Positive Relationship Between Plasma Leptin Level and Hypertension
Anoop Shankar, Jie Xiao

Abstract—Leptin is an adipose tissue-derived hormone shown to be related to metabolic, inflammatory, and hemostatic factors involved in hypertension development. Animal studies suggest that higher leptin levels may activate the sympathetic nervous system and cause elevations in blood pressure (BP). However, few studies have examined the association between leptin and hypertension in humans. Also it is not clear whether this association is present among women as well as men. Therefore, we examined the association between plasma leptin levels and hypertension in a representative sample of US adults. We examined the third National Health and Nutrition Examination Survey participants >20 years of age (n=5599; 54.7% women). Plasma leptin levels were categorized into quartiles (women: <7.68, 7.68 to 13.18, 13.19 to 21.70, >21.70 fg/L; men: <2.64, 2.64 to 4.36, 4.37 to 7.12, >7.12 fg/L). Hypertension was defined as BP-reducing medication use or having systolic BP ≥140 mm Hg and/or diastolic BP ≥90 mm Hg. We found that higher plasma leptin levels were positively associated with hypertension after adjusting for age, sex, race/ethnicity, education, smoking, alcohol intake, body mass index, diabetes mellitus, serum cholesterol, and C-reactive protein. Compared with quartile 1 of leptin (referent), the odds ratio (95% CI) of hypertension associated with quartile 4 was 1.89 (1.24 to 2.09; P for trend=0.0036). Subgroup analyses examining the relation between leptin and hypertension by sex and body mass index categories also showed a consistent positive association. In conclusion, higher plasma leptin levels are associated with hypertension both among women as well as men in a representative sample of US adults. (Hypertension. 2010;56:623-628.)

Key Words: leptin ■ hypertension ■ adipokines ■ insulin ■ nutrition surveys

Leptin is an adipose tissue-derived hormone that has been shown to be related to several metabolic, inflammatory, and hemostatic factors known to be involved in the development of hypertension and cardiovascular disease.1 Experimental animal studies suggest that higher leptin levels may activate the sympathetic nervous system and cause elevations in blood pressure (BP).2 However, in humans, relatively few studies have examined the putative association between leptin and hypertension, and their results have not been consistent. Some studies detected a significant association between plasma leptin levels and hypertension only in men,3–5 whereas some others reported a positive relationship in both men and women.6,7 Recently, Asferg et al8 reported that plasma leptin levels predicted the risk of developing hypertension in both men and women in the Copenhagen City Heart Study. In this context, we examined the association between plasma leptin levels and hypertension in a large, nationally representative sample of US adults after adjusting for major confounders. We had adequate sample size to examine this association in the whole cohort, as well as separately by sex.

Methods
The current study is based on data from the Third National Health and Nutrition Examination Survey (NHANES). Detailed description of NHANES III study design and methods are available elsewhere.11–14 In brief, the NHANES included a stratified multistage probability sample representative of the civilian noninstitutionalized US population. Selection was based on counties, blocks, households, and individuals within households and included the oversampling of non-Hispanic blacks and Mexican Americans to provide stable estimates of these groups. Subjects were required to sign a consent form before their participation, and approval was obtained from the US Department of Health and Human Services Human Subjects Committee.

The current study sample consisted of participants aged >20 years who were randomly assigned to be examined in a morning examination after an overnight fast. Plasma leptin levels were measured in 6415 participants who were examined in the morning after an overnight fast who had surplus sera available. We further excluded subjects with self-reported cardiovascular disease (n=373) and also subjects with missing data (n=443) on covariates included in the multivariable model, including systolic or diastolic BP, body mass index (BMI), or cholesterol levels. This resulted in 5599 participants (54.7% women), 1450 of whom had hypertension.

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Main Outcome of Interest: Hypertension

Seated systolic and diastolic BP s were measured using a mercury sphygmomanometer according to the American Heart Association and Seventh Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure recommendations. Up to 3 measurements were averaged for systolic and diastolic pressures. Patients were considered hypertensive if they reported current BP-reducing medication use and/or had systolic BPs ≥140 mm Hg and/or diastolic BPs ≥90 mm Hg.

Exposure Measurements

Age, sex, race/ethnicity, smoking status, alcohol intake (grams per day), level of education, history of diabetes mellitus and oral hypoglycemic intake or insulin administration, and antihypertensive medication use were assessed using a questionnaire. Individuals who had not smoked ≥100 cigarettes in their lifetimes were considered never smokers; those who had smoked ≥100 cigarettes in their lifetimes were considered former smokers if they answered negatively to the question, “Do you smoke now?” and current smokers if they answered affirmatively. BMI was calculated as weight in kilograms divided by height in meters squared.

Rigorous procedures with quality control checks were used in blood collection, and details about these procedures are provided in the NHANES Laboratory/Medical Technologists Procedures Manual. High-sensitivity C-reactive protein (CRP) was analyzed using a modification of the Behring Latex-Enhanced CRP assay on the Behring Nephelometer Analyzer System. Both within- and between-assay quality control procedures were used, and the coefficient of variation of the method was 3.2% to 16.1% through the period of data collection. Measurement of plasma leptin was performed by Linco Research, Inc. The assay was a radioimmunoassay with a polyclonal antibody raised in rabbits against highly purified recombinant human leptin. The minimum detectable concentration of the assay was 0.5 fg/L of leptin, and the limit of linearity was 100 fg/L. Recovery of leptin added to serum is 99% to 104% over the linear range of the assay. The radioimmunoassay agrees reasonably well with rough quantification by Western blots. Within- and between-assay coefficients of variation ranged from 3.4% to 8.3% and from 3.6% to 6.2%, respectively.

Serum total cholesterol was measured enzymatically. Serum glucose was measured using the modified hexokinase method at the University of Missouri Diabetes Diagnostic Laboratory. Diabetes mellitus was defined based on the guidelines of the American Diabetes Association as a serum glucose ≥126 mg/dL after fasting for a minimum of 8 hours, a serum glucose ≥200 mg/dL for those who fasted <8 hours before their NHANES visit, or a self-reported current use of oral hypoglycemic medication or insulin.

Statistical Analysis

Plasma leptin was analyzed both as a continuous variable and a categorical variable. For the analysis as a continuous variable, leptin values were log transformed (base 2) as a result of their skewed distribution. Because physiologically normal leptin levels vary in men and women, we categorized plasma leptin level as quartiles by sex (women: <7.68, 7.68 to 13.18, 13.19 to 21.70, >21.70 fg/L; men: <2.64, 2.64 to 4.36, 4.37 to 7.12, >7.12 fg/L).

The odds ratio (OR; 95% CI) of hypertension for each higher leptin quartile was calculated by taking the lowest quartile as the referent, using multivariable logistic regression models. We used 2 models, the age- and sex-adjusted model and the multivariable model, additionally adjusting for race/ethnicity (non-Hispanic whites, non-Hispanic blacks, Mexican Americans, and others), education categories (below high school, high school, and above high school), smoking (never smoker, former smoker, and current smoker), alcohol intake (grams per day), BMI (kilograms per meter squared), diabetes mellitus (absent or present), and total serum cholesterol (milligrams per deciliter). Trends in the OR of hypertension across increasing plasma leptin category were determined by modeling leptin categories as an ordinal variable. In a supplementary analysis, we additionally adjusted for serum triglyceride level (milligrams per deciliter), fasting insulin levels (microunits per milliliter), high-sensitivity CRP level (milligrams per deciliter, for inflammation), and resting supine heart rate (per minute, as a proxy measure for sympathetic nervous system activity). Sample weights that account for the unequal probabilities of selection, oversampling, and nonresponse were applied for all of the analyses using SUDAAN (version 8.0, Research Triangle Institute) and SAS (version 9.2, SAS Institute) software; SEs were estimated using the Taylor series linearization method.

Results

Table 1 presents the baseline characteristics of the study sample by increasing quartiles of plasma leptin level. Individuals in the higher plasma leptin quartiles were more likely to be older, a woman, non-Hispanic black, never or former smoker; more likely to have a higher BMI, diabetes mellitus, a higher total cholesterol level; and less likely to be a non-Hispanic white, current smoker, and current drinker. The average plasma leptin level was found to be >3 times higher among women compared with men (17.6 versus 5.9 fg/L).

Table 2 presents the association between increasing plasma leptin levels and hypertension in the whole cohort. We observed a positive association between plasma leptin levels and hypertension in both the age- and sex-adjusted model (P for trend <0.0001) and the multivariable-adjusted model (P for trend = 0.0036). The association between plasma leptin and hypertension persisted when leptin was analyzed as a continuous variable with log transformation.

Table 3 presents the association between increasing plasma leptin levels and hypertension by sex. Here also even with multivariable adjustment we observed a positive association between plasma leptin levels and hypertension both among women (P for trend = 0.0255) and men (P for trend = 0.0267).

Table 4 presents the association between increasing plasma leptin levels and hypertension by BMI categories. In the age- and sex-adjusted model, we found that plasma leptin levels were positively associated with hypertension among both normal weight (P for trend <0.0001) and overweight/obese subjects (P for trend <0.0001). With multivariable adjustment, the association was attenuated but still present among both overweight/obese subjects (P for trend = 0.0072) and normal weight subjects (P for trend = 0.0443). When we used nonparametric models to graphically examine the dose-response relationship between plasma leptin levels and hypertension without linearity assumptions involved in traditional regression models, we observed a continuous positive association between plasma leptin and hypertension without any threshold effect (Figure).

In a supplementary analysis, when we additionally adjusted for serum triglyceride level, fasting insulin levels, high-sensitivity CRP level, and resting supine heart rate the association between plasma leptin and hypertension was attenuated but still present. Compared to quartile 1 of plasma leptin (referent), the multivariable-adjusted OR (95% CI) of hypertension was 1.33 (0.95 to 1.88) in quartile 2, 1.52 (1.00 to 2.30) in quartile 3, and 1.65 (1.08 to 2.51) in quartile 4 (P for trend = 0.0236). In this same multivariable model that adjusted for leptin quartiles as a covariate, the multivariable-adjusted OR (95% CI) of hypertension for increasing serum triglyceride level (per milligram per deciliter increase) was...
1.10 (0.99 to 1.22), fasting insulin level (per microunits per milliliter increase) was 1.13 (1.00 to 1.28), and high-sensitivity CRP level (per milligram per deciliter increase) was 1.20 (1.01 to 1.43).

In a second supplementary analysis, we examined the association between leptin and hypertension after excluding subjects who were on antihypertensive medications (n = 719). We found that the magnitude of ORs were similar. Compared

Table 2. Association Between Plasma Leptin Level and Hypertension

<table>
<thead>
<tr>
<th>Plasma Leptin Level*</th>
<th>No. at Risk</th>
<th>Hypertension Cases</th>
<th>Age- and Sex-Adjusted OR (95% CI)</th>
<th>Multivariable-Adjusted OR (95% CI)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartile 1</td>
<td>1186</td>
<td>183</td>
<td>1 (referent)</td>
<td>1 (referent)</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>1345</td>
<td>294</td>
<td>1.46 (1.12 to 1.92)</td>
<td>1.33 (0.98 to 1.80)</td>
</tr>
<tr>
<td>Quartile 3</td>
<td>1533</td>
<td>412</td>
<td>2.12 (1.49 to 3.01)</td>
<td>1.58 (1.08 to 2.32)</td>
</tr>
<tr>
<td>Quartile 4</td>
<td>1535</td>
<td>561</td>
<td>3.65 (2.51 to 5.30)</td>
<td>1.89 (1.24 to 2.09)</td>
</tr>
<tr>
<td>P for trend</td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
<td>0.0036</td>
</tr>
<tr>
<td>Log-transformed leptin level</td>
<td>5599</td>
<td>1450</td>
<td>2.05 (1.69 to 2.49)</td>
<td>1.45 (1.17 to 1.80)</td>
</tr>
</tbody>
</table>

*Plasma leptin quartiles in women were as follows: quartile 1 (<7.68 fg/L), quartile 2 (7.68 to 13.18 fg/L), quartile 3 (13.19 to 21.70 fg/L), quartile 4 (>21.70 fg/L); in men: quartile 1 (<2.64 fg/L), quartile 2 (2.64 to 4.36 fg/L), quartile 3 (4.37 to 7.12 fg/L), quartile 4 (>7.12 fg/L). Data presented are row percentages or mean value±SE.

†Data were adjusted for age (years), sex (men or women), race-ethnicity (non-Hispanic whites, non-Hispanic blacks, Mexican Americans, or others), education categories (less than high school, high school, or more than high school), smoking (never, former, or current), alcohol intake (never, former, or current), BMI (normal, overweight, or obese), diabetes mellitus (absent or present), and serum total cholesterol (in milligrams per deciliter).

OR (95% CI) indicates odds ratio (95 percent confidence interval).
with quartile 1 of plasma leptin (referent), the multivariable-adjusted OR (95% CI) of hypertension was 1.16 (0.81 to 1.66) in quartile 2, 1.41 (0.93 to 2.14) in quartile 3, and 1.62 (1.04 to 2.52) in quartile 4 ($P$ for trend $<$0.0001). Finally, in a multivariable linear regression analysis, we examined the association between leptin and BP level among subjects who were not on antihypertensive medication use. In separate models, we found that increasing plasma leptin levels were positively associated with both systolic BP level ($\beta$ [SE]=0.82 (31); $P$<0.0001) and diastolic BP level ($\beta$ [SE]=0.48 (21); $P$=0.042).

**Discussion**

In a contemporary multiethnic sample of US adults, we found that higher plasma leptin levels are positively associated with hypertension, independent of traditional factors, such as age,
sex, smoking, alcohol intake, BMI, diabetes mellitus, and serum cholesterol. Although, compared with men, plasma leptin levels were higher in women, we found that the leptin-hypertension association was present both in women and men. In nonparametric graphical models, we observed a continuous positive association between increasing plasma leptin levels and hypertension without any threshold effect. We also found that the observed association between leptin and hypertension may not be fully explained by systemic inflammation as measured by high-sensitivity CRP levels.

An association between leptin and hypertension is biologically plausible. Experimental animal studies suggest that higher leptin levels may activate the sympathetic nervous system and cause chronic elevations in BP and renal dysfunction. High levels of leptin may be related to insulin resistance, which, in some recent studies, have been reported to be associated with prehypertension and hypertension. Previous reports have shown that plasma leptin levels are related to markers of inflammation, including high-sensitivity CRP levels. High levels of leptin may also affect the renin-angiotensin system. In normotensive men, the plasma angiotensinogen level has been found to be related to plasma leptin level, and a significant positive relationship between plasma leptin and plasma renin activity has been reported in hypertensive patients. Finally, a recent animal study has put forward the concept of “selective leptin resistance” as a pathogenetic mechanism in which, despite resistance to the metabolic actions of leptin, its sympathoexcitatory effects and increased arterial pressure effects are preserved.

In the current study, the magnitude of the observed association between plasma leptin and hypertension, the persistence of this association even after multivariable adjustment of confounders, and the consistency of these findings in subgroup analyses by sex and BMI suggest that these findings are less likely to be because of chance. However, the precise mechanistic link between leptin and hypertension in not clear, and, therefore, the results must be interpreted with caution. In previous studies in humans, discordant results have been obtained with regard to the relationship between leptin and BP. Some investigators detected a significant association between plasma leptin levels and BP only in men, others detected an association only in women, whereas some others reported a positive relationship in both men and women. In the current study, we found that, although plasma leptin levels are substantially higher in women (a finding that has been consistently reported in previous literature also), the leptin-hypertension association was present both in women and men.

Because leptin is an adipose tissue-derived hormone, it is possible that its association with BP is partly explained by obesity and weight gain, one of the well known risk factors for hypertension. However, in the current study, the association between leptin and hypertension was present even among subjects with normal weight (BMI <25 kg/m²), suggesting the involvement of mechanisms other than obesity, such as inflammation, as a potential explanation for the observed association. Furthermore, our findings are consistent with the findings by Galletti et al who reported an association between plasma leptin levels and hypertension that was independent of BMI in a sample of Italian men. We also found that the association between leptin and hypertension persisted even after adjusting for high-sensitivity CRP levels, indicating that there are probably underlying explanatory mechanisms that are independent of systemic inflammation.

Certain antihypertensive medications, such as angiotensin receptor blockers, angiotensin-converting enzyme inhibitors, and β-blockers, have been reported to lower plasma leptin levels. Because antihypertensive medication use was one of the criteria to define hypertension, it is possible that the observed association in our study between leptin and hypertension may be an underestimation of the true association. However, in a supplementary analysis where we examined the association between leptin and hypertension after excluding subjects who were on antihypertensive medications, we found that the magnitudes of ORs were similar, suggesting that the leptin-lowering effect of certain antihypertensive medications may not be a major bias in our study.

The main strengths of our study include its population-based nature, inclusion of a representative multietnic sample, adequate sample size, and the availability of data on confounders for multivariable adjustment. Furthermore, all of the data were collected after rigorous methodology, including a study protocol with standardized quality control checks. The main limitation of our study is the cross-sectional nature of NHANES. Therefore, despite the observed positive asso-
cation, the precise order of this association between plasma leptin and hypertension cannot be understood from our study; prospective studies are required to clarify this point. A second study limitation is that we did not have plasma adiponectin levels or a suitable direct measure of the renin-angiotensin-aldosterone system to study its role in the association between leptin and hypertension. Similarly, resting heart rate, the measure that we used as a proxy for sympathetic nervous system activity in the multivariable-adjusted model, may not be ideal; more direct measures of sympathetic activity, such as resting arterial catecholamine levels, are ideally required to study this effect.

**Perspectives**

In a multiethnic sample of US adults, we found that higher plasma leptin levels are associated with hypertension, independent of traditional factors, such as age, sex, smoking, alcohol intake, BMI, diabetes mellitus, and serum cholesterol. Although compared with men plasma leptin levels were higher in women, we found that the leptin-hypertension association was present in both men and women. We also found that the observed association between leptin and hypertension may not be fully explained by systemic inflammation, as measured by high-sensitivity CRP levels. Our results contribute to the emerging concept that suggests that plasma leptin levels may serve as a novel adipose tissue-derived biomarker for hypertension.3,10

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**Disclosures**

None.

**References**


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