Association Between Plasma Leptin Levels and Diabetes Mellitus

Pavani Bandaru, M.D., M.P.H., and Anoop Shankar, M.D., Ph.D.

Abstract

Background: Leptin is an important adipose tissue–derived hormone that has been shown to be involved in pathophysiological mechanisms related to cardiovascular disease and diabetes. However, few studies have examined the association between plasma leptin and diabetes mellitus in humans. Also, it is not clear if this association is present among women as well as in men. Therefore, we examined the association between plasma leptin levels and diabetes mellitus in a representative multiethnic sample of U.S. adults.

Methods: We examined the 1988–1994 third National Health and Nutrition Examination Survey (NHANES III) participants >20 years of age ($n = 5,599$, 54.7% women). Plasma leptin levels were categorized into quartiles (women, <7.68 fg/L, 7.68–13.18 fg/L, 13.19–21.70 fg/L, >21.70 fg/L; men, <2.64 fg/L, 2.64–4.36 fg/L, 4.37–7.12 fg/L, >7.12 fg/L). Diabetes mellitus was defined as fasting glucose ≥ 126 mg/dL, non-fasting glucose ≥ 200 mg/dL or use of oral hypoglycemic medication or insulin ($n = 395$).

Results: Higher plasma leptin levels were initially found to be associated with diabetes mellitus after adjusting for age, sex, race/ethnicity, education, smoking, alcohol intake, hypertension, serum cholesterol and C-reactive protein [odds ratio (OR), 3.79; confidence interval (CI), 2.05–7.00; P trend <0.0001]. However, when we additionally adjusted for body mass index (BMI), the association between plasma leptin levels and diabetes mellitus disappeared in both men (OR, 1.07; CI, 0.59–1.94; P trend = 0.5004) and women (OR, 0.86; CI, 0.49–1.51; P trend = 0.2819).

Conclusion: Higher plasma leptin levels are not independently associated with diabetes mellitus after adjustment for BMI. The null association was evident both in women as well as in men.

Introduction

LEPTIN IS AN IMPORTANT adipose tissue–derived hormone that has been shown to be involved in pathways influencing the risk of cardiovascular disease and diabetes.¹ Animal studies suggest that there is an inverse relationship between leptin levels and insulin secretion.^{2,3} Also, it has been suggested that the association between plasma leptin and diabetes may be a manifestation of an underlying leptin resistance mediated by obesity.⁴

However, relatively few studies have examined the putative association between leptin and diabetes, and their results have not been consistent. Some studies have reported that there is no association between plasma leptin levels and diabetes,^{5–7} whereas other studies have reported a significant positive association between plasma leptin levels and diabetes.^{8–11} However, two studies reported an inverse relation.^{12,13} Also, few studies reported that the association between

plasma leptin and diabetes mellitus was observed only in men and not in women.^{9–11}

In this context, we examined the association between plasma leptin levels and diabetes in a large nationally representative sample of U.S. adults after adjusting for major confounders. We had adequate sample size to examine this association in the whole cohort as well as separately by gender and BMI categories.

Methods

The current study is based on data from the Third National Health and Nutrition Examination Survey (NHANES III). Detailed descriptions of NHANES III study design and methods are available elsewhere.^{14–17} In brief, the NHANES survey included a stratified multistage probability sample representative of the civilian noninstitutionalized U.S. 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 Human Subjects Committee in the U.S. Department of Health and Human Service.

The current study sample consisted of participants aged greater than 20 years who were randomly assigned to be examined in the morning after an overnight fast. Plasma leptin levels were measured in 6,415 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 blood pressure, body mass index (BMI), or cholesterol levels. This resulted in 5,599 participants (54.7% women), 395 of whom had diabetes mellitus.

Main outcome of interest

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 h, a serum glucose ≥ 200 mg/dL for those who fasted < 8 h before their NHANES visit, or a self-reported current use of oral hypoglycemic medication or insulin.¹⁸

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 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. Serum total cholesterol was measured enzymatically.¹⁹

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.^{15,17} Measurement of plasma leptin was performed by Linco Research, Inc. (St. Louis, MO). 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 reasonably well with rough quantification by western blotting. Within- and between-assay CVs ranged from 3.4% to 8.3% and from 3.6% to 6.2%, respectively.¹⁷

Statistical analysis

Plasma leptin was analyzed as a categorical variable. Because physiologically normal leptin levels vary in men and women, we categorized plasma leptin level as quartiles by

gender (women, < 7.68 fg/L, 7.68–13.18 fg/L, 13.19–21.70 fg/L, > 21.70 fg/L; men, < 2.64 fg/L, 2.64–4.36 fg/L, 4.37–7.12 fg/L, > 7.12 fg/L).

The odds ratio [(OR) (95% confidence interval (CI))] of diabetes mellitus for each higher leptin quartile was calculated by taking the lowest quartile as the referent, using multivariable logistic regression models. We used three models: The age and sex-adjusted model, the multivariable model, additionally adjusting for race/ethnicity (non-Hispanic whites, non-Hispanic blacks, Mexican Americans, others), education categories (below high school, high school, above high school), smoking (never smoker, former smoker, current smoker), alcohol intake (g/day), hypertension (absent, present), total serum cholesterol (mg/dL), and C-reactive protein (CRP; mg/dL), and the multivariable model further adjusting for BMI (kg/m^2).²⁰ Trends in the OR of diabetes mellitus across the increasing plasma leptin category were determined by modeling leptin categories as an ordinal variable. Sample weights¹⁶ that account for the unequal probabilities of selection, oversampling, 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 (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, woman, non-Hispanic black, never or former smoker; more likely to have a higher BMI and 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 over three times higher among women compared to men (17.6 fg/L vs. 5.9 fg/L). The proportion of subjects with diabetes mellitus increased with increasing quartiles of plasma leptin.

Table 2 presents the association between increasing plasma leptin levels and diabetes mellitus in the whole cohort and separately by gender. Initially, we observed a positive association between plasma leptin levels and diabetes mellitus in both the age- and sex-adjusted (P trend < 0.0001) and the multivariable-adjusted model (P trend < 0.0001). However, when additionally adjusted for BMI, the association between increasing plasma leptin levels and diabetes mellitus disappeared entirely ($P = 0.7863$). This finding of a null association after additional BMI adjustment was consistently observed in separate analyses among men (P trend = 0.5004) as well as women ($P = 0.2819$).

Table 3 presents the association between increasing plasma leptin levels and diabetes mellitus by BMI categories. Consistent with the findings in Table 2, there was no association between the increasing plasma leptin levels and diabetes mellitus in normal-weight subjects (P trend = 0.8071), overweight subjects (P trend = 0.2143), or obese subjects (P trend = 0.7776).

In a supplementary analysis, we examined the association between plasma leptin levels and high-sensitivity CRP levels after adjusting for age (years), sex (men, women), race-ethnicity (non-Hispanic whites, non-Hispanic blacks, Mexican Americans, others), education categories ($<$ high school, high

TABLE 1. CHARACTERISTICS OF THE NHANES III (1988–1994) STUDY POPULATION BY CATEGORIES OF PLASMA LEPTIN LEVEL

Characteristics	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P value
Number at risk	1,186	1,345	1,533	1,535	
Age, years	40.4 ± 0.5	45.1 ± 0.5	46.4 ± 0.5	47.8 ± 0.5	<0.0001
Women, %	589 (49.7)	726 (54.0)	880 (57.4)	866 (56.4)	0.0002
Race-ethnicity, %					0.0012
Non-Hispanic whites	527 (44.4)	573 (42.6)	612 (39.9)	596 (38.8)	
Non-Hispanic blacks	328 (27.7)	331 (24.6)	393 (25.6)	501 (32.6)	
Mexican Americans	277 (23.4)	380 (28.3)	468 (30.5)	383 (25.0)	
Others	54 (4.6)	61 (4.5)	60 (3.9)	55 (3.6)	
Education categories, %					0.2926
Below high school	417 (35.2)	485 (36.1)	582 (38.0)	563 (36.7)	
High school	368 (31.0)	431 (32.0)	492 (32.1)	519 (33.8)	
Above high school	401 (33.8)	429 (31.9)	459 (39.9)	453 (29.5)	
Smoking, %					0.0261
Never smoker	588 (49.6)	703 (52.3)	834 (54.4)	822 (53.6)	
Former smoker	178 (15.0)	287 (21.3)	365 (23.8)	420 (27.4)	
Current smoker	420 (35.4)	355 (26.4)	334 (21.8)	293 (19.1)	
Alcohol intake, %					<0.0001
Current drinker	648 (54.6)	694 (51.6)	713 (46.5)	610 (39.7)	
Body mass index, kg/m ²	22.3 ± 0.1	24.7 ± 0.1	27.6 ± 0.1	32.7 ± 0.1	<0.0001
Total cholesterol, mg/dL	189.3 ± 1.2	201.8 ± 1.2	207.5 ± 1.1	212.3 ± 1.1	<0.0001
C-reactive protein, mg/L	1.8 ± 1.2	2.1 ± 1.2	2.8 ± 1.8	3.1 ± 1.9	0.002
Diabetes mellitus, %	35 (3.0)	83 (6.2)	115 (7.5)	162 (10.6)	<0.0001

Data presented are row percentages or mean values ± standard error (SE).

Plasma leptin quartiles in women: quartile 1 (<7.68 fg/L), quartile 2 (7.68–13.18 fg/L), quartile 3 (13.19–21.70 fg/L), quartile 4 (>21.70 fg/L); in men: quartile 1 (<2.64 fg/L), quartile 2 (2.64–4.36 fg/L), quartile 3 (4.37–7.12 fg/L), quartile 4 (>7.12 fg/L).

NHANES III, Third National Health and Nutrition Examination Survey.

TABLE 2. ASSOCIATION BETWEEN PLASMA LEPTIN LEVEL AND DIABETES MELLITUS IN NHANES III (1988–1994)

Plasma leptin level ^a	Number at risk (diabetes cases)	Age, sex-adjusted odds ratio (95% confidence interval)	Multivariable-adjusted odds ratio 1 (95% confidence interval) ^b	Multivariable-adjusted odds ratio 2 (95% confidence interval) ^c
Whole cohort				
Quartile 1	1,186 (35)	1 (referent)	1 (referent)	1 (referent)
Quartile 2	1,345 (83)	1.37 (0.74–2.53)	1.36 (0.71–2.59)	0.96 (0.49–1.89)
Quartile 3	1,533 (115)	2.00 (1.12–3.54)	1.90 (1.04–3.47)	0.81 (0.38–1.70)
Quartile 4	1,535 (162)	4.12 (2.32–7.30)	3.79 (2.05–7.00)	0.98 (0.56–1.72)
p-trend		<0.0001	<0.0001	0.7863
Women				
Quartile 1	589 (16)	1 (referent)	1 (referent)	1 (referent)
Quartile 2	726 (46)	1.28 (0.51–3.23)	1.29 (0.50–3.35)	0.90 (0.39–2.08)
Quartile 3	880 (62)	1.89 (0.86–4.14)	1.69 (0.78–3.65)	0.50 (0.21–1.19)
Quartile 4	866 (74)	3.32 (1.47–7.48)	2.76 (1.32–5.77)	0.86 (0.49–1.51)
p-trend		<0.0001	0.0009	0.2819
Men				
Quartile 1	597 (19)	1 (referent)	1 (referent)	1 (referent)
Quartile 2	619 (37)	1.47 (0.65–3.32)	1.37 (0.62–3.03)	1.06 (0.45–2.55)
Quartile 3	653 (53)	2.11 (1.05–4.27)	1.88 (0.91–3.90)	1.03 (0.41–2.59)
Quartile 4	669 (88)	4.89 (2.58–9.28)	4.36 (2.15–8.85)	1.07 (0.59–1.94)
p-trend		<0.0001	<0.0001	0.5004

^aPlasma leptin quartiles in women: quartile 1 (<7.68 fg/L), quartile 2 (7.68–13.18 fg/L), quartile 3 (13.19–21.70 fg/L), quartile 4 (>21.70 fg/L); in men: quartile 1 (<2.64 fg/L), quartile 2 (2.64–4.36 fg/L), quartile 3 (4.37–7.12 fg/L), quartile 4 (>7.12 fg/L).

^bAdjusted 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), hypertension (absent, present), serum total cholesterol (mg/dL), and high sensitivity C-reactive protein (mg/L).

^cAdditionally adjusted for body mass index.

NHANES III, Third National Health and Nutrition Examination Survey.

TABLE 3. ASSOCIATION BETWEEN PLASMA LEPTIN LEVEL AND DIABETES MELLITUS, BY BODY MASS INDEX IN NHANES III (1988–1994)

Plasma leptin level ^a	Number at risk	Diabetes cases	Multivariable-adjusted odds ratio (95% confidence interval) ^b
Normal weight (BMI <25 kg/m ²)			
Quartile 1	994	21	1 (referent)
Quartile 2	776	25	0.97 (0.40–2.35)
Quartile 3	348	12	1.09 (0.48–2.49)
Quartile 4	69	3	1.06 (0.34–3.30)
<i>P</i> trend			0.8071
Overweight (BMI 25–29 kg/m ²)			
Quartile 1	168	11	1 (referent)
Quartile 2	499	45	0.95 (0.31–2.91)
Quartile 3	848	58	0.61 (0.19–1.98)
Quartile 4	463	40	0.59 (0.18–1.96)
<i>P</i> trend			0.2143
Overweight/obese (BMI ≥30 kg/m ²)			
Quartile 1	24	3	1 (referent)
Quartile 2	70	13	0.74 (0.10–5.45)
Quartile 3	337	45	0.790 (0.18–3.52)
Quartile 4	1,003	119	0.96 (0.39–0.36)
<i>P</i> trend			0.7776

^aPlasma leptin quartiles in women: quartile 1 (<7.68 fg/L), quartile 2 (7.68–13.18 fg/L), quartile 3 (13.19–21.70 fg/L), quartile 4 (>21.70 fg/L). In men: quartile 1 (<2.64 fg/L), quartile 2 (2.64–4.36 fg/L), quartile 3 (4.37–7.12 fg/L), quartile 4 (>7.12 fg/L).

^bAdjusted 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), hypertension (absent, present), serum total cholesterol (mg/dL), and high sensitivity C-reactive protein (mg/L).

NHANES III, Third National Health and Nutrition Examination Survey; BMI, body mass index.

school, >high school), smoking (never, former, current), alcohol intake (never, former, current), BMI (kg/m²), hypertension (absent, present), and serum total cholesterol (mg/dL). We found that plasma leptin levels were significantly positively related to CRP levels (beta [standard error] = 2.8 [1.9]; *P* = 0.001).

Discussion

In a large, contemporary, multiethnic sample of U.S. adults, we found that higher plasma leptin levels were not independently associated with diabetes mellitus, after adjustment for BMI. In separate analysis by gender, there was no independent association between plasma leptin levels and diabetes mellitus either in women or men. Also in subgroup analysis by BMI categories, we found that the observed null association was consistently present in normal weight, overweight, and obese BMI categories.

Previous studies examining the association between serum leptin levels and diabetes mellitus were restricted to specific racial/ethnic groups and were not consistent in their findings. Some studies reported that there is no association between plasma leptin levels and diabetes,^{5–7} whereas some other studies reported significant positive associations between plasma leptin levels and diabetes only in men^{8–11}; however, two studies reported an inverse relation.^{12,13} It is possible that these differences in previous study results may be due to

differences in the inclusion or exclusion of one or two important confounding variables in the multivariable model.

To clarify the independent relation between plasma leptin levels and diabetes mellitus, we decided to adjust for confounders in an incremental fashion. We found that in the current study, in initial statistical models, a positive association was observed between leptin and diabetes mellitus even after adjusting for age, sex, race-ethnicity, education, smoking, alcohol intake, hypertension, serum total cholesterol, and high-sensitivity CRP. However, after adjusting for BMI, the association entirely disappeared. This suggests that the leptin–diabetes mellitus association may be explained by confounding due to BMI. The large sample size that is available for the current analyses, our ability to adjust for all potential confounders, and the consistency of these results after subgroup analysis by gender and BMI categories suggests that our findings are less likely due to chance.

Experimental animal and human studies suggest that leptin levels are closely related to body fatness.^{21–23} It is reported that plasma leptin may be a good biomarker of obesity.²⁴ Therefore, an association between plasma leptin and diabetes mellitus may be mediated by body fat.^{24,25} Higher leptin levels, conjunct with obesity and weight gain are probably involved in the subsequent development of diabetes.^{1,24,26} Also, consistent with previous studies,^{27,28} we have found that plasma leptin levels are positively related to CRP levels independent of BMI.

The main strengths of our study include its population-based nature, inclusion of a representative multiethnic sample, and the availability of data on confounders for multivariable adjustment. We had a large sample size that enabled us to perform separate analysis by gender. Furthermore, all data were collected following rigorous methodology, including a study protocol with standardized quality control checks. The main limitation of our study is the cross-sectional nature of NHANES, which precludes conclusions regarding the temporal nature of the association between plasma leptin and diabetes mellitus.

In summary, in a multiethnic sample of U.S. adults, we found that higher plasma leptin levels are not associated with diabetes mellitus, independent of BMI. Plasma leptin was not associated with diabetes either in men or in women.

Acknowledgments

A.S. was funded by a Research Funding Development Grant from the West Virginia University School of Medicine and a National Clinical Research Program grant from the American Heart Association. “The guarantor, A.S., accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.” All the authors contributed to the intellectual development of this paper. P.B. wrote the first draft of the paper. A.S. had the original idea for the study, supervised statistical analysis, and was involved in drafting and revising the paper.

Author Disclosure Statement

There are no conflicts of interest related to this manuscript.

References

1. Wannamethee SG, Tchernova J, Whincup P, Lowe GDO, Kelley A, Rumley A, Wallace AM, Sattar N. Plasma leptin:

- Associations with metabolic, inflammatory and haemostatic risk factors for cardiovascular disease. *Atherosclerosis* 2007; 191:418–426.
2. Kieffer TJ, Heller RS, Leech CA, Holz GG, Habener JF. Leptin suppression of insulin secretion by the activation of ATP-sensitive K⁺ channels in pancreatic beta-cells. *Diabetes* 1997;46:1087–1093.
 3. Kulkarni RN, Wang ZL, Wang RM, Hurley JD, Smith DM, Gbatei MA, Withers DJ, Gardiner JV, Bailey CJ, Bloom SR. Leptin rapidly suppresses insulin release from insulinoma cells, rat and human islets and, in vivo, in mice. *J Clin Invest* 1997;100:2729–2736.
 4. Steinberg GR, Parolin ML, Heigenhauser GJ, Dyck DJ. Leptin increases FA oxidation in lean but not obese human skeletal muscle: evidence of peripheral leptin resistance. *Am J Physiol Endocrinol Metab* 2002;283:E187–E192.
 5. Haffner SM, Stern MP, Miettinen H, Wei M, Gingerich RL. Leptin concentrations in diabetic and nondiabetic Mexican-Americans. *Diabetes* 1996;45:822–824.
 6. Sumner AE, Falkner B, Kushner H, Considine RV. Relationship of leptin concentration to gender, menopause, age, diabetes, and fat mass in African Americans. *Obes Res* 1998; 6:128–133.
 7. Maahs DM, Hamman RF, D'Agostino R Jr, Dolan LM, Imperatore G, Lawrence JM, Marcovina SM, Mayer-Davis EJ, Pihoker C, Dabelea D. The association between adiponectin/leptin ratio and diabetes type: the SEARCH for Diabetes in Youth Study. *J Pediatr* 2009;155:133–135, 135.
 8. Wannamethee SG, Lowe GD, Rumley A, Cherry L, Whincup PH, Sattar N. Adipokines and risk of type 2 diabetes in older men. *Diabetes Care* 2007;30:1200–1205.
 9. Welsh P, Murray HM, Buckley BM, de Craen AJM, Ford I, Jukema JW, Macfarlane PW, Packard CJ, Stott DJ, Westendorp RGJ, Shepherd J, Sattar N. Leptin predicts diabetes but not cardiovascular disease: results from a large prospective study in an elderly population. *Diabetes Care* 2009; 32:308–310.
 10. McNeely MJ, Boyko EJ, Weigle DS, Shofer JB, Chessler SD, Leonetti DL, Fujimoto WY. Association between baseline plasma leptin levels and subsequent development of diabetes in Japanese Americans. *Diabetes Care* 1999;22:65–70.
 11. Soderberg S, Zimmet P, Tuomilehto J, Chitson P, Gareeboo H, Alberti KGMM, Shaw JE. Leptin predicts the development of diabetes in Mauritian men, but not women: a population-based study. *Int J Obes (Lond)* 2007;31:1126–1133.
 12. Schmidt MI, Duncan BB, Vigo A, Pnakow JS, Couper D, Ballantyne CM, Hoogeveen RC, Heiss G, for the ARIC Investigators. Leptin and incident type 2 diabetes: risk or protection? *Diabetologia* 2006;49:2086–2096.
 13. Sun Q, van Dam RM, Meigs JB, Franco OH, Mantzoros CS, Hu FB. Leptin and soluble leptin receptor levels in plasma and risk of type 2 diabetes in U.S. women: A prospective study. *Diabetes* 2010 March;59(3):611–8.
 14. National Center for Health Statistics. The third National Health and Nutrition Examination Survey: reference manuals and reports [article online], 1996. Accessed November 23, 2009 at: www.cdc.gov/nchs/data/nhanes/nh3rrm.htm/.
 15. National Center for Health Statistics. Laboratory procedures used for NHANES III, 1996. Accessed November 23, 200 at: www.cdc.gov/nchs/data/nhanes/nhanes3/cdrom/nchs/manuals/labman.pdf/.
 16. National Center for Health Statistics. Analytic and reporting guidelines: NHANES III [article online], 1996. Accessed November 23, 2009 at: [www.cdc.gov/nchs/data/nhanes/nhanes3/nh3gui.pdf/](http://www.cdc.gov/nchs/data/nhanes/nhanes3/nhanes3/nh3gui.pdf/).
 17. National Center for Health Statistics. Serum leptin data and documentation: NHANES III, 2002. Accessed November 23, 2009 at: www.cdc.gov/nchs/data/nhanes/nhanes3/cdrom/nchs/manuals/labman.pdf
 18. Shankar A, Li J, Nieto FJ, Klein BE, Klein R. Association between C-reactive protein level and peripheral arterial disease among US adults without cardiovascular disease, diabetes, or hypertension. *Am Heart J* 2007;154:495–501.
 19. Syamala S, Li J, Shankar A. Association between serum uric acid and prehypertension among US adults. *J Hypertens* 2007;25:1583–1589.
 20. Shankar A, Li J, Klein BE, Javier NF, Klein R. Serum gamma-glutamyltransferase level and peripheral arterial disease. *Atherosclerosis* 2008;199:102–109.
 21. Considine RV, Sinha MK, Heimann ML, Kraucunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, Caro JF. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *NEJM* 1996; 334:292–295.
 22. Maffei M, Halaas J, Ravussin E, Tratlsey RE, Lee GH, Ahang Y, Fei H, Kim S, Lallone R, Ranganathan S, Kern PA, Friedman JM. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* 1995;1:1155–1161.
 23. Considine RV, Considine EL, Williams CJ, Nyce MR, Magosin SA, Bauer TL, Rosato EL, Colberg J, Caro JF. Evidence against either a premature stop codon or the absence of obese gene mRNA in human obesity. *J Clin Invest* 1995;95: 2986–2988.
 24. Velasquez MT, Balthena SJ, Hansen CT. Leptin and its relation to obesity and insulin in the SHR/N-corpulent rat, a model of type II diabetes mellitus. *Int J Exp Diabetes Res* 2001;2:217–223.
 25. Kanaya AM, Wassel FC, Vittinghoff E, Harris TB, Park SW, Goodpaster BH, Tyllavsky F, Cummings SR. Adipocytokines and incident diabetes mellitus in older adults: the independent effect of plasminogen activator inhibitor 1. *Arch Intern Med* 2006;136:350–356.
 26. Collier GR, Walder K, Lewandowski P, Sanigorski A, Zimmet P. Leptin and the development of obesity and diabetes in *Psammomys obesus*. *Obes Res* 1997;5:455–458.
 27. Viikari LA, Huupponen RK, Viikari JS, Marniemi J, Carita Eklund C, Hurme M, Lehtimäki T, Kivimäki M, Raitakari OT. Relationship between leptin and C-reactive protein in young Finnish adults. *J Clin Endocrinol Metab* 2007;92: 4753–4758.
 28. Shamsuzzaman AS, Winnicki M, Wolk R, Svatikova A, Phillips BG, Davison DE, Berger PB, Somers VK. Independent association between plasma leptin and C-reactive protein in healthy humans. *Circulation* 2004;109:2181–2185.

Address correspondence to:

Anoop Shankar, M.D., Ph.D.

Department of Medicine

West Virginia University School of Medicine

P.O. Box 9190

Morgantown, WV 26506-9190

E-mail: ashankar@hsc.wvu.edu