Hyperleptinemia as a Component of a Metabolic Syndrome of Cardiovascular Risk

Francisco Leyva, Ian F. Godsland, Mohammed Ghatel, Anthony J. Proudler, Stephen Aldis, Christopher Walton, Stephen Bloom, John C. Stevenson

Abstract—In humans, production of the adipocyte-derived peptide leptin has been linked to adiposity, insulin, and insulin sensitivity. We therefore considered that alterations in plasma leptin concentrations could constitute an additional component of a metabolic syndrome of cardiovascular risk. To explore this hypothesis, we employed factor analysis, a multivariate statistical technique that allows reduction of large numbers of highly intercorrelated variables to composite, biologically meaningful factors. Seventy-four men [age, 48.4±1.3 years (mean±SEM); body mass index (BMI), 25.6±0.3 kg/m²] who were free of coronary heart disease and diabetes underwent anthropometric measurements (subscapular-to-triceps [S:T] and subscapular-to-biceps [S:B] skinfold thickness ratios, measurement of fasting plasma leptin, and an intravenous glucose tolerance test (IVGTT) for assessment of insulin sensitivity. Plasma leptin concentrations were correlated with BMI (r=0.57, P<0.001), S:T (r=0.34, P=0.003), S:B (r=0.37, P<0.001), systolic and diastolic blood pressures (both r=0.24, P=0.044), fasting triglycerides (r=0.31, P=0.007), serum uric acid (r=0.35, P=0.003), fasting glucose (r=0.32, P=0.003) and insulin (r=0.33, P=0.004), and IVGTT insulin (r=0.63, P<0.001). A negative correlation was observed between leptin and insulin sensitivity (r=−0.32, P=0.006). No significant correlations emerged between plasma leptin concentrations and age, high density lipoprotein cholesterol, or IVGTT glucose. In multivariate regression analyses, BMI (standardized coefficient [SC] =0.40, P=0.001), fasting insulin (SC=0.23, P=0.036), and IVGTT insulin (SC=0.51, P<0.001) emerged as independent predictors of plasma leptin concentrations (R²=0.56, P<0.001). After adjustment for BMI, only IVGTT insulin emerged as a significant predictor of plasma leptin concentrations (SC=0.56, P<0.001, R²=0.45, P<0.001). Factor analysis of plasma leptin concentrations and the variables that are considered relevant to the insulin resistance syndrome revealed a clustering of plasma leptin concentrations with a factor dominated by insulin resistance and high IVGTT insulin, separate from a high IVGTT glucose/cenral obesity factor and a high triglyceride/low high density lipoprotein cholesterol factor. Together, these factors accounted for 55.9% of the total variance in the dataset. In conclusion, interindividual variations in plasma leptin concentrations are strongly related to the principal components of the insulin resistance syndrome. Further studies are needed to determine whether the insulin-leptin axis plays a coordinating role in this syndrome and whether plasma leptin concentrations could provide an additional measure of cardiovascular risk. (Arterioscler Thromb Vasc Biol. 1998;18:928-933.)

Key Words: leptin ■ insulin resistance ■ cardiovascular risk

The concept of a metabolic syndrome of cardiovascular risk arose from the recognition that metabolic risk factors for CHD, hypertension, and diabetes mellitus are highly intercorrelated. One such syndrome may include obesity, insulin resistance, hyperinsulinemia, glucose intolerance, hypertriglyceridemia, low HDL cholesterol, hyperuricemia, and raised blood pressure. Importantly, several prospective studies have shown hyperinsulinemia to be an independent predictor of CHD. Recently, direct measures of insulin resistance obtained by using the IVGTT have been shown to be independently related to atherosclerosis. In humans, plasma concentrations of the peptide leptin, a product of the ob gene, are directly related to body fat content, and it has been suggested that high leptin concentrations in obese individuals may reflect resistance to the effects of this hormone. There is considerable interindividual variation in plasma leptin concentrations among individuals with comparable degrees of obesity, suggesting that factors other than adiposity are involved in the regulation of leptin production. A possible regulatory role of insulin is suggested by the finding that short-term and long-term hyperinsulinemia increases ob gene expression in mice. Likewise, insulin stimulates ob gene expression in humans, and while short-term changes in plasma insulin do not affect plasma leptin concentrations, prolonged insulin infusions appear to result in slight elevations. Although some

Received December 2, 1997; revision accepted December 31, 1997.
From the Wynn Department of Metabolic Medicine, Imperial College School of Medicine at the National Heart and Lung Institute (F.L., I.F.G., A.J.P., C.W., S.A., J.C.S.); and the Department of Endocrinology, Imperial College School of Medicine, Hammersmith Campus (M.G., S.B.), London, UK.
Correspondence to Dr Francisco Leyva, MRCP, Department of Cardiology, Charing Cross Hospital, Fulham Palace Rd, London W6 8RF UK.
E-mail f.leyvaleon@ic.ac.uk
© 1998 American Heart Association, Inc.
studies in humans suggest that there is an inverse relationship between plasma leptin levels and insulin sensitivity, a positive correlation between plasma leptin and both fasting insulin and insulin sensitivity has been demonstrated by others. In the background of the emerging links between leptin, adiposity, and insulin, there are reports of mutations of the ob gene leading to non-insulin-dependent diabetes mellitus in obese ob/ob mice.

In this light, we considered that alterations in plasma leptin concentrations might constitute an additional component of the insulin resistance syndrome. To explore this hypothesis, we considered that the traditional statistical technique of multivariate regression, which aims to identify the independence of variables, is unrealistic when applied to biological systems such as the metabolic syndrome, in which the disease process is manifested in a range of intercorrelated, mutually supporting disturbances. In contrast to traditional multivariate regression techniques, the multivariate technique of factor analysis focuses on identifying interdependence between variables rather than their independence. Factor analysis thus provides a means of condensing a large number of highly intercorrelated variables to a few, composite factors that provide a quantitative “signature” for the interrelated disturbances under consideration.

**Methods**

The study group, whose characteristics are shown in Table 1, consisted of 74 participants of the Risk Indicators in a Screened Cohort Study (RISC)-2, a prospective study of metabolic risk factors for CHD and diabetes mellitus in business executives, which began in 1988 and continues to date. At the time of assessment, all of the study subjects were free of CHD, hypertension, and diabetes mellitus. To obtain a wide range of measures of adiposity, fasting insulin, and insulin sensitivity, subjects with obesity or with baseline blood pressures in the hypertensive range (systolic blood pressure >160 mm Hg or diastolic blood pressure >95 mm Hg) were included in the analyses. All patients gave written, informed consent, and the protocol was approved by the local Ethics Committee.

Studies were carried out on a metabolic day ward. Participants were asked to consume more than 200 g/d carbohydrate in their diet for 3 days prior to their visit, to have fasted for 12 hours, and to have refrained from smoking on the morning of the test. After resting for 15 minutes in a semirecumbent position, systolic and diastolic blood pressures were measured by a cuff method with a mercury sphygmomanometer. First- and fifth-phase Korotkoff sounds were recorded. A cannula was inserted into an antecubital vein in one arm for sampling, the arm having been previously rested on a heating pad to assist blood flow. Blood samples were taken for fasting plasma glucose and insulin concentrations, serum lipid and lipoprotein concentrations, and plasma leptin concentrations.

**IVGTT**

Participants then underwent an IVGTT with sampling at 16 times during 180 minutes. Dextrose was administered intravenously as a 50% solution at a dose of 0.5 g/kg body weight. Insulin sensitivity, which is inversely related to insulin resistance, was assessed using the minimal model approach, as described previously.

**Laboratory Determinations**

Leptin concentrations were measured, using a radioimmunoassay developed by Linco Research on samples stored at −20°C. The antibody used was a polyclonal rabbit antibody raised to highly purified human leptin. The standards and 125I-labeled tracer were prepared with recombinant human leptin. Standards and samples were assayed in duplicate in a single assay. One sample of each duplicate was diluted to confirm reproducibility at a second point in the standard curve. Serum (100 mL) was mixed with 125I-labeled leptin and incubated with leptin antibody overnight at 4°C. Anti-rabbit IgG was added to the samples and incubated at 4°C for 20 minutes to precipitate the antigen-antibody complex. Samples were centrifuged at 2000g for 15 minutes at 4°C. Supernatants were decanted, and radioactivity in both the pellet and the supernatant was counted to determine bound and unbound radioactivity. The sensitivity of the assay is 0.5 mg/L, and the intra-assay and interassay coefficients of variation were 3.4% to 8.3% and 3.6% to 6.2%, respectively.

Plasma glucose was determined on the same day by using glucose oxidase procedures, with 4-aminophenazone as the chromogen. Plasma insulin concentrations were measured on samples stored at −20°C by using a radioimmunoassay procedure. Fully enzymatic assays were employed for measurement of total cholesterol and triglycerides. Concentrations of HDL were measured by sequential precipitation with heparin/manganese ions. Within- and between-batch precision was monitored throughout the study by using frozen plasma and serum pools and commercially available lyophilized sera and by participation in national quality assurance schemes.

**Body Fat**

BMI was taken as an index of overall adiposity. Centrality of body fat was expressed as the ratios S:T, S:B, and I:S of skinfold thicknesses. The mean of three measurements of skinfold thickness from the iliac, subscapular, and triceps regions, which was per-
formed with the volunteer in the standing position, was employed in calculations.

Statistical Analyses

All results are presented as mean±SEM. Differences between the groups were analyzed by ANOVA, and significant differences were further analyzed by Fisher’s exact test. Interrelationships were assessed using multivariate and stepwise regression analyses (SYSTAT, SYSTAT Inc). A value of P<0.05 was considered statistically significant. Due to a skewed distribution, fasting insulin levels were logarithmically transformed, and insulin sensitivity was square-root transformed. Owing to a skewed distribution in the pooled sample, leptin concentrations were also logarithmically transformed.

Factor Analysis

The clustering of plasma leptin concentrations in the background of variables that are relevant to the insulin resistance syndrome was assessed by factor analysis. The multivariate technique has been previously used in the study of the insulin resistance syndrome in healthy individuals and in patients with heart failure. In brief, factor analysis provides a means of condensing a large number of highly intercorrelated variables to a few, composite entities. Whether or not the factors identified by factor analysis merely represent statistical associations depends on whether pathophysiological meaning can be ascribed to the different factors. Therefore, only those variables that are considered relevant to the metabolic syndrome were considered in the analyses. Factor analysis comprises the following steps.

Correlation Analysis

This procedure consists of calculating appropriate measures of associations for a set of relevant variables. In this study, a correlation analysis on the variables (R-factor analysis) rather than on the individuals under study (Q-factor analysis) was performed.

Principal-Component Analysis

This step aims to identify linear combinations of the variables that account for the maximum proportion of the total variance in the set of variables. The principal components are derived in such a way that one component is independent of the other; ie, they are uncorrelated (the Pearson correlation coefficient between them is predetermined to be zero) or orthogonal.

Rotation

The aim of rotation is to facilitate interpretation of the components obtained by principal-component analysis. The pattern of loadings obtained is thereby simplified by reducing a considerable percentage of its elements to values of or near zero. With the varimax method of rotation (an orthogonal method), the loadings are made large or small so that most variables have a high loading on a small number of factors.

Interpretation

This step involves the assessment of the magnitude of loadings of each variable on each derived factor. Factor loadings are equivalent to Pearson’s correlation coefficients between each variable and the factor to which it has been assigned. The variables that should carry the most weight in interpretation are those with high loadings. The first factor accounts for a maximum amount of the communal variance of the variables, the second for a maximum amount after the first has been removed, and so on. As recommended by Stevens, only components sharing at least 15% of the variance with the factor, equivalent to a factor loading of 0.40, will be considered in interpretation.

Results

As shown in Table 2, strong correlations were found between plasma leptin concentrations and BMI and measures of central adiposity, namely, S:T and S:B skinfold ratios. In addition, plasma leptin was also correlated positively with systolic and diastolic blood pressures, fasting triglycerides, serum uric acid, fasting glucose and insulin, and IVGTT insulin. A negative correlation was observed between leptin and insulin sensitivity. No significant correlations emerged between plasma leptin concentrations and age, HDL cholesterol, or IVGTT glucose. With the exception of blood pressure, the strength of the observed associations with plasma leptin concentrations was not appreciably changed after adjustment for BMI.

Variables that on univariate analyses were found to be correlated with plasma leptin concentrations were used in further analyses. In multivariate regression analyses (Table 3), BMI, fasting insulin, and IVGTT insulin emerged as independent predictors of plasma leptin concentrations. Stepwise linear regression analysis using the same independent variables yielded similar results, with BMI (SC=0.38, P<0.001), fasting insulin (SC=0.20, P=0.026), and IVGTT insulin (SC=0.37, P<0.001) emerging as independent predictors of plasma leptin concentrations (multiple R²=0.49, P<0.001). With BMI-adjusted leptin concentrations as the dependent variable, IVGTT insulin emerged as the only significant predictor of plasma leptin concentrations.

Factor analysis of plasma leptin concentrations and the variables that are considered relevant to the metabolic syndrome reduced 12 highly intercorrelated variables to three uncorrelated factors (Table 4). The first factor, which explained 22.9% of the total variance in the dataset, was dominated by insulin resistance and high IVGTT insulin; the second factor was dominated by high IVGTT glucose and central obesity (percent variance=19.2); whereas the third factor consisted of a negative relationship between high triglycerides and low HDL cholesterol (percent variance=13.8). Together these factors accounted for 55.9% of the total variance in the dataset. Plasma leptin concentrations

---

**TABLE 2. Univariate Correlations for Log₁₀ Plasma Leptin Concentrations, With and Without Adjustment for BMI**

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>BMI Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>BMI</td>
<td>0.57</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S:T</td>
<td>0.34</td>
<td>0.33</td>
</tr>
<tr>
<td>S:B</td>
<td>0.37</td>
<td>0.36</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.24</td>
<td>0.23</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.24</td>
<td>0.23</td>
</tr>
<tr>
<td>Triglycerides*</td>
<td>0.31</td>
<td>0.30</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.06</td>
<td>NS</td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.35</td>
<td>0.34</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>0.32</td>
<td>0.31</td>
</tr>
<tr>
<td>IVGTT glucose area</td>
<td>0.13</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting insulin*</td>
<td>0.33</td>
<td>0.33</td>
</tr>
<tr>
<td>IVGTT insulin area*</td>
<td>0.63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin sensitivity†</td>
<td>-0.32</td>
<td>-0.32</td>
</tr>
</tbody>
</table>

---

*Log₁₀ transformed.†Square-root transformed data.
TABLE 3. Results of Multiple Linear Regression Analysis With Fasting Plasma Leptin (Log_{10} Transformed) and Log_{10} Plasma Leptin Concentration, Adjusted for BMI, as the Dependent Variables

<table>
<thead>
<tr>
<th>Dependent variable: log_{10} plasma leptin concentration</th>
<th>SC</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.40</td>
<td>0.001</td>
</tr>
<tr>
<td>S:T</td>
<td>−0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>−0.12</td>
<td>NS</td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.07</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides*</td>
<td>−0.11</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>−0.07</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting insulin*</td>
<td>0.23</td>
<td>0.036</td>
</tr>
<tr>
<td>IVGTT insulin*</td>
<td>0.51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin sensitivity†</td>
<td>0.03</td>
<td>NS</td>
</tr>
</tbody>
</table>

$R^2 = 0.56, P < 0.001$

Dependent variable: log_{10} plasma leptin concentration adjusted for BMI

<table>
<thead>
<tr>
<th>SC</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>S:T</td>
<td>0.03</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>−0.05</td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.15</td>
</tr>
<tr>
<td>Triglycerides*</td>
<td>−0.00</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>0.00</td>
</tr>
<tr>
<td>Fasting insulin*</td>
<td>0.17</td>
</tr>
<tr>
<td>IVGTT insulin*</td>
<td>0.56</td>
</tr>
<tr>
<td>Insulin sensitivity†</td>
<td>0.03</td>
</tr>
</tbody>
</table>

$R^2 = 0.45, P < 0.001$

*Log_{10} transformed.
†Square-root transformed data.

loaded on two separate factors; the insulin resistance/high IVGTT insulin factor and the glucose intolerance (high IVGTT glucose)central obesity factor. Factor analysis using BMI-adjusted leptin concentrations represented graphically in the Figure revealed a similar pattern of factor loadings. Exclusion of obese subjects (BMI>27 kg/m², n=19) led to the emergence of a predominant factor (percent variance=19.5), which comprised BMI-adjusted plasma leptin concentrations (loading=0.63), IVGTT insulin (loading=0.82), and insulin sensitivity (loading=−0.67). A similar factor (percent variance=21.3) emerged after excluding obese and hypertensive subjects (systolic blood pressure $>$160 mm Hg or diastolic blood pressure $>$95 mm Hg, n=6) (factor loadings: IVGTT insulin=0.90, insulin sensitivity=−0.85, and BMI-adjusted leptin=0.62).

Discussion

In this study we have found that in healthy male subjects, plasma leptin concentrations are related to the metabolic disturbances that constitute the metabolic syndrome, including overall and central obesity, raised blood pressure, insulin resistance, hyperinsulinemia, high plasma triglycerides, and elevated serum uric acid. In multivariate regression analyses, both fasting insulin and IVGTT insulin emerged as significant predictors of plasma leptin concentrations, independent of overall and central body fat. When the panel of metabolic disturbances that constitute the insulin resistance syndrome was considered together in factor analysis, plasma leptin concentrations formed part of a cluster that was dominated by insulin resistance and hyperinsulinemia, core metabolic disturbances of the metabolic syndrome. These relationships were also evident after correcting for body fat.

In both univariate and multivariate analyses, plasma insulin concentrations and in particular IVGTT insulin emerged as significant positive predictors of plasma leptin concentrations. Interestingly, the predominant factor that emerged in our analysis was dominated by insulin measures and leptin, whereas central fat, blood pressure, and both triglycerides and HDL cholesterol were represented in other factors, adding further support for the existence of an insulin-leptin axis. These observations are consistent with our previous finding that in both healthy individuals and patients with chronic heart failure (an insulin-resistant, hyperinsulinemic state), elevations in plasma leptin were positively related to eleva-
rather than positively with changes in plasma leptin concentrations. The mechanisms underlying the suppressing effect of troglitazone on leptin production in humans are still underdetermined. The possibility still remains that treatment with these agents may lead to decreases in circulating leptin levels through decreases in plasma insulin concentrations, rather than through their direct effects on insulin sensitivity.

In conclusion, this study demonstrates that interindividual variations in plasma leptin concentrations are strongly related to the components of a metabolic syndrome of cardiovascular risk. The statistical strength of the relationship between plasma leptin and both fasting and postglucose insulin concentrations suggests that the insulin-leptin axis may be important in the coordination of the metabolic disturbances that constitute this syndrome.

Acknowledgments

We are grateful for the support of the Heart Disease and Diabetes Research Trust and the Cecil Rosen Foundation.

References


Hyperleptinemia as a Component of a Metabolic Syndrome of Cardiovascular Risk
Francisco Leyva, Ian F. Godslan, Mohammed Ghatei, Anthony J. Proudler, Stephen Aldis, Christopher Walton, Stephen Bloom and John C. Stevenson

doi: 10.1161/01.ATV.18.6.928
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/18/6/928

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/