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Plasma Leptin Levels Are Associated with Coronary Atherosclerosis in Type 2 Diabetes

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Leptin signaling may promote atherothrombosis and lead to cardiovascular disease. However, whether leptin is associated with human atherosclerosis, distinct from thrombosis, is unknown. We determined the association of plasma leptin levels with coronary artery calcification (CAC), a measure of coronary atherosclerosis, in a cross-sectional study of type 2 diabetes. Leptin levels were associated with CAC after adjusting for established risk factors [odds ratio (95% confidence interval) for 5 ng/ml leptin increase: 1.31 (1.10–1.55); $P = 0.002$]. Leptin remained associated with CAC after further controlling for body mass index (BMI) [1.29 (1.07–1.55); $P = 0.008$], waist circumference [1.30 (1.09–1.57); $P = 0.003$], C-reactive protein (CRP) levels [1.28 (1.07–1.55); $P = 0.008$], and subclinical vascular disease [1.30 (1.08–1.57); $P = 0.006$]. Addition of BMI ($P = 0.97$), waist ($P = 0.55$), or CRP ($P = 0.39$) to a model with leptin failed to improve the model's explanatory power, whereas addition of leptin to a model with BMI ($P = 0.029$), waist ($P = 0.006$), or CRP ($P = 0.005$) improved the model significantly. Plasma leptin levels were associated with CAC in type 2 diabetes after controlling adiposity and CRP. Whether leptin signaling promotes atherosclerosis directly or represents a therapeutic target for the prevention of atherosclerotic cardiovascular disease remains to be explored. (*J Clin Endocrinol Metab* 89: 3872–3878, 2004)

THE RISK OF atherosclerotic cardiovascular disease (CVD) in type 2 diabetes mellitus (DM) is 2- to 4-fold higher than in nondiabetics (1). Obesity is a strong risk factor for the development of type 2 DM and CVD (2). However, the pathophysiological mechanisms that link obesity and CVD are poorly defined (2). The clustering of central obesity with insulin resistance, metabolic dyslipidemia, and chronic inflammation may account for part of the proatherosclerotic effects of adiposity (3, 4). Adipocytes respond to metabolic and inflammatory stimuli by secreting a variety of molecules known as adipokines (5, 6), including leptin, that are thought to modulate atherosclerosis and are candidate risk factors for CVD (6–8).

Obesity is associated with a marked increase in circulating leptin concentrations (9, 10). However, plasma leptin displays a strong association with cardiovascular risk factors, including insulin resistance, metabolic syndrome, and inflammatory markers, even after controlling for measures of body fat mass (11–14). Peripheral actions of leptin that may promote atherosclerosis include endothelial activation and migration (15, 16), smooth muscle cell proliferation and calcification (17), and activation of monocytes and adaptive immune responses (18, 19). Leptin receptors are expressed in atherosclerotic lesions (20), and leptin signaling has been implicated in the promotion of both thrombosis and atherosclerosis in mice models, suggesting a role for leptin in atherothrombosis *in vivo* (21–23).

No previous studies have examined a link between plasma leptin and atherosclerosis in type 2 DM. Wallace *et al.* (24) found that plasma leptin levels were predictors of CVD events, after adjusting for traditional risk factors, BMI, and plasma C-reactive protein (CRP), in a case-control study of hypercholesterolemic patients in the West of Scotland Coronary Prevention Study (WOSCOPS). However, other studies have shown no association of plasma leptin with cardiovascular events (25). Furthermore, a relationship between leptin and measures of atherosclerosis as the basis for the link to clinical events has not been established (26, 27). Given conflicting results, there is a need to explore further the pathophysiology of the relationship between leptin and CVD in humans.

Coronary artery calcification (CAC), measured at electron beam tomography (EBT), is correlated with the degree of atherosclerosis at histopathology and coronary angiography (28, 29). Several studies have shown that CAC scores are increased in type 2 DM compared with nondiabetic controls (30–32), and the degree of CAC in DM is related to the degree of angiographic atherosclerosis and the presence of clinical CVD (33, 34). Because the mechanisms of CVD in type 2 DM remain poorly defined, we addressed the hypotheses that plasma leptin levels, which are elevated in obesity and type

Abbreviations: ABI, Ankle brachial indices; BMI, body mass index; CAC, coronary artery calcification; CRP, C-reactive protein; CVD, cardiovascular disease; DM, diabetes mellitus; EBT, electron beam tomography; EKG, electrocardiogram; HbA1c, hemaglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LR, likelihood ratio; MI, myocardial infarction; OR, odds ratio; PAOD, peripheral arterial obstructive disease.

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2 DM, were associated with CAC in a sample of type 2 DM patients.

Subjects and Methods

Study subjects

Subjects with type 2 DM, identified through the medical clinics of the Veterans Affairs Medical Center and the Hospital of University of Pennsylvania, both in Philadelphia, Pennsylvania, were invited to participate in a cross-sectional study of novel risk factors for coronary atherosclerosis in type 2 DM. Inclusion criteria included: 1) age 35–75 yr; 2) clinical diagnosis of type 2 DM (defined as fasting blood glucose ≥126 mg/dl, 2-h postprandial glucose ≥200 mg/dl, or use of oral hypoglycemic agents/insulin in a subject greater than age 40 yr); and 3) negative pregnancy test if female of child-bearing potential. Exclusion criteria were: 1) history of clinical CVD [myocardial infarction (MI), documented angiographic coronary artery disease, positive stress test, percutaneous coronary or peripheral intervention, coronary artery or peripheral artery bypass grafting, stroke, or transient ischemic attack]; 2) clinical diagnosis of type 1 DM (diagnosis of DM and insulin use before age 35); 3) serum creatinine level above 2.5 mg/dl; 4) active infection or malignancy; and 5) weight more than 300 lb (limit of EBT scanner). Screening was performed by telephone interview using a standardized telephone questionnaire. The University of Pennsylvania Institutional Review Board approved the study protocol, and all subjects gave written informed consent. The 200 subjects recruited for this analysis provided 80% power to detect a difference in plasma leptin levels of 0.4 SD between subjects with CAC scores below the median compared with those with scores above the median, based on the difference and SD reported in the WOSCOPS (controls, 5.04 ± 2.09 ng/ml *vs.* cases, 5.87 ± 2.04 ng/ml) (24).

Data collection

Study subjects were evaluated at the General Clinical Research Center (GCRC) after a 12-h overnight fast. A questionnaire regarding medical, family, and social history, medication use, and cardiac history was completed. Hypertension was defined as taking antihypertensive medications or blood pressure higher than 130/80 mm Hg. Height, weight, waist and hip circumference, resting bilateral systolic and diastolic blood pressure, electrocardiogram (EKG), and Doppler ankle brachial indices (ABI) were performed. Pathological Q waves in two contiguous EKG leads defined MI and an ABI less than 0.9 defined peripheral arterial obstructive disease (PAOD). A sample of whole blood was drawn. Leptin levels were measured, in duplicate, in stored (−80 C) plasma samples using a human leptin RIA assay (Linco Research, Inc., St. Charles, MO) (35). The limit of sensitivity is 0.5 ng/ml. There is no cross-reactivity with human insulin, pro-insulin, or C-peptide. The within-assay and between-assay coefficients of variation were 5.5 and 9.0%, respectively.

Complete blood count, routine chemistries, including glucose and hemaglobin A1c (HbA1c) assays, and microalbuminuria assays were performed at the clinical laboratories at the hospital. Total low-density lipoprotein (LDL), high-density lipoprotein (HDL), very low-density lipoprotein cholesterol, and triglycerides were measured by the ultracentrifugation technique (β-quantification) in a Centers for Disease Control-certified lipid laboratory (36). Plasma insulin levels were measured using a commercially available RIA (Linco Research, Inc.). Plasma samples were assayed for CRP using an ultra-high-sensitivity latex turbidimetric immunoassay (Wako Pure Chemicals, Ltd., Osaka, Japan) on a Cobas Fara II analyzer (Roche Diagnostics, Indianapolis, IN) (37). Laboratory test results were generated by personnel blinded to the clinical characteristics and CAC scores of research subjects.

Global CAC scores were determined as described (31, 38) according to the method of Agatston *et al.* (39) from 40 continuous 3-mm-thick computed tomograms collected on an EBT scanner (Imatron, San Francisco, CA). Scoring was performed by a single experienced radiological technologist, blinded to clinical and laboratory characteristics, using customized software (Imatron).

Statistical analysis

Data distributions are reported as median and range or mean ± SD. For unadjusted analyses, leptin levels were divided into quartiles, and median CAC scores were compared across leptin quartiles using the Kruskal-Wallis rank test and a Wilcoxon nonparametric test for trend.

We wished to include a number of variables that are correlated with plasma leptin (BMI, waist, plasma insulin levels, CRP levels) in multivariable models examining factors associated with CAC. Therefore, we determined the degree of collinearity among these variables using the matrix of variance decomposition proportions for variables in a linear regression model (40). A condition number more than 30 was considered evidence of excessive correlation between variables. Log-transformed plasma leptin levels (ln-leptin) were used as the dependent variable in linear regression models to determine factors associated with plasma leptin.

Multivariable analyses of CAC data. Multivariable analysis of CAC data is challenging because the absence of CAC in many subjects and the presence of a few large scores (Fig. 1A) violate the assumptions of linear regression, even after log transformation (Fig. 1B). Recently, a number of groups, including ours, have shown that ordinal logistic regression (31, 37, 38, 41, 42) with the CAC outcome expressed as grouped continuous data (ordinal categories) is a useful method for the analysis of CAC data in this setting. Ordinal regression permits logistic regression to be applied to nonnormal data without the loss of information associated with collapsing continuous data to a binary outcome (43). There-

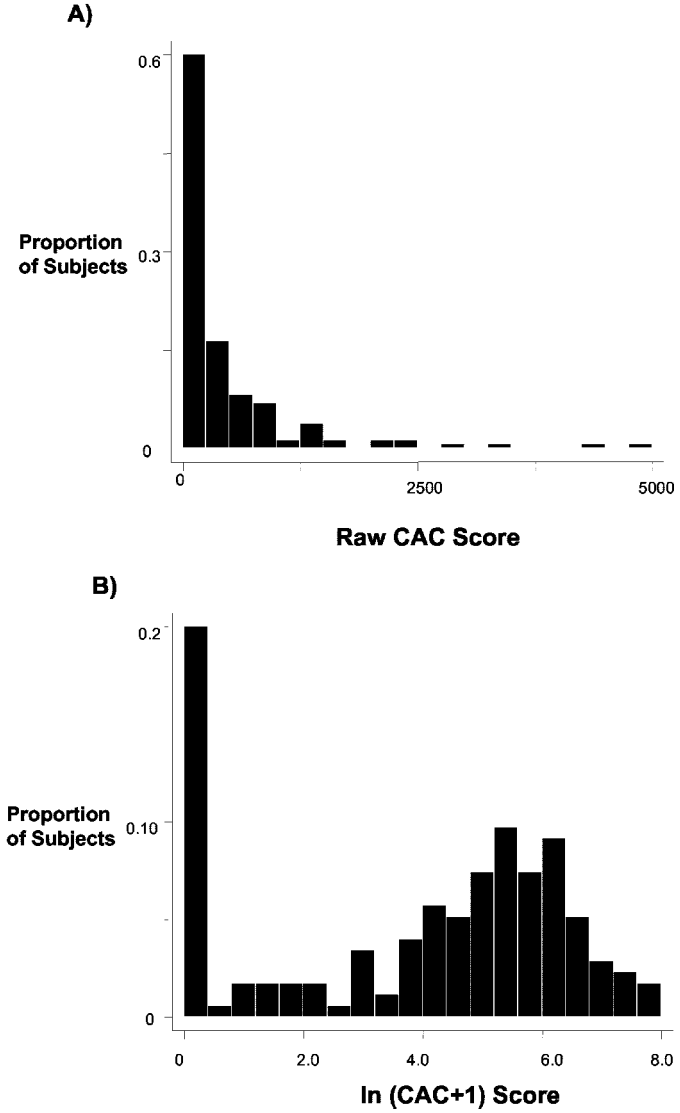

FIG. 1. Distribution of (A) raw CAC scores and (B) natural log (ln) transformation of (CAC + 1) scores in the study sample.

fore, we applied ordinal regression using, as the outcome, CAC categories that were based on published criteria that approximate no, mild, moderate, and severe coronary atherosclerosis (CAC scores of 0, 1–100, 101–400, >400) (29).

As our primary approach, we included plasma leptin levels as a continuous independent variable in multivariable models. Leptin quartiles (<6.25, 6.25 to <10.57, 10.57 to <16.10, and ≥16.10 ng/ml) were used in confirmatory analyses. The association of leptin with CAC was examined in a series of ordinal regression models that contained: 1) leptin, age, and gender; 2) leptin, age, gender, and a set of established risk factors excluding BMI, waist, CRP, EKG, and ABI data; 3) BMI (or waist circumference) in addition to leptin, age, gender, and established risk factors; 4) plasma CRP levels in addition to leptin, BMI (or waist circumference), age, gender, and established risk factors; and 5) EKG and ABI data, in addition to leptin, CRP levels, BMI (or waist circumference), age, gender, and established risk factors. The set of established risk factors, including potential confounders, included race (Caucasian, African-American, other), systolic blood pressure, fasting lipoproteins (LDL and HDL cholesterol and triglycerides), fasting plasma glucose, HbA1c, plasma insulin levels, family history of premature CVD, cigarette smoking status, exercise (none, <3 d/wk, >3 d/wk, daily), alcohol intake (number of drinks: less than one a week, one to six a week, one a day, more than one a day), white blood cell count, urinary microalbuminuria, serum creatinine, and use of the following medications: daily aspirin, statins, insulin, metformin, sulfonylureas, thiazolidinediones, β-blockers, angiotensin-converting enzyme inhibitors. Interaction of leptin with gender and established risk factors was determined in fully adjusted models using the likelihood-ratio (LR) χ^2 test. Because gender, established subclinical vascular disease, insulin therapy, adiposity, and diabetic control may influence both leptin levels and CAC, we performed sensitivity analyses in subjects with 1) no evidence of MI or PAOD (n = 181); 2) no insulin therapy (n = 157); 3) BMI higher than 25 kg/m^2 (n = 182); and 4) HbA1c more than 6.0% (n = 171), as well as in men and women separately, to determine whether results were consistent with the full study sample. The LR test was also used in nested models to determine whether the addition of BMI, waist circumference, or CRP levels improved the model performance compared with a model with leptin alone.

Results of ordinal logistic regression are presented as the odds ratio (OR) of being in a higher CAC category for a 5-ng/ml change in leptin levels or for the highest *vs.* lowest leptin quartile. The proportional odds assumption of ordinal regression was satisfied in all models (43). Statistical analyses were performed using Stata 8.0 software (Stata Corp., College Station, TX).

Results

The demographic and laboratory characteristics, including plasma leptin levels, in men and women are shown in Table 1. The study sample was predominantly male (87%), consistent with the large representation from a Veterans Administration population (71%). Median BMIs were greater than 30 kg/m^2 in both men and women. Consistent with type 2 DM and overweight state, plasma leptin levels were elevated compared with reported values for healthy control subjects (44, 45). Leptin levels were more than 2-fold higher in women than men, despite equivalent body mass. This finding has been noted previously (46) and is thought to reflect the influence of female sex hormones on adipose expression and secretion of leptin (47). EKG documented Q-wave MI (n = 13; 6.5%), and Doppler evidence of PAOD (n = 7; 3.5%) was present only in a small proportion of subjects, consistent with the recruitment of asymptomatic subjects.

The bimodal distribution of CAC data in our sample (Fig. 1 and Table 2) is similar to previous reports in type 2 DM and is consistent with increased CAC in asymptomatic type 2 DM subjects compared with nondiabetic sample subjects (30–32). Almost 35% of men and women had scores higher than the 75th percentile based on a gender-specific, age-standardized database of CAC scores (derived from CAC scores in 30,000 asymptomatic subjects) (48). Absolute CAC scores of more than 400, considered to represent extensive subclinical coronary atherosclerosis, were prevalent in men (27.4%), despite exclusion of subjects with clinical CVD.

Female gender, waist, BMI, systolic blood pressure, and

TABLE 1. Characteristics of the study sample

Characteristic	Men (n = 174) [median (range)]	Women (n = 26) [median (range)]
Age (yr)	61.3 (36–75)	56.9 (40–72)
Race (%)		
Caucasian	64.2	76.9
African-American	27.7	7.7
Other	8.1	15.4
Duration of diabetes (yr)	7.0 (1–33)	7.5 (1–18)
Plasma lipoproteins		
Total cholesterol		
(mmol/liter)	4.72 (2.62–9.90)	5.13 (3.13–7.12)
(mg/dl)	182 (101–382)	198 (121–275)
LDL-cholesterol		
(mmol/liter)	2.75 (0.83–6.48)	2.69 (1.17–4.61)
(mg/dl)	106 (32–250)	104 (45–178)
HDL-cholesterol		
(mmol/liter)	1.01 (0.52–2.28)	1.22 (0.67–2.49)
(mg/dl)	39 (20–88)	47 (26–96)
Triglycerides		
(mmol/liter)	1.58 (0.43–11.71)	1.69 (0.52–6.26)
(mg/dl)	139 (38–1032)	149 (46–552)
Fasting serum glucose		
(mmol/liter)	7.39 (2.11–20.6)	7.00 (4.17–15.1)
(mg/dl)	133 (38–371)	126 (75–271)
Hemoglobin A1c (%)	6.85 (4.8–12.8)	6.9 (5.3–9.5)
Insulin (pmol/liter)		
No insulin therapy	107 (20–923)	103 (29–272)
Insulin therapy	141 (38–513)	53 (43–303)
BMI (kg/m^2)	31.5 (17.8–46.3)	31.2 (23–46.2)
Waist circumference (cm)	108 (62–155)	109 (75–144)
Leptin (ng/ml)	9.6 (1.6–47.0)	23.7 (5.0–79)
CRP (mg/liter)	1.3 (0.1–11)	2.7 (0.1–8.7)
Blood pressure (mm Hg)		
Systolic	135 (93–178)	125 (103–162)
Diastolic	80 (50–103)	73 (55–101)
Cigarette smokers (%)		
Current	10.9	11.5
Ex	68.5	34.6
Never	25.9	53.9
Alcohol (no. of drinks) (%)		
<1 per week	56.4	50
1–6 per week	25.4	38.5
1 per day	7.8	7.7
>1 per day	10.4	3.8
Exercise (%)		
None	31.2	28.0
<3 per week	25.6	28.0
>3 per week	23.0	28.0
Daily	20.2	16.0
Postmenopausal (%)	NA	73
Medications (%)		
Sulfonylureas	55.2	30.8
Metformin	54.1	57.7
TZDs	17.1	26.9
Insulin	20.1	30.8
Statins	43.9	39.5
Aspirin	38.5	26.9
ACE-inhibitor	61.1	43.3
HRT	NA	15.4

ACE, Angiotensin-converting enzyme; HRT, hormone replacement therapy; NA, not applicable; TZD, thiazolidinedione.

TABLE 2. CAC scores in study sample

CAC score or category	Men (n = 174)	Women (n = 26)
CAC median (range)	145 (0–4100)	1.5 (0–3129)
CAC mean ± SD	402 ± 695	163 ± 615
CAC > 75th percentile, n (%)	56 (32.2%)	9 (34.6%)
CAC = 0, n (%)	37 (21.3%)	13 (50%)
CAC 1–100, n (%)	43 (24.7%)	9 (34.6%)
CAC 101–400, n (%)	46 (26.4%)	2 (7.7%)
CAC >400, n (%)	48 (27.6%)	2 (7.7%)

TABLE 3. CAC scores across quartile categories of plasma leptin

Plasma leptin (quartile)	CAC score in men [median (IQR)]	CAC score in women [median (IQR)]	*P* for trend
1st	70 (0–358)	0 (0–11)	0.02
2nd	103 (0–598)	0 (0–5)	
3rd	164 (15–433)	3 (0–19)	
4th	325 (45–536)	52 (0–349)	

IQR, Interquartile range.

plasma levels of insulin and CRP were associated positively with leptin, whereas use of metformin therapy was negatively associated with leptin in multivariable linear regression models using log-transformed plasma leptin levels (ln-leptin) as the outcome (data not shown). Collinearity diagnostics for BMI, waist circumference, leptin, insulin, and CRP showed marked correlation (condition number = 34.6) of BMI and waist (Spearman coefficient, +0.83). For this reason, these two variables were not included in multivariable models together. Although there was modest correlation of leptin with BMI (Spearman coefficient, +0.60) and insulin (Spearman coefficient, +0.33), the condition numbers were less than 30 and, therefore, these variables were included in models as outlined above.

In unadjusted analysis, CAC scores increased significantly across plasma leptin quartiles (χ^2 for trend = 5.86; $P = 0.02$) (Table 3). Although leptin levels were higher in women than men, there was no interaction between leptin levels and gender in the association with CAC (LR test $\chi^2 = 0.07$; interaction $P = 0.80$ for fully adjusted model). Therefore, results of multivariable analyses are presented for men and women together.

Plasma leptin levels were significantly associated with CAC scores after controlling for age, gender, and established risk factors (Table 4). Further adjustment for BMI (or waist circumference), plasma CRP levels, and measures of subclinical vascular disease (MI at EKG or PAOD at ABI) did not attenuate the association of leptin with CAC. Results of fully adjusted analysis in men [OR, 1.39 (1.04–1.91); $P = 0.03$], subjects without MI or PAOD [OR, 1.29 (1.06–1.57); $P = 0.01$], subjects not on insulin therapy [OR, 1.17 (1.02–1.38); $P = 0.03$)], subjects with BMI greater than 25 kg/m^2 [OR, 1.30 (1.06–1.58); $P = 0.03$], and subjects with HbA1c higher than 6.0% [OR, 1.40 (1.13–1.73); $P = 0.002$] were consistent with findings in the full study sample. In age-adjusted analysis, the association of leptin levels with CAC was similar in men [OR, 1.30 (1.07–1.57); $P = 0.008$] and women [OR, 1.22 (1.03–1.45); $P = 0.027$]. Fully adjusted analysis in women was not possible because of limited sample size. In a confirmatory ordinal regression, use of leptin quartiles instead of leptin levels provided similar results [OR, 3.40 (1.13–10.2); $P = 0.03$ for highest *vs.* lowest leptin quartile].

TABLE 4. Association of plasma leptin levels with CAC in fully adjusted models

Model adjusted for	OR (CI)	*P* value
1) Age, gender	1.26 (1.12–1.43)	<0.001
2) Age, gender, RF[a]	1.31 (1.10–1.55)	0.002
3a) BMI, age, gender, RF[a]	1.29 (1.07–1.55)	0.008
3b) Waist, age, gender, RF[a]	1.30 (1.09–1.57)	0.005
4a) CRP, BMI, age, gender, RF[a]	1.28 (1.07–1.55)	0.008
4b) CRP, waist, age, gender, RF[a]	1.30 (1.08–1.57)	0.006
5a) MI, PAOD, CRP, BMI, age, gender, RF[a]	1.30 (1.08–1.57)	0.006
5b) MI, PAOD, CRP, waist, age, gender, RF[a]	1.32 (1.09–1.58)	0.004

Results of ordinal regression are presented as the OR of being in higher CAC category for a 5-ng/ml increase in plasma leptin levels. CAC categories: 0, 1–100, 101–400, and >400. CI, Confidence interval.

[a] The set of established risk factors (RF) included race (Caucasian, African-American, other), systolic blood pressure, fasting lipoproteins (LDL and HDL cholesterol and triglycerides), fasting plasma glucose, HbA1c, plasma insulin levels, family history of premature CVD, cigarette smoking status, exercise, alcohol intake, urinary microalbuminuria, serum creatinine, and use of the following medications: daily aspirin, statins, insulin, metformin, sulfonylureas, thiazolidinediones, β-blockers, angiotensin-converting enzyme inhibitors.

TABLE 5. Association of plasma leptin levels with CAC: comparison of models with BMI, waist circumference, and CRP levels

Model[a] including	LR test χ^2	*P* value
Leptin + BMI *vs.* BMI alone	4.80	0.029
Leptin + BMI *vs.* leptin alone	0.01	0.97
Leptin + waist *vs.* waist alone	7.29	0.006
Leptin + waist *vs.* leptin alone	0.40	0.55
Leptin + CRP *vs.* CRP alone	7.91	0.005
Leptin + CRP *vs.* leptin alone	0.73	0.39

[a] All models included race, systolic blood pressure, fasting lipoproteins (LDL and HDL cholesterol and triglycerides), fasting plasma glucose, HbA1c, plasma insulin levels, family history of premature CVD, cigarette smoking, exercise, alcohol intake, urinary microalbuminuria, serum creatinine, and use of medications (aspirin, statins, insulin, metformin, sulfonylureas, thiazolidinediones, β-blockers, angiotensin-converting enzyme inhibitors).

In contrast to the improved explanatory power with the addition of leptin to a model that contained BMI, waist, or CRP, adding BMI, waist, or CRP to a model that already contained leptin failed to improve the model fit for CAC (Table 5).

Discussion

In the first study of its kind, we examined the association between plasma levels of leptin and CAC, a measure of subclinical coronary atherosclerosis in a sample of asymptomatic type 2 DM subjects. Plasma leptin levels were positively associated with CAC even after controlling for BMI, waist circumference, plasma CRP levels, or measures of subclinical vascular disease. In fact, BMI, waist, and plasma CRP provided no additional explanatory power for CAC beyond that provided by plasma leptin levels.

Obesity is a strong risk factor for the development of type 2 DM and atherosclerotic CVD (2). Leptin plays a role in the

long-term regulation of body weight (9, 49). Paradoxically, obesity is associated with markedly increased plasma leptin levels, most likely due to resistance to its actions in target organs in the setting of increased production by adipose tissue. However, there is considerable evidence supporting the importance of leptin hormonal signaling beyond its capacity to reflect body fat mass. Increased leptin levels are associated with lower insulin sensitivity, more metabolic syndrome features, and higher inflammatory markers independent of body fat mass (11–13, 45). In particular, leptin levels have been linked to inflammatory and fibrinolysis markers that predict future CVD events, including CRP and plasminogen activator inhibitor-1 (14, 50).

In vitro studies demonstrate direct effects of leptin on vascular and inflammatory cells that may promote atherothrombosis independent of the centrally mediated metabolic effects of leptin. Leptin activates multiple signal transduction pathways in human monocytes and vascular cells including phosphoinositide 3-kinase, protein kinase A, and MAPKs (15, 17, 18, 51), and leptin receptors are expressed in these cells in human atherosclerotic lesions (17, 20). Studies in mice have implicated leptin signaling in both arterial thrombosis (23, 52) and atherosclerosis (21, 22).

Some (24, 53, 54) but not all (25, 55) studies have found positive associations between plasma leptin and clinical CVD. Soderberg *et al.* (53) found a positive association of plasma leptin levels with first MI (n = 62 cases), independent of traditional risk factors, body mass, and plasma insulin levels in a case-control study. In the largest study of clinical events to date, plasma leptin levels positively predicted cardiovascular events even after adjusting for traditional risk factors, BMI, and plasma CRP levels in a case (n = 377) control (n = 783) study nested within the WOSCOPS clinical trial (24). However, plasma leptin levels were not associated with CVD in a nested case (n = 86) control (n = 95) study from the Quebec Cardiovascular Study cohort (25). In fact, leptin levels were negative predictors of CVD mortality [hazards ratio 0.88 (0.80–0.97); $P = 0.02$] in a recent study of 207 women (55). Given conflicting results, it is of substantial interest to explore the pathophysiology of the relationship between leptin and CVD in humans.

Few data are available on the association between leptin and direct measures of atherosclerosis in humans. Several small studies have failed to establish a convincing association. van den Beld *et al.* (27) found no association between plasma leptin levels and carotid intima media thickness in 403 healthy elderly men. Ciccone *et al.* (26) reported an association of leptin with intima media thickness in 126 healthy Italians that was lost after adjustment for BMI. Our study provides the first evidence that leptin may be linked to clinical CVD through an association with human coronary atherosclerosis. Indeed, we found an association of plasma leptin with CAC even after controlling for waist circumference and plasma levels of CRP, an inflammatory marker that is linked to both the metabolic syndrome and CVD events (4). Thus, plasma leptin levels may represent an integrated marker of adiposity, insulin resistance, and vascular dysfunction that could prove useful in future approaches to cardiovascular risk stratification in clinical practice.

This study has several possible limitations. The study sample is small and cross-sectional. Thus, causal inferences cannot be made. Plasma biomarkers, such as leptin and other metabolic variables, are subject to physiological influences. For this reason, blood sampling was performed between 0800 and 1000 h in a fasting state using validated assays for all laboratory variables, including leptin (35). Plasma leptin levels in both men and women in our sample were consistent with published values for diabetic subjects of similar body mass (44, 45). Given the small number of women, we cannot exclude an association of plasma leptin with CAC that is modified by gender. The generalizability of our findings is limited mostly to urban type 2 DM men. Confounding is always possible due to the effect of unmeasured or unknown confounders. However, we collected information on multiple potential confounders, including medications, and our findings were consistent across multivariable analyses. In particular, the association of leptin with CAC was consistent across all subgroup analyses. This study is not capable of determining whether plasma leptin levels are a marker for other unknown risk factors or whether leptin is a direct or indirect cause of atherosclerosis. Although not a direct measure of coronary atherosclerosis, autopsy and coronary angiography suggest that CAC provides a quantitative measure of coronary atherosclerosis in both nondiabetic (28, 29) and type 2 diabetic (32–34) samples, and recent studies support its utility as a predictor of CVD events in asymptomatic subjects (56).

In summary, we found an association between plasma leptin levels and CAC in type 2 DM after controlling for traditional measures of obesity and plasma CRP. Our findings suggest that leptin may provide greater insight into the proatherosclerotic risk associated with adiposity than established measures such as BMI or waist circumference. The utility of plasma leptin levels in predicting progression of atherosclerosis and cardiovascular events needs to be examined in additional, community-based prospective studies. Whether leptin signaling promotes atherosclerosis directly or represents a therapeutic target for the prevention of atherosclerotic CVD remains to be explored.

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