Leptinemia Is Not a Risk Factor for Ischemic Heart Disease in Men

Prospective results from the Quebec Cardiovascular Study

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OBJECTIVE — To investigate the possibility that leptin levels may be predictive of the risk of ischemic heart disease (IHD) through the relationship of leptin to body fat.

RESEARCH DESIGN AND METHODS — The Quebec Cardiovascular Study cohort consisted of 2,103 French-Canadian men without IHD in 1985 who were followed until 1990, by which time 114 had experienced an IHD event. These men were then individually matched for age, BMI, cigarette smoking, and alcohol intake with 114 subjects who were free of IHD at follow-up. After exclusion of diabetic patients and those in whom leptin levels could not be measured, we were able to compare the initial metabolic profiles of 86 men in the IHD group and of 95 control subjects.

RESULTS — Plasma leptin concentrations were positively correlated with BMI \( r = 0.67, P < 0.0001 \) and with fasting insulin concentrations \( r = 0.46, P < 0.0001 \) in the overall sample. These significant associations were also observed when men with IHD and the control subjects were examined separately (control subjects: \( r = 0.68 \) for BMI and \( r = 0.45 \) for insulin; IHD subjects: \( r = 0.65 \) for BMI and \( r = 0.50 \) for insulin). With the exception of plasma triglyceride \( r = 0.25, P < 0.001 \), no significant association was found between leptin and plasma lipoprotein and lipid concentrations. Furthermore, plasma insulin remained significantly associated with leptin levels even after adjustment for BMI \( r = 0.22, P < 0.005 \). There was no difference in baseline leptin levels among men who developed IHD versus men who remained IHD-free during the 5-year follow-up \( (5.56 \pm 3.12 \text{ vs. } 5.36 \pm 2.90 \text{ ng/ml, respectively}) \). Thus, although significantly correlated with the BMI and fasting insulin levels, plasma leptin concentration was not a significant predictor of the 5-year incidence of IHD. This lack of a relationship to IHD was noted when leptin levels were analyzed as tertiles and when leptin concentration was analyzed as a continuous variable.

CONCLUSIONS — These prospective results suggest that leptinemia, despite being a strong correlate of obesity, does not appear to be an independent risk factor for the development of IHD in men.

Obesity has long been recognized to have detrimental effects on health, and a higher rate of metabolic complications are found in overweight compared with lean individuals \( (1) \). These complications contribute to increased risk of NIDDM and of ischemic heart disease (IHD) in obese patients \( (1) \). In this regard, the recent cloning of the human ob gene and the characterization of its protein product, leptin \( (2) \), have been major breakthroughs in investigating the etiology of at least some forms of obesity. These studies have shown that leptin is essentially produced by adipose tissue and that plasma leptin levels are strongly and positively correlated with amount of body fat \( (3-9) \). Furthermore, excessive insulin, a risk factor for IHD, has also been reported to upregulate the expression of the obese gene in adipose cells \( (3-5,9-14) \). Accordingly, plasma insulin concentration is an independent correlate of leptinemia, even after control for the effect of body fat \( (6,10) \). In the present study, we tested the hypothesis that this newly discovered hormone may be related to IHD through its relationship to obesity and hyperinsulinemia. The 5-year follow-up results of the Quebec Cardiovascular Study have allowed us to examine this question.

RESEARCH DESIGN AND METHODS

Study subjects

A complete and extensive description of the Quebec Cardiovascular Study cohort has been published elsewhere \( (15-17) \). Briefly, in 1985, a sample of 2,103 men aged 47–76 years were characterized as being free of IHD and underwent a complete profile evaluation for IHD risk factors. Over a 5-year follow-up period, 114 of these men had an IHD event. These 114 IHD subjects were then individually matched with men who remained healthy for the following variables over the 5-year follow-up period: age, BMI, smoking, and alcohol consumption \( (16,17) \). After excluding IHD subjects who could not be matched because of extreme smoking habits, diabetic subjects, and subjects who were unavailable for plasma leptin assays, we were able to obtain data on 86 IHD subjects and 95 control subjects.

Definition of events

The definition of a first IHD event was typical effort angina, coronary insufficiency, nonfatal myocardial infarction, or coronary death. Myocardial infarction was diagnosed according to the electrocardiographic crite-
Table 1—Baseline characteristics of 86 men who developed a first IHD event over a 5-year follow-up period versus 95 subjects who remained free of IHD: The Quebec Cardiovascular Study

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>IHD subjects (n=86)</th>
<th>Control subjects (n=95)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>86</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>59.3 ± 7.8</td>
<td>58.9 ± 6.9</td>
<td>—</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.0 ± 12.3</td>
<td>75.8 ± 11.1</td>
<td>—</td>
</tr>
<tr>
<td>Smoking (cigarettes/day)</td>
<td>10.4 ± 15.1</td>
<td>10.0 ± 14.0</td>
<td>—</td>
</tr>
<tr>
<td>Alcohol consumption (oz/week)</td>
<td>5.27 ± 7.98</td>
<td>5.25 ± 8.30</td>
<td>—</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.1 ± 3.7</td>
<td>26.2 ± 3.5</td>
<td>—</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>136 ± 17</td>
<td>133 ± 18</td>
<td>0.3</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>6.07 ± 1.00</td>
<td>5.51 ± 0.94</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>2.02 ± 0.78</td>
<td>1.70 ± 0.66</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>0.95 ± 0.22</td>
<td>1.02 ± 0.25</td>
<td>0.06</td>
</tr>
<tr>
<td>Apo B (mg/dl)</td>
<td>130 ± 30</td>
<td>112 ± 27</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Cholesterol-to-HDL cholesterol ratio</td>
<td>6.73 ± 1.93</td>
<td>5.73 ± 1.66</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>92.3 ± 27.6</td>
<td>78.1 ± 29.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fasting leptin (ng/ml)</td>
<td>5.56 ± 3.12</td>
<td>5.36 ± 2.90</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Data are means ± SD. The variables used in matching IHD and control subjects were age, BMI, smoking status, and alcohol consumption.

Alcohol consumption (oz/week) was obtained to review relevant hospital status, and alcohol consumption. Evaluation of risk factors

In 1985, data on demographic and lifestyle variables, medical history, and medication were obtained through a standardized questionnaire, administered by trained nurses and further reviewed by a physician. Body weight and height were recorded. Resting blood pressure measurements were performed after a 5-min rest in a sitting position, using phases I and V of Korotkoff sounds for systolic and diastolic blood pressure, respectively. The mean of two blood pressure measurements obtained 3 min apart was used. The following information was compiled from the questionnaire: history of diabetes (both family and personal), smoking habits, alcohol consumption, and medication use. The use of hypolipidemic drugs was not prevalent in 1985, as it was reported in only ~1% of the subjects with and without IHD. The regular use of β-blockers, mainly propranolol and metoprolol, was observed in 11% of the men with IHD and in 6.5% of those without IHD. The proportion of men using diuretics on a regular basis was 7.5 and 2.8% in IHD and control subjects, respectively. Alcohol intake, expressed in ounces per week, was computed from the type of beverage (beer, wine, or spirits) consumed and then standardized as an absolute quantity, with 1 oz of absolute alcohol being equivalent to 22.5 g of alcohol (15). Family history of IHD was considered positive if at least one parent and/or sibling had a history of IHD.

Laboratory analyses

In 1985, 12-h fasting blood samples were obtained from antecubital veins while participants were sitting. A tourniquet was used but was released before blood withdrawal into Vacutainer tubes (Becton Dickinson, Mountain View, CA) containing EDTA. Plasma was separated from blood cells by centrifugation and immediately used for lipid and apolipoprotein measurements. Aliquots of fasting plasma were frozen at −80°C at time of collection for subsequent analyses. Plasma cholesterol and triglyceride (TG) levels were determined on an Auto Analyzer II (Technicon, Tarrytown, NY), as previously described (19). HDL cholesterol was measured in the supernatant fraction after precipitation of apolipoprotein B (apoB)-containing lipoproteins with heparin-MnCl₂ (20). Plasma apoB levels were measured using the rocket immunoelctrophoresis method of Laurell (21), as previously described (19). Serum standards for apoB determination were prepared in our laboratory and calibrated against sera from the Centers for Disease Control (Atlanta, GA). Peak heights between 15 and 35 mm yielded linear and reliable results. The cumulative coefficients of variation for total cholesterol, HDL cholesterol, TG, and apoB measurements were all <3%. Fasting insulin concentrations were measured by a commercial double-antibody radioimmunoassay (RIA) (Human Insulin Specific RIA Method, Linco, St. Louis, MO). This assay shows little cross-reactivity (<0.2%) with human proinsulin (22). Finally, plasma leptin concentrations were determined with a highly sensitive commercial double-antibody RIA (Human Leptin Specific RIA Kit, Linco, St. Louis, MO), which detects relatively low leptin levels of 0.5 ng/ml and does not cross-react with human insulin, proinsulin, glucagon, pancreatic polypeptide, or somatostatin. Our coefficients of variation ranged from 4.0 to 5.5% for lower leptin concentrations and from 6.5 to 8.5% for higher plasma leptin concentrations.

Statistical analyses

The SAS statistical package (SAS Institute, Cary, NC) was used for all analyses. Student t tests were used to compare means between men with IHD and those without IHD. Associations among variables were assessed using Spearman’s correlation coefficients. Odds ratios (ORs) of IHD were calculated using the coefficients (β) obtained from the various logistic models. ORs of the continuous variables were computed as the increase or decrease in risk of IHD associated with an elevation of 1 SD in the concentration of the various risk factors. Also, the men were subdivided into tertiles of plasma leptin and of total cholesterol–HDL cholesterol ratio.
Leptin and ischemic heart disease

Model 1: Leptin
Model 2: CHOL/HDL-cholesterol

Figure 1—Odds ratios of developing IHD among tertiles of leptinemia and of the cholesterol (CHOL)-to-HDL cholesterol ratio in the overall sample of 181 men. Adjustment of risk for systolic blood pressure, medication use, and family history of IHD failed to alter these results. *P < 0.003 for the total cholesterol-to-HDL cholesterol ratio model.

and the risk of IHD among the tertiles was assessed by logistic regression analyses using the tertile with the lowest value as reference, which, by definition, was assigned a risk of 1.0. Relative odds of developing IHD were adjusted for potential confounders other than those used in the matching procedures, namely systolic blood pressure, medication use, and family history of IHD. Adding the matching factors to the logistic models had essentially no impact on the parameter estimates.

RESULTS — Baseline characteristics of men who developed a first IHD event during the follow-up period and those who remained free of IHD are shown in Table 1. Men with IHD had significantly higher fasting plasma cholesterol, TG, apoB, and insulin concentrations compared with control subjects. Men who developed IHD also had a higher initial average cholesterol-to-HDL cholesterol ratio compared with men who remained IHD-free. However, the two groups showed no difference in plasma leptin concentrations.

Multiple logistic regression analyses were performed to examine the association between plasma leptin concentration and the risk of developing IHD. No significant change in the risk of IHD was associated with elevated plasma leptin concentration (the OR for IHD for every 1-SD increase in leptin concentration was 1.0 [95% CI, 0.8-1.4]). Figure 1 compares the risk of developing IHD according to tertiles of leptin levels and the risk associated with the total cholesterol-to-HDL cholesterol ratio, a well-known risk factor for IHD, in the same sample of men.

Figure 2 illustrates the highly significant relationship between plasma leptin levels and BMI in the overall sample. This relationship was also observed when men with IHD (r = 0.65, P < 0.0001) and men without IHD (r = 0.68, P < 0.0001) were examined separately.

Finally, plasma leptin concentrations were positively associated with plasma TG and insulin levels; all other metabolic variables studied showed no association with leptinemia (Fig. 3). When plasma insulin levels were adjusted for concomitant variation in adiposity (i.e., BMI), their association with leptin levels remained significant (r = 0.22, P < 0.005).

CONCLUSIONS — Leptin, the protein product of the obese gene, is a hormone expressed and secreted by adipose cells (2,12). In the present study, the BMI (a crude correlate of adiposity) was positively correlated with leptin levels, and these results are in accordance with the frequently reported close relationship of plasma leptin levels to adiposity (3–9). This association remained significant when we separately analyzed men with IHD and men without IHD. Furthermore, the slope of the regression of leptinemia over the BMI was similar for both control and IHD subjects. Thus, for a given BMI, men with IHD had plasma leptin levels that were fully comparable to those of men without IHD.

Despite significant differences in plasma cholesterol, TG, apoB, and insulin levels between men with and without IHD, leptinemia was not associated with plasma lipoprotein and lipid concentrations other than plasma TG levels. Thus, plasma leptin, in addition to not being an independent risk factor for IHD, is not a good correlate of known lipid risk factors for IHD, with the exception of TG levels. However, we found an association between leptin and fasting insulin concentrations, a relationship that was independent of adiposity (BMI). These results support the notion that hyperinsu-
linemia is related to increased leptin concentrations, a finding concordant with previously published observations (6,10). However, this relationship with insulin does not appear to cause bias regarding IHD risk.

Thus, results of the present study suggest that fasting leptinemia is not an independent risk factor for IHD in men. Obesity is considered by many investigators to be a risk factor for the development of IHD because of the numerous metabolic disturbances resulting from excess accumulation of adipose tissue (1). The matched design in the present study did not allow us to examine the contribution of the BMI as a risk factor for IHD. When analyses were conducted in the whole cohort of the Quebec Cardiovascular Study, the BMI was not found to be associated with IHD risk (23). However, given that BMI is not necessarily a good correlate of body composition (24) whereas leptin, being selectively produced by adipose tissue, appears to be fairly closely associated with level of body fat (3-9), the possibility remained that leptinemia (through its association with body fat) would be predictive of IHD risk. On the other hand, it has been suggested that abdominal visceral adipose tissue may represent the critical factor associated with an increased risk of IHD among obese subjects (25). The lack of association between fasting plasma leptin concentrations and the 5-year incidence of IHD observed in the present study would tend to support that notion. Indeed, it has been reported that abdominal subcutaneous adipose cells show an increased expression of the obese gene compared with intra-abdominal adipocytes (12). In the present prospective study, computed tomography was not used; thus, we were not able to obtain measurements of total abdominal visceral versus subcutaneous adipose tissue accumulation. However, hyperinsulinemia resulting from insulin resistance has been shown to be strongly correlated with an increased visceral adipose tissue accumulation (26). In the present study, men who developed IHD had significantly higher fasting plasma insulin concentrations compared with control subjects (P < 0.001). The fasting hyperinsulinemic state of IHD patients may suggest that they also had a higher visceral adipose tissue accumulation than men who remained healthy over the 5-year follow-up.

Because we report no association between plasma leptin levels and the risk of IHD, the question of study power is a very important issue that requires discussion. We have performed additional analyses to determine the power of the present study to detect a significant and possibly clinically relevant difference in plasma leptin concentrations between IHD and control subjects. As mentioned above, the discovery of leptin is fairly recent. Given that fact, along with the exploratory nature of the study, it is difficult at this point to determine a clinically and biologically relevant difference between the two study groups. Based on the relative case-control difference in plasma total cholesterol (10%), TG (19%), and apoB (16%) concentrations—which were all statistically significant in the Quebec Cardiovascular Study (23)—we can speculate that a 15% relative case-control difference in plasma leptin concentrations would indeed represent a clinically and biologically relevant difference. Additional analyses revealed that the difference in leptinemia between IHD and control subjects observed in the present study would reach statistical significance (P = 0.05, with a power of 80%) only if the sample size exceeded 40,000 subjects. Finally, the analysis of risk across tertiles of leptin levels presented in this report, which essen-

**Figure 3**—Relationships between plasma leptin concentrations and metabolic variables in the overall sample of 181 men. CHOL, cholesterol; ●, men with IHD; ○, men without IHD.
Leptin and ischemic heart disease

...tially eliminates the potential negative impact of the broad distribution of leptin levels on the study power, shows no increase in the risk of IHD in the group of men with the highest leptin concentrations (OR = 1.0 [95% CI, 0.8–1.4]) compared with men in the lowest tertile of the distribution. For these reasons, we believe that the lack of association between plasma leptin concentrations and the risk of IHD reported herein is not likely to be attributable to insufficient statistical power.

In summary, results of the present study indicate that plasma leptinemia is unlikely to be a major independent risk factor for the development of IHD in men. Moreover, the lack of association between leptin and plasma lipoprotein or lipid levels excludes leptin as being a major modulator or a correlate of a dyslipidemic state predictive of an increased IHD risk. However, a significant association between increased leptin levels and hyperinsulinemia was found and was independent of the concomitant variation in the BMI. Thus, although the discovery of leptin will allow us to obtain new insights regarding factors involved in the regulation of energy balance, results of the present study do not support the measurement of fasting leptinemia in the evaluation of IHD risk.

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References