Nonobese Patients With Familial Combined Hyperlipidemia Are Insulin Resistant Compared With Their Nonaffected Relatives


Abstract Familial combined hyperlipidemia (FCH) is a heterogeneous lipid disorder, characterized by the presence of a multiple-type hyperlipidemia with elevated plasma cholesterol and/or plasma triglyceride levels and the frequent occurrence of premature cardiovascular disease in first-degree relatives. Originally, FCH was supposed to be caused by the variable expression of a single autosomal dominant gene primarily regulating plasma triglyceride levels and secondarily affecting cholesterol levels. However, several metabolic and biochemical defects that have recently been related to the trait suggest that the genetic basis of FCH is more heterogeneous. As a consequence, the spectrum of FCH also comprises other related phenotypes, such as hyperapobetalipoproteinemia, the LDL subclass pattern B phenotype, familial dyslipidemic hypertension, and some features of the insulin resistance syndrome.

Insulin resistance, defined as a decreased ability of insulin to stimulate glucose uptake, is increasingly recognized as a common factor underlying various conditions, all of which predispose to coronary heart disease. Resistance to normal action of insulin is increasingly recognized as a common factor underlying various conditions, all of which predispose to coronary heart disease. Resistance to normal action of insulin is increasingly recognized as a common factor underlying various conditions, all of which predispose to coronary heart disease. Resistance to normal action of insulin is increasingly recognized as a common factor underlying various conditions, all of which predispose to coronary heart disease.

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concentrations of glucose, insulin, and NEFA, possibly confounded by obesity of the study group, or obtained by steady state plasma glucose and insulin concentration determinations after somatostatin administration. Somatostatin in itself may exhibit vascular effects. Therefore, in this study we have determined the sensitivity to the metabolic and hemodynamic effects of insulin directly by means of the euglycemic hyperinsulinemic clamp technique in combination with skeletal blood flow measurements. The studies were performed in a carefully selected sample of well-defined nonobese normotensive male FCH patients, which were compared with matched nonaffected relatives.

Methods

Subjects

For this study, initially 12 unrelated nonobese normotensive male FCH patients, selected from an available sample of well-defined Dutch FCH families, were compared with one of their nonaffected male relatives. To anticipate environmental influences, we carefully matched related individuals (i.e., affected and unaffected) for age, BMI, and waist-to-hip ratio. In addition, all subjects were apparently healthy and met the following inclusion criteria: BMI <27 kg/m²; blood pressure <165/90 mm Hg (measured in the supine position after 5 minutes’ rest); absence of diabetes mellitus, a medical history of vascular disease, and use of any medication except lipid-lowering drugs, which were discontinued at least 1 month before participation. Two FCH patients and two relatives were habitual smokers.

The FCH diagnosis was based on the following criteria: (1) the presence of a multiple-type hyperlipidemia in first-degree relatives, with at least two first-degree relatives with a different hyperlipidemia (i.e., hypertriglyceridemia, hypercholesterolemia, or a combined hyperlipidemia). The assessment of hyperlipidemia was based on the presence of constant elevated cholesterol and/or triglyceride levels above the 90th percentile, in spite of dietary advice; (2) a positive family history of premature cardiovascular disease before the age of 60 years.

Participating FCH patients had both total plasma cholesterol and triglyceride concentrations above the 90th percentile for age and sex after dietary advice and after withdrawal of lipid-lowering drugs for at least 4 weeks. In contrast, unaffected relatives (nine were brothers; two were cousins) had both total plasma cholesterol and triglyceride concentrations below the 75th percentile for age and sex. None of the subjects was homozygous for the apoE2 allele.

Protocol and Procedures

All participants were asked to visit the department twice after an overnight fast. During a first visit, a questionnaire was filled out to collect information on medical status, and supine blood pressure, BMI, and waist-to-hip ratio were measured. Furthermore, blood was sampled for determination of baseline values for lipids, lipoproteins, HbA1c, and C-peptide and for determination of the individual distribution of LDL subfractions in the LDL subtraction profile. Moreover, a 75-g OGTT was performed to exclude impaired glucose tolerance or overt diabetes mellitus. In these tests, glucose and insulin concentrations were determined at baseline and 30, 60, 90, and 120 minutes after the glucose load. Only when participants showed 2-hour postload glucose levels ≤7.8 mmol/L was a hyperinsulinemic euglycemic clamp performed, combined with hemodynamic measurement of blood pressure, heart rate, and FBF, during a second visit, 1 to 2 weeks later. After OGTT, one FCH patient and his related control subject were excluded from further analyses due to impaired glucose tolerance of this FCH patient (2-hour glucose level of 10.2 mmol/L). The experimental protocol was approved by the ethical committee of our hospital, and all participants gave their written informed consent.

Euglycemic Hyperinsulinemic Clamp

Experiments were performed with the participants in the supine position in a quiet, temperature-controlled room (24°C to 25°C). Under local anesthesia (0.3 to 0.4 mL lidocaine HCl, 20 mg/mL), a 20-gauge catheter (Angiocath, Deseret Medical Inc, Becton Dickinson) was inserted into the left brachial artery and connected with an arterial pressure monitoring line (Viggo Sorensen, No. 992399A/14368) to a Hewlett Packard monitor (type 78335B, Hewlett Packard GmbH). MAP was determined by an electronically integrated area under the brachial arterial pulse wave-form. The arterial line was kept patent with saline infusion (with 2 U heparin per milliliter added). On the contralateral side, an identical venous catheter was inserted into a large forearm vein for infusion of insulin and glucose. Insulin (Actrapid, Novo-Nordisk) was infused in a dose of 60 mU/m² per minute for 180 minutes. Insulin was diluted in 50 mL 0.9% NaCl with addition of 2 mL albumin, to a concentration of 1 U/mL. According to the clamp technique, plasma glucose concentration was kept at a euglycemic level by a variable infusion of glucose 20% solution, adjusted by arterial plasma glucose levels measured at 5-minute intervals. All subjects were clamped at fasting glucose levels minus 0.3 mmol/L. The glucose infusion rate was calculated as the given amount of glucose in milliliters per kilogram body weight per minute, which equals M. The glucose uptake per plasma unit of insulin (I) (insulin sensitivity index, calculated as M/I) was determined as an additional measure of insulin sensitivity. In addition, the concentrations of NEFAs were determined before and at the end.

FBF Measurement

FBF was measured using a mercury-in-silicone elastomer strain-gauge venous occlusion plethysmography on both arms as previously described. One minute before the start of the measurements (which were taken every 30 minutes), a wrist cuff was inflated to 100 mm Hg above the systolic blood pressure, to be sure that the measurement referred only to the forearm skeletal muscle vascular bed. The collecting cuff around the upper arm was inflated to a pressure of 45 mm Hg during 8 to 10 heart cycles by using a rapid cuff inflator (Hokanson E20) rapid cuff inflator, Hokanson Inc). The strain gauges were connected to a plethysmograph (Hokanson EC4 plethysmograph, Hokanson Inc). Changes in FBF during the clamp procedure were calculated with the means of the left and right FBF.

Analytical Methods

Glucose concentrations were measured in duplicate by using the oxidation method (Beckman Glucose Analyzer2, Beckman Instruments Inc). Plasma insulin concentrations were determined using an "in-house" assay (double antibody method) with an interassay variability of 6%. C-peptide was determined using a commercial double antibody kit (Diagnostic Products Corp, catalog No. KPD1 double antibody), with an interassay variability of 4.3%. HbA1c was determined with a high-performance liquid chromatography technique (Biorad Laboratories), with a reference range of 4.8% to 6.2%. The concentrations of NEFAs were determined using a commercially available ACS-ACOD method (Waco Chemicals GmbH, catalog No. 994-
TABLE 1. Baseline Anthropomorphic Measurements; BP; and Lipid, Lipoprotein, and ApoB Levels in FCH Patients and Their Unaffected Relatives

<table>
<thead>
<tr>
<th></th>
<th>Affected</th>
<th>Unaffected</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>Age, y</td>
<td>41.9±9.0</td>
<td>44.9±11.5</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.9±1.2</td>
<td>24.3±1.5</td>
<td></td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.99±0.05</td>
<td>1.00±0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>122.7±11.7</td>
<td>132.7±16.5</td>
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<tr>
<td>Diastolic BP, mm Hg</td>
<td>79.5±6.9</td>
<td>81.8±8.4</td>
<td></td>
</tr>
<tr>
<td>Total chol*</td>
<td>7.7±1.01</td>
<td>5.59±0.85</td>
<td>.003</td>
</tr>
<tr>
<td>Total TG*</td>
<td>3.73±2.50</td>
<td>1.24±0.43</td>
<td>.003</td>
</tr>
<tr>
<td>VLDL chol*</td>
<td>1.90±1.40</td>
<td>0.50±0.29</td>
<td>.003</td>
</tr>
<tr>
<td>VLDL TG*</td>
<td>2.80±1.40</td>
<td>0.71±0.41</td>
<td>.003</td>
</tr>
<tr>
<td>LDL chol*</td>
<td>4.90±1.39</td>
<td>4.03±0.88</td>
<td>NS</td>
</tr>
<tr>
<td>HDL chol*</td>
<td>0.89±0.17</td>
<td>1.20±0.15</td>
<td>.006</td>
</tr>
<tr>
<td>ApoB, mg/dL</td>
<td>178.9±45.3</td>
<td>122.8±26.8</td>
<td>.003</td>
</tr>
<tr>
<td>K value</td>
<td>-0.92±0.13</td>
<td>-0.08±0.24</td>
<td>.004</td>
</tr>
</tbody>
</table>

BP indicates blood pressure; chol, cholesterol; TG, triglyceride; and NS, not significant. All values except n and waist-to-hip ratio are mean±SD.

*Values in mmol/L.

TABLE 2. Baseline Concentrations of Parameters of Glucose Metabolism in 11 FCH Patients and 11 Unaffected Relatives

<table>
<thead>
<tr>
<th></th>
<th>Affected</th>
<th>Unaffected</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/L</td>
<td>5.45±0.12</td>
<td>5.22±0.15</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin, μU/mL</td>
<td>8.55±0.95</td>
<td>7.00±0.98</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.45±0.10</td>
<td>5.36±0.08</td>
<td>NS</td>
</tr>
<tr>
<td>C peptide, nmol/L</td>
<td>0.65±0.03</td>
<td>0.47±0.04</td>
<td>.02</td>
</tr>
<tr>
<td>insulin-to-glucose ratio</td>
<td>1.57±0.18</td>
<td>1.35±0.18</td>
<td>NS</td>
</tr>
<tr>
<td>NEFA</td>
<td>0.86±0.16</td>
<td>0.75±0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS indicates not significant. All values except those for l-to-glucose ratio are mean±SEM.

Statistical Analysis

Differences in baseline characteristics and baseline values for lipid, lipoproteins, glucose, and HbA1c were tested by the non-parametric two-sample Wilcoxon signed rank test. The results of the glucose tolerance test were tested by comparing areas under the curve of both the glucose excursion curve and the insulin response. To compare M and the insulin sensitivity index of the affected with that of the unaffected subjects, controlling for possible influence of confounding variables, an ANOVA was performed, with age, BMI, and waist-to-hip ratio as covariates. To investigate differences in the time of action of insulin on hemodynamic parameters between the two groups, the time/group interaction was calculated by repeated-measures ANOVA, with insulin as the dependent factor. All statistical

Results

Baseline Characteristics

Baseline characteristics of both 11 FCH patients and their 11 nonaffected relatives are presented in Table 1. Age, BMI, waist-to-hip ratio, and blood pressure were not different between the groups. As a consequence of the selection procedure, all lipid and lipoprotein concentrations (except the LDL cholesterol concentration) were

Analysis were performed using the SPSS/PC+ program (SPSS Inc). Results in tables and figures are presented as mean±SEM unless otherwise indicated. Differences with a test value <.05 were considered to be significant.

Fig 1. Results of the 75-g OGTT in 11 FCH patients and 11 unaffected relatives. Upper panel, mean glucose curves of FCH patients (■) and unaffected control subjects (□). Calculated areas under the curve were not significantly different, but mean glucose concentration at 120 minutes was significantly higher in patients than in control subjects. Lower panel, mean insulin response curves of FCH patients (■) and unaffected control subjects (□). Calculated areas under the curve were significantly higher in FCH.
significantly higher in FCH patients than in the unaffected relatives. Total plasma apoB was also higher in FCH patients than in unaffected relatives. The K value, as a description of the LDL subfraction profile, was more negative in FCH patients than in unaffected relatives, corresponding with the predominance of small, dense LDL particles in FCH patients.

**Responses to Oral 75-g Glucose Load**

Baseline values of fasting glucose, fasting insulin, HbA1c, C-peptide, and the calculated insulin-to-glucose ratio are presented in Table 2. C-peptide concentrations were significantly higher in FCH patients than in their unaffected relatives. Concentrations of glucose, insulin, HbA1c, and insulin-to-glucose ratio all tended to be higher in FCH patients than in the unaffected relatives, but these differences did not reach significant levels. Glucose concentrations after oral glucose load were comparable in both groups (Fig 1), although a significantly higher glucose concentration after 2 hours was observed in FCH patients compared with the control subjects (5.73±0.52 mmol/L versus 4.36±0.29 mmol/L, P=.019). Plasma insulin responses during the 2 hours after the oral glucose load, also presented in Fig 1, were significantly higher in patients than control subjects. All included participants had a normal glucose tolerance by exhibiting plasma glucose levels <7.8 mmol/L 2 hours after an oral 75-g glucose load.

**Metabolic Response to Clamp Procedure**

Plasma glucose values during the last 60 minutes of the clamp procedure were stable in patients and unaffected relatives (coefficient of variation, 3.4±0.3% and 4.1±0.3%, respectively). Plasma insulin concentrations increased in all subjects and were similar after 3 hours of insulin infusion in both groups (94.6±4.3 mU/L in FCH patients and 93.8±4.8 mU/L in unaffected relatives). M, calculated from the glucose infusion rate of both groups during the clamp procedure, is presented in Fig 2 and was significantly different over the last 60 minutes between FCH patients and unaffected relatives (6.89±0.31 versus 8.94±0.76 mg·kg⁻¹·min⁻¹, respectively; P=.01 by ANOVA). The M value of the control relatives was very similar to previously obtained values of healthy volunteers (8.8±1.3 mg·kg⁻¹·min⁻¹). The glucose uptake during the last 60 minutes of the clamp procedure per plasma unit of insulin (insulin sensitivity index) also differed between the groups (M/I, 7.46±0.50 versus 9.51±0.53, respectively; P=.009 by ANOVA). When the results of the related couples were compared, it was found that in nine FCH patients M was lower, in one FCH patient it was equal, and in one FCH patient it was higher than in their respective normolipidemic relatives (Fig 3). To avoid the possible confounding effect of smoking, the rates of uptake were also compared in nonsmokers. In nonsmokers, the rates of M were also significantly lower in FCH patients (n=9) than in unaffected relatives (n=9) (6.90±0.66 versus 9.00±0.93 mg·kg⁻¹·min⁻¹, respectively; P=.019 by ANOVA). Although fasting plasma NEFA concentrations were higher in the FCH patients than in control subjects, this difference appeared not to be significant (Table 2). Both NEFA concentrations were suppressed in a similar manner during the clamp procedure (from 0.86±0.16 to 0.12±0.04 mmol/L in FCH patients, P=.003, and from 0.75±0.12 to 0.10±0.05 mmol/L in unaffected relatives, P=.003), without differences between the groups.

**Effects on FBF**

At baseline, the mean values of hemodynamic parameters (FBF and MAP) of both groups were similar. Only unaffected relatives exhibited a significant increase of 31.8±14.3% in FBF (FBF from 1.85±0.16 to 2.45±0.35 mL·min⁻¹·dL⁻¹, ANOVA P=.025), whereas FCH patients showed no significant change in FBF (1.87±0.15 to 2.06±0.24). MAP did not change in either group (FCH patients: 92.7±2.3 to 93.0±3.6 and relatives: 95.3±3.0 to 98.4±2.7 mm Hg). The time course of the changes in FBF showed the major and statistically significant changes during the last hour of the clamp, reflecting a phenomenon with slow onset.

**Relationship Between LDL Subfraction Distribution and M**

The individual LDL subfraction profile, described as parameter K, correlated significantly with the glucose
uptake during the clamp procedure (Pearson's $r=.51$, $P=.008$; Fig 4). When the groups were compared separately, FCH patients were more insulin resistant and exhibited a predominance of small, dense LDL subfractions. The unaffected relatives, with the exception of one individual, were characterized by the presence of more buoyant LDL particles.

**Discussion**

In this study, we demonstrated directly by means of the euglycemic clamp technique a reduced insulin sensitivity in well-defined male FCH patients compared with one of their normolipidemic relatives. Since it has been established that insulin resistance is also associated with obesity, age, and sex alone, it is important to emphasize that these observations were not confounded by differences in sex or age and were registered in the absence of obesity, hypertension, and impaired glucose tolerance in the participants. Furthermore, we selected the affected and unaffected participants in genetically related pairs, presuming that by this study design, differences in environmental or genetic influences were reduced. Apart from the metabolic resistance to insulin (reduced glucose uptake), FCH patients also exhibited resistance to the vasodilator effects of insulin. Finally, diminished $M$ correlated with the presence of small, dense LDL subfractions.

Because of the similarity in presenting lipid disturbances and the association with increased cardiovascular risk, diminished insulin sensitivity may be related to FCH. This observation is further supported by the finding that insulin resistance affects both hepatic VLDL production and lipoprotein lipase action features also met in FCH. Previous studies indicate that insulin resistance is associated with elevated concentrations of VLDL triglyceride. Recent studies, however, showed no improvement in insulin sensitivity after substantial reduction of plasma triglyceride levels with fibrate treatment in both nondiabetic men with type IIb hyperlipidemia and in type II diabetic patients with hypertriglyceridemia. This factor may indicate that in some patients insulin resistance is of an inherited nature, not dependent on plasma triglyceride concentrations but probably preceding VLDL triglyceride overproduction.

A proposed mechanism responsible for VLDL overproduction is the impaired postprandial suppression of NEFA release in adipose tissue due to resistance to the normal suppressive effect of insulin on hormone-sensitive lipase. An elevated concentration of NEFA is associated with increased hepatic VLDL triglyceride secretion, possibly due to reduced intracellular apoB degradation. Furthermore, when normal insulin-mediated activation of lipoprotein lipase in adipose tissue is diminished, an impaired postprandial clearance of triglyceride-rich lipoproteins may occur. Several reports mentioned an impaired lipoprotein lipase activity as one of the factors contributing to FCH. A postprandial status with prolonged circulating triglyceride-rich lipoproteins is a provoking situation for the formation of small, dense LDL particles. In the Kaiser Permanente Women Twin study and other studies, small, dense LDL was found to be an integral feature of the insulin resistance syndrome. Others, however,
showed a significant correlation between LDL particle size and triglyceride levels but not insulin resistance in mildly hypertriglyceridemic subjects. Here, the authors considered small, dense LDL a feature of the insulin resistance syndrome more as a consequence of abnormalities in VLDL metabolism. Our observed correlation between glucose uptake as a measure of insulin sensitivity and LDL density is in accordance with these reports but cannot discriminate between metabolic influences, such as the correlation with hypertriglyceridemia, causing a dense LDL subfraction profile, or primarily genetic effects related to diminished insulin sensitivity and small, dense LDL in FCH. The recently reported major gene effect on LDL subfraction size in FCH may possibly be found in mutations causing insulin resistance underlying hypertriglyceridemia and the formation of small, dense LDL. On the other hand, reported linkage of small, dense LDL to a locus near the LDL receptor and the insulin receptor gene on chromosome 19p45 may indicate that the allele responsible for expression of small, dense LDL also predisposes to insulin resistance.

Disorders related to insulin resistance seem to exhibit their major defect on the level of glucose uptake in skeletal muscle rather than at the level of the liver or the adipose tissue. Furthermore, several groups, including ourselves, have demonstrated the vasodilator effect of systemic hyperinsulinemia, which was reduced in non-insulin-dependent diabetes mellitus. It is hypothesized that decreased insulin sensitivity in humans may be due not only to lower insulin-mediated glucose extraction in insulin-sensitive tissues but also to a lower blood flow to these tissues, due to a decreased ability of insulin to stimulate skeletal muscle blood flow. In accordance with other studies in which comparable systemic insulin concentrations were reached, we observed a 30% increase in FBF in nonaffect ed subjects but not FCH patients. Although part of the present reported insulin resistance may be related to this diminished insulin-induced vasodilation, this factor could in fact only account for the last 60 to 80 minutes of the clamp, since differences in FBF between the groups became statistically significant in this period.

The insulin-induced vasodilation seems to be endothelium dependent. Reduced insulin-induced vasodilation may be due to direct endothelium-disturbing action of elevated lipid levels. Therefore, in FCH patients, the elevated lipoprotein concentration may directly influence the endothelium function, resulting in decreased nitric oxide release during hyperinsulinemia. Because insulin resistance itself may underlie VLDL overproduction, the reduced hemodynamic effects of insulin in hyperlipidemia can amplify metabolic disturbances found in FCH patients.

Recently, the Insulin Resistance Atherosclerosis Study demonstrated an inverse association between insulin sensitivity and atherosclerosis, as assessed by B-mode ultrasonography of the carotid artery wall. This association was eliminated, although not completely, by correction for traditional cardiovascular risk factors. Insulin resistance in our FCH patients, who were exposed to elevated lipid levels for a relatively short period of time (mean age <42 years) and without signs of atherosclerosis, represents more likely a metabolic defect rather than the consequence of existing atherosclerosis.

In conclusion, this study suggests that FCH patients exhibit a diminished insulin sensitivity. This insulin resistance may underlie the observed hyperlipidemia, characterized by elevated concentrations of VLDL cholesterol and triglycerides and a predominance of small, dense LDL in the affected subjects, and may be partly explained by a reduced capacity of insulin to induce vasodilation in skeletal muscle of the patients. Therefore, interactions between insulin action and lipid metabolism in FCH warrant further investigation.

Acknowledgments

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