Gender Differences in Postprandial Ketone Bodies in Normolipidemic Subjects and in Untreated Patients With Familial Combined Hyperlipidemia


Objective—An increased hepatic flow of free fatty acids (FFAs) is associated with impaired peripheral FFA trapping by malfunctioning of the complement component 3 (C3)/acylation-stimulating protein system and overproduction of VLDL in familial combined hyperlipidemia (FCHL). Postprandial ketone bodies reflect FFA oxidation in the liver, but the postprandial changes in male and female patients separately have not been determined yet. Gender differences in postprandial ketone bodies and C3 changes were investigated in normolipidemic patients and patients with untreated FCHL.

Methods and Results—Thirty-two normolipidemic patients (16 female and 16 male) and 19 patients with untreated normolipidemia (9 female and 10 male) underwent an oral fat-loading test. Total and incremental areas under the curves (AUC and dAUC, respectively) after the oral fat load were calculated. Triglyceride AUC was similar between genders in each group. Normolipidemic female subjects showed a higher levels of dAUC-hydroxybutyric acid than male subjects (1.37 ± 0.49 and 0.98 ± 0.43 mmol · h/L). In FCHL, a similar trend was observed in female (1.92 ± 0.38) compared with male (1.55 ± 0.87) subjects. In contrast to normolipidemia, FCHL did not show a postprandial increase in C3, although C3 was higher in FCHL.

Conclusions—Women have higher postprandial ketone bodies than men, probably reflecting enhanced postprandial hepatic FFA oxidation. In FCHL, both genders have higher postprandial ketone bodies and therefore higher hepatic FFA delivery. The higher fasting and postprandial C3 levels in FCHL may reflect resistance of the C3/acylation-stimulating protein system to promote peripheral fatty acid trapping. (Arterioscler Thromb Vasc Biol. 2003;23:1875-1880.)

Key Words: familial combined hyperlipidemia ■ insulin resistance ■ apolipoprotein B ■ abdominal obesity

Disturbed fatty acid metabolism has been suggested to play a central role in different metabolic disorders, such as insulin resistance, diabetes mellitus, and familial combined hyperlipidemia (FCHL). An increased flow of free fatty acids (FFAs) into the portal system has been postulated as a major contributor of dyslipidemia in those disorders because it could lead to hepatic overproduction of VLDL particles and interfere with hepatic glucose metabolism. One of the hypothetical mechanisms causing increased hepatic FFA flux is impaired action of the complement component 3 (C3)/acylation-stimulating protein (ASP) pathway, linking postprandial lipoprotein metabolism to the complement system.

Enhanced postprandial ketogenesis has been used to illustrate increased postprandial hepatic FFA flux in hyperlipidemic patients with FCHL. Animal models have shown gender-related differences in hepatic FFA oxidation, but this has never been confirmed in humans. The aim of the present study was to investigate postprandial changes of FFA and ketone bodies in relation to the C3/ASP system in normolipidemic nonobese and nondiabetic subjects and in patients with untreated FCHL.

Methods

Subjects
Fifty-one subjects participated in the present study. Seventeen subjects were normolipidemic patients with premature coronary artery disease (CAD) without any characteristics of FCHL. Data on these patients have been published in part elsewhere. Nineteen participants were patients with FCHL. Baseline characteristics of a subset of these patients have been published elsewhere.

Exclusion criteria for all participants were the presence of diabetes (according to the American Diabetes Association criteria), the presence of apolipoprotein (apo) E-2/E-2 genotype, body mass index (BMI) > 30 kg/m², renal or liver failure, and the use of more than 3 U of alcohol per day. All patients with CAD had fasting plasma...
cholesterol ≤6.5 mmol/L and fasting plasma triglycerides ≤2.3 mmol/L (not taking lipid-lowering medication) and were free of a cardiac event or revascularization procedure during the 6 months before the start of the study. Patients with FCHL presented with primary hyperlipidemia and elevated plasma apoB concentrations (>1.2 g/L) at the time of diagnosis; at least 1 first-degree relative had a different hyperlipidemic phenotype, and each had a positive family history of premature CAD. None of the patients with FCHL had a personal history of CAD. Both FCHL patients and CAD subjects were studied off lipid-lowering medication for at least 5 weeks. Healthy normolipidemic volunteers did not use any medication. All subjects gave written informed consent. The study was approved by the Medical Ethical Committee of the University Medical Center Utrecht, and all participants gave written informed consent.

## Oral Fat-Loading Test

All subjects visited our department after a 12-hour fast and underwent a standardized oral fat-loading test containing 50 g fat and 3.75 g dextrose per square meter of body surface. 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 (t=0 hours) and at 2 hourly intervals until 10 hours after the meal. Peripheral blood samples for the determination of hydroxybutyric acid (HBA) were collected in tubes containing lithium-heparin. HBA was the only ketone body measured, because in previous studies it was found that HBA and acetoacetate showed identical postprandial responses. Blood samples were kept on ice and centrifuged immediately for 15 minutes at 800 g at 4°C. Postprandial insulin levels in patients with FCHL were only available for 7 male and 4 female subjects. C3 levels were measured in blood samples by an enzymatic colorimetric method (Wako Chemicals GmbH and Neuss). For FFA measurement, a lipase inhibitor (Orlisat) was added to the plasma to block ex vivo lipolysis. Glucose was measured by glucose oxidase dry chemistry (Vitros GLU slides) and colorimetry, whereas insulin was measured by competitive radioimmunoassay using polyclonal antibodies. C3 was measured by nephelometry (Dade Behring Nephelometer type II). LDL cholesterol was calculated using the Friedewald formula. HBA was measured spectrophotometrically by the principle of converting NADH to NAD⁺ after adding 3-hydroxybutyrate dehydrogenase. For this purpose, 0.5 mL lithium-heparin blood was deproteinized by adding 1 mL of 0.7 mol/L HClO₄ immediately after collection.

## Table 1. Demographic Characteristics and Baseline Fasting Biochemical Characteristics: Normolipidemic Versus FCHL Subjects

<table>
<thead>
<tr>
<th></th>
<th>Normolipidemic Subjects</th>
<th>FCHL Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men (n=16)</td>
<td>Women (n=16)</td>
</tr>
<tr>
<td></td>
<td>Men (n=10)</td>
<td>Women (n=9)</td>
</tr>
<tr>
<td>Age, y</td>
<td>49±6</td>
<td>51±8</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.1±3.2</td>
<td>24.9±2.8</td>
</tr>
<tr>
<td>WHR</td>
<td>0.93±0.06</td>
<td>0.85±0.08*</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>121±16</td>
<td>127±17</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>83±15</td>
<td>85±11</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.5±0.7</td>
<td>5.2±0.9†</td>
</tr>
<tr>
<td>HDL Chol, mmol/L</td>
<td>1.21±0.21†</td>
<td>1.25±0.20†</td>
</tr>
<tr>
<td>LDL Chol, mM</td>
<td>3.7±0.6</td>
<td>3.4±0.9</td>
</tr>
<tr>
<td>Fasting TG, mM</td>
<td>1.36±0.53†</td>
<td>1.28±0.50†</td>
</tr>
<tr>
<td>Apo B, g/L</td>
<td>1.02±0.22</td>
<td>0.88±0.21†</td>
</tr>
<tr>
<td>FFA, mmol/L</td>
<td>0.54±0.18</td>
<td>0.48±0.18</td>
</tr>
<tr>
<td>Insulin, IE</td>
<td>5.5±3.2†</td>
<td>8.0±3.6</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.5±0.4</td>
<td>5.1±0.5*</td>
</tr>
<tr>
<td>Complement component 3, g/L</td>
<td>1.03±0.22†</td>
<td>0.93±0.25†</td>
</tr>
</tbody>
</table>

Values are mean±SD. Insulin levels were known in 7 male FCHL and 4 female FCHL patients. *P<0.05 compared with men. †P<0.05 compared with gender-matched FCHL patients.

## Statistical Analysis

Data are given as mean±SD in Tables and text and as mean±SEM in the Figures. The area under the curve (AUC) was calculated by the trapezoidal rule. The incremental AUC (dAUC) was calculated after correction for fasting values. Differences between male and female subjects were tested by unpaired t test. Plasma TG, C3, insulin, NEFA, and HBA concentrations 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 between dAUC-HBA, dAUC-C3, and other variables were calculated using Spearman’s correlation coefficients. All significantly correlated variables were used as independent variables in stepwise multiple regression analysis with dAUC-HBA and dAUC-C3 as dependent variables. Insulin and TG concentrations were log transformed before testing. Differences were considered statistically significant if P<0.05 (2-tailed). Analyses were performed with SPPS/PC 10.0 (SPSS).

## Results

### General Characteristics

Twenty-five women (8 with normolipidemia and CAD, 9 with FCHL) and 26 men (9 with normolipidemia and CAD, 10 with FCHL) were enrolled in the study. Table I (available online at http://atvb.ahajournals.org) shows data of all normolipidemic patients on the basis of their CAD status.
When male patients with CAD were compared with healthy male control subjects, the patients had a higher BMI, waist to hip ratio (WHR), and diastolic blood pressure than controls (Table 1). Female patients with CAD did not differ from healthy female control subjects. When data for all normolipidemic subjects were pooled, men had a higher WHR and fasting glucose levels compared with women (Table 1). Other biochemical and anthropometric variables were not significantly different between male and female patients with normolipidemia. Compared with gender-matched normolipidemic subjects, male patients with FCHL had lower fasting HDL cholesterol and higher fasting plasma TG, insulin, and C3 concentrations. Female patients with FCHL had lower HDL cholesterol and higher plasma cholesterol, TG, apoB, and C3 levels than normolipidemic female subjects.

**Postprandial Lipemia and Markers of FFA Metabolism**

Because there were no major differences in baseline characteristics between normolipidemic patients with CAD and control subjects, these data were pooled and are presented in Figures 1 through 3 and online Figures I and II (available at http://atvb.ahajournals.org). After fat ingestion, plasma TG increased, reaching a maximum at t=4 hours in all subjects (Figure 1). At all time points, patients with FCHL had higher TG concentrations than normolipidemic subjects. Plasma C3, insulin, FFA, and HBA concentrations are given in Figure 1. In concordance with earlier reports, patients with FCHL did not show an early postprandial increase of plasma C3 levels after ingestion of the meal in contrast to normolipidemic subjects.

In the normolipidemic group, there were no gender differences except for AUC-insulin, AUC-HBA, and dAUC-HBA, which were all increased in female compared with male subjects (Table 2). Within the FCHL group, no gender differences were present. However, in male patients with FCHL, AUC-TG, dAUC-TG, dAUC-HBA, and AUC-C3 were higher and dAUC-C3 was lower than in normolipidemic male subjects. In female patients with FCHL, AUC-TG, and dAUC-TG were higher and dAUC-C3 was lower compared with normolipidemic female subjects (Table 2).

**Determinants of Postprandial HBA Increase**

In normolipidemic male subjects, dAUC-HBA was significantly correlated to AUC-C3 (r=0.86), fasting C3 (r=0.72), fasting glucose (r=0.66), BMI (r=0.62), diastolic blood pressure (r=0.61), dAUC-C3 (r=0.53), WHR (r=0.53), and apoB (r=0.52). In normolipidemic female subjects, dAUC-HBA was correlated only with the WHR (r=0.51). Using multiple regression analysis, the only determinant of dAUC-HBA in normolipidemic female subjects was the WHR (adjusted R=0.32, β=0.61, P<0.05). The best determinant of dAUC-HBA in normolipidemic male subjects was AUC-C3 (adjusted R=0.43, β=0.68, P<0.01). Addition of fasting glucose improved the model significantly (adjusted R=0.57). Other variables from Tables 1 and I did not add statistical significance and were not entered into the model.

In male patients with FCHL, dAUC-HBA was significantly correlated with AUC-FFA (r=0.67) and FFA levels at 2 and 10 hours after fat ingestion (r=0.66 and 0.65, respectively). However, dAUC-HBA was inversely correlated with age (r=-0.86) and fasting insulin levels (r=-0.79). In female patients with FCHL, no significant correlations were found. Only WHR tended to correlate to dAUC-HBA (r=0.63; P=0.07).

**Discussion**

The present study confirms earlier results of increased postprandial hepatic FFA flux in patients with FCHL compared with control subjects based on a higher increase in postprandial HBA (dAUC-HBA in Table 2) and is in line with results in mouse models of FCHL showing higher ketone body production. The present study extends those observations by showing this biological phenomenon for both male and female patients with FCHL. Furthermore, it is shown for the first time that female subjects with normolipidemia and those with FCHL have higher postprandial HBA responses than male subjects. This was only statistically significant for normolipidemic subjects, showing the same trend in patients with FCHL without reaching statistical significance because of the relatively small number of subjects with FCHL included. It should be underlined that all subjects were matched for age and BMI. However, WHRs were lower in female than in male subjects, both in those with normolipidemic subjects.
demia and those with FCHL. This may in some way be linked to the exaggerated postprandial HBA response in female subjects. One possible explanation could be that women have a lower intraabdominal fat mass than men. It is likely that a greater proportion of plasma FFAs are trapped in this intraabdominal fat compartment in men than in women and therefore less FFA reaches the liver in men than in women, thus explaining the higher HBA levels in the latter. Why are these FFAs not incorporated into VLDL, and why is insulin resistance not increased in female subjects? One of the reasons could be that estrogens may decrease VLDL production by enhancing hepatic FFA oxidation. Indeed, there is in vivo human data available suggesting that estrogens induce fatty acid availability and oxidation, which is supported by our data. Alternatively, the positive correlation between WHR and dAUC-HBA in male subjects with the same trend in female subjects found here suggests that this fat compartment may also be a source of FFA shunted to the liver in the postprandial situation. To answer these questions, quantitative data on hepatic FFA flux in humans are necessary. Our study is the first to suggest a significantly increased hepatic postprandial FFA flux in female subjects and improved intrahepatic FFA oxidation compared with male subjects.

Alternatively, a less efficient utilization of ketone bodies by peripheral tissues in women than in men would also explain the higher postprandial HBA curves in the former. However, this is not likely, because 6 hours postprandially, tissues do not use ketone bodies as a fuel.

Gender differences and the effects of intraabdominal fat accumulation with respect to postprandial lipemia have been reported previously. In the present study, all participants were of similar age and BMI. Moreover, WHR between men and women did not differ largely. These findings may explain the lack of gender differences of the postprandial triglyceridemia in each group. The most striking difference was the postprandial HBA response, especially in the normolipidemic subjects. In addition, fasting plasma triglycerides, which are the best determinants of postprandial lipemia, were very similar between genders in each group, in contrast to other studies in which fasting plasma TGs were already different between male and female subjects in the fasting state.

In FCHL, gender and BMI are major determinants of the plasma apoB levels and of subclinical atherosclerosis estimated by intima-media thickness. However, in a recent study by the Seattle group, neither visceral obesity nor insulin resistance could fully explain the elevated levels of apoB in FCHL. Our data are in line with these observations, because men and women with FCHL had higher plasma apoB concentrations than their gender-matched controls despite similar WHR.

A second novel finding in this study is that although the postprandial plasma FFA concentrations were similar at t=4 hours in male and female patients with FCHL, the late postprandial FFA concentrations were highest in the female patients with FCHL at t=8 and 10 hours. This was also the case in normolipidemic female subjects compared with normolipidemic male subjects. In this period, part of the FFA may originate from intracellular lipolysis in adipose tissue. A recent study has provided convincing evidence for stimulation of intracellular lipolysis in adipocytes by estrogens and phytoestrogens. This could explain the differences in late postprandial FFA concentrations and HBA concentrations in women compared with men found in the present study. Ten hours after the meal, FFA concentrations in female subjects were still significantly higher than fasting levels, whereas

![Figure 3. Mean (±SEM) plasma HBA concentrations in response to an acute oral fat-loading test, ingested at t=0 (see Methods section for details). ○, normolipidemic male subjects; □, normolipidemic female subjects; ■, male subjects with FCHL; and ◆, female subjects with FCHL. *P<0.01, †P<0.05 vs t=0 hours.](attachment:image.png)

**Table 2. Postprandial AUCs for TG, FFA, HBA, and C3**

<table>
<thead>
<tr>
<th></th>
<th>Normolipidemic Subjects</th>
<th>FCHL Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men (n=16)</td>
<td>Women (n=16)</td>
</tr>
<tr>
<td></td>
<td>Men (n=10)</td>
<td>Women (n=9)</td>
</tr>
<tr>
<td>AUC-TG, mmol/h per L</td>
<td>19.1±7.1†</td>
<td>17.2±6.8‡</td>
</tr>
<tr>
<td></td>
<td>41.2±14.8</td>
<td>39.4±20.0</td>
</tr>
<tr>
<td>dAUC-TG, mmol/h per L</td>
<td>5.5±2.8‡</td>
<td>4.4±4.2‡</td>
</tr>
<tr>
<td></td>
<td>8.4±4.5</td>
<td>10.5±8.7</td>
</tr>
<tr>
<td>AUC-FFA, mmol/h per L</td>
<td>6.3±0.9</td>
<td>6.7±1.9</td>
</tr>
<tr>
<td></td>
<td>6.5±1.7</td>
<td>7.0±1.0</td>
</tr>
<tr>
<td>AUC-HB, mmol/h per L</td>
<td>1.59±0.57</td>
<td>1.94±0.69*</td>
</tr>
<tr>
<td></td>
<td>1.78±0.86</td>
<td>2.14±0.39</td>
</tr>
<tr>
<td>dAUC-HBA, mmol/h per L</td>
<td>0.98±0.43‡</td>
<td>1.37±0.49*</td>
</tr>
<tr>
<td></td>
<td>1.55±0.87</td>
<td>1.92±0.38</td>
</tr>
<tr>
<td>AUC-C3, g/h per L</td>
<td>11.7±2.6†</td>
<td>10.6±3.0</td>
</tr>
<tr>
<td></td>
<td>13.9±1.4</td>
<td>13.6±2.3</td>
</tr>
<tr>
<td>dAUC-C3, g/h per L</td>
<td>1.4±0.9†</td>
<td>1.2±0.9‡</td>
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<tr>
<td></td>
<td>−0.8±2.1</td>
<td>−0.8±0.8</td>
</tr>
<tr>
<td>AUC-insulin, IE/h per L</td>
<td>49±24†</td>
<td>76±35*</td>
</tr>
<tr>
<td></td>
<td>109±67</td>
<td>74±17</td>
</tr>
</tbody>
</table>

*Values are mean±SD *P<0.05 compared with men. †P<0.05 compared to gender matched FCHL patients;
male FFA levels had returned to baseline levels (Figure 1) both in normolipidemic and FCHL subjects. It should be noted that our results are in contrast to those by Couillard et al., who showed higher postprandial FFA and insulin concentrations in male compared with female subjects. The authors did not provide data on postprandial ketone bodies. One of the explanations may be the different test meal used and the differences in fasting lipid profile in male and female subjects in that study. Finally, in vitro lipolysis may have influenced the results, because an inhibitor of lipolysis was not used.

The third novel finding in this study is that an impaired postprandial C3 response in FCHL, which has been reported earlier by our group, is a characteristic of both genders. We have previously shown that patients with CAD and fasting normolipidemia and healthy controls show an early increase of C3 as a response to an acute oral fat load. This rapid and significant increase was only observed in the normolipidemic subjects in the present study, with higher concentrations in male than in female patients. In contrast, male and female patients with FCHL showed a similar abnormal postprandial C3 pattern. C3 is the precursor molecule of ASP, which is supported by a limited number of studies showing a positive correlation between plasma C3 and ASP28 and by observations in our laboratory (unpublished data, 2003). The mechanism linking higher fasting and postprandial C3 levels to postprandial hepatic fatty acid flux in FCHL could be ASP resistance, as proposed earlier. If postprandial fatty acid trapping is disturbed by impaired postprandial ASP action, FFAs will accumulate after an oral fat load. Increased plasma levels of FFAs will result in detachment of LPL from the endothelial surface, resulting in slower catabolism of triglyceride-rich lipoproteins and their remnants in the circulation. Because triglyceride-rich lipoproteins are the most potent stimulator of ASP synthesis and therefore of C3 secretion by adipocytes, this could lead to increased plasma C3 levels, whereas the plasma FFAs will be taken up by the liver, leading to an increased HBA production. The fact that such correlations were not found in normolipidemic women is in agreement with a report in which male but not female authors did not provide data on postprandial ketone bodies. Finally, in vivo lipolysis may have influenced the results, because an inhibitor of lipolysis was not used.

The reference section is included in the document.

References


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Figure II

![Graph showing insulin levels over time](image)