Plasma Leptin and the Risk of Cardiovascular Disease in the West of Scotland Coronary Prevention Study (WOSCOPS)

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Background—Leptin plays a role in fat metabolism and correlates with insulin resistance and other markers of the metabolic syndrome, independent of total adiposity. Therefore, we hypothesized that raised leptin levels may identify men at increased risk of a coronary event in the West of Scotland Coronary Prevention Study (WOSCOPS).

Methods and Results—Plasma leptin levels were measured at baseline in 377 men (cases) who subsequently experienced a coronary event and in 783 men (controls) who remained free of an event during the 5-year follow-up period of the study. Controls were matched to cases on the basis of age and smoking history and were representative of the entire WOSCOPS cohort. Leptin levels were significantly higher in cases than controls (5.87±2.04 ng/mL versus 5.04±2.09 ng/mL, P<0.001). In univariate analysis, for each 1 SD increase in leptin, the relative risk (RR) of an event increased by 1.25 (95% confidence interval [CI], 1.10 to 1.43; P<0.001). There was minimal change in this RR with correction for body mass index (RR, 1.24; 95% CI, 1.06 to 1.45; P=0.006) or with further correction for classic risk factors, including age, lipids, and systolic blood pressure (RR, 1.20; 95% CI, 1.02 to 1.42; P=0.03). Leptin correlated with C-reactive protein (r=0.24, P<0.001) and, even with this variable added to the model, leptin retained significance as a predictor of coronary events (RR, 1.18; 95% CI, 1.00 to 1.39; P=0.05) at the expense of C-reactive protein.

Conclusions—We show, for the first time, in a large prospective study that leptin is a novel, independent risk factor for coronary heart disease. (Circulation. 2001;104:3052-3056.)

Key Words: adipocytes ■ insulin resistance ■ inflammation ■ cardiovascular diseases

Since the discovery that the adipocyte ob gene encodes leptin, a secreted protein that regulates body weight in mice,1 there has been intense interest in its potential metabolic role in humans. Leptin is hypothesized to be an “adiposity signal” for the long-term regulation of body weight by the brain. In accordance with this postulated role, leptin concentrations increase with obesity and correlate strongly with percent body fat in men and women.2

As a result of leptin’s role in fat metabolism and obesity, its potential association with insulin action has undergone intense investigation. Data from several different populations suggest strong positive correlations between leptin and insulin concentrations,3–5 and insulin-resistant men have higher leptin concentrations than those who are insulin-sensitive, independent of body fat mass.6 Similarly, a large epidemiological study of 2537 men and women from Mauritius showed a strong association between fasting insulin level and leptin concentration, independent of obesity (body mass index [BMI] and waist-hip ratio).7 Leptin also seems to correlate with other markers of the metabolic syndrome, such as plasma triglyceride and apolipoprotein B levels, and with systolic blood pressure, independent of BMI and glucose disposal rate.8 It is also positively associated with markers of impaired fibrinolysis.9 Further, preliminary data from our group10 have linked leptin to C-reactive protein (CRP), a marker of low-grade chronic inflammation and a robust predictor of risk for coronary heart disease (CHD). Finally, higher leptin levels predict subsequent development of obesity or type 2 diabetes in Japanese-Americans.11,12

In light of these observations, we hypothesized that raised leptin levels might signal an increased risk of vascular events; however, to date this relationship has been sparsely studied. We examined whether plasma leptin levels were linked with the risk of a coronary event in the West of Scotland Coronary Prevention Study (WOSCOPS), a landmark primary prevention trial that demonstrated the effectiveness of pravastatin in

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This report was prepared on behalf of the WOSCOPS Executive Committee; members of this committee can be found in the Appendix.

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The baseline characteristics of cases and controls are shown in Table 1. Subjects who experienced a coronary event during the 5 years of trial follow-up were older, more likely to be smokers, had higher blood pressures and LDL cholesterol levels, and lower HDL cholesterol levels. A history of hypertension and nitrate use predicted coronary events in the trial itself, and the number of subjects with these characteristics differed between cases and controls in the present study (Table 1). Baseline leptin levels were 16% higher in the cases than controls (P<0.001). Further, in addition to BMI, lipids, systolic blood pressure, and CRP, leptin was found to be a univariate predictor of risk of a coronary event when tested as a continuous variable (Table 2). In univariate analysis, the RR of log leptin was identical in those given pravastatin and in those given placebo (RR=1.27; data not shown).

When the group was divided into quintiles of baseline leptin level, it can be seen that risk rose 2-fold when comparing the highest 2 quintiles with the lowest quintile (Figure 1). Risk levels associated with quintiles 4 and 5 remained significant at 1.68 (95% CI, 1.08 to 2.62) and 1.70 (95% CI, 1.05 to 2.76) after adjustment for classic risk factors. The possibility of a threshold effect on risk cannot be excluded by the present analysis because no increment in risk was evident between the lowest quintile (quintile 1) and the subsequent 2 cohorts (quintiles 2 and 3).

**Methods**

The design of the original WOSCOPS and the prospective, nested, case-control study drawn from it have been previously described in detail. Briefly, moderately hypercholesterolemic men (LDL cholesterol, 4.5 to 6.0 mmol/L) who had not had a myocardial infarction and who did not exhibit other major clinical manifestations of coronary artery disease were randomized to a 5-year trial of pravastatin versus placebo. Pravastatin treatment significantly reduced a range of coronary events, including morbidity (myocardial infarction) and mortality and the need for revascularization.

In the case-control study, men who experienced a coronary event during the trial were compared with age- and smoking-matched controls (on a 1:2 basis; hence, n=1160) in a series of studies designed to identify novel risk factors. In the present study, a reduced set of samples was available for leptin analysis (the remainder were exhausted); therefore, we used samples from 377 of the 580 cases (65%) and 783 of the 1160 controls (68%). The characteristics of the truncated groups of cases and controls were virtually identical to those seen in the complete set (compare Table 1 with Table 1 in reference 14). Again, the controls seemed to be a representative sample of the entire WOSCOPS cohort (Table 1). Plasma samples from all subjects were taken at screening (immedi-ately before randomization) and stored frozen at −70°C. Aliquots of these were used for the measurement of potentially novel risk factors, including CRP and leptin, as described here.

**Laboratory Analyses**

Major risk factors were assessed during recruitment. Plasma total cholesterol, triglyceride, very low-density lipoprotein (VLDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol levels were measured before randomization, and the baseline level was taken as the average. Plasma leptin was measured by an in-house radioimmunoassay that was validated thoroughly against the commercially available Linco assay. The intra-assay and interassay coefficients of variation were <7% and <10%, respectively, over the sample concentration range.

The detection limit of the assay was 0.5 ng/mL. Details of the CRP assay are given in Reference 14.

**Statistical Analyses**

The baseline factors were compared individually between cases and controls using means and SDs for continuous variables and counts and percentages for categorical variables. Both leptin and CRP values were log-transformed to correct their skewed distributions. Univariate analyses were carried out by conditional logistic regression, which was suitable for the matched design. Results are presented in the form of relative risks (RR) and 95% confidence intervals (CI) for an incident coronary event over the 5 years of follow-up. For continuous variables, this is the change in risk for a 1 SD change, as calculated from the distribution of the variable in control subjects. In addition, RRs were calculated for quintiles of leptin at baseline (the cut-off points were calculated from the control subjects). The quintile of lowest leptin level was used as reference. Multivariate conditional regression models were also generated to test the independence of leptin as a predictor of risk. Associations of both leptin and CRP with the other risk factors were calculated for the control subjects using Spearman rank correlations. Adjusted partial correlations were also used.

**Results**

The baseline characteristics of the cases and controls in the present study are shown in Table 1. Subjects who experienced a coronary event during the 5 years of trial follow-up were older, more likely to be smokers, had higher blood pressures and LDL cholesterol levels, and lower HDL cholesterol levels. A history of hypertension and nitrate use predicted coronary events in the trial itself, and the number of subjects with these characteristics differed between cases and controls in the present study (Table 1). Baseline leptin levels were 16% higher in the cases than controls (P<0.001). Further, in addition to BMI, lipids, systolic blood pressure, and CRP, leptin was found to be a univariate predictor of risk of a coronary event when tested as a continuous variable (Table 2). In univariate analysis, the RR of log leptin was identical in those given pravastatin and in those given placebo (RR=1.27; data not shown).

When the group was divided into quintiles of baseline leptin level, it can be seen that risk rose 2-fold when comparing the highest 2 quintiles with the lowest quintile (Figure 1). Risk levels associated with quintiles 4 and 5 remained significant at 1.68 (95% CI, 1.08 to 2.62) and 1.70 (95% CI, 1.05 to 2.76) after adjustment for classic risk factors. The possibility of a threshold effect on risk cannot be excluded by the present analysis because no increment in risk was evident between the lowest quintile (quintile 1) and the subsequent 2 cohorts (quintiles 2 and 3).
Plasma leptin levels correlated strongly \((P<0.001)\) with BMI, as expected, but they also correlated with plasma triglycerides, CRP, and fasting plasma glucose (Table 3). The associations of leptin with CRP, glucose, and triglycerides remained significant after adjustment for BMI (data not shown). Both leptin and CRP were weakly associated with systolic blood pressure (Table 3).

The independence of leptin as a predictor of coronary events is explored in Table 4 and Figure 2. Adjustment for BMI hardly attenuated the association with risk for leptin \((RR, 1.24; 95\% \text{ CI}, 1.06 \text{ to } 1.45; P=0.006; \text{ Figure 2})\). Further adjustment for baseline lipids and systolic blood pressure also had only a minor effect \((RR, 1.21; 95\% \text{ CI}, 1.02 \text{ to } 1.42; P=0.02; \text{ Table 4 and Figure 2})\), as did adding glucose to this model \((RR, 1.20; 95\% \text{ CI}, 1.02 \text{ to } 1.42; P=0.03; \text{ Table 4})\). The addition of CRP to the model including classic risk factors and leptin (model A) resulted in the association of leptin with coronary risk shifting to borderline significance \((RR, 1.18; 95\% \text{ CI}, 1.00 \text{ to } 1.39; P=0.05; \text{ Table 4 and Figure 2})\), although CRP achieved borderline significance \((P=0.05)\) as a predictor of CHD in a model including other major risk factors but excluding leptin (data not shown), the addition of leptin into the model resulted in CRP’s loss as an independent predictor \((P=0.12; \text{ Table 4})\).

**Discussion**

This is the first prospective study to show that higher plasma leptin concentrations in hypercholesterolemic men are associated with an increased risk of a future coronary event. This was true whether leptin was considered as a continuous variable or in quintiles of baseline value. Indeed, the significance of leptin was as strong as classic risk factors in univariate analysis \((P<0.001)\), and the RR for a 1 SD change was as high as the RR for a 1 SD change in systolic blood pressure or HDL cholesterol. More importantly, we found that the predictive value of leptin was hardly altered by adjustment for BMI and was even maintained after adjustment for classic CHD risk factors. Finally, we note that after further adjustment for CRP, a marker of low-grade chronic inflammation, leptin retained a borderline significant association with risk, at the expense of CRP.

**Table 2. Univariate Analysis of Association Between Leptin, CRP, Traditional Risk Factors, and CHD Risk**

<table>
<thead>
<tr>
<th>Variable</th>
<th>RR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>3.81</td>
<td>1.29 to 11.23</td>
<td>0.015</td>
</tr>
<tr>
<td>BMI</td>
<td>1.04</td>
<td>1.01 to 1.30</td>
<td>0.035</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>1.26</td>
<td>1.11 to 1.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma triglycerides</td>
<td>1.18</td>
<td>1.05 to 1.34</td>
<td>0.008</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>1.18</td>
<td>1.04 to 1.33</td>
<td>0.008</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.74</td>
<td>0.64 to 0.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP</td>
<td>1.26</td>
<td>1.11 to 1.44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leptin</td>
<td>1.25</td>
<td>1.10 to 1.43</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Expanded end point includes the primary end point (ie, myocardial infarction/coronary heart disease \([n=321]\) plus revascularization [ie, coronary artery bypass graft/percutaneous transluminal coronary angioplasty; \([n=56]\) as a first event).†Refers to the RR for the expanded end point of a 1 SD increase in the variable.

**Table 3. Associations of Baseline Leptin and CRP With Other Major Risk Factors in Control Subjects**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Leptin, r*</th>
<th>CRP, r*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>BMI</td>
<td>0.59†</td>
<td>0.13†</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.12†</td>
<td>0.12†</td>
</tr>
<tr>
<td>Plasma cholesterol</td>
<td>0.15†</td>
<td>0.07</td>
</tr>
<tr>
<td>Plasma triglycerides</td>
<td>0.34†</td>
<td>0.19†</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.06</td>
<td>0.09</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>−0.12†</td>
<td>−0.21†</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.29†</td>
<td>0.03</td>
</tr>
<tr>
<td>CRP</td>
<td>0.24†</td>
<td>…</td>
</tr>
</tbody>
</table>

*Spearman rank correlation coefficients.†P<0.001.

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**Figure 1.** Association of leptin with the risk of a coronary event. Levels of leptin at baseline in the patients were divided according to the quintile values in the control subjects. In each case, the group of patients with the lowest risk serves as the reference group \((RR=1.0)\). Circles indicate RR, and vertical bars denote 95% CIs.

**Figure 2.** Association of leptin with the risk of a coronary event, with and without adjustment for other risk factors. The predictive value of leptin was initially tested in univariate analysis, with results presented for a change in risk of 1 SD. Subsequent analysis used multivariable conditional regression models to test the independence of leptin as a predictor of coronary events after adjustment for BMI, BMI and other traditional risk factors (age, lipids, and systolic blood pressure), and finally with further adjustment for CRP. Circles indicate RRs, and vertical bars denote 95% CIs.
TABLE 4. Multivariate Models Incorporating Leptin

<table>
<thead>
<tr>
<th>Variable</th>
<th>RR†</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>4.61</td>
<td>1.52 to 14.0</td>
<td>0.008</td>
</tr>
<tr>
<td>BMI</td>
<td>0.95</td>
<td>0.81 to 1.11</td>
<td>0.53</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>1.25</td>
<td>1.09 to 1.42</td>
<td>0.001</td>
</tr>
<tr>
<td>Plasma triglycerides</td>
<td>1.00</td>
<td>0.87 to 1.16</td>
<td>0.98</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>1.14</td>
<td>1.01 to 1.29</td>
<td>0.04</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.74</td>
<td>0.63 to 0.87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leptin</td>
<td>1.21</td>
<td>1.02 to 1.42</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Variations to model A

Addition of glucose

Glucose | 1.02 | 0.90 to 1.15 | 0.81 |
Leptin   | 1.20 | 1.02 to 1.42 | 0.03 |

Addition of CRP

CRP      | 1.12 | 0.97 to 1.29 | 0.12 |
Leptin   | 1.18 | 1.00 to 1.39 | 0.05 |

*In Model A leptin and traditional risk factors were included in the conditional logistic regression model.
†Relative risk of a coronary event using the expanded endpoint associated with 1 SD change in variable. SD is given in Table 1.

The results of this prospective study concur with a recent small, retrospective, case-control study from Northern Europe that reported plasma leptin concentration as an independent risk factor for first-ever acute myocardial infarction and first-ever hemorrhagic stroke.18,19 In contrast to the results of our study, a high plasma leptin concentration was not a predictor for ischemic heart disease in the Quebec cardiovascular study.20 The reason for this difference is unclear. However, it is noteworthy that although only cases with events (myocardial infarction or coronary revascularization) were included in our analysis, the Quebec study included “softer” end points, such as effort angina and coronary insufficiency. These may not be as strongly associated with leptin. The Quebec study was also smaller (86 cases and 95 controls).

We originally speculated that leptin might predict the risk of future coronary events after adjustment for BMI because, as noted previously, leptin correlates with established risk factors, such as plasma triglycerides and systolic blood pressure, independently of BMI.8 It is more surprising, however, that leptin retains its significance as a predictive marker once these traditional risk factors and fasting glucose are included in a multivariate analysis (model A). Part of the explanation for this latter observation may relate to the association of leptin with insulin resistance. Several investigators have reported correlations between plasma leptin and fasting plasma insulin independent of BMI,7,21,22 whereas others have shown that hyperleptinemia correlates with insulin resistance independent of changes in body weight.23 In line with these observations, leptin seems to predict subsequent development of type 2 diabetes, at least in populations tested thus far.12 Of course, associations between insulin resistance, diabetes, and cardiovascular risk are well-established.24 It would be of interest in future studies to examine leptin as a predictor of vascular risk in models incorporating more direct markers of insulin resistance, such as fasting insulin, although because of the needs for rapid sample centrifugation, separation, and storage, such studies are much more difficult to execute.

Leptin is an extremely robust circulating marker of percent fat mass (correlation coefficient >0.8 in many studies). Thus, its correlation with CRP is biologically plausible because both leptin and cytokines such as interleukin-6 (which promotes CRP secretion) are produced by adipocytes.2 Alternative, because leptin increases as part of the acute phase response, hyperleptinemia may also be a sequel to low-grade chronic inflammation. In that regard, recent evidence suggests that CRP, like leptin, correlates with insulin resistance independently of BMI26 and that markers of inflammation predict risk of diabetes.27 Whatever the mechanism, leptin’s continued significance as a predictor of risk in the multivariate model at the expense of CRP is of considerable interest.

The mechanistic implications of our results are that leptin, perhaps as a circulating marker of percent fat mass or an independent correlate of insulin resistance or as a marker of some as-yet-unknown activity of adipose tissue, may yield important information with respect to risk of vascular disease. It is unlikely that leptin has direct atherogenic properties. Because our report represents a post-hoc analysis in an albeit large database, our results do not currently justify adding leptin to risk factor assessment. Instead, our data must be confirmed and extended to different sectors of the population, such as women, individuals with existing CHD, those with diabetes, and different ethnic groups. With respect to the latter, it is noteworthy that immigrant South Asian men and women have significantly higher leptin concentrations relative to individuals of European descent with similar BMIs. Thus, high leptin levels may be a part of the explanation for the high rates of CHD in this population. Our data also reinforce the benefits of exercise and improved diet, because both measures have been shown to reduce plasma leptin concentrations, independent of changes in body mass or, indeed, fat mass.28 Extension of our findings to other cohorts is readily possible because leptin is an extremely stable protein in serum or plasma, is not influenced by thawing, and is stable over long periods of time.

In conclusion, we have shown, for the first time, in a large prospective study that plasma leptin concentration is a novel, independent risk factor for coronary events. Our data provide further support for a link between adipocyte function and/or mass (rather than total BMI) and cardiovascular disease.

Appendix

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References
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