Addition of glucose to an oral fat load reduces postprandial free fatty acids and prevents the postprandial increase in complement component 3\textsuperscript{1–3}

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ABSTRACT

Background: Elevated fasting plasma concentrations of complement component 3 (C3) are associated with elevated fasting and postprandial triacylglycerol concentrations, insulin resistance, obesity, and coronary artery disease. C3 is the central component of the complement system and the precursor of acylation-stimulating protein (ASP). Insulin and ASP are principal determinants of free fatty acid (FFA) trapping by adipose tissue.

Objective: Because controversy exists concerning postprandial changes in C3 and because meal composition may influence complement activation, we studied postprandial lipemia in relation to changes in plasma C3.

Design: After an overnight fast, 6 healthy men (\(\bar{x} \pm SD\) age: 23 \(\pm\) 2 y) underwent 4 oral liquid challenges: fat (50 g/m\(^2\) body surface), glucose (37.5 g/m\(^2\)), fat and glucose (mixed test), and water (as a control test) in a random, crossover design.

Results: Plasma ASP concentrations did not change postprandially in any test. Changes in C3 concentration were observed only after the fat challenge: elevated concentrations occurred between 1 and 3 h, and a maximum increase of 11% occurred at 2 h (\(P = 0.05\)). Postprandial triacylglycerolemia did not differ significantly between the fat and mixed tests. The FFA response after the fat challenge was the highest of all the tests (\(P < 0.05\) for all comparisons) and was accompanied by an increase in ketone bodies (maximum at 6 h); this increase did not occur after the mixed test, which suggests less hepatic FFA delivery.

Conclusions: When glucose is added to an oral fat load, the postprandial FFA response is reduced, and the fat-specific increase in C3 is prevented. After ingestion of fat without glucose, the lack of insulin response may lead to C3-mediated peripheral FFA trapping, which probably serves as a backup system in case of insufficient or inefficient insulin-dependent FFA trapping.


KEY WORDS Complement component 3, free fatty acids, insulin, triacylglycerol, acylation-stimulating protein, healthy men

INTRODUCTION

The complement system is a major component of the immune system and consists of 3 different pathways: the alternative pathway, the classical pathway, and the mannose-binding lectin pathway (1). Complement activation leads to cleavage of complement component 3 (C3). The resulting cleavage products initiate a cascade that produces various physiologically active molecules (1). Elevated fasting serum C3 concentrations are related to coronary artery disease (CAD) (2, 3) and to several established CAD risk factors, such as fasting and postprandial dyslipidemia, obesity, hypertension, and insulin resistance (3–7). C3 is produced in the liver and in extrahepatic cells like fibroblasts, mononuclear cells, endothelial cells, and adipocytes (8, 9). The relation between CAD, lipemia, and complement has received attention since the recognition of acylation-stimulating protein (ASP) in 1989 by Cianflone et al (10). ASP is identical to the desarginated form of the C3 split-product C3a and is immunologically inactive (11). ASP stimulates reesterification of free fatty acids (FFAs) into triacylglycerol in adipocytes and fibroblasts (11), reduces endogenous FFA production by inhibiting hormone-sensitive lipase (12), and stimulates intracellular uptake of glucose by adipocytes, fibroblasts, and muscle cells (11, 13). ASP is thought to be of clinical significance because elevations of FFAs, particularly in the postprandial phase, are a common characteristic of various metabolic disorders linked to CAD, eg, type 2 diabetes, obesity, and familial combined hyperlipidemia (14, 15). Therefore, it has been suggested that ASP-mediated FFA trapping could be an important determinant of a “healthy” lipoprotein phenotype (15, 16).

Chylomicrons are the strongest activators of adipocyte C3 production in vitro (9). However, oral-fat-loading studies in humans showed that plasma ASP concentrations do not change postprandially (6, 17, 18). On the other hand, some studies described an increase in C3 after meal consumption (7, 19), whereas other studies did not (17, 18). One of the reasons for this discrepancy could be the different study meals used by the various groups. Therefore, the aim of the present study was to in-

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investigate the effects of different test meals on postprandial plasma C3 and ASP concentrations.

SUBJECTS AND METHODS

Subjects

Six healthy, normolipidemic men aged 20-25 y were recruited by advertisement. The exclusion criteria were as follows: fasting dyslipidemia (plasma cholesterol > 6.5 mmol/L, plasma triacylglycerol > 2.0 mmol/L), fasting plasma glucose > 6.5 mmol/L, body mass index (in kg/m\(^2\)) > 30, smoking, alcohol intake > 2 U/d [1 U = 12 fl oz (360 mL) beer, 4 fl oz (120 mL) wine, or 1 oz (30 mL) hard liquor], the presence of renal and liver diseases, apolipoprotein E2/E2 genotype, and a family history of premature myocardial infarction or type 2 diabetes mellitus. None of the subjects consumed special diets or took vitamins, antioxidants, or medication. All subjects gave written informed consent. The study was approved by the Independent Ethics Committee of the Institutional Review Board of the University Medical Center Utrecht.

Study design

The subjects visited the hospital 4 times at intervals of \(\approx 4\) wk. On each occasion, the subjects fasted overnight for \(\geq 12\) h and did not drink alcohol on the day before the test. After a cannula was inserted into a vein for blood sampling, the subjects rested for 1 h before administration of the liquid load. On the morning of the first visit, blood pressure and waist-to-hip ratio were measured, and blood samples were taken to measure baseline plasma concentrations of lipids and glucose. Each challenge was ingested within 5 min. The participants remained supine during each test and were allowed to drink only mineral water. Peripheral blood samples were obtained in sodium EDTA (2 mg/mL) and lithium-heparin before the meal and at regular time intervals up to 10 h after the meal.

Four liquid challenges were randomly administered to the subjects: fat, glucose, fat and glucose combined (mixed test), and water. For the fat test, fresh cream was used; the fresh cream was a 40% (wt:vol) fat emulsion (polyunsaturated:saturated fat ratio of 0.10) that contained 0.001% (wt:vol) cholesterol and 3% (wt:vol) carbohydrates and had a total energy content of 3700 kcal/L. The cream was ingested at a dose of 50 g fat and 3.75 g glucose/m\(^2\) body surface. The glucose test consisted of a 30% (wt:vol) glucose solution (1200 kcal/L) that was ingested in a final dose of 37.5 g/m\(^2\) body surface. For the mixed test, a combination of the fat and glucose tests was used. For the mixed challenge, the final dose ingested was 50 g fat and 37.5 g glucose/m\(^2\) body surface. Finally, as a blank test, distilled water was given in a volume equal to that of the other meals (125 mL/m\(^2\) body surface).

Analytic methods

All EDTA blood samples were chilled, centrifuged immediately for 15 min at 800 \(\times\) g and 4 °C, and stored at \(-80\) °C. For FFA measurements, a lipase inhibitor (Orlistat; Roche, Mijdrecht, Netherlands) was added to the plasma to block ex vivo lipolysis (20, 21). Total serum C3 concentrations were measured by using nephelometry (Dade Behring Nephelometry type II; Dade Behring, Marburg, Germany) (7, 19). The total plasma C3 measured in our study represented C3, C3b, or C3c production. Because C3a is the least immunogenic part of C3 and is much smaller than the complete C3 molecule, the contribution of C3a or ASP to the total C3 measured in our study was negligible. ASP was measured by using a sandwich enzyme-linked immunosorbent assay as described previously (19, 22). Concentrations of total cholesterol and HDL cholesterol obtained after precipitation with heparin–manganese chloride were measured in duplicate by using colorimetric assays with the CHOD-PAP kit (Roche Diagnostics, Mannheim, Germany), and triacylglycerol concentrations were also measured in duplicate by using a colorimetric assay with the GPO-PAP kit (Roche Diagnostics). FFA concentrations were measured by using an enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany) (23). Total plasma apolipoprotein B concentrations were measured by using immunoturbidimetry as described previously (24). Hydroxybutyric acid (HBA) concentrations were measured spectrophotometrically according to the principle of NADH to NAD\(^+\) conversion after the addition of 3-hydroxybutyrate dehydrogenase (21). For this purpose, 0.5 mL blood from the lithium-heparin tubes was denaturated by adding 1 mL of a 0.7-mol HClO\(_4\)/L solution immediately after collection. Glucose concentrations were measured by using glucose oxidase dry chemistry (Yellow Springs Instruments, Yellow Springs, OH). Insulin concentrations were measured by using an enzyme-linked immunosorbent assay (Mercodia, Uppsala, Sweden). For estimation of insulin sensitivity, the homeostasis model assessment index (glucose concentration \(\times\) insulin concentration/22.5) was calculated.

Statistics

Data are given as means \(\pm\) SDs in the text and tables, but for the sake of clarity, SEM bars are shown in the figures. Differences in baseline values between the study meals were tested by using one-factor analysis of variance (ANOVA) with Bonferroni adjustment for multiple comparisons. Study meal \(\times\) time interactions were tested by using two-factor repeated-measures ANOVA with study meal and time as within-subjects factors and with Bonferroni adjustment. The interaction was significant, values at individual time points were compared with the baseline value by using one-factor repeated-measures ANOVA with time as the within-subjects factor and with Bonferroni adjustment. If there were significant differences between baseline values, these were used as covariates. Differences between the study meals were studied by using one-factor repeated-measures ANOVA comparing incremental areas under the curve (AUCs), with study meal as the within-subjects factor and with Bonferroni adjustment. The sequence of study meal administration was not introduced as an additional factor in the repeated-measures ANOVA because of the low number of subjects in the study. For the same reason, correlation studies were not performed. AUCs were calculated by using the trapezoidal rule with GraphPad PRISM version 3.0 (GraphPad Software, San Diego). Incremental AUCs were calculated by subtracting the baseline value from each following measured value. For triacylglycerol, HBA, homeostasis model assessment index, and insulin, calculations were performed after logarithmic transformation. The sample size was chosen on the basis of previous observations in our department showing statistically significant plasma C3 increments after oral fat challenges in \(\geq 6\) subjects (7, 19). For statistical analysis, SPSS version 10.0 (SPSS Inc, Chicago) was used. \(P\) values < 0.05 (two-tailed) were considered significant.
### RESULTS

#### Subject characteristics and postprandial lipemia

Baseline characteristics of the 6 participants are shown in Table 1. All the participants were healthy, normolipidemic, insulin-sensitive, nonobese students from the University Medical Center Utrecht.

Baseline triacylglycerol concentrations did not differ significantly between the meals. The fat test increased triacylglycerol concentrations from 1.04 ± 0.26 mmol/L to a maximum value of 2.29 ± 0.86 mmol/L at 4 h (P < 0.005), but concentrations returned to baseline values at 8 h (Figure 1). The mixed test also increased triacylglycerol concentrations from 0.91 ± 0.45 mmol/L at baseline to a maximum value of 1.82 ± 1.34 mmol/L at 2 h (P = 0.04). The incremental triacylglycerol response after the fat test was not significantly different from that after the mixed test (5.32 ± 2.33 compared with 3.08 ± 1.44 mmol · h/L, respectively), whereas the incremental triacylglycerol responses after both tests were significantly higher than those after water and the glucose test (P < 0.01).

Baseline FFA concentrations did not differ significantly between the tests (Figure 1). All study challenges significantly increased FFA concentrations at 10 h (158% higher than the baseline value after the fat test, 215% higher after the glucose test, 85% higher after the mixed test, and 191% higher after water; P < 0.005 for each). FFA concentrations after the fat test reached a maximum of 0.92 ± 0.12 mmol/L at 5 h (P = 0.02) and decreased afterward. The incremental FFA response after the fat test was the highest (P < 0.05 for all comparisons), whereas the FFA responses after the other tests did not differ significantly from one another.

Baseline HBA concentrations did not differ significantly between the tests (Figure 1). A significant postprandial increase in HBA concentration was seen only after the fat test, with a maximum value reached at 6 h (0.39 ± 0.36 compared with 0.06 ± 0.09 mmol/L at baseline; P = 0.02). The incremental HBA responses were not significantly different between the tests.

#### Table 1

Baseline characteristics of the study group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>23 ± 2 (20–25)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72 ± 6 (63–80)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.2 ± 1.3 (19.6–22.5)</td>
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<tr>
<td>Waist (cm)</td>
<td>0.80 ± 0.06 (0.73–0.88)</td>
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<tr>
<td>Waist-to-hip ratio</td>
<td>0.85 ± 0.06 (0.78–0.91)</td>
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<tr>
<td>Blood pressure (mm Hg)</td>
<td>120 ± 8 (111–130)</td>
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<tr>
<td>Systolic</td>
<td>76 ± 9 (65–90)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>4.1 ± 0.2 (3.9–4.5)</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>3.8 ± 1.6 (1.6–5.9)</td>
</tr>
<tr>
<td>HOMA index</td>
<td>0.71 ± 0.30 (0.28–1.02)</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/L)</td>
<td>0.13 ± 0.11 (1.01–1.70)</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.40 ± 1.17 (2.72–5.69)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.13 ± 0.20 (0.44–1.00)</td>
</tr>
<tr>
<td>Apolipoprotein B (g/L)</td>
<td>0.85 ± 0.11 (0.69–0.98)</td>
</tr>
<tr>
<td>ASP (ng/mL)</td>
<td>231 ± 90 (193–367)</td>
</tr>
</tbody>
</table>

* ± SD; range in parentheses. n = 6. HOMA, homeostasis model assessment; C3, complement component 3; ASP, acylation-stimulating protein.

#### FIGURE 1

Mean (+ SEM) concentrations of triacylglycerol (TG), free fatty acids (FFAs), and hydroxybutyric acid (HBA) in 6 healthy men before and after ingestion of 4 study meals: ○, fat meal; ■, glucose meal; □, mixed meal; ▲, water. There was a significant meal × time interaction for TG, FFAs, and HBA (P < 0.005, two-factor repeated-measures ANOVA with Bonferroni correction). TG concentrations between 2 and 6 h after the fat meal and between 1 and 5 h after the mixed meal were significantly higher than the respective baseline concentrations (all P < 0.01, one-factor repeated-measures ANOVA with Bonferroni correction). FFA concentrations from 1 h after the fat meal, from 5 h after glucose, and from 4 h after the mixed meal and water were significantly higher than the respective baseline concentrations (all P < 0.01, one-factor repeated-measures ANOVA with Bonferroni correction). Glucose resulted in an initial decrease in FFA concentration for the first 2 h (P < 0.05, one-factor repeated-measures ANOVA with Bonferroni correction). HBA concentrations significantly higher than the respective baseline concentrations were observed only after the fat meal between 4 and 10 h (P < 0.01, one-factor repeated-measures ANOVA with Bonferroni correction).

#### Postprandial complement component 3 and acylation-stimulating protein

Baseline C3 concentrations did not differ significantly between the tests. C3 concentrations between 1 and 3 h after the fat test were higher than those at baseline. Maximum C3 concentrations were seen 2 h after the fat test (11% higher than the baseline value; 0.94 ± 0.15 compared with 0.85 ± 0.15 g/L at baseline; P = 0.05) (Figure 2). The incremental C3 response after the fat test (0.51 ± 0.42 g · h/L) was significantly higher than the incremental C3 responses after the glucose (-0.25 ± 0.30 g · h/L;
Postprandial glucose and insulin

Baseline glucose concentrations did not differ significantly between the tests (Figure 3). The glucose test resulted in a significant plasma glucose increase at 30 min (5.54 ± 1.05 compared with 4.18 ± 0.27 mmol/L at baseline; \( P = 0.01 \)), which was followed by a nadir at 2 h (3.32 ± 0.56 mmol/L; \( P = 0.02 \) compared with baseline). The glucose response after the mixed test was blunted: maximal concentrations were reached at 45 min (4.72 ± 0.79 compared with 3.98 ± 0.28 mmol/L at baseline; \( P = 0.01 \)) and were followed by a nadir at 1.5 h (3.42 ± 1.31 mmol/L; \( P = 0.01 \) compared with baseline). The incremental glucose responses were not significantly different between the tests.

Baseline plasma insulin concentrations did not differ significantly between the tests (Figure 3). Plasma insulin concentrations increased significantly in the first hour after the glucose and mixed tests (from 5.7 ± 2.6 to 40.9 ± 27.2 mU/L after the glucose test and from 3.5 ± 2.3 to 49.8 ± 30.2 mU/L after the mixed test; \( P = 0.006 \) and \( P = 0.02 \), respectively). The incremental insulin response after the mixed test (65.3 ± 37.0 mU · h/L) was significantly higher than that after the fat test (1.8 ± 9.3 mU · h/L; \( P < 0.05 \)) but was not significantly different from that after the glucose test (19.5 ± 44.8 mU · h/L).

Discussion

The present study was performed to investigate postprandial lipemia and serum concentrations of ASP and C3 in relation to different nutrients in young healthy men. Plasma C3 concentrations increased significantly after a standardized fat challenge, but addition of glucose to the fat load abolished that significant change.

The C3-ASP system is a potentially important mediator of peripheral FFA handling and is of particular importance in the postprandial state because chylomicrons are produced in the postprandial state and are the strongest activators of this system (9, 10, 15). In the postprandial state, plasma ASP and C3 concentrations are hypothesized to increase because of activation of the C3-ASP system. However, postprandial plasma ASP concentrations did not change in healthy lean and obese subjects or in patients in 3 studies (6, 17, 18) and decreased in obese subjects.
in another study (25, 26). In the present study, we confirmed this lack of postprandial increase in ASP concentration and ruled out the possibility that the results of the above-mentioned studies were confounded because of the use of different types of nutrients.

In contrast with the results for ASP, postprandial changes in plasma C3 have been reported; however, the findings were inconsistent. We hypothesized that this was due to the use of different study meals. Two independent groups reported unchanged postprandial plasma C3 concentrations in healthy subjects (17, 18), but a small but significant 2-h postprandial increase in C3 concentrations was found in obese subjects (18). In those studies, mixed meals were administered. In agreement with those data, we did not detect any significant C3 increment after a standardized mixed challenge in healthy subjects. Studies from our department showed postprandial lipemia-associated increases in plasma C3 concentration in healthy subjects, normolipidemic patients with CAD, and patients with familial combined hyperlipidemia after ingestion of a standardized oral fat load (7, 19). In the present study, the observed increase in C3 of 11% in the first 2 h after the fat load was comparable with the C3 load (7, 19). In the present study, the observed increase in C3 of patients with CAD, and patients with familial combined hyperlipidemia after ingestion of a standardized oral fat load (7, 19). In the present study, the observed increase in C3 of 11% in the first 2 h after the fat load was comparable with the C3 response in the control group from one study (13% increase at 4 h) (19) but was lower than the responses observed earlier in CAD patients (7) and patients with familial combined hyperlipidemia (19) and in a different group of older control subjects (7) (30%, 23%, and 25% maximum increase in C3, respectively). This discrepancy could be explained by a lower waist circumference and body mass index, higher insulin sensitivity, and more favorable fasting lipid profile of the participants in the present study because all of these variables have been shown to determine fasting and postprandial C3 concentrations (3–7, 18). In contrast with our previous studies, the present study did not include females (7, 19). Fasting C3 concentrations in females, especially after menopause, have been shown to be higher than those in males (4).

It was remarkable that when glucose was added to the oral fat load, the postprandial FFA increment was blunted, and the increase in C3 was prevented. Postprandial lipemia is determined by the hydrolysis of triacylglycerol, which is dependent on lipoprotein lipase activity, hepatic production and reuptake of lipoproteins, and FFA trapping (14). It is well known that insulin acts on these processes, thereby resulting in reduced postprandial lipemia (27). Because of the small number of subjects in the present study, postprandial triacylglycerolemia after the mixed meal was not different from that after the fat meal. Enhanced peripheral FFA trapping after the mixed meal was likely because the postprandial increase in HBA was blunted after this meal, which probably reflected fewer FFAs reaching the liver after the mixed meal than after the fat meal. Alternatively, insulin may have decreased hepatic HBA production, as has been shown at supraphysiologic concentrations (28). A different possibility is enhanced peripheral HBA clearance by insulin (28). However, how long these effects would have persisted in our study remains uncertain because insulin concentrations were elevated in the first part of the postprandial phase only. Note that we did not directly measure peripheral FFA trapping in vivo but instead extrapolated the observed changes in plasma FFA and HBA concentrations.

We hypothesize that postprandial peripheral FFA trapping is more effective after the mixed meal in the presence of insulin and excess glucose than after the fat meal because glucose is necessary as a glycerol source for FFA esterification into triacylglycerol. Under these conditions, chylomicron-induced activation of the C3-ASP system for peripheral FFA trapping may not be necessary and may thereby explain the lack of postprandial increase in C3 in the present study and in earlier reports (17, 18). We cannot rule out the possibility that after a mixed meal, the C3-ASP system may be stimulated but only act in the vicinity of the adipocytes and therefore not cause any changes in plasma C3 concentration. The clinical consequence of effective FFA trapping would be reduced hepatic FFA flux and therefore lower VLDL synthesis. On the other hand, in the long term, effective FFA trapping will lead to obesity, with its well-known, harmful metabolic consequences. To directly investigate the mechanism linking FFA trapping to a C3 response in vivo, studies with labeled fatty acids and measurement of uptake in different tissues should be performed.

The plasma C3 increment after a fat meal seems to be a physiologic process because it occurs in healthy people. This phenomenon could reflect less effective FFA trapping because of a lack of sufficient plasma glucose and insulin, which results in stimulation of the C3-ASP system. Consequently, in insulin resistance, impaired peripheral glucose and FFA uptake could explain the higher fasting and postprandial plasma C3 concentrations observed in patients with type 2 diabetes and subjects with familial combined hyperlipidemia than in healthy, insulin-sensitive subjects (19, 29, 30). Under these conditions, we cannot rule out the possibility that sources other than adipose tissue may be responsible for the postprandial C3 increment in vivo.

The present study shows that in insulin-sensitive subjects, the postprandial FFA response is reduced and complement changes are prevented when glucose is added to an oral fat load. These data may explain the controversy in the literature about postprandial C3 concentrations because they depend on the type of test meal administered. We postulate that when glucose is available as a glycerol source for intracellular triacylglycerol synthesis, peripheral postprandial FFA trapping is more efficient than when glucose is not available, and the C3-ASP system is overruled.

REFERE N C E S