Delayed and Exaggerated Postprandial Complement Component 3 Response in Familial Combined Hyperlipidemia


Abstract—Very low density lipoprotein overproduction is the major metabolic characteristic in familial combined hyperlipidemia (FCHL). Peripheral handling of free fatty acids (FFAs) in vitro may be impaired in FCHL by decreased action of acylation-stimulating protein (ASP), which is identical to the immunologically inactive complement component 3a (C3adesArg). Because decreased FFA uptake by impaired complement component 3 (C3) response (as the precursor for ASP) may result in enhanced FFA flux to the liver in FCHL, we have evaluated postprandial C3 changes in vivo in FCHL patients. Accordingly, 10 untreated FCHL patients and 10 matched control subjects underwent an oral fat loading test. Fasting plasma C3 and ASP levels were higher in FCHL patients (1.33±0.09 g/L and 70.53±4.37 mmol/L, respectively) than in control subjects (0.91±0.03 g/L and 43.21±8.96 mmol/L, respectively; P<0.01 and P<0.05). In control subjects, C3 concentrations increased significantly after 4 hours (to 1.03±0.04 g/L). In FCHL, plasma C3 was unchanged after 4 hours. The earliest postprandial C3 rise in FCHL patients occurred after 8 hours (1.64±0.12 g/L). The maximal apolipoprotein B-48 concentration was reached after 6 hours in FCHL patients and control subjects. Postprandial FFA and hydroxybutyric acid (as a marker of hepatic FFA oxidation) were significantly higher in FCHL patients than in control subjects, and the early postprandial C3 rise was negatively correlated with the postprandial FFA and hydroxybutyric acid concentrations. The present data suggest an impaired postprandial plasma C3 response in FCHL patients, most likely as a result of a delayed response by C3, as the precursor for the biologically active ASP, acting on FFA metabolism. Therefore, an impaired postprandial C3 response may be associated with impaired peripheral postprandial FFA uptake and, consequently, lead to increased hepatic FFA flux and very low density lipoprotein overproduction. (Arterioscler Thromb Vasc Biol. 2002;22:811-816.)

Key Words: familial combined hyperlipidemia ▪ complement component 3 ▪ acylation-stimulating protein ▪ free fatty acids

Familial combined hyperlipidemia (FCHL) is the most frequent dominantly inherited disorder of lipid metabolism, leading to increased risk for atherosclerosis.1–5 The diagnosis is based on clinical criteria such as the presence of “multiple type hyperlipidemia,”2–5 increased concentrations of plasma apoB, and a positive family history of premature coronary heart disease (CHD). The genetic basis of FCHL has not been elucidated, although several groups have provided evidence suggesting that different genes are involved in the pathogenesis of this disorder.6–14 Recently, we have reported that postprandial hepatic free fatty acid (FFA) flux in vivo is increased in FCHL,15 providing further evidence for the role of disturbed FFA metabolism in this disorder.16,17

In vitro and in vivo experiments have demonstrated that the uptake of FFA by peripheral cells is stimulated by acylation-stimulating protein (ASP).18,19 ASP is identical to complement component 3a (C3adesArg), which is one of the immunologically inactive cleavage products of complement component 3 (C3).18 Adipose tissue has been shown to produce C3, factor B, and factor D on incubation with chylomicrons, all of which are necessary for the generation of ASP.18 These in vitro data suggest that during postprandial lipemia, C3 production by adipose tissue in vivo may occur. However, there are no data supporting this concept, and in fact, unchanged postprandial plasma C3 concentrations after a mixed meal have been reported in healthy volunteers.20 In contrast to that study, recent work from our laboratory has shown that plasma C3 concentrations increase postprandially after an acute oral fat load in control subjects and patients with premature coronary artery disease.21 In addition, it has been shown that the postprandial C3 response is closely associated with postprandial lipemia.21

In FCHL, ASP-mediated FFA uptake in vitro is blunted,22 possibly explaining in part the increased hepatic FFA flux.
postulated in FCHL.16,17 Fasting plasma C3 concentrations are elevated in affected FCHL relatives and are well correlated with different lipid parameters, such as plasma triglycerides (TGs), cholesterol, and apoB.23

Postprandial plasma ASP concentrations in normolipidemic non-FCHL subjects have been reported to remain unchanged.20 In FCHL subjects, postprandial plasma ASP does not increase.24 ASP is mainly active in the microenvironment of adipose tissue, and adipose tissue blood flow is relatively low compared with the cardiac output, explaining that systemic ASP levels do not increase postprandially.19,25 Because diminished postprandial FPA uptake by peripher al cells and, consequently, increased hepatic FFA flux may also be explained by impaired activation of ASP and because C3 is a precursor of ASP, the aim of the present study was to determine postprandial C3 changes in FCHL patients and matched healthy control subjects.

Methods

Subjects

The study protocol was approved by the Human Investigations Review Committee of the University Medical Center Utrecht. All participants gave written informed consent. Ten unrelated FCHL patients were recruited from the Lipid Clinic of the University Medical Center Utrecht. FCHL subjects met the following criteria: primary hyperlipidemia with varying phenotypic expression and at least 1 first-degree relative with a different hyperlipidemic phenotype, elevated plasma apoB concentrations (>1.2 g/L), and a positive family history of premature CHD (defined as myocardial infarction or cerebrovascular disease before the age of 60 years) in at least 1 blood-related subject of the index patient. In addition, the patients fulfilled the following inclusion criteria: absence of xanthomas; absence of secondary factors associated with hyperlipidemia as demonstrated by normal thyroid, renal, and liver function tests; diabetes mellitus type II; body mass index (BMI) <30 kg/m2; absence of apoE2/E2 genotype; no use of drugs affecting lipid metabolism; and consumption of <3 U alcohol per day. All FCHL patients stopped lipid-lowering drugs 4 weeks before the oral fat load. Ten normolipidemic healthy control subjects without a family history of cardiovascular disease, with the absence of the apoE2/E2 genotype, and with no use of drugs known to affect lipid metabolism were recruited by advertisement. Body fat was estimated with a Body Impedance Analyzer (RJL Systems) according to instructions provided by the manufacturer.26,27

Control subjects were matched to FCHL patients by age, BMI, and waist-to-hip ratio.

Oral Fat Loading Test

Cream was used as the fat source, which was a 40% (wt/vol) fat emulsion with a P/S ratio of 0.06 and containing 0.001% (wt/vol) cholesterol and 2.8% (wt/vol) carbohydrates. To the cream, 60 g/L dextrose and vitamin A were added.16 After an overnight fast of 12 hours, the subjects ingested the fresh cream in a dose of 50 g fat per square meter body surface and 7.5 g dextrose per square meter body surface. After ingestion of the fat load, subjects were allowed to drink only water and sugar-free tea during the following 24 hours. Peripheral blood samples were obtained in sodium EDTA (2 mg/mL) before (0 hours) and at hourly intervals up to 10 hours after the fat load and after 12 and 24 hours. For measurement of hydroxybutyric acid (HBA), every 2 hours peripheral blood samples were obtained in heparin tubes. Blood was placed on ice and centrifuged immediately for 15 minutes at 800g and 4°C. Plasma samples were stored at −20°C immediately after centrifugation.

Analytical Methods

TGs and cholesterol were measured in duplicate by commercial colorimetric assay (GPO-PAP and Monotest Cholesterol, respectively; Boehringer-Mannheim). FFA was measured in plasma samples by an enzymatic colorimetric method (Wako Chemicals GmbH). HBA was measured spectrophotometrically by the principle of converting NADH to NAD+ after adding 3-hydroxybutyrate dehydrogenase. For this purpose, 0.5 mL of the blood samples in heparin tubes was denatured by adding 1 mL of 0.7 mol/L HClO4.15 The detection limit of HBA is 0.02 mmol/L. C3 was measured by nephelometry (Dade Behring Nephelometry type II),22 and ASP was measured by using an ELISA as previously described.18,19 HDL cholesterol was determined as described.20 The quantitative assay of apoB has been described in detail.20 ApoB-48 concentrations in chylomicron fractions (S >400) were determined according to the method described by Karpe and Hamsten.30 Glucose was measured with the use of glucose oxidase by dry chemistry (Vitros GLU slides) and colorimetry, and insulin was measured by commercial ELISA (Mercodia). For estimation of insulin sensitivity, the homeostasis model assessment was calculated as follows: (glucose × insulin)/22.5.

Statistical Analysis

All values are expressed as mean±SEM. The area under the curve (AUC) and incremental AUC (dAUC) were calculated by the trapezoidal rule and after correction for fasting values, respectively. The first 8 hours after ingestion of the fat load represents the postprandial period.13 Mean fasting differences between control subjects and FCHL patients were calculated by unpaired t test. Mean differences of fasting plasma TGs and insulin were calculated with the Mann-Whitney U test. For statistical analysis of changes in C3, TG, FFA, and HBA concentrations, repeated measures ANOVA was used, with the least significant difference test used as a post hoc test. For the calculation of correlations, the Spearman correlation coefficient was determined. Calculations were performed by using SPSS/PC+ 9.0 (SPSS Inc). A value of P<0.05 (2-tailed test) indicated statistical significance.

Results

General Characteristics

FCHL patients and healthy control subjects did not differ in anthropometric characteristics (Table 1). The data of 7 of the FCHL patients, concerning postprandial hepatic FFA flux, have been previously reported.15 However, a different control group was used to match the FCHL patients. Fasting concentrations of plasma TGs, cholesterol, apoB, FFA, and glucose were significantly higher in FCHL patients. HDL cholesterol was higher in control subjects (Table 2). Insulin sensitivity, determined by homeostasis model assessment, was not different between the groups.
Postprandial C3 and Chylomicron Changes

Fasting plasma C3 and ASP were significantly higher in FCHL patients than in control subjects (Table 2). In FCHL patients, no increase in C3 concentrations was seen after 4 hours (1.32±0.07 g/L at 4 hours, *P*=NS compared with fasting concentrations); however, in control subjects, a 13% increase was found (to 1.03±0.04 g/L at 4 hours, *P*<0.01 compared with fasting concentrations). The earliest rise in C3 concentrations in FCHL patients was found after 8 hours (to 1.64±0.12 g/L, *P*<0.05 compared with fasting concentrations, representing a 23% increase). The percentile increase from fasting to peak value tended to be higher in FCHL patients (*P*=0.07). In control subjects, 8 hours after fat ingestion, C3 concentrations returned to fasting levels (0.89±0.05 g/L). The time curve was significantly different between FCHL patients and control subjects (*P*<0.05).

In both groups, C3 concentrations increased from 12 to 24 hours after fat ingestion: in FCHL patients, by 24% (from 1.51±0.09 to 1.82±0.12 g/L, *P*<0.01), and in control subjects, by 8% (from 0.88±0.04 to 0.95±0.05 g/L, *P*<0.05). The relative C3 increase was higher in FCHL patients compared with control subjects (*P*<0.05). The total C3-AUC (37.58±2.14 versus 21.93±0.99 g·h⁻¹·L⁻¹ for FCHL versus control, respectively; *P*<0.01) and the postprandial C3-AUC (11.22±0.63 versus 7.37±0.52 g·h⁻¹·L⁻¹ for FCHL versus control, respectively; *P*<0.01) were higher in FCHL patients (Figure 1A).

Fasting apoB-48 in the chylomicron fraction (chyl-o-B48) was higher in FCHL patients than in control subjects (Table 2). Fasting chyl-o-B48 increased postprandially, reaching maximal concentrations after 6 hours (1.05±0.38 mg/L, *P*<0.05 compared with fasting concentrations). In control subjects, a similar pattern was found, albeit reaching lower concentrations (postprandial rise to 0.16±0.02 mg/L after 6 hours, *P*<0.05; Figure 1B). There were no significant corre-

Table 2. Fasting Laboratory Values at the Time of the Oral Fat Loading Test of 10 Untreated FCHL Patients and 10 Matched Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>FCHL</th>
<th>Controls</th>
<th><em>P</em></th>
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<tr>
<td>Cholesterol, mmol/L</td>
<td>6.97±0.84</td>
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<td>TG, mmol/L</td>
<td>2.94±0.42</td>
<td>1.21±0.16</td>
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<td>HDL-C, mmol/L</td>
<td>0.78±0.07</td>
<td>1.12±0.09</td>
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<td>LDL-C, mmol/L</td>
<td>4.05±0.74</td>
<td>2.11±0.28</td>
<td>&lt;0.05</td>
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<tr>
<td>Chylo apoB-48, mg/L</td>
<td>0.30±0.13</td>
<td>0.02±0.01</td>
<td>&lt;0.01</td>
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<tr>
<td>ApoB, g/L</td>
<td>1.52±0.14</td>
<td>0.83±0.06</td>
<td>&lt;0.01</td>
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<tr>
<td>ASP, mmol/L</td>
<td>70.53±4.37</td>
<td>43.21±8.96</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Complement 3, g/L</td>
<td>1.33±0.09</td>
<td>0.91±0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Insulin, IU/L</td>
<td>10.6±1.6</td>
<td>9.4±1.1</td>
<td>NS</td>
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<tr>
<td>Glucose, mmol/L</td>
<td>5.7±0.3</td>
<td>4.9±0.1</td>
<td>&lt;0.05</td>
</tr>
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<td>HOMA</td>
<td>2.70±0.44</td>
<td>2.04±0.22</td>
<td>NS</td>
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<td>2/3: N=2</td>
<td></td>
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<td></td>
<td>3/3: N=5</td>
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<td></td>
<td>3/4: N=4</td>
<td>3/4: N=1</td>
<td></td>
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</table>

Data are mean±SEM.
HOMA indicates homeostasis model assessment.

Figure 1. Mean changes of plasma C3 concentrations (A) and mean changes of chylomicron apoB-48 concentrations (B) in 10 untreated FCHL patients (solid circles) compared with 10 matched control subjects (open circles). Data are mean±SEM. In panel A, y axis starts at 0.8 g/L.

Relationship Between Postprandial C3 Response and FFA and HBA Changes

Fasting plasma C3 concentrations were significantly correlated with fasting plasma TGs (Spearman *r*=0.86, *P*<0.001), TG, mmol/L 2.94±0.84 1.21±0.16 37.58±2.14 21.93±0.99 1.05±0.38 0.83±0.06 70.53±4.37 1.33±0.09 10.6±1.6 5.7±0.3 2.70±0.44 2/3: N=1 3/3: N=5 3/4: N=4

Plasma TG, FFA, and HBA Changes

In FCHL patients and control subjects, plasma TG levels increased significantly to a maximal concentration at 5 hours after fat ingestion (from 2.94±0.42 to 5.36±0.63 mmol/L in FCHL patients [*P*<0.01] and from 1.15±0.13 to 2.36±0.53 mmol/L in control subjects [*P*<0.01], Figure 2A). FFA concentrations increased in FCHL patients from 0.40±0.05 mmol/L to a maximum of 0.99±0.07 mmol/L at 5 hours (*P*<0.01) and in control subjects from 0.31±0.03 to 0.62±0.03 mmol/L (*P*<0.01), reaching the peak at 7 hours (Figure 2B).

HBA concentrations increased in FCHL patients from fasting (0.02±0.001 mmol/L) to a maximum of 0.41±0.02 mmol/L at 6 hours (*P*<0.01). In control subjects, the maximal HBA concentration was found at 8 hours (from 0.02±0.001 to 0.18±0.05 mmol/L, *P*<0.01). The maximal postprandial HBA increase (1813%) and the postprandial HBA-AUC (1.72±0.09 mmol·h⁻¹·L⁻¹) were significantly higher in FCHL patients than in control subjects (721% HBA increase and 0.95±0.16 mmol·h⁻¹·L⁻¹, respectively; Figure 2C).
apoB (r=0.62, P<0.01), total cholesterol (r=0.53, P=0.02), and fasting glucose (r=0.76, P<0.001). There were no significant correlations with BMI (r=0.12, P=NS) or insulin (r=0.02, P=NS), but a trend was found with the waist-to-hip ratio (r=0.47, P=0.05).

Postprandial FFA changes from 0 to 8 hours, calculated as AUC, were significantly associated with postprandial HBA changes in the whole group (r=0.46, P=0.04; Figure 3A). The C3 increase at 4 hours was significantly negatively associated with total postprandial (0 to 8 hours) FFA increase (r=-0.55, P=0.01; Figure 3B) and the maximal HBA increase (r=-0.49, P=0.03; Figure 3C).

**Discussion**

The present study shows a delayed, albeit exaggerated, postprandial C3 response to an acute oral fat load in untreated FCHL patients. One of the major characteristics of FCHL is VLDL overproduction, which may be caused, in part, by an increased postprandial hepatic FFA flux. Because C3 is the precursor of ASP, postprandial C3 concentrations were measured to evaluate the changes in FCHL patients compared with healthy non-FCHL volunteers.

ASP plays an important role in FFA uptake by adipocytes after lipolysis of chylomicrons. Chylomicrons stimulate the production of ASP by adipocytes in vitro, which results in increased adipocyte FFA trapping, with consequently less FFA remaining in the circulation as a substrate for hepatic VLDL production. However, hyper-apoB/FCHL patients have elevated fasting plasma ASP concentrations and an increased hepatic FFA flux. It has been shown that ASP-mediated FFA uptake in vitro is impaired in peripheral cells of FCHL patients, suggesting a receptor defect rather than decreased ASP production.

Different studies, aimed at investigating the relationship between C3 and lipids, showed that fasting C3 concentrations were correlated strongly not only with lipid parameters but also with several features of the insulin resistance syndrome. Ylitalo et al. also found a correlation between fasting lipids and insulin concentration with plasma ASP levels. However, stronger correlations were demonstrated between C3 and the same parameters. Our data point in the same direction, although we...
did not find a correlation with insulin, probably because both groups were carefully matched, and similar insulin sensitivity indexes were present. Furthermore, in accordance with the present data, it has been demonstrated that CHD and FCHL patients, compared with healthy age-matched control subjects, have elevated levels of fasting plasma ASP and C3. It remains to be shown whether these high ASP and C3 levels are caused by higher C3 production by adipocytes. In future studies addressing this issue, mRNA expression in adipose tissue should be determined, as has been published recently for type 2 diabetic men and obese subjects. In those patient groups, increased C3 mRNA expression was found compared with the expression in lean nondiabetic men.

In the present study, we demonstrate that not only fasting C3 concentrations but also postprandial C3 responses differ between untreated FCHL patients and control subjects. Four hours after fat ingestion, an increase in C3 concentration was seen exclusively in control subjects. C3 increase occurred later and was more pronounced in FCHL patients than in control subjects. Because C3 is the precursor of ASP, we propose that the delayed but exaggerated C3 response in FCHL may be due to resistance at the level of adipose tissue for ASP. This view is in line with recent publications. Another explanation for the difference in C3 response could be that intestinal lipid absorption may be delayed in FCHL patients. However, the rapid rise of plasma TGs after ingestion of the fat load does not support such an explanation. In our view, the ASP resistance, indicated by delayed and exaggerated postprandial C3 responses and elevated fasting concentrations of C3 and ASP in a nonobese patient group, is a more likely candidate to explain the increased postprandial FFA flux in FCHL patients. Furthermore, the delayed TG clearance and increased postprandial FFA and apoB-48 as well as increased postprandial HBA concentrations in FCHL patients also point to an increased hepatic postprandial FFA flux, which theoretically may enhance VLDL secretion.

Chylomicrons may induce C3 production by adipocytes, leading to increased local concentrations of ASP. Our data in control subjects support this chylomycin-induced C3 rise in vivo. In FCHL patients, the tie pattern of chylo-B48 and C3 suggests a delayed response by C3-producing cells exposed to chylomicrons. We did not measure postprandial ASP concentrations in the present study, because it has been shown that systemic ASP levels do not increase postprandially because ASP is mainly active in the microenvironment of adipose tissue. However, when ASP action is diminished, as shown in vitro in hyper-apoB patients, this could lead to increased postprandial FFA concentrations and, subsequently, enhanced FFA flux to the liver. The significant negative associations between early postprandial C3 changes and FFA and HBA changes as found in the present study support this view. In the late postprandial period (from 4 to 8 hours) in FCHL patients, the exaggerated C3 increase may have been necessary to overcome the C3/ASP resistance, resulting in adequate peripheral FFA uptake, which was reflected by a decrease in plasma FFA concentrations during that period, whereas the postprandial plasma FFA rise in control subjects stabilized earlier.

VLDL particles compete with chylomicrons for the action of lipoprotein lipase (LPL) and will diminish the rate at which chylomicrons are hydrolyzed by LPL, thus explaining the delayed chylomicron clearance in FCHL patients. Furthermore, the increased postprandial FFA concentrations can lead to premature detachment of chylomicron remnants from adipose tissue LPL, which could also contribute to decreased clearance. The high concentrations of chylomicrons in FCHL patients will lead to higher production of C3 and, consequently, ASP, leading to a further increase in C3 and ASP. As far as we know, no negative-feedback system has been described for the production of ASP.

In a recent report, it has been suggested that the lack of ASP response after an oral fat load indicates that chylomicrons do not serve as a physiological trigger for ASP formation in plasma. We are not convinced that this is the case, inasmuch as ASP is mainly active at the level of adipocytes; thus, ASP changes in the systemic circulation are not likely to be detected. This may explain why different studies did not detect postprandial ASP changes. Furthermore, a postprandial ASP increase has been demonstrated in vivo by using arteriovenous techniques.

After 12 hours, a second increase of C3 in FCHL patients and control subjects was seen. It is not clear what mechanism could be responsible for this postabsorptive C3 increase. Because C3 is also formed in other cells, such as hepatocytes and macrophages, it could be hypothesized that the postabsorptive C3 increase is not adipocyte-derived but that it may have its origin in the liver. Persistently increased concentrations of chylomicron remnants may lead to enhanced C3 production by the liver, as has been demonstrated for cytokines. Alternatively, chylomicron remnants are taken up by macrophages, and the late C3 increase may originate from these cells.

In conclusion, the present study shows increased fasting plasma ASP and C3 concentrations and exaggerated late postprandial and postabsorptive C3 responses in FCHL patients, which may be a feature of C3/ASP resistance. The absence of an early postprandial increase of plasma C3 after an oral fat load in FCHL patients may be linked to decreased trapping of FFA by adipocytes and, consequently, may lead to an increased hepatic FFA flux and VLDL overproduction.

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References
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