Relationship of Insulin Sensitivity and ApoB Levels to Intra-abdominal Fat in Subjects With Familial Combined Hyperlipidemia

Jonathan Q. Purnell, Steven E. Kahn, Robert S. Schwartz, John D. Brunzell

Abstract—Familial combined hyperlipidemia (FCHL) is one of the most common familial dyslipidemias associated with premature heart disease. Subjects with FCHL typically have elevated apolipoprotein B (apoB) levels, variable elevations in cholesterol and/or triglycerides, and a predominance of small, dense, low density lipoprotein particles. It is thought that insulin resistance is important in the expression of the combined hyperlipidemia phenotype. To further characterize the relationship between insulin resistance and increased apoB levels, 11 subjects from well-characterized FCHL families and normal control subjects matched for weight and/or age underwent measurement of intra-abdominal fat (IAF) and subcutaneous fat (SQF) by CT scan, insulin sensitivity (Si) by the frequently sampled intravenous glucose tolerance test, and lipoprotein levels. Body mass index and IAF were higher and Si was lower (more insulin resistant) in the FCHL group than in the age-matched group, but the values were similar in the FCHL group and the age- and weight-matched control group. When the relationship between body fat distribution and Si was tested with multiple linear regression, only IAF was significantly correlated with Si after the addition of SQF and body mass index as independent variables. For any level of insulin sensitivity or IAF, however, apoB levels remained higher in the FCHL subjects than in the control groups. In conclusion, in FCHL, visceral obesity is an important determinant of insulin resistance. Visceral obesity and insulin resistance, however, do not fully account for the elevated levels of apoB in this disorder, and this study provides physiological support for separate, but additive, genetic determinants in the etiology of the lipid phenotype. (Arterioscler Thromb Vasc Biol. 2001;21:567-572.)

Key Words: obesity ■ insulin resistance ■ hyperlipidemia ■ visceral fat ■ apolipoproteins

Familial combined hyperlipidemia (FCHL) was first described in families of myocardial survivors when elevations in triglycerides, total cholesterol, or both were found in affected relatives. Subsequent reports confirmed these findings and the association of FCHL with premature coronary artery disease. Compared with normal control subjects, subjects with FCHL characteristically have elevations in apoB levels and an increased amount of small dense LDL particles, which persist even after reduction of triglyceride levels with gemfibrozil. Kinetic studies have suggested that 1 mechanism for the elevated apoB levels in FCHL subjects is through an increased production rate of apoB-containing lipoprotein particles. Although initially described as a monogenic disorder, inheritance of the lipid phenotype has been shown to be more complex. Segregation and linkage analysis have provided evidence of the influence of major gene effects on the elevation in apoB levels and the presence of small dense LDL particles in FCHL families. Further evidence of genetic heterogeneity is derived from studies showing that 36% of subjects with FCHL have reduced postheparin lipoprotein lipase (LpL) activity. FCHL subjects with this diminished LpL activity have higher triglyceride levels than do FCHL subjects with normal LpL activity, and by DNA sequencing, several mutations of the apoA-IV gene and regulatory elements of the LpL gene that could contribute to the diminished LpL activity and variable hyperlipidemia in this group have been described. In addition, several groups have shown polymorphisms of the LpL gene to be associated with higher lipid levels, specifically triglyceride levels, in FCHL subjects who carry these mutations than in noncarriers (for review, see Aouizerat et al). In the general population, small dense LDL particles are common, with an estimated prevalence rate of 30%. Subjects with small dense LDL have a number of other lipid

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abnormalities in common with FCHL subjects, including elevated triglyceride levels, apoB production rates, and apoB levels.21–23 Insulin resistance has also been reported in subjects with small dense LDL particles,24,25 and recent studies have shown that subjects with FCHL are also insulin resistant.26–32

Given these similarities in metabolic phenotype, it has been hypothesized that insulin resistance is a major determinant of the hyperlipidemia phenotype in FCHL, including elevated apoB levels.27,31,33 However, whether the increased apoB levels in FCHL can be entirely accounted for by the finding of insulin resistance in this population remains to be determined. In a study of FCHL families, Jarvik et al34 suggested that mechanisms resulting in the dense LDL phenotype (such as insulin resistance) may contribute to the lipoprotein phenotype of FCHL, but they do not fully explain the elevated apoB levels in this disorder. Therefore, the present study sought to examine the relationship between insulin resistance and apoB levels in subjects with FCHL. In addition, the relationship of visceral fat (intra-abdominal fat [IAF]) accumulation, an important determinant of insulin resistance in the general population,24,35,36 to the expression of insulin resistance in FCHL subjects is described.

Methods

Subjects With FCHL

Eleven affected subjects from families with FCHL were recruited for the present study. A family was defined as having FCHL if elevated levels of total cholesterol and triglyceride greater than the 90 percentile of age- and sex-matched controls (as reported in the Lipid Research Clinic data set57) were present in the proband and at least 2 other first-degree relatives. Participating subjects were screened with a history and physical examination, and all medications affecting lipid levels were stopped 4 weeks before the study. All other medications were continued, and thyroid status was confirmed to be normal if subjects were diagnosed with hypothyroidism and were taking replacement doses of hormones.

Control Subjects

Fifteen and 22 subjects recruited for other studies of weight loss or hormonal supplementation at the University of Washington served as the age-matched and the age- and weight-matched control groups, respectively. Men and women participating in these studies were considered to be healthy nonsmokers and nonexercisers and to be free of chronic diseases such as cancer, cardiac disease, lung disease, and kidney disease. Data obtained at the baseline visit before the intervention were used for the present study. Informed consent was obtained in all subjects before they entered the study, and the Human Subjects Committee of the University of Washington approved all procedures.

Lipids and Apolipoproteins

After a 12- to 16-hour overnight fast, blood was collected in 0.1% EDTA and immediately centrifuged at 4°C at 3000 rpm for 15 minutes, and measurements were made on fresh plasma within 2 days. Plasma total cholesterol, triglycerides, HDL cholesterol, and apoB were measured at the Northwest Lipid Research Laboratory as previously described.38,39

Insulin Sensitivity

The tolbutamide-modified frequently sampled intravenous glucose tolerance test was performed as previously described40. 3 basal samples were drawn for insulin and glucose at 5-minute intervals; glucose (11.4 g/m²) was injected at time 0 as a bolus over 60 seconds; tolbutamide (125 mg/m²) was injected at the 20-minute time point after the glucose injection over 30 seconds; and blood samples for glucose and insulin measurements were drawn at 32 time points over 4 hours. Plasma glucose concentrations were measured in triplicate by using the glucose oxidase method. Plasma insulin was measured in duplicate by using a modification of a double-antibody radioimmunoassay.41 Insulin sensitivity (Si) was quantified by using the minimal model of glucose kinetics of Bergman et al.42

Body Composition and Distribution

IAF and subcutaneous abdominal fat (SQF) depots were manually separated and quantified by a blinded reader using single abdominal CT images obtained on inspiration at the level of the umbilicus. The CT image was analyzed for cross-sectional area of fat by use of a density contour program available in the standard GE computer software as described previously.43 A single blinded observer measured all the CT measurements of IAF and SQF. The coefficient of variation of reading the same scan is <2%.

Statistical Methods

For comparisons between groups, the t test was used unless the data were nonnormally distributed, in which case the rank sum test was used. Correlations were tested by linear regression. The independence of linear relationships was tested by multiple linear regression.

Results

All groups were matched for age (Table 1). In the FCHL group, no effect of sex on lipid levels, IAF, or SQF was found (data not shown). Compared with the age-matched group, the FCHL group and the age- and weight-matched group had higher body mass index (BMI), IAF, and SQF, but the FCHL and age- and weight-matched group values were not different from each other (Table 1).

FCHL subjects had significantly higher levels of total cholesterol, triglycerides, VLDL cholesterol, LDL cholesterol, and apoB compared with both control groups (Table 2). Except for a lower HDL cholesterol level in the age- and weight-matched control group compared with the age-matched control group, lipid levels were similar between these 2 groups (Table 2).

Fasting glucose levels were similar in all groups (Table 3). Insulin levels were significantly elevated in the age- and weight-matched control group compared with the age-matched control group and slightly higher than levels in the FCHL group. Si was lower (the subjects were more insulin resistant) in the FCHL group compared with the age-matched control group, but Si was not different in the

<table>
<thead>
<tr>
<th>Subjects</th>
<th>FCHL (N=11)</th>
<th>Age-Matched (N=15)</th>
<th>Age- and Weight-Matched (N=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males/Females, n/n</td>
<td>5/6</td>
<td>10/5</td>
<td>21/1</td>
</tr>
<tr>
<td>Age, y</td>
<td>59±11</td>
<td>64±5.9</td>
<td>65±4.2</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>30±5.3*</td>
<td>26±2.6</td>
<td>31±2.9‡</td>
</tr>
<tr>
<td>IAF, cm²</td>
<td>173±88*</td>
<td>113±56</td>
<td>194±61†</td>
</tr>
<tr>
<td>SQF, cm²</td>
<td>297±135†</td>
<td>173±52</td>
<td>274±56‡</td>
</tr>
</tbody>
</table>

Values are mean±SD.

*P<0.05, †P<0.01, and ‡P<0.001 vs age-matched control subjects.
FCHL group compared with the age- and weight-matched group (Table 3). Finally, acute insulin response to glucose was not different between groups, although results tended to be lower for the more insulin-resistant FCHL group and age- and weight-matched group compared with the age-matched group (Table 3).

When the relationship between Si and body composition was tested in the FCHL group, Si was inversely associated with IAF \( (r = -0.668, P < 0.05) \) and BMI \( (r = -0.642, P < 0.05) \) but not SQF \( (r = -0.443, P = 0.17) \). After all groups were combined, Si was inversely related to BMI \( (r = -0.521, P < 0.001) \), SQF \( (r = -0.407, P = 0.005) \), and IAF \( (r = -0.582, P < 0.001; \text{Figure 1}) \). Multiple linear regression analysis of Si as the dependent variable in the combined groups showed that only IAF remained significantly related to Si as an independent variable after the addition of BMI and SQF (Table 4).

To explore the relationships between the Si or IAF accumulation and apoB levels in the FCHL group, scatterplots were generated with a line to indicate the 90th percentile for apoB levels in the combined control groups (Figure 2). Nine of 11 subjects with FCHL had apoB levels at or above the 90th percentile of the control groups at any level of Si (Figure 2) and IAF (Figure 3). ApoB levels were not related to Si in the FCHL group alone \( (r = 0.201, P = 0.553) \), in the combined control groups alone \( (r = 0.203, P = 0.236) \), or in the FCHL and control groups combined \( (r = 0.165, P = 0.16) \). Similarly, apoB levels were not related to IAF in the FCHL subjects \( (r = 0.236, P = 0.484) \) or in the FCHL and control groups combined \( (r = 0.157, P = 0.285) \); however, a nonsignificant trend between apoB levels and IAF was found in the combined control groups \( (r = 0.301, P = 0.07) \).

**Discussion**

A number of studies have demonstrated greater insulin resistance in subjects with FCHL than in control groups and unaffected family members. Elevated fasting insulin levels, a marker of insulin resistance, were found in FCHL subjects compared with normolipidemic control subjects. Castro Cabezas et al. found that FCHL subjects with the highest triglyceride and cholesterol levels also had significantly higher insulin and nonesterified fatty acid levels compared with FCHL subjects with lesser degrees of hyperlipidemia. In these studies, BMI tended to be higher in the FCHL subjects with elevated insulin levels, but no measurements of abdominal fat were made. From these early studies, impaired suppression of fatty acid levels in FCHL subjects was proposed as a mechanism to explain the insulin resistance and the lipid phenotype.

Using the euglycemic hyperinsulinemic clamp, Aitman et al. demonstrated reduced Si and higher steady-state free fatty acid levels in FCHL subjects than in control subjects. The FCHL subjects in that study were slightly heavier (although not significantly) and had significantly greater waist fat, as measured by DEXA scan, than did the control subjects. Similar findings using the frequently sampled intravenous glucose tolerance test were reported by Ascaso and colleagues. Subsequent studies using the clamp technique confirmed that whole-body glucose uptake is reduced in FCHL subjects compared with control subjects. When the FCHL subjects in these studies were subdivided by lipid phenotype (high cholesterol, high triglycerides, or combined hyperlipidemia), Si was found to be reduced only in those with elevated triglycerides and cholesterol.

**Table 2. Lipid Levels of Subjects**

<table>
<thead>
<tr>
<th></th>
<th>FCHL Subjects</th>
<th>Age-Matched Subjects</th>
<th>Age- and Weight-Matched Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.41±1.0*</td>
<td>4.78±0.78</td>
<td>5.02±0.62</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>3.30±2.29†</td>
<td>1.19±0.80</td>
<td>1.54±0.58</td>
</tr>
<tr>
<td>VLDL-C, mmol/L</td>
<td>1.42±1.11‡§</td>
<td>0.52±0.39</td>
<td>0.70±0.26</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>4.09±0.93‡§</td>
<td>3.08±0.83</td>
<td>3.36±0.59</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>0.91±0.22‡</td>
<td>1.19±0.41</td>
<td>0.96±0.21</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>1.37±0.22‡</td>
<td>0.98±0.25</td>
<td>1.07±0.18</td>
</tr>
</tbody>
</table>

Values are mean±SD. VLDL-C, LDL-C, and HDL-C indicate VLDL, LDL, and HDL cholesterol, respectively.

**Table 3. Levels of Fasting Glucose, Insulin, and Si**

<table>
<thead>
<tr>
<th></th>
<th>FCHL Subjects</th>
<th>Age-Matched Subjects</th>
<th>Age- and Weight-Matched Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/L</td>
<td>5.2±0.40</td>
<td>5.2±0.67</td>
<td>5.4±0.43</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>94.2±70.8*</td>
<td>55.2±22.2</td>
<td>126±90†</td>
</tr>
<tr>
<td>Si, ( \times 10^{-1} \cdot \text{min}^{-1} \cdot \text{pmol}^{-1} \cdot \text{L} )</td>
<td>2.9±1.9†</td>
<td>4.6±2.0</td>
<td>2.8±1.8§</td>
</tr>
<tr>
<td>AIRglu, pmol/L</td>
<td>503±362</td>
<td>634±636</td>
<td>401±371</td>
</tr>
</tbody>
</table>

Values are mean±SD. AIRglu indicates acute insulin response to glucose.

\* P<0.05 vs age- and weight-matched control subjects; † P<0.001, ‡ P<0.05, and § P<0.01 vs age-matched control subjects.

**Figure 1. Regression relationship between IAF and Si in FCHL and control subjects combined \( (r = -0.582, P < 0.001) \). Values of FCHL subjects are shown as black circles; those of the control subjects are shown as open circles.**
Visceral adiposity has been associated with a number of metabolic abnormalities, including insulin resistance, increased triglyceride levels, lower HDL cholesterol, more cholesterol in small dense LDL particles, increased apoB production rates, and increased apoB levels. To determine whether accumulation of IAF is similarly associated with insulin resistance and dyslipidemia in FCHL, subjects in the present study underwent measurement of IAF and SQF by CT scan. Consistent with previous reports, FCHL subjects in the present study were more insulin resistant than were the age-matched control subjects. When FHCL subjects were compared with control subjects matched for age, BMI, and amount of IAF, however, Sı was not different. Indeed, Sı was inversely related to the amount of IAF but not to the amount of SQF in the FCHL subjects, and when the FCHL and control groups were combined, only IAF remained correlated with insulin resistance after including SQF and BMI on multiple regression analysis. So although the present study confirms the presence of insulin resistance in subjects with FCHL, it extends previous observations to show that FCHL subjects are viscerally obese and that their insulin resistance is appropriate for their degree of visceral adiposity. Although this conclusion may appear at odds with the report of reduced Sı in nonobese FCHL subjects by Bredie et al, a study by Fujimoto et al has demonstrated that even in a nonobese population, IAF levels measured by CT scan can vary up to 10-fold and are a major determinant of insulin resistance. However, confirmation of this would require measurement of Sı and IAF by CT in nonobese FCHL and control subjects.

One potential confounder in the present study is the predominance of men in the age- and weight-matched control group compared with the FCHL group. Men are known to have a greater amount of IAF and higher triglyceride and lower HDL cholesterol levels on average compared with age-matched women, and this may have introduced a bias, making some differences with the FCHL group more difficult to detect (ie, lipid levels) or other differences more pronounced (ie, IAF). In fact, neither of these was found in the present study. Lipid levels (and apoB) were still higher, and IAF was no different between these groups. With the added analysis showing no sex differences in the FCHL group for levels of lipids and IAF, it is unlikely that bias introduced by having a greater proportion of men in one control group is important in this analysis.

An important observation from the present study is that although increased production of apoB particles and elevated levels of apoB have been described in viscerally obese subjects, neither the amount of IAF nor insulin resistance could fully account for the elevation in apoB levels reported in the FCHL subjects in the present study. In >80% of the FCHL subjects, apoB levels were higher than the 90th percentile of controls at any level of Sı or amount of IAF. These data provide support for genetic models describing a major, but separate, gene(s) for elevated apoB distinct from genes with effects on triglyceride and small dense LDL in subjects with FCHL. To date, no candidate genes, including the apoB gene, have been found to account for the increased apoB in FCHL. On the other hand, many genes seem to contribute to the increase in triglyceride and other lipid levels in FCHL.

In summary, subjects with FCHL are viscerally obese and insulin resistant compared with the age-matched control subjects. However, when compared with age-, weight-, and IAF-matched control subjects, their insulin resistance is appropriate for their degree of visceral obesity. Levels of apoB are higher in FCHL subjects at any level of Sı and IAF accumulation compared with levels in control subjects. Taken together, the present study suggests that the visceral obesity/insulin resistance syndrome contributes to the FCHL lipid phenotype but that a separate genetic regulator(s) likely controls the increased apoB levels above those found in the control subjects.
Insulin Resistance, ApoB, and IAF in FCHL

Purnell et al

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