s adjustment).
‡ Percentage change from fat alone after the addition of casein.
§ Percentage change from fat + oligosaccharides after the addition of casein.
¶ ± SEM (all such values).
arginine-leucine and arginine-phenylalanine with glucose results in the largest increase in plasma insulin concentrations. This synergistic effect of the combined intake of carbohydrate and protein on plasma insulin concentrations was later confirmed in both healthy subjects (22) and those with type 2 diabetes (23–25). This effect was considerably less pronounced in nondiabetic persons (26). Van Loon et al (27–29) performed a series of studies in healthy young subjects in whom he determined the in vivo insulinotropic potential of various proteins, hydrolysates, and free amino acids in combination with carbohydrates. He found the highest insulin secretion after a mixture containing wheat protein hydrolysate, free leucine, and phenylalanine. In contrast, arginine, in an amount likely to be ingested in a high-protein meal, does not stimulate insulin secretion (30). Note that the above studies focused only on 2–3 h after the oligosaccharides intake. Our data, also obtained in nondiabetic subjects, show an increase in the insulin AUC by 29% up to 3 h; insulin secretion, however, did not affect glucose when casein was added to the fat alone. This casein-induced rise in insulin also occurred when oligosaccharides were given in addition, leading to a lowering of the peak glucose concentration by 10% after 60 min (P < 0.05). Thus, our data confirm early insulinotropic action of casein in nondiabetic persons.

In contrast with the above-mentioned studies, the present study investigated the postabsorption phase up to 8 h. During this second phase, concentrations of C-peptide and insulin are elevated almost during the entire period from 4 to 8 h and decline only slowly. The reason for these late elevations is probably again the release of insulinotropic amino acids from casein. The kinetics of arginine as a representative amino acid are shown in Figure 3. Interestingly enough, the variable reacting most sensitively to this late and continuous insulin elevation was not glucose but FFAs. Although glucose concentrations do not react at all with the administration of casein, the FFA AUCs were reduced by 20% and 24% when casein was added to fat alone and to fat plus oligosaccharides, respectively. The observation that FFAs are more sensitive to insulin variations than is glucose is in line with a clamp study by Boden et al (31) that showed a dose-dependent lowering of FFAs by insulin before glucose was affected.
This lowering of FFAs may bring about certain beneficial consequences. Because FFAs fuel hepatic triacylglycerol production, a lowering of VLDL may be a long-term consequence when fat-rich meals are regularly combined with arginine-rich proteins such as casein. This assumption is in line with an observation by Hurson et al (32), who supplemented 17 g arginine/d isocalorically to humans for 14 d and reported a significant 18% lowering of the serum triacylglycerol. Further advantages of reductions in FFAs might relate to a decrease in the LDL transfer into endothelial cells (33), improvements of the antioxidative protection of endothelial cells (34, 35), and inhibition of platelet aggregation (36).

Regarding the delay and reduction of postprandial lipemia after the addition of oligosaccharides to a fatty meal, another mechanism is in operation besides the increase in insulin. The pronounced delays of chylomicrons and VLDL are caused by the well-known effects of glucose and insulin on gastric emptying (37, 38). When casein was added, there was a further delay without an accompanying delay in the 13C breath test. We speculate that this additional postponement may have been caused by gastric precipitation of casein (39, 40) slowing down the subsequent fat absorption in the intestine. In addition to these delays, there were also reductions, mainly in chylomicrons. The chylomicron reduction may be secondary to the increased insulin concentrations, because insulin activates lipoprotein lipase (41, 42), which might degrade chylomicron triacylglycerol more rapidly.

In conclusion, casein added to an oligosaccharide-containing fatty meal reduces the chylomicron response. Casein also markedly suppresses FFAs in the presence and absence of oligosaccharides in the fatty meal. The FFA suppression occurring in both the postprandial and postabsorption phases may be beneficial.

We are indebted to Eva Maria Gittel, Katrin Deneser, and Marlies Kania for excellent assistance with the laboratory work.

SW designed the study and wrote the manuscript, SK organized and carried out the clinical study, ET organized the measurements and collection of data, AD carried out the breath tests, JD performed the statistical analyses, and CL helped in study design, evaluation, and manuscript writing. None of the authors had a personal or financial conflict of interest.

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23. Gannon MC, Nutall FQ, Lane JT, Burmeister LA. Metabolic response to cottage cheese or egg white protein, with or without glucose, in type II diabetic subjects. Metabolism 1992;41:1137–45.