Abstract—Familial combined hyperlipidemia (FCHL) is characterized by hyperlipidemia and insulin resistance, but intracellular defect in insulin action is unknown. Therefore, we investigated insulin action by applying the hyperinsulinemic euglycemic clamp technique with indirect calorimetry in 58 FCHL family members (28 with FCHL; 30 without dyslipidemia; aged 49±12 years; body mass index [BMI], 25.2±4.0 kg/m²) and in 72 healthy control subjects (aged 54±6 years; BMI, 26.3±3.1 kg/m²). In the fasting state, FCHL patients had higher levels of total cholesterol, total triglycerides, and apolipoprotein B than control subjects (P<0.001 after adjustment for gender, age, and BMI). During the euglycemic clamp, FCHL patients had lower rates of glucose oxidation (15.93±3.55 versus 19.65±4.60 μmol/kg/min; P=0.001) and higher rates of lipid oxidation (0.15±0.13 versus 0.01±0.25 mg/kg/min; P=0.024), as well as higher levels of serum-free fatty acids (FFA) (0.24±0.17 versus 0.06±0.06 mmol/L; P<0.001) compared with those of control subjects. Relatives without dyslipidemia differed similarly from control subjects with respect to rates of glucose and lipid oxidation and FFA suppression during the hyperinsulinemic clamp. In FCHL family members, during the euglycemic clamp FFAs correlated negatively with the rates of glucose oxidation (P=0.001) but not with the rates of glucose nonoxidation (P=0.408). In FCHL family members without dyslipidemia and in control subjects, FFAs during the clamp correlated positively with levels of total triglycerides (P=0.001) and very low density lipoprotein cholesterol (P=0.008). We conclude that in patients with FCHL, and also in their first-degree relatives, insulin’s suppressive effect on FFA levels is impaired, which may precede dyslipidemia in FCHL. (Arterioscler Thromb Vasc Biol. 1998;18:1548-1553.)

Key Words: familial combined hyperlipidemia ■ insulin resistance ■ insulin ■ glucose oxidation ■ nonoxidative glucose disposal
consecutive male patients who suffered from an acute myocardial infarction from 1978 to 1980 at the Kuopio University Hospital were included. For each proband, an age- and sex-matched male control subject without coronary heart disease (based on clinical examination and ECG) was selected from the population register of the Kuopio Province. Both groups of probands (75 with and without CHD) and their first-degree relatives (siblings and children) were used to define FCHL families. Subjects having total cholesterol ≥5.0 mmol/L were excluded as well as subjects under 22 years of age in the follow-up study (to match the age range in the baseline and follow-up studies). The control population described above consisted of 250 persons (161 males and 89 females). The cut-off points for abnormal lipids were defined as 80th percentile for total cholesterol and total triglycerides and 90th percentile for total triglycerides. The 80th percentile for total cholesterol was used because of high cholesterol level among subjects living in eastern Finland. After adjustment for age with linear regression analysis the values for the median age (55 years) of subjects in this population were used as cut-off points for abnormal lipids. These values were 7.7 mmol/L for total cholesterol in both men and women and 2.2 mmol/L for total triglycerides in women and 2.4 mmol/L in men.

To meet the criteria for FCHL, each family had to have at least 3 affected members with different types of dyslipidemia and at least 1 affected member in 2 different generations. Altogether, 25 families with FCHL and 162 family members met the criteria and were included in this study.

A random sample of probands, their siblings, and children of the FCHL families who participated in the follow-up visit from 1993 to 1995 were invited for the hyperinsulinemic euglycemic clamp. Altogether, 58 subjects participated: 30 (17 men, 13 women) who did not have dyslipidemia and 28 (18 men, 10 women) with FCHL defined by total cholesterol ≥7.7 mmol/L and/or total triglycerides ≥2.2 mmol/L in women and ≥2.4 mmol/L in men (3 probands, 20 siblings of probands, 5 children of probands). Participants were somewhat younger (50 versus 61 years) and leaner (body mass index [BMI] 25 versus 27 kg/m²) than nonparticipants, but total cholesterol (6.92 versus 7.08 mmol/L) or triglycerides (1.94 versus 2.41 mmol/L) did not differ between these groups. Control subjects in this study were healthy unrelated men from our previous population study who had total cholesterol <7.7 mmol/L and total triglycerides <2.4 mmol/L. They had a normal glucose tolerance according to the World Health Organization criteria and no chronic disease, hypertension, symptoms or signs of coronary heart disease, nor continuous drug treatment. All probands, family members, and controls had normal liver, kidney, and thyroid function tests and none had a history of excessive alcohol intake.

Informed consent was obtained from all subjects after the purpose and potential risks of the study were explained to them. The protocol

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**Table 1. Clinical Characteristics of Control Subjects and Subjects With and Without FCHL**

<table>
<thead>
<tr>
<th></th>
<th>FCHL Families</th>
<th>Control (n=72)</th>
<th>Without FCHL (n=30)</th>
<th>With FCHL (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>53.6±5.5</td>
<td>44.1±10.8*</td>
<td>54.4±11.0†</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.3±3.1</td>
<td>23.8±3.8‡</td>
<td>26.8±3.6‡</td>
<td></td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.96±0.05</td>
<td>0.90±0.09§</td>
<td>0.93±0.07§</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>136±12</td>
<td>124±15</td>
<td>132±17</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>85±7</td>
<td>82±12</td>
<td>88±10</td>
<td></td>
</tr>
<tr>
<td>Hypertensives, No. of subjects</td>
<td>0</td>
<td>5</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.76±0.95</td>
<td>6.08±0.78</td>
<td>7.70±1.16</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.88±0.82</td>
<td>4.08±0.72</td>
<td>5.10±1.27</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.29±0.26</td>
<td>1.35±0.23</td>
<td>1.30±0.25</td>
<td></td>
</tr>
<tr>
<td>VLDL cholesterol, mmol/L</td>
<td>0.58±0.23</td>
<td>0.65±0.26</td>
<td>1.22±0.49</td>
<td></td>
</tr>
<tr>
<td>Total triglycerides, mmol/L</td>
<td>1.32±0.42</td>
<td>1.41±0.48</td>
<td>2.60±1.02</td>
<td></td>
</tr>
<tr>
<td>Apolipoprotein A1, g/L</td>
<td>1.56±0.26</td>
<td>1.50±0.18</td>
<td>1.56±0.25</td>
<td></td>
</tr>
<tr>
<td>Apolipoprotein B, g/L</td>
<td>0.97±0.21</td>
<td>0.99±0.17</td>
<td>1.34±0.19</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.5±0.5</td>
<td>5.3±0.4</td>
<td>5.5±0.5</td>
<td></td>
</tr>
<tr>
<td>Fasting insulin, pmol/L</td>
<td>53.3±33.6</td>
<td>49.8±25.2</td>
<td>67.8±25.2</td>
<td></td>
</tr>
<tr>
<td>Fasting free fatty acids, mmol/L</td>
<td>0.46±0.17</td>
<td>0.48±0.16</td>
<td>0.58±0.23</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SD.

*P<0.01, †P<0.001 family members without dyslipidemia vs. control subjects.

§P<0.05, †P<0.01 FCHL patients vs. family members without dyslipidemia.

¶P<0.01, †P<0.001 FCHL patients vs. family members without dyslipidemia or control subjects after adjustment for age, gender, and BMI.

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**Figure 1.** The rates of fasting lipid oxidation (mg/kg/min) and fasting glucose oxidation (μmol/kg/min) in control subjects (open bars), relatives without FCHL (hatched bars), and relatives with FCHL (filled bars). *P<0.01 (control subjects vs other groups).
glucose, and lipid oxidation rates were calculated according to
the first 10 minutes of each measurement were discarded, and the
baseline blood drawing, a priming dose of insulin (Actrapid 100
IU/mL, Novo Nordisk) was administered during the initial 10
minutes to raise insulin concentration quickly to the desired level,
where it was maintained by a continuous insulin infusion of 480
pmol/m²/min (80 mU/m²/min). Under these study conditions, hepatic
metabolism (mg/kg/min) during the hyperinsulinemic clamp in con-
and was in accordance with the Helsinki Declaration.
Metabolic Studies
The degree of insulin resistance was evaluated with the euglycemic
clamp technique after a 2-hour fast as previously described. After
baseline blood drawing, a priming dose of insulin (Actrapid 100
IU/mL, Novo Nordisk) was administered during the initial 10
minutes to raise insulin concentration quickly to the desired level,
where it was maintained by a continuous insulin infusion of 480
pmol/m²/min (80 mU/m²/min). Under these study conditions, hepatic
glucose production is suppressed completely in nondiabetic sub-
jects. Blood glucose was clamped at 5.0 mmol/L for the next 180
minutes by the infusion of 20% glucose at varying rates according to
blood glucose measurements performed at 5-minute intervals. The
mean value for the last hour was used to calculate the rates of
insulin-stimulated whole body glucose uptake (WBGU).
Indirect calorimetry was performed with a computerized flow-
through can gas analyzer system (Deltatrac, Datex) as previously
described. Gas exchange was measured for 30 minutes after a
12-hour fast and during the last 30 minutes of the euglycemic clamp.
The first 10 minutes of each measurement were discarded, and the
mean value of the last 20 minutes was used in calculations. Protein,
glucose, and lipid oxidation rates were calculated according to
Ferrannini. The rate of nonoxidative glucose disposal during the
euglycemic clamp was estimated by subtracting the carbohydrate
oxidation rate (as determined by indirect calorimetry in the last 20
minutes of the euglycemic clamp) from the glucose infusion rate.
Analytical Methods
Plasma glucose levels in the fasting state and after an oral glucose
load, as well as blood glucose and plasma lactate levels during the
euglycemic clamp, were measured by the glucose oxidase method
(2300 Stat Plus, Yellow Springs Instrument Co Inc). For the
determination of plasma insulin, blood was collected in EDTA-
containing tubes, and after centrifugation the plasma was stored at
−20°C until the analysis was performed. Plasma insulin concen-
tration was determined by a commercial double-antibody solid-phase
radioimmunoassay (Phadezym Insulin RIA 100, Pharmacia Diag-
nostics AB). Lipoprotein fractionation was performed by ultracen-
trifugation and selective precipitation as previously described.22
Cholesterol and triglyceride levels from whole serum and lipoprotein
fractions were assayed by automated enzymatic methods (Boehringer-
er-Mannheim). ApoB and apolipoprotein A1 were determined by a
commercial immunoturbidimetric method (Kone Instruments) and
serum FFAs from fresh frozen samples by an enzymatic method
(Wako Chemicals GmbH). Nonprotein urinary nitrogen was mea-
sured by an automated Kjeldahl method.21
Statistical Analysis
All calculations were done with the SPSS/Win programs (SPSS Inc).
The differences between the 3 study groups were evaluated with
ANCOVA after adjustment for age, gender, and BMI. If the
difference was statistically significant (P<0.05), pairwise compari-
sions of age, gender, and BMI between the study groups were done
using ANCOVA. Correlations between the variables were deter-
mined as Pearson correlations. VLDL cholesterol, total triglycerides,
inulin, and FFA levels were transformed logarithmically to obtain
normal distribution before statistical analyses. P values <0.05 were
considered statistically significant. All data are presented as
mean±SD.
Results
Clinical Characteristics and Fasting State Glucose
and Lipid Metabolism
First-degree relatives of FCHL patients without dyslipidemia
(n=30) were younger and had lower BMI and waist-to-hip ratio
than did control subjects (n=72) and FCHL patients (n=28), but
blood pressure did not differ between the groups. VLDL and
LDL cholesterol, total triglycerides, and apoB levels were higher
in relatives with FCHL than in controls or in relatives without
FCHL. In the fasting state glucose, insulin, and FFA levels did not
 differ between the study groups (Table 1). However, the
rates of lipid oxidation were higher in FCHL family members
with (0.83±0.25 mg/kg/min) or without dyslipidemia (0.90±0.25 mg/kg/min) when compared with those of controls
(0.57±0.35 mg/kg/min; P=0.007 and P=0.002, respectively).
The rates of glucose oxidation were lower in FCHL family
members with or without dyslipidemia than in controls
(3.83±2.36 versus 4.00±2.31 versus 7.54±4.17 μmol/kg/min; 
P=0.001 and P=0.002; Figure 1). No differences in FFA levels
or in the rates of lipid and glucose oxidation were present
between first-degree relatives of FCHL patients without dyslip-
idemia and patients with FCHL.
Lipid and Glucose Metabolism During the
Hyperinsulinemic Euglycemic Clamp
Glucose (5.0±0.1 mmol/L in controls, 5.0±0.1 in relatives
without dyslipidemia, and 5.0±0.1 in FCHL patients), insulin
(1073±215 versus 941±169 versus 1004±190 pmol/L), and
lactate levels (1.15±0.27 versus 1.19±0.27 versus
1.13±0.23 mmol/L) did not differ significantly between the
groups during the last hour of the euglycemic clamp. During
the clamp relatives both with and without FCHL had higher
levels of FFAs (0.24±0.17 versus 0.13±0.11 versus

Figure 2. Free fatty acids (mmol/L) and the rates of lipid oxy-
genation (mg/kg/min) during the hyperinsulinemic clamp in con-
sults (open bars), relatives without FCHL (hatched bars), and
FCHL patients (filled bars). *P<0.001 (control subjects vs
other groups); †P<0.05 (control subjects vs other groups).

Figure 3. Rates of whole body glucose uptake during the hy-
perinsulinemic clamp in control subjects, relatives without
FCHL, and relatives with FCHL. Oxidative glucose disposal
(filled portion of bars) and nonoxidative glucose disposal (open
portion of bars) are shown. *P<0.05 (the rates of nonoxidative
glucose disposal between relatives without FCHL and control
subjects); †P<0.01 (the rates of oxidative glucose disposal
between relatives without FCHL and control subjects); ‡P<0.01
(the rates of oxidative glucose disposal between FCHL patients
and control subjects).
0.06±0.06 mmol/L; \( P<0.001 \) and higher rates of lipid oxidation (0.15±0.13 versus 0.13±0.17 versus 0.007±0.25 mg/kg/min; \( P=0.011 \) and \( P=0.024 \)) than did control subjects (Figure 2). Both groups of FCHL family members also had lower rates of WBGU (49.73±12.10 versus 55.50±14.31 versus 58.50±14.65 mmol/kg/min; \( P=0.001 \) and \( P=0.035 \)) than did control subjects (Figure 3). This difference was due mainly to lower rates of glucose oxidation in FCHL family members with or without dyslipidemia (15.93±3.55 versus 17.70±4.16 versus 19.65±4.60 mmol/kg/min; \( P=0.003 \) and \( P=0.001 \)) compared with those in controls. Furthermore, FCHL family members without dyslipidemia had lower rates of nonoxidative glucose disposal (33.80±11.65 versus 38.79±12.76 109 \( \mu \)mol/kg/min; \( P=0.024 \)) when compared with control subjects. No difference in the rates of nonoxidative glucose disposal was observed between FCHL patients and controls (unadjusted, \( P=0.067 \); adjusted for age, gender, and BMI, \( P=0.217 \)) (Figure 3). Serum FFAs during the euglycemic clamp in subjects with FCHL tended to be higher when compared with those of their relatives without dyslipidemia (\( P=0.078 \); Figure 2), but no significant difference was present in the rates of lipid oxidation, WBGU, glucose oxidation, and nonoxidative glucose disposal between FCHL family members with or without dyslipidemia.

**Correlations of FFA Levels During the Hyperinsulinemic Clamp With Insulin-Stimulated Glucose Uptake and Lipid Metabolism**

Because the impaired suppressive effect of insulin on FFAs could explain both dyslipidemia and insulin resistance via increased FFA levels, correlations between FFA levels during the euglycemic clamp and other variables were calculated separately for controls and FCHL families (Table 2). In control subjects, FFA levels had a positive correlation with VLDL cholesterol (\( P=0.041 \)) and total triglycerides (\( P=0.008 \)) but no correlation with the levels of total, LDL or HDL cholesterol levels or the rates of lipid oxidation during the euglycemic clamp. Similarly, in first-degree relatives of FCHL patients without dyslipidemia, FFA levels correlated positively with VLDL cholesterol (\( P=0.042 \)) and total triglycerides (\( P=0.031 \)) but not with total, LDL, or HDL cholesterol levels or the rates of lipid oxidation. However, in FCHL patients, no significant correlations were found between these variables. In controls, FFAs did not correlate with the rates of glucose oxidation or with the rates of nonoxidative glucose disposal. In contrast, in FCHL families, FFAs correlated negatively with the rates of glucose oxidation (\( P<0.001 \)) both in patients without (\( P=0.002 \)) and with
dyslipidemia (P=0.010, Figure 4). All partial correlations with FFA levels remained statistically significant (P<0.05) when age, gender, and BMI were controlled.

Discussion
This study demonstrated that insulin’s effect on glucose metabolism is impaired in families with FCHL. More specifically, this study is the first to show that the defect in insulin-stimulated glucose uptake in FCHL family members is more pronounced in glucose oxidation than in nonoxidative glucose disposal. Moreover, during hyperinsulinemia, the suppression of FFA levels was impaired and the rates of lipid oxidation elevated in FCHL patients compared with those in controls. Defects in both glucose oxidation and FFA suppression also were observed in first-degree relatives of FCHL patients without dyslipidemia.

Insulin resistance has been associated with most of the characteristic metabolic disorders in FCHL (high triglyceride levels, combined hyperlipidemia, high apoB levels, hepatic overproduction of lipoproteins, and small LDL size), as well as with the disorder itself. In addition to dyslipidemia, insulin resistance in these patients may also contribute to increased risk of atherosclerosis. Therefore, studies on the mechanisms that could potentially cause both insulin resistance and dyslipidemia in these patients are of great importance. Because FFA levels affect both glucose uptake in peripheral tissues and hepatic production of lipoproteins, changes in serum FFA levels could explain impaired glucose metabolism as well as dyslipidemia in FCHL patients.

The combination of low rates of insulin-stimulated glucose oxidation and high rates of lipid oxidation in FCHL family members differs from the pattern of insulin resistance in non-insulin–dependent diabetes mellitus. In this disease, a defect in insulin’s action on nonoxidative glucose disposal is likely to be primary metabolic disorder, and insulin’s action on lipid oxidation has been reported to be normal. Interestingly, in obese subjects, insulin-stimulated glucose oxidation is decreased and lipid oxidation increased similarly as in FCHL family members in this study. In our study, FCHL family members had BMIs and waist-to-hip ratios similar to healthy controls; therefore, obesity or its central distribution cannot explain the findings.

Because the rates of lipid oxidation were higher in FCHL family members compared with controls during the hyperinsulinemic euglycemic clamp, simultaneously high FFA levels in FCHL family members cannot be explained by a defect in FFA removal from the plasma. Therefore, high FFA levels likely are explained by higher rates of FFA release from fat cells during hyperinsulinemia compared with those in controls. The activity of hormone-sensitive lipase has been reported as low in patients with FCHL. Therefore, high FFA levels in this study are not necessarily explained by impaired antilipolytic effect of insulin, but decreased effect on triglyceride synthesis in fat cells (and compensatory FFA release from fat cells) may play a significant role. Regardless of the cause for high FFA levels, these are likely to impair the rates of glucose oxidation further according to Randle’s cycle by increasing the rates of lipid oxidation. Therefore, the defect in glucose oxidation in FCHL family members could be secondary to high FFA levels. The possibility that the defect in insulin’s action in FCHL is localized both in skeletal muscle and adipose tissue cannot be excluded, because during hyperinsulinemia, a major portion of glucose uptake is in skeletal muscle.

Opposite to findings in the study by Bredie et al, relatives with FCHL and without dyslipidemia had similar defects in insulin action in our study. In the study by Bredie et al, relatives were more normolipidemic (lipids <75th percentile) than relatives in this study, which may have led to opposite findings. At least 3 possibilities can be presented to explain the association of insulin resistance with dyslipidemia in FCHL according to our findings. First, insulin resistance may be independent of dyslipidemia in FCHL families, implying that insulin resistance and dyslipidemia have different etiologies in this disease. Secondly, in addition to insulin resistance, some other defect may be needed for dyslipidemia to develop (additive effect for example with the apoB locus). Finally, insulin resistance may be an inherited characteristic of FCHL and precede dyslipidemia. In favor of this notion are our results that the relatives of FCHL patients without dyslipidemia were as insulin resistant as relatives with FCHL, but 10 years younger. Therefore, these subjects may develop dyslipidemia with aging.

In FCHL family members, FFA levels during the euglycemic clamp correlated with the rates of glucose oxidation independent of dyslipidemia, whereas in controls no correlation was found. Twenty-three percent of the variation in the rates of glucose oxidation was due to FFA levels in FCHL families even after controlling for age, gender, and BMI (r=−0.48; P<0.001). This confirms the finding that FFAs have an important role in insulin resistance in FCHL families. In normal individuals, FFA levels regulate VLDL production. Therefore, it is not surprising that FFA levels during the euglycemic clamp were correlated with the levels of VLDL cholesterol and total triglycerides in controls and in relatives of FCHL patients without dyslipidemia. The lack of significant correlation between FFA levels and dyslipidemia in FCHL patients indicates that some dyslipidemia is caused by defects independent of insulin resistance.

Rare mutations in the lipoprotein lipase gene can cause FCHL; furthermore, lipid levels in FCHL patients have been associated with the apo A1-CIII-AIV gene cluster. However, genetic background for FCHL remains unknown in most of the patients. Because of the complex nature of this disorder, genome-wide random search is evidently the method for resolving this problem. However, wrong negative results cannot be avoided in the random mapping because of a heterogenous background of FCHL. Therefore, candidate gene approach will still remain as an useful method for studying the genetic background for the typical traits in these patients. Our study suggests that genes that regulate lipolysis and fat cell metabolism are potentially important candidate genes for FCHL, and they may play a role already in early stages of the development of dyslipidemia in these patients.

On the basis of our findings, a hypothesis can be presented that explains the simultaneous occurrence of insulin resistance and dyslipidemia. A defect in insulin’s ability to suppress the FFA release from the adipose tissue leads to
elevated levels of FFAs. In the peripheral tissues, particularly in skeletal muscle, high levels of FFA block glucose oxidation, causing insulin resistance. In the liver, high flux of FFAs is used for triglyceride synthesis, resulting in an elevated level of LDL cholesterol in FCHL.

In summary, this study shows that the impaired effect of insulin on the suppression of FFA levels and on the stimulation of glucose oxidation is an essential part of disturbed glucose metabolism in patients with FCHL. Because these findings also were observed in first-degree relatives of FCHL patients, defects in adipose tissue and fat cell metabolism may precede lipid disorders that characterize FCHL. Screening for defects in genes that regulate fat cell metabolism are potentially important in resolving the genetic background for FCHL.

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References
Impaired Insulin-Stimulated Glucose Oxidation and Free Fatty Acid Suppression in Patients with Familial Combined Hyperlipidemia: A Precursor Defect for Dyslipidemia?
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