Clinical Study

Relationship between Plasma Leptin Level and Chronic Kidney Disease

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Introduction

Leptin is an adipose tissue-derived hormone that has been shown to be related to several metabolic, inflammatory, and hemostatic factors related to chronic kidney disease. Recent animal studies have reported that infusion of recombinant leptin into normal rats for 3 weeks fosters the development of glomerulosclerosis. However, few studies have examined the association between leptin and CKD in humans. Therefore, we examined the association between plasma leptin levels and CKD in a representative sample of US adults.

Methods

The current study is based on data from the Third National Health and Nutrition Examination Survey (NHANES III).
Detailed description of NHANES III study design and methods are available elsewhere [8–13]. In brief, NHANES III 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 in order to provide stable estimates of these groups. Subjects were required to sign a consent form before their participation, and approval was obtained from the Human Subjects Committee in the US Department of Health and Human Service. Secondary data analysis was approved by the West Virginia University Institutional Review Board.

The sample included in the current analysis consisted of participants aged greater than 20 years who were randomly assigned to complete an examination in the morning after an overnight fast. Plasma leptin levels were measured in 6415 of these participants. We further excluded participants with self-reported cardiovascular disease (n = 434), missing serum creatinine (n = 60) or who were missing data (n = 101) on covariates included in the multivariable model, including systolic or diastolic blood pressure, body mass index (BMI), or cholesterol levels. This resulted in 5820 participants (53.6% women).

2.1. Main Outcome of Interest: Presence of Chronic Kidney Disease. Serum creatinine was measured using the Jaffe kinetic alkaline picrate method performed on a Roche Hitachi 737 analyzer [13]. The laboratory coefficient of variability ranged from 0.2% to 1.4%. Serum creatinine values in NHANES III were calibrated to the standard creatinine values from the Cleveland Clinic Foundation (CCF) laboratory who used a Roche coupled enzymatic assay method that was traceable to an isotope dilution mass spectrometric method using the following Deming regression equation: Standard Creatinine in mg/dL = 0.960 × NHANES measured serum creatinine (in mg/dL) − 0.184 [14]. Glomerular filtration rate (eGFR) was estimated from serum creatinine using the 4-variable Modification of Diet in Renal Disease (MDRD) study equation as follows: eGFR = 175 × (serum creatinine in mg/dL)^−1.154 × (age in years)^−0.203 × (0.742 if female) × (1.21 if black) [15]. CKD was defined as an eGFR of <60 mL/min/1.73 m², consistent with National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI) ≥ Stage 3 chronic kidney disease [16].

2.2. Exposure Measurements. Age, gender, race/ethnicity, smoking status, alcohol intake (g/day), level of education, history of diabetes and oral hypoglycemic intake or insulin administration, and antihypertensive medication use were assessed using standardized questionnaires. 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.

Using height and weight measured during the study examination, body mass index (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 [8, 11]. Measurement of plasma leptin was performed by Linco Research, Inc., St. Louis, Mo, USA. The assay was a radioimmunoassay (RIA) 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 leptin, and the limit of linearity was 100 Fg/L. Recovery of leptin added to serum is 99–104% over the linear range of the assay. The RIA agrees reasonable well with rough quantification by Western blot. Within- and between-assay CVs ranged from 3.4% to 8.3% and from 3.6% to 6.2%, respectively [11, 12].

Serum total cholesterol was measured enzymatically at the Johns Hopkins Lipid Laboratory. Serum glucose was measured using the modified hexokinase method at the University of Missouri Diabetes Diagnostic Laboratory. Diabetes 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, a glycosylated hemoglobin value ≥6.5%, or a self-reported current use of oral hypoglycemic medication or insulin. Seated systolic and diastolic blood pressures were measured using a mercury sphygmomanometer according to the American Heart Association and Seventh Joint National Committee (JNC7) recommendations [17]. Up to 3 measurements were averaged for systolic and diastolic pressures. Participants were considered to have hypertension if they reported current blood pressure-reducing medication use and/or had systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg [17].

2.3. Statistical Analysis. Plasma leptin was analyzed both as a continuous variable as well as a categorical variable. For the analysis as a continuous variable, leptin values were log transformed (base e) as a result of their skewed distribution. Using the distribution present in the NHANES III population, we categorized plasma leptin level into quartiles (≤4.3 Fg/L, 4.4–8.7 Fg/L, 8.8–16.9 Fg/L, ≥16.9 Fg/L). The multivariable-adjusted odds ratio [(OR) (95% confidence interval (CI))] of CKD associated with leptin quartile was calculated with the lowest quartile as the referent, using logistic regression models. Odds ratios were calculated initially after age and sex adjustment and subsequently after additional adjusting for race/ethnicity (non-Hispanic whites, non-Hispanic blacks, Mexican Americans, and others), education categories (below high school, high school, above high school), smoking (never smoker, former smoker, current smoker), alcohol intake (continuous), BMI (continuous), diabetes mellitus (absent, present), hypertension (absent, present), and total serum cholesterol (continuous). Trends in the OR of CKD across increasing plasma leptin category were determined by modeling median within-quartile leptin
level as a continuous variable. To examine the dose-response relationship of the observed association between plasma leptin level and CKD without linearity assumptions, we used flexible nonparametric logistic regression employing the generalized additive modeling approach (R system for statistical computing, available from Comprehensive R Archive Network [http://www.cran.r-project.org/]) to calculate odds ratio of CKD’s mellitus, adjusting for all covariates in the multivariable model; the predicted odds ratio of CKD was then plotted against increasing leptin levels (on the log scale). Previous studies have shown that serum leptin levels are associated with increased systemic inflammation as measured by C-reactive protein levels [18], hyperglycemia [12], high insulin levels [19], and increased systolic blood pressure [20], factors that have also been shown to be associated with CKD[17, 21, 22]. Therefore, in a supplementary analysis, to examine if the observed association between plasma leptin and CKD was explained by C-reactive protein levels, fasting insulin, glucose levels, or systolic blood pressure, we adjusted and oversampled, and nonresponse were applied for all analyses using SUDAAN (version 8.0; Research Triangle Institute, Research Triangle Park, NC) and SAS (version 9.2; SAS Institute, Cary, NC) software; Standard errors (SE) were estimated using the Taylor series linearization method.

3. Results

Table 1 presents the characteristics of the study population included in the current analysis. Overall, this study included a broad age range, multiethnic sample of Americans with an approximately equal number of men and women. The mean eGFR of the study participants was 94 mL/min/1.73 m², and 3.5% had CKD.

Table 2 presents the association between quartile of plasma leptin and CKD. A positive association between higher leptin quartiles and CKD was present in the age, sex-adjusted model as well as the multivariable model. When analyzed as a continuous variable after log transformation, a positive association was present between leptin and CKD.

Table 3 presents the association between plasma leptin levels and CKD within subgroups defined by gender, BMI, categories, and diabetes and hypertension. Overall, the association between leptin and CKD was consistently present within these subgroups. Although some of the ORs failed to reach conventional levels of statistical significance due to limited sample size and therefore statistical power, tests for interaction were not statistically significant (each \( P > 0.10 \) for all stratified analyses).

When we employed nonparametric models to graphically examine the dose-response relationship between plasma leptin levels and CKD without linearity assumptions involved in traditional regression models, we observed an overall positive association between plasma leptin and CKD, consistent with the results in Tables 1, 2, and 3. However, there was a steeper association with CKD for plasma leptin levels \( >16 \) Fg/L (Figure 1).

In a supplementary analysis, to examine if the observed association between leptin and CKD was explained by C-reactive protein, a marker of inflammation, or fasting insulin or glucose levels, or systolic blood pressure, we additionally adjusted for these variables to the multivariable-adjusted model. The positive association between leptin and CKD was attenuated, but still present. Compared to quartile 1 of plasma leptin (referent), the multivariable OR (95% CI) of CKD was 1.31 (0.70 to 2.44) in quartile 2, 1.22 (0.57 to 2.62) in quartile 3, and 2.72 (1.14 to 6.48) in quartile 4; \( P \)-trend = 0.0475.

4. Discussion

In a multi-ethnic, population-based sample of US adults, we found that higher plasma leptin levels were positively associated with CKD. This association appeared to be independent of confounders such as age, race-ethnicity, education, BMI, diabetes, and hypertension and appeared to be consistently present in both men and women. Furthermore, the observed
### Table 2: Association between plasma leptin level and prevalence of chronic kidney disease (CKD).

| Plasma leptin level | N   | CKD weighted % | Age, sex-adjusted odds ratio (95% confidence interval) | Multivariable-adjusted odds ratio (95% confidence interval)* |
|---------------------|-----|----------------|--------------------------------------------------------|----------------------------------------------------------|
| Quartile 1 (≤4.3 Fg/L) | 1453 | 1.8            | 1 (referent)                                            | 1 (referent)                                             |
| Quartile 2 (4.4–8.7 Fg/L) | 1541 | 3.2            | 1.42 (0.80 to 2.52)                                      | 1.35 (0.73 to 2.52)                                      |
| Quartile 3 (8.8–16.9 Fg/L) | 1464 | 3.5            | 1.40 (0.74 to 2.62)                                      | 1.34 (0.63 to 2.87)                                      |
| Quartile 4 (>16.9 Fg/L) | 1452 | 6.3            | 3.25 (1.61 to 6.55)                                      | 3.31 (1.41 to 7.78)                                      |
| P-trend              |     |                | 0.0019                                                  | 0.0135                                                   |
| Log-transformed leptin | 5820 | 3.6            | 1.57 (1.23 to 2.01)                                      | 1.74 (1.27 to 2.38)                                      |

*Adjusted for age (years), sex (men, women), race-ethnicity (non-Hispanic whites, non-Hispanic blacks, Mexican Americans, others), education categories (<high school, high school, >high school), smoking (never, former, current), alcohol intake (never, former, current), body mass index (normal, overweight, obese), diabetes (absent, present), hypertension (absent, present), and serum total cholesterol (mg/dL).

### Table 3: Association between plasma leptin level and chronic kidney disease (CKD), by subgroups.

| Subgroups of interest | No. at risk | CKD weighted % | Multivariable-adjusted odds ratio of CKD associated with log-leptin (95% confidence interval)* |
|-----------------------|-------------|----------------|--------------------------------------------------------------|
| **Age**               |             |                |                                                              |
| < 60                  | 4324        | 0.9            | 2.06 (1.01 to 4.20)                                          |
| ≥ 60                  | 1496        | 15.7           | 1.36 (1.01 to 1.83)                                          |
| **Gender**            |             |                |                                                              |
| Men                   | 2627        | 2.8            | 1.43 (0.96 to 2.12)                                          |
| Women                 | 3193        | 4.3            | 1.77 (1.09 to 2.88)                                          |
| **Race-ethnicity, %** |             |                |                                                              |
| Non-Hispanic whites   | 2403        | 3.9            | 1.63 (1.10 to 2.41)                                          |
| Non-Hispanic blacks   | 1615        | 2.5            | 3.31 (1.86 to 5.90)                                          |
| Mexican Americans, others | 1802 | 2.5            | 1.40 (0.80 to 2.45)                                          |
| **Body mass index (BMI)** |           |                |                                                              |
| BMI <25 kg/m²         | 2273        | 2.9            | 1.38 (0.62 to 3.07)                                          |
| 25 kg/m² ≤ BMI <30 kg/m² | 2058 | 3.6            | 2.44 (1.48 to 4.04)                                          |
| BMI ≥30 kg/m²         | 1489        | 4.9            | 3.30 (1.91 to 5.70)                                          |
| **Diabetes**          |             |                |                                                              |
| Absent                | 5358        | 3.2            | 1.70 (1.23 to 2.35)                                          |
| Present               | 462         | 10.1           | 1.41 (0.42 to 4.73)                                          |
| **Hypertension**      |             |                |                                                              |
| Absent                | 3908        | 1.6            | 1.42 (0.66 to 3.07)                                          |
| Present               | 1912        | 8.6            | 1.73 (1.20 to 2.49)                                          |

*Adjusted for age (years), sex (female, male), race-ethnicity (non-Hispanic whites, non-Hispanic blacks, Mexican Americans, others), education categories (<high school, high school, >high school), smoking (never, former, current), current alcohol intake (absent, present), body mass index (normal, overweight, obese), diabetes (absent, present), hypertension (absent, present), serum total cholesterol (mg/dL), glucose (mg/dL), insulin (uU/mL) and C-reactive protein (mg/dL), except the stratifying variable.
Odds ratio of CKD according to serum leptin level

Table 1: Multivariable-adjusted odds ratio of chronic kidney disease (CKD) according to plasma leptin level (Fg/L). Solid thick line represents the predicted odds of CKD from nonparametric logistic regression; dashed lines, 95% confidence limits for the nonparametric logistic regression estimates. The nonparametric logistic regression was adjusted for age (years), sex (men, women), race-ethnicity (non-Hispanic whites, non-Hispanic blacks, Mexican Americans, others), education categories (<high school, high school, >high school), smoking (never, former, current), body mass index (normal, overweight, obese), diabetes (absent, present), and serum total cholesterol (mg/dL). The median value of quartile 1 of leptin (1.285 Fg/L) was used as the referent category to calculate odds ratios.

| Serum leptin (fg/L) | Odds ratio of CKD |
|---------------------|-------------------|
| 0                   | 0.14              |
| 200                 | 0.37              |
| 400                 | 1.00              |
| 600                 | 2.72              |
| 800                 | 7.39              |
| 1000                |                   |
| 1200                |                   |
| 1400                |                   |
| 1600                |                   |
| 1800                |                   |
| 2000                |                   |
| 2200                |                   |
| 2400                |                   |
| 2600                |                   |
| 2800                |                   |
| 3000                |                   |

There is a significant association between plasma leptin levels and CKD that is independent of traditional factors such as age, sex, smoking, alcohol intake, BMI, diabetes, hypertension, and serum cholesterol. Our results suggest that leptin may explain part of the reported association between obesity and kidney disease. However, future prospective cohort studies are needed to confirm or refute our findings.

**Authors’ Contributions**

All authors contributed to the intellectual development of this paper. AS had the original idea for the study, wrote the paper and is the guarantor. JX performed the statistical analyses. PM was involved in critical revisions to the manuscript.

**Disclosure**

“The guarantor, AS, accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.”

**Conflicts of Interests**

There is no conflicts of interests to this manuscript.

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