Postprandial Increase of Complement Component 3 in Normolipidemic Patients With Coronary Artery Disease
Effects of Expanded-Dose Simvastatin


Abstract—Plasma concentrations of the third complement component (C3) predict the risk of myocardial infarction. Because chylomicrons stimulate C3 production by adipocytes in vitro, we investigated plasma C3 changes in vivo after an oral fat load. Thirty-seven subjects (20 normolipidemic patients with coronary artery disease [CAD] and 17 healthy control subjects) underwent an oral fat load (50 g/m²). C3 was measured at baseline and at 2-hour intervals after fat intake for 10 hours. The effects of lipid lowering by simvastatin were evaluated in 16 patients. Fasting plasma C3 was 1.06±0.26 and 0.90±0.12 g/L in CAD patients and control subjects, respectively. Fasting C3 was correlated with several parameters associated with insulin resistance. The best determinant of fasting C3 was waist circumference (adjusted \( R^2 = 0.48, \beta = 0.71, P < 0.001 \)); the addition of postprandial triglyceridemia to the model improved it (adjusted \( R^2 = 0.63 \)). Plasma C3 levels at 2, 4, and 6 hours after fat ingestion were significantly higher than fasting levels in patients and control subjects. C3 increased maximally to 1.39±0.33 g/L in patients and to 1.11±0.18 g/L in control subjects (\( P < 0.01 \) for patients versus control subjects). Total postprandial triglyceridemia was the best determinant of maximal C3 increase (adjusted \( R^2 = 0.47, \beta = 0.70; P < 0.001 \)). Treatment with simvastatin decreased fasting and postprandial C3 by 6% and 39%, respectively (\( P < 0.05 \) for both versus no treatment). Postprandial plasma C3 concentrations increase in CAD patients and control subjects. Fasting C3 is associated with waist circumference, but postprandial C3 increment is associated with postprandial lipemia. Fasting and postprandial C3 concentrations decrease after treatment with simvastatin. (Arterioscler Thromb Vasc Biol. 2001;21:1526-1530.)

Key Words: atherosclerosis ■ chylomicrons ■ complement component 3 ■ apolipoprotein B ■ simvastatin

The plasma level of the third complement component (C3) is a powerful indicator of the risk of myocardial infarction in men.\(^1\) Fasting plasma concentrations of C3 are associated with several risk factors for myocardial infarction, among which fasting plasma insulin levels\(^2\) and apolipoprotein B have been identified recently.\(^3\) In vitro studies have shown that in the presence of chylomicrons, adipocytes secrete C3.\(^4\) In vivo studies have shown a postprandial increase in factor VII activity\(^5\) and C4b binding protein levels,\(^6\) linking postprandial lipoprotein metabolism to thrombotic and inflammatory processes. Adipose tissue may be a source of different cytokines,\(^7\) and increased secretion of interleukin-6 (IL-6) by adipose tissue after a meal has been described.\(^8\)

We investigated whether plasma C3 increases in vivo after an oral fat load in healthy subjects and in normolipidemic patients with premature coronary sclerosis. In addition, we intended to evaluate which parameters are associated with postprandial plasma C3 changes. For this purpose, we measured fasting and postprandial C3 levels in a group of normolipidemic patients with premature coronary artery disease (CAD) and normolipidemic control subjects. To evaluate the influence of aggressive lipid-lowering therapy on postprandial lipemia and the postprandial response of C3, a subgroup of the CAD patients was studied after treatment with expanded-dose simvastatin.

Methods

Subjects

Seventeen healthy control subjects and 20 normolipidemic patients with coronary sclerosis established by coronary angiography at a young age (before the age of 55 years in men and 65 years in women) were included. Exclusion criteria were as follows: the presence of diabetes, body mass index (BMI) >30 kg/m², renal and/or liver failure, fasting plasma cholesterol >6.5 mmol/L or fasting triglycerides (TGs) >2.3 mmol/L (off lipid-lowering medication), use of alcohol of >3 U/d, and a cardiac event or revascularization procedure during the 6 months before the start of the study. Control subjects were included if they had a negative family history of
significant. Differences at the post hoc analysis test. Bivariate correlations were calculated between plasma TG concentrations by using repeated-measures ANOVA with Bonferroni correction. The use of GraphPad Prism, version 3.0. Differences between 2 groups were tested by the independent-sample t test. Differences before and after treatment were analyzed by paired t test. Plasma TG and C3 during the oral fat load were compared with fasting values. Mean maximal postprandial C3 increase was 0.33±0.13 g/L in patients and 0.22±0.11 g/L in control subjects (P<0.01, patients versus control subjects). AUC-C3 and dAUC-C3 were higher in patients compared with control subjects (bottom right panel).

**Differences among C3 levels**

All significant correlations between AUC-TG, dAUC-TG, fasting and maximal postprandial C3 levels, absolute maximal C3 increase, and the characteristics from Table 1 are listed in Table 2. Multiple regression analysis was performed by using all significantly correlated parameters from Table 2. The best determinant of fasting C3 in all groups was calculated by the trapezoidal rule with the use of GraphPad Prism, version 3.0. Differences between 2 groups were tested by the independent-sample t test. Differences before and after treatment were analyzed by paired t test. Plasma TGs and C3 during the oral fat load were compared with fasting concentrations by using repeated-measures ANOVA with Bonferroni as the post hoc analysis test. Bivariate correlations were calculated by using Pearson correlation coefficients. All significantly correlated variables were used as independent variables in stepwise multiple regression analysis with fasting C3 levels and postprandial C3 increase as dependent variables. In the case of plasma TG and insulin, the calculations were performed after logarithmic transformation. For statistical analysis, SPSS, version 10.0, was used. Differences at P<0.05 (2-tailed) were considered to be statistically significant.

**Oral Fat-Loading Test**

All subjects underwent a standardized oral fat-loading test containing 50 g fat per square millimeter body surface to which vitamin A was added. On the morning of the test, anthropometric parameters were measured. After ingestion of the fat load, subjects were only allowed to drink water and tea during the following 10 hours. Peripheral blood samples were obtained in sodium EDTA (2 mg/mL) before (time 0) and at 2-hour intervals until 10 hours after the meal. Blood samples were kept on ice and centrifuged immediately for 15 minutes at 800g at 4°C.

**Analytical Methods**

C3 was measured by nephelometry (Dade Behring Nephelometry type II). TGs and cholesterol in plasma and HDL cholesterol obtained after precipitation with dextran sulfate/MgCl2 were determined by using a Vitros 250 analyzer (Johnson & Johnson). Plasma apoB was measured by nephelometry with the use of apoB monoclonal antibodies (Behring Diagnostics NV, OSAN 14/15). Glucose was measured by glucose oxidase dry chemistry (Viros GLU slides) and colorimetry, and insulin was measured by competitive radioimmunoassay with the use of polyclonal antibodies. LDL cholesterol was calculated by using the Friedewald formula (LDL=total cholesterol−(HDL+TG/2.2)).

**Statistical Analysis**

Data are given as mean±SD. The total and incremental areas under the TG and C3 curve (AUC-TG and dAUC-TG and AUC-C3 and dAUC-C3, respectively) were calculated by the trapezoidal rule with the use of GraphPad Prism, version 3.0. Differences between 2 groups were tested by the independent-sample t test. Differences before and after treatment were analyzed by paired t test. Plasma TGs and C3 during the oral fat load were compared with fasting concentrations by using repeated-measures ANOVA with Bonferroni as the post hoc analysis test. Bivariate correlations were calculated by using Pearson correlation coefficients. All significantly correlated variables were used as independent variables in stepwise multiple regression analysis with fasting C3 levels and postprandial C3 increase as dependent variables. In the case of plasma TG and insulin, the calculations were performed after logarithmic transformation. For statistical analysis, SPSS, version 10.0, was used. Differences at P<0.05 (2-tailed) were considered to be statistically significant.

**Results**

**General Characteristics**

Baseline characteristics of patients and controls are shown in Table 1. Because no differences were present between males and females (data not shown), data were presented for both sexes combined. Controls had a lower waist circumference and diastolic blood pressure. Fasting glucose, apoB, TG, and C3 concentrations were higher in patients compared with control subjects.

**Postprandial TG Changes**

Postprandial plasma TG values are presented in the top panel of Figure 1 for patients and control subjects. AUC-TG was higher in patients compared with control subjects, but the difference in dAUC-TG was not statistically significant (top right panel).

**Table 1. Demographic Characteristics and Baseline Fasting Biochemical Characteristics in 20 Normolipidemic CAD Patients (Off Treatment) and 17 Healthy Control Subjects**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients (n=20)</th>
<th>Control Subjects (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>50±8</td>
<td>49±7</td>
</tr>
<tr>
<td>Sex (male/female), n</td>
<td>12/8</td>
<td>11/6</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.0±3.3</td>
<td>23.8±2.7</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>93±12</td>
<td>84±7*</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>126±14</td>
<td>118±16</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>88±9</td>
<td>78±14*</td>
</tr>
<tr>
<td>Fasting C3, g/L</td>
<td>1.06±0.26</td>
<td>0.90±0.12*</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.5±0.8</td>
<td>5.1±0.8</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.16±0.18</td>
<td>1.24±0.27*</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.8±0.8</td>
<td>3.4±0.8</td>
</tr>
<tr>
<td>Fasting TG, mmol/L</td>
<td>1.4±0.5</td>
<td>1.1±0.3*</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>1.06±0.24</td>
<td>0.88±0.19*</td>
</tr>
<tr>
<td>Insulin, mU/L</td>
<td>10.3±5.8</td>
<td>9.0±4.2</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.5±0.4</td>
<td>5.1±0.4*</td>
</tr>
</tbody>
</table>

Values are mean±SD. *P<0.05 vs patients.
subjects was the waist circumference (adjusted $R^2=0.48$, $\beta=0.71$, $P<0.001$; Figure 2, top panel). Addition of AUC-TG improved the model significantly (adjusted $R^2=0.63$). Other variables from Table 2 did not enter into the model. The postprandial C3 peak was best predicted by fasting C3 (adjusted $R^2=0.89$, $\beta=0.95$, $P<0.001$; Figure 2, middle panel). Addition of apoB and AUC-TG improved the model significantly (adjusted $R^2=0.91$ and 0.92, respectively). Other variables from Table 2 did not enter into the model. The absolute postprandial C3 increase was best predicted by AUC-TG (adjusted $R^2=0.47$, $\beta=0.70$, $P<0.001$; Figure 2, bottom panel). Addition of apo B improved the model significantly (adjusted $R^2=0.55$).

**Effects of Treatment With Expanded-Dose Simvastatin**

During treatment, anthropometric variables like BMI and waist circumference did not change (data not shown). After treatment, fasting apoB, total cholesterol, and LDL cholesterol decreased by 48±9%, 42±8%, and 58±11%, respectively ($P<0.001$ for each, Table 3). Fasting TG decreased by 20±29% ($P=0.009$), and C3 decreased by 6±12% ($P=0.049$, Table 3). Figure 3 shows that AUC-TG decreased significantly from 21.0±7.7 to 15.3±6.1 mmol·h·L$^{-1}$ ($P<0.001$). Changes of dAUC-TG did not reach statistical significance (from 6.1±4.2 to 4.4±3.4 mmol·h·L$^{-1}$, $P=0.18$). dAUC-C3 decreased from 1.6±1.0 to 0.8±1.0 g·h·L$^{-1}$ ($P<0.05$). Treatment with simvastatin suppressed the maximal postprandial C3 increase from 0.31±0.14 to 0.19±0.15 g/L ($P<0.01$). The change in dAUC-TG was the only variable that correlated significantly with the change in fasting C3 ($r=0.51$, $P=0.044$).

**Discussion**

The present study shows a postprandial increase in plasma C3 in normolipidemic subjects with and without cardiovascular disease. The use of expanded-dose simvastatin suppressed this increase. The correlation between fasting C3 and dAUC-TG supports the hypothesis that increased TG levels stimulate the postprandial C3 increase.

**Table 2. Pearson Correlation Coefficients Between Fasting and Postprandial Plasma C3 Levels and Anthropometric and Biochemical Variables**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fasting C3</th>
<th>Maximal Postprandial C3</th>
<th>Maximal C3 Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.34</td>
<td>0.54</td>
<td>0.55</td>
</tr>
<tr>
<td>BMI</td>
<td>0.61</td>
<td>0.54</td>
<td>0.55</td>
</tr>
<tr>
<td>Waist</td>
<td>0.70</td>
<td>0.73</td>
<td>0.55</td>
</tr>
<tr>
<td>SBP</td>
<td>0.43</td>
<td>0.36</td>
<td>0.46</td>
</tr>
<tr>
<td>DBP</td>
<td>0.53</td>
<td>0.46</td>
<td>0.55</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.57</td>
<td>0.58</td>
<td>0.38</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.57</td>
<td>0.58</td>
<td>-0.37</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.41</td>
<td>0.46</td>
<td>0.37</td>
</tr>
<tr>
<td>Fasting TGs</td>
<td>0.60</td>
<td>0.68</td>
<td>0.57</td>
</tr>
<tr>
<td>ApoB</td>
<td>0.53</td>
<td>0.62</td>
<td>0.55</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.60</td>
<td>0.53</td>
<td>0.55</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.63</td>
<td>0.61</td>
<td>0.33</td>
</tr>
<tr>
<td>C3</td>
<td>ND</td>
<td>0.92</td>
<td>0.44</td>
</tr>
<tr>
<td>AUC-TG</td>
<td>0.63</td>
<td>0.74</td>
<td>0.64</td>
</tr>
<tr>
<td>dAUC-TG</td>
<td>0.39</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; and ND, not determined. Only significant correlations are shown.
disease. This postprandial C3 increase was correlated with postprandial triglyceridemia and was altered by aggressive lipid-lowering therapy, providing further evidence for previous data from in vitro and in vivo studies, suggesting that TG metabolism and C3 levels are closely linked.1,4

In the present study, fasting C3 levels were associated with several features of the insulin resistance syndrome, such as fasting levels of glucose, TGs, and insulin, BMI, blood pressure, and waist circumference, in line with earlier work of Muscari et al.2 However, by multiple regression analysis, waist circumference was the strongest determinant of the fasting C3 levels. Waist circumference is strongly associated with the amount of intra-abdominal adipose tissue and with several metabolic disorders seen in insulin resistance.10,11 A possible explanation for this finding may be enhanced production of C3 by intra-abdominal adipocytes explaining, in part, the correlations between fasting C3 levels and variables linked to insulin resistance, as proposed by Yudkin.12

Compared with healthy control subjects, CAD patients in the present study had higher fasting and postprandial C3 levels. AUC-TG was the best predictor of the postprandial C3 increase in patients and control subjects. In vitro studies have shown that chylomicrons increase C3 production in adipocytes.4 Therefore, enhanced postprandial triglyceridemia may directly increase C3 levels. Because humans are almost always in a postprandial condition, it could be hypothesized that exposure to chylomicrons leads to upregulation of C3 levels. This could be an explanation for the correlation between fasting C3 concentrations and AUC-TG in the present study. Because patients with premature coronary sclerosis have enhanced postprandial triglyceridemia,14–18 this could explain the increased fasting C3 levels seen in our patients compared with matched control subjects.

The major source of circulating C3 is the liver.19 We cannot exclude postprandial C3 increase due to enhanced C3 synthesis in hepatocytes. We are not aware of any report describing enhanced hepatic C3 production by chylomicrons. Baldo et al20 have suggested a metabolic link between postprandial lipemia and the complement system, involving the acylation stimulating protein (ASP), a metabolic product of C3. ASP strongly stimulates TG synthesis and transmembrane transport of glucose in human adipocytes.20 An attenuated postprandial ASP production from adipose tissue may play a role in the impaired clearance of postprandial TGs from plasma.21 Systemic postprandial ASP levels do not change,22 suggesting that ASP generation is a local process occurring in the vicinity of adipocytes. Because spillover into the systemic circulation may be low, we have focused on total postprandial plasma C3 changes. In their first report, Baldo et al20 reported a postprandial plasma ASP increase. However, this finding seemed to be incorrect because of the nonspecificity of the antibody used. The present finding could explain their findings if that antibody had cross-reacted with C3, because ASP is a 76–amino acid fragment of C3. In a previous report by Charlesworth et al,22 no postprandial changes of C3 or ASP levels were found, although no details on C3 were given in that report. In that study,22 a mixed meal was used, in contrast with the present study, which may explain, in part, the contrasting results in both studies.

It has been suggested that inefficient postprandial TG clearance in patients with hyper-apoB may be linked with reduced postprandial fatty acid trapping by ASP.23 Enhanced postprandial flux of free fatty acid (FFA) toward the liver could lead to increased hepatic production of apoB-containing particles.9,24 Interestingly, in the present study, postprandial plasma concentrations of C3 were associated with fasting plasma levels of apoB. If postprandial fatty acid trapping is disturbed by impaired postprandial ASP generation, plasma levels of FFA will accumulate in plasma after an oral fat load. Increased plasma levels of FFA will result in detachment of lipoprotein lipase from the endothelial surface,25–27 resulting in slower catabolism of chylomicrons in the circulation. Because chylomicrons in vitro lead to increased C3 secretion by adipocytes, it could be hypothesized that an increase in circulating chylomicrons leads to higher plasma levels of C3. Thus, hyper-apoB and increased postprandial C3 levels could be due to impaired postprandial fatty acid metabolism. Whether this is caused by “ASP resistance” or decreased conversion of C3 to ASP is not clear.12

After treatment with expanded-dose simvastatin, fasting and postprandial C3 concentrations decreased significantly in the present study. The magnitude of these changes was correlated with changes in postprandial TG metabolism, underlining the relationship between postprandial triglyceridemia and C3. Statins are known to improve postprandial lipoprotein metabolism by upregulating LDL receptors and by decreasing hepatic VLDL synthesis.28,29 Both mechanisms lead to less competition for the clearance mechanisms shared by chylomicrons and VLDL.30 It was not feasible to perform >2 oral fat-loading tests in these patients, so we chose to investigate the effects of the maximally allowed dose of simvastatin. Whether treatment with lower doses of statins results in similar changes in postprandial TG and C3 metabolism in similar patients remains to be investigated.

In conclusion, plasma C3 levels increase after an oral fat load. This increase is best predicted by postprandial TG levels and is associated with fasting apoB levels. Treatment with expanded-dose simvastatin mitigates this postprandial increase, probably by improving postprandial lipoprotein metabolism.

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