Relationships of Abdominal Obesity and Hyperinsulinemia to Angiographically Assessed Coronary Artery Disease in Men With Known Mutations in the LDL Receptor Gene

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Background—Patients with a mutation in the LDL receptor gene (familial hypercholesterolemia, or FH) are characterized by substantial elevations in plasma LDL cholesterol and are at higher risk of developing coronary artery disease (CAD). Correlates of abdominal obesity may also contribute to the risk of ischemic cardiac events. Whether the hyperinsulinemic–insulin-resistant state of abdominal obesity affects coronary atherosclerosis among FH patients has not been determined.

Methods and Results—The relation of abdominal adiposity and hyperinsulinemia to angiographically assessed CAD was evaluated in a sample of 120 French Canadian men aged ≤60 years who were heterozygotes for FH and in a group of 280 men without FH. In the present study, the risk of CAD associated with abdominal obesity, as estimated by the waist circumference, was largely dependent on the concomitant variation in plasma lipoprotein and insulin concentrations. In contrast, the association between fasting insulin and CAD was independent of variations in waist girth, triglyceride, HDL, and apolipoprotein B concentrations (odds ratio, 1.86; \( P = .0005 \)). However, the most substantial increase in the risk of CAD was observed among abdominally obese (waist circumference >95 cm) and hyperinsulinemic FH patients (odds ratio, 12.9; \( P = .0009 \)). This increase in risk remained significant even after adjustment for LDL cholesterol or apolipoprotein B concentrations.

Conclusions—Results of the present study provide support for the notion that the hyperinsulinemic–insulin-resistant state of abdominal obesity is a powerful predictor of CAD in men, even in a group of patients with raised LDL cholesterol concentrations due to FH. (Circulation. 1998;97:871–877.)

Key Words: hyperinsulinemia ■ obesity ■ hypercholesterolemia ■ coronary disease
Prospective Cardiovascular Study cohort suggested that the relation of hyperinsulinemia to ischemic heart disease may be largely independent of alterations in body weight, blood pressure, and plasma lipoprotein concentrations. It has been suggested that visceral obesity may be a common component of the cluster of metabolic abnormalities found in insulin-resistant subjects. This metabolic cluster also includes hypertension, low HDL-C, elevated apo B, elevated TG concentrations, impaired fibrinolysis, and impaired insulin-mediated glucose uptake. In this regard, it is likely that the components of the metabolic syndrome may add to the atherogenic potential of hypercholesterolemia due to raised LDL-C concentrations. However, the contribution of abdominal adiposity and hyperinsulinemia to CAD in patients with substantial increases in plasma LDL-C and apo B concentrations has never been specifically examined. In this regard, the present study focused on the effect of important correlates of the metabolic syndrome (namely, abdominal obesity and hyperinsulinemia) on the expression of CAD among well-characterized patients with raised LDL-C and apo B concentrations due to FH.

Methods

Study Design

The present study is a case-control analysis of the relationships of abdominal adiposity to angiographically assessed CAD among men with or without a mutation in the LDL receptor gene. The study population was derived from all unrelated patients aged 60 years or who have been monitored at the Chicoutimi Hospital Lipid Clinic (Chicoutimi, Quebec, Canada) between January 1991 and January 1996 and who underwent coronary angiography for the investigation of ischemic heart disease (typical angina or a positive exercise tolerance test) or both (N = 1014). The diagnosis of FH was based on the following: (1) the presence of a mutation in the LDL receptor gene; (2) the presence of a mutation in the LDL receptor gene in a first-degree relative and either typical tendinous xanthomata or plasma LDL-C above the 95th percentile in the absence of a secondary cause of hypercholesterolemia; or (3) LDL-C above the 95th percentile as well as tendinous xanthomata and a family history of raised plasma LDL-C transmitted in an autosomal dominant pattern. Patients with a mutation in the LDL receptor gene (case subjects) were matched on the basis of age and smoking with 2 or 3 patients without FH who underwent coronary angiography over the same period (control subjects). The mean difference for age between matched case and control subjects was 0.7 year. All nonsmokers were matched with nonsmokers. Patients were excluded if they were homozygous for FH (n = 1), if they had a known mutation in the LDL gene (n = 3), or if they had a diagnosis of diabetes mellitus before angiography (n = 105), or if they were receiving lipid-lowering or thyroid medication during the month preceding the lipid-lipoprotein measurements (n = 188). According to these criteria, 120 patients with raised plasma LDL-C concentrations were included in the FH group whereas 280 patients remained in the non-FH group. To control for spurious associations that may arise from population admixture, all patients selected were born in the SLSJ region located in the eastern part of the province of Quebec, Canada. The prevalence of FH in the SLSJ population is ~6-fold higher than what has been reported in most other populations (1.2% versus 0.2%). Regarding the assessment of coronary stenosis, four coronary arteries were considered: left main coronary, left anterior descending, circumflex, and right coronary. Patients with ≥1 lesion leading to a narrowing of at least 50% of the lumen of any of these four coronary arterial segments were considered as having coronary stenosis. Results of coronary angiograms were also analyzed by use of a score based on the number of diseased vessels. This score ranged from 0 (no vessel with at least 50% narrowing) to 4 (all four vessels with at least 50% narrowing). Interpretation of coronary angiograms was performed independently by two cardiologists and one radiologist who were not aware of the patients’ inclusion in the study. This project received the approval of the Chicoutimi Hospital Ethics Committee.

Mutations in the LDL Receptor Gene

All patients from the present study were screened for the presence of mutations in the LDL receptor gene after obtaining their informed written consent. Almost 90% of FH cases in the SLSJ could be attributed to only two mutations: (1) a missense mutation in exon 3 (W666G), leading to an impaired lipoprotein binding (class III mutation), and (2) a deletion of >15 kb in the promoter and exon 1, which is a receptor negative mutation due to the absence of transcriptional signal (class I mutation). However, screening of FH included detection of the six mutations explaining the majority of cases in the province of Quebec, namely, two deletions (5 kb and >15 kb) and four point mutations in exons 3, 4, and 14 was detected by dot-blot hybridization of genomic DNA amplified by PCR with allele-specific oligonucleotide probes, according to Leitersdorf et al. or by PCR-based restriction fragment analysis according to Vohl et al. The presence of the nonsense stop 468 mutation in exon 10 was detected by PCR-based restriction fragment analysis according to Vohl et al.

Estimation of Abdominal Fat Deposition and Evaluation of Other Risk Factors

Biological and lifestyle variables as well as medical and nutritional histories were obtained through questionnaires and physical exams performed at the Chicoutimi Hospital Lipid Clinic by trained nurses, dietitians, and physicians. Waist and hip circumferences were measured according to the procedures recommended at the Anlke Conference. Body weight and height were recorded, and the body mass index was calculated in kilograms per meter squared. Fasting plasma glucose was enzymatically measured, whereas fasting insulinemia was measured by radioimmunoassay with polyethylene glycol separation. Individuals diagnosed as having NIDDM were excluded when one of the following criteria was met: (1) previously established diagnosis of NIDDM; (2) two fasting glucose values >7.8 mmol/L obtained before the coronary angiogram; or (3) at least one value >11.1 mmol/L during a 75-g oral glucose tolerance test. Smoking habits were defined as follows: (1) men who never smoked and (2) men who ever smoked. Men who ever smoked were further classified into three categories (0 to 10 cigarettes/d, 11 to 25 cigarettes/d, and >25 cigarettes/d). A subject was considered hypertensive if diagnosis of essential hypertension had been previously established or when three values of systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg were recorded in the patient’s medical chart. Resting blood pressure measurements were performed after the subjects had a 5-minute rest in a sitting position, phases I and V of Korotkoff sounds being used for systolic and diastolic blood pressures, respectively. Alcohol consumption was defined in two categories: (1) regular drinkers (>5 ounces of absolute alcohol/wk) and (2) nonregular drinkers. A family history of premature coronary atherosclerosis was defined as the presence of symptomatic ischemic heart disease or

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**Selected Abbreviations and Acronyms**

- apo = apolipoprotein
- CAD = coronary artery disease
- FH = familial hypercholesterolemia
- HDL-C = HDL cholesterol
- LDL-C = LDL cholesterol
- Lp(a) = lipoprotein(a)
- NIDDM = non–insulin-dependent diabetes mellitus
- PCR = polymerase chain reaction
- SLSJ = Saguenay-Lac-St-Jean
- TG = triglycerides

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coronary death in a first-degree relative aged <55 years (male relative) or <60 years (female relative).

**Plasma Lipid-Lipoprotein Measurements**

Blood samples were obtained the morning after a 12-hour overnight fast from an antecubital vein into evacuated container tubes containing EDTA. Total cholesterol, TG, and HDL-C levels were measured with the use of enzymatic assays. TC was determined in serum and HDL-C was measured in the supernatant after precipitation of the apo B–containing lipoproteins with dextran sulfate and magnesium chloride. LDL-C was calculated by use of the Friedewald formula when TG levels were <5 mmol/L, and subjects with TG >5 mmol/L were excluded from the present study. Plasma apo B levels were measured according to the rocket immunoelectrophoretic method of Laurell.

Serum standards for apo B determinations were prepared in our laboratory and calibrated against sera from the Centers for Disease Control (Atlanta, Ga). Standards were lyophilized and stored at −85°C until use. Peak heights between 15 and 35 mm yielded linear and reliable results. Lp(a) levels were measured on an Array 360 system using LPA reagent (P/N 465360) and LPA Cal (P/N 465365) obtained from Beckman Instruments Inc.

**Statistical Analyses**

Differences in continuous variables between groups were compared either by Student’s unpaired two-tailed test or by ANOVA with the use of the Bonferroni procedure for pairwise comparisons. Categorical variables were compared with the χ² test. Associations between potential correlates of abdominal fat deposition were quantified by use of Pearson or Spearman correlation coefficients for parametric and nonparametric variables, respectively. Finally, conditional logistic regression models were constructed to investigate the independent relationship between CAD, considered as the dependent variable, and correlates of abdominal adiposity. The effect of risk factors for CAD as possible confounders was taken into account in the different analyses, and adjustments were used for any significant (P<.1) effect of class of mutation in the LDL receptor gene, hypertension, fasting glucose concentration, alcohol consumption, or use of medication (β-blockers and diuretics). The matching variables did not modify the estimates of the association between other risk factors and CAD. Furthermore, conditional logistic regression analysis, which allows the removal of the confounding factors introduced by the matching, yielded results that were the same as those of the unconditional regression analyses. For these reasons, age and smoking were excluded from the regression models. In all analyses, plasma TG and insulin data were log10-transformed to normalize their distribution. Analyses were performed with the use of the SPSS package (release 6.1, SPSS Inc).

**Results**

Characteristics of men with or without FH who underwent coronary angiography are presented in Table 1. Compared with non-FH men, men with a mutation in the LDL receptor gene tended to be younger at the time of first coronary angiography and more severely affected by CAD as assessed by the proportion of patients having four diseased vessels. Furthermore, FH patients had higher plasma Lp(a), LDL-C, and apo B concentrations. However, plasma HDL-C levels were similar in the two groups, whereas plasma TG concentrations were significantly higher among patients without FH (P=.03). Patients without FH also had higher fasting insulin levels and were more obese as a group than FH patients.

**TABLE 1. Characteristics of the Study Patients According to the Presence (FH) or Absence (Non-FH) of a Mutation in the LDL Receptor Gene**

<table>
<thead>
<tr>
<th></th>
<th>FH</th>
<th>Non-FH</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>n=120</td>
<td>n=280</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>46.7±0.7</td>
<td>46.9±0.4</td>
<td>.58</td>
</tr>
<tr>
<td>Age at first coronary angiography</td>
<td>43.6±0.7</td>
<td>45.6±0.4</td>
<td>.04</td>
</tr>
<tr>
<td>Number of diseased vessels (% of patients)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 vessels with &gt;50% stenosis</td>
<td>6 (5.0%)</td>
<td>31 (11.0%)</td>
<td>.0001</td>
</tr>
<tr>
<td>1 vessel with &gt;50% stenosis</td>
<td>27 (22.5%)</td>
<td>98 (35.0%)</td>
<td>.005</td>
</tr>
<tr>
<td>2 vessels with &gt;50% stenosis</td>
<td>30 (25.0%)</td>
<td>72 (25.7%)</td>
<td>.96</td>
</tr>
<tr>
<td>3 vessels with &gt;50% stenosis</td>
<td>28 (23.3%)</td>
<td>58 (20.7%)</td>
<td>.65</td>
</tr>
<tr>
<td>4 vessels with &gt;50% stenosis</td>
<td>29 (24.1%)</td>
<td>21 (7.5%)</td>
<td>.0001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.0±0.3</td>
<td>27.9±0.3</td>
<td>.0001</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>92.3±0.8</td>
<td>97.6±0.7</td>
<td>.0001</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.92±0.01</td>
<td>0.96±0.01</td>
<td>.0001</td>
</tr>
<tr>
<td>Mean blood pressure, mm Hg</td>
<td>93.0±13.6</td>
<td>95.4±13.4</td>
<td>.15</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>8.3±1.6</td>
<td>5.9±0.6</td>
<td>.0001</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>6.4±0.2</td>
<td>4.0±0.1</td>
<td>.0001</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>0.89±0.02</td>
<td>0.89±0.01</td>
<td>.77</td>
</tr>
<tr>
<td>TG, mmol/L*</td>
<td>2.1±0.1</td>
<td>2.3±0.1</td>
<td>.03</td>
</tr>
<tr>
<td>Apo B, g/L</td>
<td>1.51±0.03</td>
<td>1.17±0.01</td>
<td>.0001</td>
</tr>
<tr>
<td>Lp(a), mg/dL*</td>
<td>43.3±3.9</td>
<td>32.2±1.9</td>
<td>.005</td>
</tr>
<tr>
<td>Fasting insulin, mU/L*</td>
<td>16.2±0.8</td>
<td>19.0±0.7</td>
<td>.02</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L*</td>
<td>5.5±1.1</td>
<td>5.5±0.9</td>
<td>.62</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; TC, total cholesterol.

*Geometric mean and approximate SEM. P values after log-transformations.
bution of abdominal obesity. The contribution of an increased waist girth to the variation of common metabolic risk factors is illustrated in Fig 1. In both FH and non-FH patients, a bigger waistline tended to be associated with elevated plasma insulin and TG levels as well as with a reduction in plasma HDL-C concentration. However, for a given waist girth, no difference in plasma insulin, TG, or HDL-C levels was noted between FH and non-FH patients. Nevertheless, plasma apo B concentrations were obviously higher in FH patients, irrespective of the waist circumference. In the present study, 60% of men with FH presented a receptor defective mutation (W66G) in exon 1 and the promoter), and 12.5% presented other mutations in exons 4, 7, 10, and 14, respectively. However, the nature of the mutation in the LDL receptor gene did not alter the relationships of correlates of abdominal fat deposition and hyperinsulinemia to coronary stenosis. Thus, on the basis of these results, all FH patients were pooled for the different analyses presented.

Univariate analyses revealed that fasting insulin was a significant correlate of waist girth ($r=0.20; P=0.01$) and showed a highly significant association with coronary stenosis among both FH ($r=0.28; P=0.0001$) and non-FH ($r=0.22; P=0.003$) patients. The importance of fasting insulin as a predictor of CAD was also evident in the different multivariate regression models tested. Indeed, Table 2 presents multivariate logistic analyses performed to determine the contribution of FH to CAD before and after adjustment for waist girth and plasma lipid and insulin levels. In the first model, in which the contribution of FH was examined before the inclusion of correlates of abdominal adiposity, the relative odds of expressing $\geq 50\%$ stenosis in at least one coronary artery among FH patients was twofold higher than among the non-FH group ($P=0.002$). Further adjustment for the potentially confounding effects of the correlates of abdominal fat deposition did not substantially weaken the relation of FH per se to the risk of CAD (models 2 through 5 in Table 2). However, the risk of CAD associated with abdominal obesity per se, as estimated by the waist circumference, was largely dependent on the variation in plasma lipoproteins and insulin concentrations. In contrast, results of model 5 revealed that the association between plasma insulin concentration and the risk of coronary stenosis was, to a certain extent, independent of variations in the waist girth, TG, and HDL-C concentrations ($P=0.005$). As shown in model 6, the relationship of FH to the risk of coronary stenosis was attenuated when we accounted for the contribution of apo B concentrations to CAD. Finally, when we tested for multiplicative interaction between waist girth and insulin in FH and non-FH patients considered separately, the interaction term was significant in FH ($P=0.01$) but not in non-FH patients (data not shown).

Because FH and insulin were strong predictors of CAD in the present study, we further investigated the combined effects of FH, fasting insulin, and abdominal fat deposition on the risk of coronary stenosis. As shown in Fig 2, the absolute effect of hyperinsulinemia on CAD among FH patients appeared to be significantly affected by a bigger waistline, which was not the case among non-FH patients. In this regard, the results presented in Fig 2 indicate that the most substantial increase in the risk of CAD was observed among men with FH, abdominal obesity (waist circumference $\geq 95$ cm), and elevated insulin ($P=0.0005$). This increase in CAD risk among abdominally obese and hyperinsulinemic FH patients remained significant even after adjustment for plasma LDL-C and apo B concentrations (odds ratio, 7.6; 95% CI, 1.8 to 28.7). In the absence of hyperinsulinemia and before adjustment for LDL-C and apo B, abdominal fat deposition increased the odds of having CAD among men with a mutation in the LDL receptor gene (odds ratio, 3.32; 95% CI, 1.01 to 12.19) but not among patients without FH ($P=0.87$).

**Discussion**

Results of the present study indicate that the combination of hyperinsulinemia and abdominal obesity substantially increased the risk of coronary stenosis among FH patients. However, the risk associated with an increased waist girth in FH was largely explained by the lipid abnormalities that are common among obese, insulin-resistant men, whereas the association of insulin with CAD was at least partly independent of these lipid abnormalities. Overall, men with FH were characterized by a higher level of coronary stenosis and a lower prevalence of modifiable cardiovascular risk factors than non-FH men, suggesting that mutations in the LDL receptor remain a major cause of CAD among young adults.

FH is a monogenic trait due to mutations in the LDL receptor gene, characterized by raised plasma LDL-C concentrations and tendinous xanthomas. CAD is an early event in heterozygous FH, and 45% to 48% of men and 20% to 21% of women develop coronary atherosclerosis before age 50. It is well known that the nature of the mutation in the LDL receptor gene may affect the expression of coronary athero-
sclerosis. Indeed, recent studies in homozygous as well as heterozygous FH patients have shown that null (class 1) mutations are associated with higher plasma cholesterol levels and with earlier manifestations of CAD than missense (class III) mutations.37–39 However, the expression of CAD in FH patients not only is determined by the nature of the gene defect but also is influenced by other risk factors, an issue that has been emphasized by many investigators.8–10,38 In the present study, however, the nature of the mutation did not alter the relation of abdominal adiposity to CAD. Furthermore, abdominal obesity, as assessed by waist girth, was associated with numerous metabolic alterations among FH patients: lower plasma HDL-C, higher TG levels, and higher fasting insulin concentrations. The cosegregation of obesity, dyslipidemia, and hyperinsulinemia among families of obese subjects is well documented,30–42 and numerous studies have shown that a preferential accumulation of abdominal fat is associated with a metabolic cluster that may contribute to substantially increase the risk of atherosclerotic cardiovascular disease.14–17

In the present study, although average waist circumference and fasting insulin values tended to be significantly lower in FH than in non-FH patients, the contribution of hyperinsulinemia, abdominal obesity, and CAD was evident in FH, and the absolute effect of hyperinsulinemia on CAD among FH patients appeared to be significantly affected by a higher waistline. Small, dense LDL particles, hypertriglyceridemia, and low plasma HDL-C are conditions that are quite prevalent among abdominally obese insulin-resistant patients, and this dyslipidemic profile has been reported to be associated with an increased risk of CAD.43–52 In this regard, obese and hyperinsulinemic FH patients are potentially exposed over time to both the quantitative atherogenicity and qualitative alterations of apo B–containing lipoproteins. Indeed, the combination over time of an increased number of LDL particles in circulation, which is a feature of FH, together with the presence of a greater proportion of denser and otherwise modified LDL, which often accompanies the abdominally obese, insulin-resistant state, is likely to increase the risk of CAD in FH.
Indeed, previous studies\(^4\) clearly demonstrated that in addition to elevated LDL-C concentrations, elevated plasma TG levels (type IIB dyslipidemia) contribute to the severity of coronary ischemic disease among FH heterozygotes. Furthermore, the density of LDL has been proposed recently as an independent predictor of CAD risk in the Quebec Cardiovascular Study.\(^4\) However, in the FH patients of the present study, fasting TG concentration was not independently associated with CAD, a finding generally consistent with most of the literature on non-FH patients.\(^4\)

The increased CAD risk associated with abdominal obesity and hyperinsulinemia found in FH patients of the present study could not be explained by a concomitant elevation in Lp(a) levels. The contribution of abdominal obesity and hyperinsulinemia to CAD also could not be explained by the presence of known defects in the LPL gene that are quite prevalent among French Canadians, because patients bearing these mutations were excluded from the present study.

In conclusion, results of the present study provide further support to the notion that hyperinsulinemia is a powerful predictor of coronary heart disease in men,\(^3\) even among heterozygous FH patients with well-defined genetic defects and substantial elevations in plasma LDL-C concentrations. Furthermore, abdominal obesity and hyperinsulinemia appear to act synergistically to substantially increase the odds of CAD among men with FH. This finding suggests that the estimation of abdominal adipose tissue deposition, at least by measurement of waist circumference, must be considered important in the evaluation of the cardiovascular risk profile, even among FH patients. Furthermore, it is proposed that results of the present study have important therapeutic implications. Indeed, because abdominal obesity and hyperinsulinemia appear to be important risk factors for CAD even among well-characterized FH patients and even after controlling for LDL-C and apo B concentrations, emphasis should not only be placed on the relevant and justified lowering of LDL concentrations but also on the treatment of abdominal obesity and the related insulin-resistant hyperinsulinemic state for an optimal reduction of CAD risk. Finally, further studies are required to verify whether these conclusions derived from the study of male FH patients are also valid for women with FH.

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