Impaired Free Fatty Acid Suppression During Hyperinsulinemia Is a Characteristic Finding in Familial Combined Hyperlipidemia, but Insulin Resistance Is Observed Only in Hypertriglyceridemic Patients

Jussi Pihlajamäki, Leena Karjalainen, Pauli Karhapää, Ilkka Vauhkonen, Markku Laakso

Abstract—Insulin resistance has been associated with hypertriglyceridemia, combined hyperlipidemia, and familial combined hyperlipidemia (FCHL). Whether all FCHL patients with different types of dyslipidemia have low insulin sensitivity has not been evaluated. We measured insulin sensitivity by the hyperinsulinemic euglycemic clamp with indirect calorimetry in 110 healthy controls and in 105 nondiabetic, FCHL family members: in 50 without dyslipidemia, in 19 with hypercholesterolemia (total cholesterol ≥7.7 mmol/L), in 22 with hypertriglyceridemia (total triglycerides ≥2.4 mmol/L in men 2.4 mmol/L in women), and in 14 with combined hyperlipidemia. During the hyperinsulinemic clamp, FCHL family members had higher free fatty acid levels than did controls (0.06±0.06 [mean±SD] in controls versus 0.16±0.11 in relatives without dyslipidemia versus 0.15±0.07 in hypercholesterolemic patients versus 0.29±0.14 in hypertriglyceridemic patients versus 0.27±0.17 mmol/L in patients with combined hyperlipidemia; P<0.001 after adjustment for age, sex, and body mass index). Relatives without dyslipidemia (16.4±4.4 μmol·kg⁻¹·min⁻¹, P=0.001) and patients with hypertriglyceridemia (12.8±3.8 μmol·kg⁻¹·min⁻¹, P<0.001) had lower rates of insulin-stimulated glucose oxidation than did controls (19.4±4.7 μmol·kg⁻¹·min⁻¹). Also, the rates of nonoxidative glucose disposal were lower in patients with hypertriglyceridemia (P=0.001) and combined hyperlipidemia (P=0.011) than in controls. In contrast, subjects with hypercholesterolemia and control subjects had similar rates of insulin-stimulated glucose uptake. We conclude that a defect in free fatty acid suppression during hyperinsulinemia, probably located in adipose tissue, is characteristic for all FCHL patients with varying types of dyslipidemia, whereas insulin resistance in skeletal muscle is observed only in FCHL patients with elevated triglyceride levels. (Arterioscler Thromb Vasc Biol. 2000;20:164-170.)

Key Words: familial combined hyperlipidemia ■ insulin resistance ■ insulin ■ free fatty acids

Familial combined hyperlipidemia (FCHL) is the most common familial dyslipidemia, occurring in 10% to 20% of patients with premature coronary artery disease.1-3 Hepatic overproduction of apoB-containing lipoproteins has been suggested to cause different dyslipidemias in FCHL patients4-6 in combination with a defect in lipoprotein lipase suggested to cause different dyslipidemias in FCHL patients.4-6 Originally, FCHL was described as a monogenic, dominantly inherited disease,1 but polygenic inheritance is more likely.8 Although the linkage to locus 1q21-23 has been recently reported, no major genes for this disorder have been identified.9

Recent studies have indicated that insulin resistance is a central part of FCHL.10-12 Obese FCHL patients are more resistant to insulin than are nonobese patients,13 but even lean FCHL patients seem to have low rates of insulin-stimulated glucose uptake.11-13 This observation indicates that overweight is only a modifying factor for insulin sensitivity in FCHL.14 A defect in insulin action in adipose tissue is likely to play a significant role in FCHL because insulin’s suppressive effect on free fatty acid (FFA) levels is impaired in FCHL patients.10,12,15 High FFA levels may in turn lead to both a decrease in insulin-stimulated glucose uptake in adipose tissue and skeletal muscle, according to the scheme proposed by Randle et al.16 and to an increase in the synthesis of lipoproteins in the liver.17

Whether low insulin sensitivity is a characteristic feature for all FCHL patients or only a subgroup of FCHL patients, depending on the type of dyslipidemia, has not been investigated with direct measurements of insulin sensitivity. Furthermore, it is not known whether insulin’s impaired action on FFA suppression10,12 is a typical finding for all FCHL patients or only for a certain dyslipidemic phenotype. To address these questions, we measured the rates of whole-body glucose uptake (WBGU) and FFA levels during the hyperinsulinemic clamp in 110 control subjects and in 105 FCHL family members with varying dyslipidemias.

Received February 23, 1999; revision accepted June 28, 1999.
From the Department of Medicine, University of Kuopio, Kuopio, Finland.
Correspondence to Markku Laakso, MD, Professor and Chair, Department of Medicine, University of Kuopio, 70210 Kuopio, Finland. E-mail markku.laakso@uku.fi
© 2000 American Heart Association, Inc.
Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org

164
TABLE 1. Clinical Characteristics of Control Subjects and Members of Families With FCHL With or Without Different Types of Dyslipidemia

<table>
<thead>
<tr>
<th></th>
<th>FCHL Family Members</th>
<th>Controls (n=110)</th>
<th>No FCHL (n=50)</th>
<th>High Cholesterol (n=19)</th>
<th>High Triglycerides (n=22)</th>
<th>Combined Hyperlipidemia (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M/F</td>
<td>82/28</td>
<td>29/21*</td>
<td>14/5</td>
<td>16/6</td>
<td>8/6</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>50.6±7.6</td>
<td>48.5±11.9</td>
<td>52.2±11.8</td>
<td>49.6±12.1</td>
<td>56.4±5.0*</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.1±3.6</td>
<td>25.6±4.5</td>
<td>26.9±3.3</td>
<td>29.7±5.7†</td>
<td>29.5±5.2‡</td>
<td></td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.94±0.09</td>
<td>0.91±0.09</td>
<td>0.95±0.07</td>
<td>0.98±0.06*</td>
<td>0.93±0.07</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>132±14</td>
<td>131±19</td>
<td>136±15</td>
<td>138±18</td>
<td>132±13</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>84±7</td>
<td>83±10</td>
<td>86±6</td>
<td>89±15</td>
<td>84±9</td>
<td></td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>5.5±0.5</td>
<td>5.4±0.5</td>
<td>5.7±0.6</td>
<td>5.6±0.5</td>
<td>5.6±0.5</td>
<td></td>
</tr>
<tr>
<td>Fasting plasma insulin, pmol/L</td>
<td>55.8±33.0</td>
<td>65.4±43.8</td>
<td>60.0±20.4</td>
<td>84.6±40.8‡</td>
<td>75.0±25.8‡</td>
<td></td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.78±1.13</td>
<td>6.19±0.89*</td>
<td>8.28±0.53‡</td>
<td>6.22±0.66*</td>
<td>8.46±0.64‡</td>
<td></td>
</tr>
<tr>
<td>VLDL cholesterol</td>
<td>0.63±0.61</td>
<td>0.59±0.25</td>
<td>0.79±0.33</td>
<td>1.43±0.56‡</td>
<td>1.57±0.37‡</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>3.83±1.00</td>
<td>4.28±0.83</td>
<td>6.06±0.63‡</td>
<td>3.71±0.79</td>
<td>5.71±0.58‡</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.33±0.29</td>
<td>1.34±0.24</td>
<td>1.43±0.28</td>
<td>1.09±0.21‡</td>
<td>1.19±0.22*</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.38±0.98</td>
<td>1.42±0.45†</td>
<td>1.63±0.43</td>
<td>3.57±1.31‡</td>
<td>3.28±0.65‡</td>
<td></td>
</tr>
<tr>
<td>VLDL triglycerides</td>
<td>0.83±0.89</td>
<td>0.92±0.43</td>
<td>1.01±0.34</td>
<td>2.72±1.33‡</td>
<td>2.44±0.59†</td>
<td></td>
</tr>
<tr>
<td>LDL triglycerides</td>
<td>0.31±0.13</td>
<td>0.31±0.13</td>
<td>0.42±0.13</td>
<td>0.46±0.21†</td>
<td>0.63±0.12†</td>
<td></td>
</tr>
<tr>
<td>HDL triglycerides</td>
<td>0.20±0.10</td>
<td>0.21±0.06</td>
<td>0.20±0.05</td>
<td>0.29±0.09*</td>
<td>0.27±0.06*</td>
<td></td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>0.99±0.27</td>
<td>1.04±0.21</td>
<td>1.38±0.14‡</td>
<td>1.32±0.23‡</td>
<td>1.68±0.27†</td>
<td></td>
</tr>
<tr>
<td>ApoA1, g/L</td>
<td>1.57±0.25</td>
<td>1.54±0.22</td>
<td>1.60±0.25</td>
<td>1.36±0.20*</td>
<td>1.46±0.23</td>
<td></td>
</tr>
<tr>
<td>FFAs, mmol/L</td>
<td>0.59±0.21</td>
<td>0.58±0.28</td>
<td>0.55±0.19</td>
<td>0.64±0.22</td>
<td>0.71±0.26</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SD.
*P<0.05, †P<0.01, ‡P<0.001 when compared with controls.

Methods

Subjects

Twenty-five of the probands with FCHL were selected from the myocardial infarction survivor family study carried out at our department. Selection of these subjects has been previously described. In brief, cutoff points for lipids 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 family members with different types of dyslipidemia and at least 1 affected family member in 2 generations. Additionally, 9 FCHL probands and their families were identified from the Coronary Angiography Register of the Kuopio University Hospital according to the same lipid criteria. None of the study subjects had tendon xanthomas or defects of the LDL receptor, which explain ~90% of all cases of familial hypercholesterolemia in this area.

All nondiabetic family members with dyslipidemia and a random sample of relatives without dyslipidemia, after exclusion of subjects <30 years of age and those with severe chronic disease, were invited for the hyperinsulinemic, euglycemic clamp. Results on the first 58 family members (30 without dyslipidemia and 28 with FCHL) have been previously reported. The final study population consisted of 105 family members, which allowed us to divide them into subgroups: 50 relatives without dyslipidemia (29 men, 21 women), 19 with hypercholesterolemia (14 men, 5 women), 22 with hypertriglyceridemia (16 men, 6 women), and 14 with combined hyperlipidemia (8 men, 6 women).

Control subjects were 110 healthy, unrelated subjects from our previous population studies, members of the control families in the myocardial infarction survivor study, or offspring of subjects who had a repeatedly normal glucose tolerance during 10-year follow-up. All controls and FCHL family members had normal glucose tolerance according to the World Health Organization criteria; normal liver, kidney, and thyroid function tests; no history of excessive alcohol intake; and no severe chronic disease. In addition, control subjects did not have hypertension, symptoms or signs of coronary heart disease, or continuous drug treatment. Clinical characteristics of the study groups according to the phenotypes of dyslipidemia are shown in Table 1.

Consent was obtained from all subjects after the purpose and potential risks of the study were explained to them. The protocol was approved by the Ethics Committee of the University of Kuopio and was in accordance with the Declaration of Helsinki.

Metabolic Studies

The degree of insulin resistance was evaluated with the euglycemic clamp technique after a 12-hour fast as previously described. After a baseline blood draw, 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, which was maintained by a continuous insulin infusion of 480 pmol/m² per minute. Under these study conditions, hepatic glucose production is completely suppressed in nondiabetic subjects. 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 WBGU.

Indirect calorimetry was performed with a computerized flow-through canopy 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. The rates of glucose oxidation were calculated according to Ferrannini and colleagues (1989) (determined by indirect calorimetry in the last 20 minutes of the euglycemic clamp). The rates of nonoxidative glucose disposal during the euglycemic clamp were estimated by subtracting the carbohydrate oxidation rate from the rates of WBGU.

### 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 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. Plasma insulin concentration was determined by an antibody-antibody, solid-phase radioimmunoassay (Phadeseph Insulin RIA 100, Pharmacia Diagnostics AB). Lipoprotein fractionation was performed by ultracentrifugation and sequential precipitation as previously described. Cholesterol and triglyceride levels from whole serum and lipoprotein fractions were assayed by automated enzymatic methods (Boehringer-Mannheim). ApoB and apolipoprotein A1 levels were determined by a commercial immunoassay (Phadeseph Insulin RIA 100, Pharmacia Diagnostics AB). Nonprotein urinary nitrogen was measured by an automated Kjeldahl method.

### Statistical Analysis

All calculations were done with the SPSS/Win programs (version 7.5, SPSS Inc). The differences in insulin sensitivity among the 3 study groups were tested by ANCOVA, with age, sex, and body mass index (BMI) as covariates. If the difference was statistically significant (P<0.05), pairwise comparisons between the study groups were done with ANCOVA, adjusting for confounding factors. Correlations between the variables were determined as Pearson correlations with 2-sided tests. VLDL cholesterol, total triglycerides, all subfractions of triglycerides, insulin, and FFA levels were logarithmically transformed to obtain normal distributions before statistical analyses. Probability value <0.05 were considered statistically significant. All data are presented as means ± SD.

### Results

#### Clinical Characteristics and Glucose and Lipid Metabolism in the Fasting State

In FCHL family members without FCHL, there were more women than in control subjects (P=0.036). No significant difference in sex distribution was observed between controls and groups of FCHL patients. FCHL subjects with combined hyperlipidemia were older than control subjects (P=0.035). Relatives of FCHL patients without dyslipidemia (n=50) had higher levels of total (P=0.018) and LDL (P=0.006) cholesterol than did control subjects. FCHL patients with hypercholesterolemia had higher total and LDL cholesterol and apolipoprotein B levels than did control subjects (P<0.001). Both patients with hypertriglyceridemia and combined hyperlipidemia had a higher BMI (P<0.001), and patients with hypertriglyceridemia also had a higher waist-to-hip ratio (P=0.019) than did control subjects. In addition, patients with hypertriglyceridemia and combined hyperlipidemia had higher fasting insulin (P<0.001 and P=0.006, respectively), total cholesterol (P<0.013 and P<0.001), VLDL cholesterol (P<0.001), total triglycerides (P<0.001), VLDL triglycerides (P<0.001), LDL triglycerides (P=0.002 and P<0.001), HDL triglycerides (P=0.015 and P=0.032), and apoB (P<0.001) levels and lower HDL cholesterol levels (P<0.001 and P=0.042) than did control subjects. Patients with hypertriglyceridemia also had lower levels of apoA1 (P=0.015) than did controls. No differences in the fasting FFA levels were observed (Table 1).

### Table 2. FFA Levels and Rates of WBGU During the Hyperinsulinemic, Euglycemic Clamp in Control Subjects and Members of Families With FCHL With or Without Different Types of Dyslipidemia

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=110)</th>
<th>No FCHL (n=50)</th>
<th>High Cholesterol (n=19)</th>
<th>High Triglycerides (n=22)</th>
<th>Combined Hyperlipidemia (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFAs, mmol/L</td>
<td>0.06±0.06</td>
<td>0.16±0.11†</td>
<td>0.15±0.07‡</td>
<td>0.29±0.14‡</td>
<td>0.27±0.17‡</td>
</tr>
<tr>
<td>Lipid oxidation, mg · kg⁻¹ · min</td>
<td>0.01±0.23</td>
<td>0.18±0.20‡</td>
<td>0.17±0.19‡</td>
<td>0.29±0.26‡</td>
<td>0.25±0.10‡</td>
</tr>
<tr>
<td>WBGU, μmol · kg⁻¹ · min</td>
<td>57.7±14.9</td>
<td>52.0±15.5</td>
<td>53.6±12.7</td>
<td>40.7±11.8†</td>
<td>41.4±13.8‡</td>
</tr>
<tr>
<td>Glucose oxidation</td>
<td>19.4±4.7</td>
<td>16.4±4.4‡</td>
<td>17.7±3.1</td>
<td>12.8±3.8‡</td>
<td>13.7±3.1‡</td>
</tr>
<tr>
<td>Glucose nonoxidation</td>
<td>38.3±13.3</td>
<td>35.6±12.3</td>
<td>35.9±10.8</td>
<td>27.9±10.4†</td>
<td>27.7±11.4*</td>
</tr>
</tbody>
</table>

Values are mean±SD. *P<0.05, †P<0.01, ‡P<0.001 when compared with controls after adjustment for age, sex, and BMI.

#### FFA Levels and Rates of Glucose Oxidation and Glucose Nonoxidation During the Hyperinsulinemic, Euglycemic Clamp

Glucose and insulin infusions during the clamp resulted in similar levels of glucose (5.0±0.1 [mean±SD]) in control subjects, 5.0±0.1 in relatives without dyslipidemia, 5.0±0.1 in patients with hypercholesterolemia, 5.0±0.1 in patients with hypertriglyceridemia, and 5.0±0.2 mmol/L in patients with combined hyperlipidemia, insulin (1073±215 versus 941±169 versus 990±185 versus 1008±201 versus 1004±190 pmol/L, respectively), and lactate (1.15±0.27 versus 1.19±0.27 versus 1.17±0.25 versus 1.18±0.30 versus 1.13±0.23 mmol/L) in the study groups. During the clamp, all FCHL family members with or without dyslipidemia had higher levels of FFAs (Table 2 and the Figure; P<0.001 after adjustment for age, sex, and BMI) and the rates of lipid oxidation (P<0.001) than did the controls. FFA levels and the rates of lipid oxidation did not differ between subjects without dyslipidemia and patients with hypercholesterolemia, but patients with hypertriglyceridemia (P<0.001) and combined hyperlipidemia (P=0.027) had even higher levels of FFAs than did the relatives without dyslipidemia. The rates of insulin-stimulated WBGU were lower in patients with hypertriglyceridemia and combined hyperlipidemia than...
in control subjects (P<0.001). No difference in the rates of WBGU were observed between controls and relatives without dyslipidemia or patients with hypercholesterolemia (Table 2). The rates of insulin-stimulated glucose oxidation were lower in relatives without dyslipidemia (P<0.001) and patients with hypertriglyceridemia (P<0.001) compared with controls, whereas patients with hypercholesterolemia did not differ from controls (Table 2 and the Figure). Similar to FFA levels, patients with hypertriglyceridemia and combined hyperlipidemia had even lower rates of glucose oxidation than did relatives without dyslipidemia (P<0.001 and P<0.022). The rates of nonoxidative glucose disposal were lower in subjects with hypertriglyceridemia (P=0.001) and combined hyperlipidemia (P=0.011) than in control subjects. No difference in the rates of glucose nonoxidation was observed when relatives without dyslipidemia and patients with hypercholesterolemia were compared with controls (Table 2 and the Figure).

Correlations Between FFA Levels During the Hyperinsulinemic Clamp and Serum Lipids and Lipoproteins

Because FFA levels could serve as a link between insulin resistance and dyslipidemias, their correlations with the levels of serum lipids and lipoproteins were calculated in control subjects, relatives without dyslipidemia, and FCHL patients separately (Table 3). FFA levels were correlated positively with VLDL cholesterol in controls (r=0.308, P=0.005; after controlling for age, sex, and BMI) and positively with total triglyceride levels in all study groups (r=0.261 to 0.338; P<0.01 in all groups) but not with total, LDL, and HDL cholesterol levels and apoB levels in any of the study groups.

Discussion

Our study shows that FCHL patients have impaired FFA suppression during hyperinsulinemia, independent of the type of dyslipidemia. In contrast, insulin-stimulated glucose uptake was impaired only in patients with hypertriglyceridemia (hypertriglyceridemia or combined hyperlipidemia). Thus, impaired insulin action on FFA suppression is a central part of FCHL in all patients, whereas impaired insulin action on glucose metabolism is selective and dependent on the type of dyslipidemia.

Originally, FCHL was suggested to be a dominantly inherited, monogenic disease, but polygenic inheritance is more likely. Segregation analyses have indicated a major locus for apoB; small, dense LDL; and triglyceride levels. However, so far only gene defects with a modifying, but not a major, effect on lipid and lipoprotein levels have been found in the lipoprotein lipase gene, in the intestinal fatty acid–binding protein 2 gene, and in the apoE gene. In addition, the apoAI–CHI–AIV gene complex has been implicated in the etiology of FCHL, but negative results have also been published. The locus in 1q21–q23 is a promising locus for FCHL because the identical locus has

| TABLE 3. Partial Pearson Correlations After Controlling for Age, Sex, and BMI Between FFA Levels During Hyperinsulinemic Clamp and Fasting Serum Lipids and Lipoproteins in Controls, Relatives Without FCHL, and Patients With FCHL |
|---------------------------------|-----------------|-----------------|
|                                 | Controls (n=110)| No FCHL (n=50) | FCHL (n=55) |
| Total cholesterol               | 0.139           | 0.188           | -0.211 |
| VLDL cholesterol               | 0.308*          | 0.088           | 0.144 |
| LDL cholesterol                | -0.045          | 0.188           | -0.240 |
| HDL cholesterol                | -0.002          | -0.037          | -0.089 |
| Total triglycerides            | 0.372†          | 0.261*          | 0.338* |
| ApoB                           | 0.157           | 0.179           | -0.062 |

*P<0.01, †P<0.001.
been linked with dyslipidemias in mice. However, it is doubtful that this locus could totally explain the etiology of FCHL, as linkage with hypercholesterolemia was not found in humans.

Low rates of insulin-stimulated glucose uptake are typical for patients with FCHL. This is the first study to indicate that insulin’s action on glucose metabolism is selectively impaired only in hypertriglyceridemic FCHL patients. The latter result is in accordance with previous studies, which have demonstrated that total triglycerides, but not total cholesterol levels, are correlated with insulin sensitivity or fasting insulin levels in FCHL families. In contrast, FFA levels during hyperinsulinemia were poorly suppressed in all FCHL patients with varying dyslipidemias and not only in patients with high triglyceride levels. This defect seems to be specific for FCHL, because by utilizing an identical methodology and laboratory determinations, we have previously shown that the suppression of FFA levels during hyperinsulinemia is normal in familial and nonfamilial hypercholesterolemia, in nonfamilial combined hyperlipidemia, and in patients with isolated low HDL cholesterol levels without hypertriglyceridemia.

Why are FFA levels poorly suppressed by insulin in patients with FCHL? FFAs are released into the plasma either by lipoprotein lipase from triglyceride-rich lipoproteins in adipocyte capillaries or from adipocyte triglyceride storage by hormone-sensitive lipase (HSL). On the other hand, FCHL is characterized by low lipoprotein lipase activity and low HSL activity. Therefore, impaired uptake of FFAs into fat cells rather than increased lipolysis has been suggested to explain high serum FFA levels in FCHL. At least 3 mechanisms could explain the combination of low HSL activity and impaired uptake of FFAs into fat cells. First, although no linkage of the HSL gene with FCHL was found in the Finnish population, defects in the HSL gene are possible in this disorder. This hypothesis is supported by novel findings suggesting an association between a polymorphic marker in this gene and the metabolic syndrome and an association between a polymorphism in the promoter region of this gene and reporter gene activity in vitro. Second, low HSL activity could be secondary to impaired uptake of FFAs into fat cells by fatty acid transporter proteins. Third, low rates of triglyceride synthesis and consequent low uptake of FFAs from plasma could explain these findings.

The defect in FFA suppression was partly dependent on the type of dyslipidemia, because it was greater in FCHL patients with high triglyceride levels (hypertriglyceridemia or combined hyperlipidemia) than in patients with pure hypercholesterolemia. The difference is not likely to be explained by obesity in patients with hypertriglyceridemia, because the difference was statistically significant after adjustment for BMI. Higher FFA levels in patients with hypertriglyceridemia may explain, at least in part, the simultaneous occurrence of impaired glucose oxidation via increased lipid oxidation and consequently, an increase in intracellular acyl-CoA/CoA and NADH/NAD+ ratios, which inhibit pyruvate dehydrogenase, as suggested by Randle et al. However, the rates of nonoxidative glucose metabolism were also impaired in hypertriglyceridemic FCHL patients. Because 80% of insulin-stimulated glucose uptake occurs in skeletal muscle, this finding indicates a defect in skeletal muscle cells proximal to the phosphorylation of glucose or separate defects in both oxidative and nonoxidative glucose metabolism. A defect in the translocation of glucose transporters to the plasma membrane or in the phosphorylation of glucose could be caused not only by defects in the insulin-signaling pathway (eg, by defects in the genes coding for the insulin receptor substrates phosphatidylinositol-3-kinase or hexokinase II) but also by an increased supply of inhibitory mediators released from adipose tissue, such as tumor necrosis factor-α or FFAs. The possibility of 2 separate defects also cannot be excluded: 1 in adipose tissue, causing impaired FFA suppression during hyperinsulinemia, eg, by defects in the HSL gene, leading to decreased rates of glucose oxidation in skeletal muscle, and another in skeletal muscle due to impaired insulin-stimulated glycogen synthesis.

Because we used a rather high cutoff point for total cholesterol (7.70 mmol/L), one could ask whether family members with lower cholesterol values were truly unaffected. Therefore, we formed a new group of family members with cholesterol levels <6 mmol/L and triglyceride levels <2 mmol/L. These normolipidemic FCHL family members also had higher levels of FFAs (0.12 ± 0.08 versus 0.06 ± 0.06 mmol/L; P = 0.003 after adjustment for age, sex, and BMI) and lower levels of glucose oxidation (17.2 ± 4.1 versus 19.4 ± 4.7 μmol · kg⁻¹ · min⁻¹; P = 0.041) than did the control subjects. Although there were proportionally more women in FCHL family members without FCHL than in controls, it is unlikely that this explains our findings, since we adjusted for sex. Therefore, we think that impaired FFA suppression and glucose oxidation during hyperinsulinemia are characteristic findings also in relatives at risk to develop FCHL, as proposed in our previous study.

High apoB levels are characteristic of FCHL patients with different types of dyslipidemia. This finding could be explained by impaired insulin-mediated FFA suppression, since FFAs also stimulate apoB and VLDL production in the liver. However, in the present study, FFA levels during hyperinsulinemia did not correlate with fasting apoB levels in FCHL patients, suggesting that mechanisms other than insulin resistance are needed to explain the high apoB levels in FCHL. Because no difference was observed in the rates of insulin-stimulated WBGU between relatives without dyslipidemia and patients with hypercholesterolemia, mechanisms independent of insulin action are likely to determine high levels of VLDL and LDL cholesterol in FCHL patients. However, FFA levels may partly determine the lipid content of VLDL particles by stimulating triglyceride synthesis in the liver independently of apoB synthesis.

Some of our FCHL family members were relatives, and therefore we analyzed our results by including family status as a covariate. This additional analysis did not change the results. We did not apply a family-based analysis (segregation or linkage studies) because direct measurement of insulin sensitivity with the hyperinsulinemic, euglycemic clamp technique is possible only in healthy adults and not in very old or very young people. Therefore, studies that apply indirect measurements of insulin sensitivity, such as fasting or postprandial insulin levels, in young and old people are needed to demonstrate whether a common locus determines insulin resistance and dyslipidemias in FCHL.
In conclusion, the impaired effect of insulin in adipose tissue to suppress FFA levels during hyperinsulinemia is likely to be 1 of the primary metabolic disorders in FCHL because it occurred in all of our FCHL patients. In addition, FCHL patients with hypertriglyceridaemia have a separate defect in skeletal muscle glucose uptake. Identification of these defects in adipose tissue, in skeletal muscle, or in the mediators that regulate these target tissues is needed to fully explain varying phenotypes in FCHL.

Acknowledgments
This study was supported by grants from the Medical Research Council of the Academy of Finland (to M.L.), the Finnish Heart Research Foundation (to J.P., L.K.), and the Aare and Aili Turunen Foundation (to L.K.).

References


Impaired Free Fatty Acid Suppression During Hyperinsulinemia Is a Characteristic Finding in Familial Combined Hyperlipidemia, but Insulin Resistance Is Observed Only in Hypertriglyceridemic Patients

Jussi Pihlajamäki, Leena Karjalainen, Pauli Karhapää, Ilkka Vauhkonen and Markku Laakso

Arterioscler Thromb Vasc Biol. 2000;20:164-170
doi: 10.1161/01.ATV.20.1.164
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/20/1/164

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at: http://atvb.ahajournals.org//subscriptions/