

Plasma Leptin Levels and Incidence of Heart Failure, Cardiovascular Disease, and Total Mortality in Elderly Individuals

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OBJECTIVE— Obesity predisposes individuals to congestive heart failure (CHF) and cardiovascular disease (CVD). Leptin regulates energy homeostasis, is elevated in obesity, and influences ventricular and vascular remodeling. We tested the hypothesis that leptin levels are associated with greater risk of CHF, CVD, and mortality in elderly individuals.

RESEARCH DESIGN AND METHODS— We evaluated 818 elderly (mean age 79 years, 62% women) Framingham Study participants attending a routine examination at which plasma leptin was assayed.

RESULTS— Leptin levels were higher in women and strongly correlated with BMI ($P < 0.0001$). On follow-up (mean 8.0 years), 129 (of 775 free of CHF) participants developed CHF, 187 (of 532 free of CVD) experienced a first CVD event, and 391 individuals died. In multivariable Cox regression models adjusting for established risk factors, log-leptin was positively associated with incidence of CHF and CVD (hazard ratio [HR] per SD increment 1.26 [95% CI 1.03–1.55] and 1.28 [1.09–1.50], respectively). Additional adjustment for BMI nullified the association with CHF (0.97 [0.75–1.24]) but only modestly attenuated the relation to CVD incidence (1.23 [1.00–1.51], $P = 0.052$). We observed a nonlinear, U-shaped relation between log-leptin and mortality ($P = 0.005$ for quadratic term) with greater risk of death evident at both low and high leptin levels.

CONCLUSIONS— In our moderate-sized community-based elderly sample, higher circulating leptin levels were associated with a greater risk of CHF and CVD, but leptin did not provide incremental prognostic information beyond BMI. Additional investigations are warranted to elucidate the U-shaped relation of leptin to mortality.

Diabetes Care 32:612–616, 2009

Excess adiposity is a key risk factor for congestive heart failure (CHF) (1) and cardiovascular disease (CVD) (2), in part because it is a fundamental precursor of

several vascular risk factors including high blood pressure, dyslipidemia, and impaired glucose homeostasis. Increased BMI is often used as a surrogate measure of excess adi-

posity. However, BMI reflects the combined mass of adipose and nonadipose tissue (including muscles and bones). Therefore, leptin, an anorexogenic hormone that is predominantly produced in adipose tissue, may be a better risk marker for CVD than current BMI.

Several lines of evidence indicate a putative role for leptin in cardiac (3) and vascular remodeling (4,5), although reports are divided on whether leptin has predominantly adverse or beneficial effects on the heart (6,7) and the vascular system (4,8,9). Likewise, clinical studies on the association with CVD have yielded inconsistent results (10,11).

Interestingly, previous studies suggested that leptin levels are not only determined by the current adipose tissue volume but also by the weight history of an individual (12). Leptin might, therefore, be a better marker of CVD risk than current BMI, particularly in normal-weight individuals with a history of obesity, who might have subclinical CVD (due to antecedent obesity). Conversely, because obesity is associated with relative leptin resistance that often persists after weight reduction, “current” leptin levels may misclassify the biological effects of leptin exposure, leaving BMI with a stronger association to CVD outcomes (relative to leptin).

On the basis of the clinical and experimental data summarized above, we hypothesized that leptin levels are associated with the incidence of CHF, CVD, and all-cause mortality in elderly individuals. We tested this hypothesis by relating leptin levels to the occurrence of CVD (including CHF) and death prospectively in a moderate-sized elderly community-based sample; we assessed specifically whether leptin levels offer incremental prognostic information over established risk factors and BMI. We also sought to examine the cross-sectional clinical correlates of leptin and evaluated whether leptin correlates with measures of past BMI (i.e., serves as an indicator of an individual’s weight history).

RESEARCH DESIGN AND METHODS

The design and the selection criteria of the Framingham Heart Study have been published elsewhere

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Received 29 August 2008 and accepted 22 December 2008.

Published ahead of print at <http://care.diabetesjournals.org> on 29 December 2008. DOI: 10.2337/dc08-1596.

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(13). Participants in the original cohort are seen in the Heart Study clinic every 2 years. At each examination, participants undergo laboratory testing for CVD risk factors, anthropometry, and an examination by a physician that includes a standardized medical history and a physical examination. All attendees at the 22nd examination cycle of the original cohort (referred to as the “index” examination; $n = 1,173$) were eligible for the present investigation; we excluded individuals with nonavailable leptin levels ($n = 355$). After exclusions, 818 participants remained eligible for the analysis of clinical correlates and relations to all-cause mortality. For analyses of new-onset CHF, we excluded 43 individuals with prevalent CHF so that 775 remained eligible. For analyses of first CVD events the participants with prevalent CVD were excluded ($n = 286$), which left 532 eligible. All participants provided written informed consent, and the study protocol was approved by the institutional review board at the Boston University Medical Center.

Leptin assay

At examination cycle 22, blood was drawn from nonfasting participants, usually between 1:00 and 2:00 P.M. Plasma leptin levels were measured using a commercial radioimmunoassay (Linco Research, St. Louis, MO). The interassay coefficient of variation ranged from 3.0 to 6.2%. The lower sensitivity limit was 0.5 ng/ml.

Definitions of outcome events

All Heart Study participants are under continuous surveillance for the incidence of CVD (including CHF) and death. Outcome events are adjudicated by a team of three physicians who reviews all relevant medical information, hospitalization records, and physician office visits using standardized criteria. A separate group consisting of neurologists reviews and adjudicates all suspected cerebrovascular events. Criteria for CVD have been published and include coronary heart disease (CHD) (recognized and unrecognized myocardial infarction, coronary insufficiency, angina, and CHD death), cerebrovascular disease (stroke and transient ischemic attack), intermittent claudication, and CHF (14). A diagnosis of CHF requires the presence of at least two major criteria or one major and two minor criteria in the absence of a condition that can explain the findings. The same definitions have been used in the Framingham Heart Study over the years.

In our primary analyses, we focused on three end points: incident CHF, CVD, and total mortality. In secondary analyses, we related leptin with incident “hard” CVD, a composite end point that includes fatal and nonfatal myocardial infarction, coronary insufficiency, stroke, or CHF.

Statistical analyses

Given a right-skewed distribution, leptin levels were logarithmically transformed. We evaluated the correlation of log-leptin with BMI measured at the index examination, in the decade preceding the examination and over the interval of 10–20 years preceding the index examination (average of available values for the respective decades). Next, we evaluated the clinical correlates of log-leptin using multivariable linear regression. Eligible covariates included age, sex, BMI, systolic and diastolic blood pressure, antihypertensive treatment, total-to-HDL cholesterol ratio, triglycerides, diabetes, smoking, and prevalent CVD.

Separate analyses were conducted for each of the three outcomes. Analysis demonstrated a striking sexual dimorphism in leptin levels, as reported previously (15). Therefore, we standardized log-leptin values separately within sex. We performed pooled sex analyses relating sex-standardized log-leptin to the incidence of outcome events because we did not observe effect modification by sex ($P \geq 0.75$ for interactions for all three outcomes).

We evaluated the cumulative incidence of outcome events for leptin levels greater than or equal to versus less than the sex-specific median value. The assumption of proportionality of hazards was confirmed. We used Cox regression to relate log-leptin to each outcome. Two sets of models were evaluated: 1) with adjustment for established risk factors; and 2) with additional adjustment for BMI. We built models using this hierarchical schema because leptin is strongly correlated with BMI and also to assess the incremental information provided by leptin over BMI alone. Covariates in the first model evaluating CHF incidence and mortality included age, sex, total/HDL cholesterol, diabetes, systolic and diastolic blood pressure, antihypertensive treatment, current smoking, history of atrial fibrillation, and prevalent CVD. For analyses of CVD incidence, covariates were similar with the exception that participants with prevalent CVD were excluded. To gain insights into potential nonlinearity of associations between lep-

tin and risk of outcomes, we examined generalized additive models using penalized splines.

We tested for effect modification by age, hypertension status, and obesity separately for each of the outcomes. In secondary analyses, we related leptin levels to incidence of a first hard CVD event on follow-up (covariates as noted above for analyses of CVD). In secondary analyses, we assessed the association of leptin with CHF, CVD, and mortality in individuals with and without a history of obesity (defined as BMI ≥ 30 kg/m² at any time in the 25 years preceding baseline examination cycle 22 at which leptin was assayed).

$P < 0.05$ was used to indicate statistical significance.

RESULTS— The baseline characteristics of our elderly study sample are shown in Table 1. Three-fifths of the sample comprised women, and there was a high prevalence of antihypertensive treatment, diabetes, and CVD.

Clinical correlates of leptin including relations to antecedent BMI

Leptin was strongly correlated with BMI at the index examination ($r = 0.67$, $P < 0.0001$). More modest correlations were noted with BMI in the decade preceding the index examination (average of readings obtained 1–10 years before baseline; $r = 0.60$, $P < 0.0001$) and with BMI 10–20 years before baseline ($r = 0.46$, $P < 0.0001$). Of note, BMI at the index examination was more strongly correlated (relative to leptin) with BMI in the previous decade ($r = 0.93$, $P < 0.0001$) and in the prior 10- to 20-year period ($r = 0.83$, $P < 0.0001$).

In multivariable analyses evaluating the clinical correlates of log-leptin, BMI (partial $R^2 = 0.26$) and sex (partial $R^2 = 0.28$) were the strongest correlates. Women (median 17.4 [quartile 1, quartile 3: 10.6, 28.7]) had higher leptin levels than men (7.2 [4.5, 11.4]; $P < 0.001$), as noted earlier. Positive associations of leptin were also noted with the ratio of total-to-HDL cholesterol (Table 2). Although participants with diabetes (13.4 [7.9, 23.2]) had higher leptin levels in unadjusted analyses than participants without diabetes (12.6 [6.5, 22.4]), these differences were no longer statistically significant upon adjustment for BMI.

Table 1—Baseline characteristics of the study sample

Characteristic	Value
Clinical features	
Women (%)	62.3
Age (years)	79.0 ± 4.5
Systolic blood pressure (mmHg)	143 ± 21
Diastolic blood pressure (mmHg)	72 ± 11
BMI (kg/m ²)	26.8 ± 4.7
Antihypertensive treatment (%)	48.9
Current smoking (%)	8.3
Diabetes (%)	27.4
History of atrial fibrillation (%)	10.6
Prevalent heart failure (%)	5.3
History of CVD (%)	35.0
Biochemical features	
Total-to-HDL cholesterol ratio (mg/dl)	4.54 ± 1.51
Leptin (ng/ml)	12.8 (7.0, 22.7)
Log-leptin	2.51 ± 0.81

Data are % for binary traits and means ± SD for continuous traits, except for leptin for which data are median (quartile 1, quartile 3). *n* = 818. Log, natural logarithmically transformed.

Association of leptin levels with incidence of CHF

During a mean follow-up of 8.5 years (range 0.2–10), 129 participants (73 women) developed CHF. Figure A1a (available in an online appendix at <http://care.diabetesjournals.org/cgi/content/full/dc08-1596/DC1>) shows that the cumulative incidence of CHF in individuals with leptin levels greater than or equal to the sex-specific median value was higher than that in individuals with leptin levels below the median (log-rank *P* = 0.038). In multivariable models without BMI, leptin was positively associated with incident CHF (Table 3, model I). Figure A1b (available in an online appendix) displays the results of splines relating leptin to CHF risk: a continuous gradient of increasing CHF risk is noted without evidence of a threshold. However, the association of leptin with CHF risk was rendered nonsignificant after additional adjustment for BMI (Table 3, model I).

Association of leptin with CVD and hard CVD

During a mean follow-up of 7.8 years, 187 participants experienced a first CVD event (121 women). Figure A2a (available in an online appendix) shows a higher cumulative incidence of CVD events in individuals with leptin levels greater than or equal to the sex-specific median compared with participants with levels below the median (log-rank *P* = 0.0030). Leptin was associated with CVD incidence in a multivariable-adjusted model without BMI, a finding confirmed by analysis of splines (Fig. A2b, available in an online appendix). However, the relations were attenuated, becoming borderline statistically significant, upon adjustment for BMI (Table 3, model II).

In secondary analyses, leptin levels were also associated with hard CVD events (141 events in, on average, 8.3 years of follow-up) in multivariable-adjusted models without BMI (hazard ratio [HR] 1.26 [95%

CI 1.04–1.52], *P* = 0.016). However, this association was rendered statistically nonsignificant upon additional adjustment for BMI (1.05 [0.83–1.33], *P* = 0.70).

Based on the width of the confidence intervals presented in Table 3, we can exclude (with 95% confidence) a >24% increase in the hazard of developing CHF and a >51% increase in the hazard of developing CVD per 1 SD increase in sex-standardized log-leptin in models assessing incremental contribution of leptin levels over BMI. More modest incremental associations (over BMI) of leptin with CHF and CVD outcomes would require analyses of much larger samples.

Association of leptin levels with total mortality

During a mean follow-up of 8.0 years, 391 participants (217 women) died. The cumulative incidence of death in participants did not seem to differ for participants with leptin levels more than or equal to versus less than the sex-specific median (log-rank *P* = 0.55) (Fig. A3a, available in an online appendix). Overall, leptin levels were not related to total mortality in multivariable models without (HR 1.00 [95% CI 0.89–1.12]) and with (0.97 [0.84–1.12]) adjustment for BMI. Analyses of splines revealed a U-shaped relation of log-leptin levels with mortality (Fig. A3b, available in an online appendix). Adding log-leptin squared into the model confirmed a significant association with log-leptin squared and mortality in multivariable models without (*P* = 0.002) and with (*P* = 0.005) adjustment for BMI. Given the U-shaped relation of log-leptin and mortality, we repeated the analysis excluding participants who died within the first year of follow-up (*n* = 19) to exclude the possibility that the association between lower leptin levels and mortality may be due to reverse causality (i.e., comorbidity leads to low leptin levels and is associated with higher mortality). Our results were essentially unchanged in these analyses (*P* = 0.0099 for log-leptin squared).

To further explore the contributions of cardiovascular versus noncardiovascular mortality to the U-shaped relations, in post hoc analyses we examined the two outcomes separately. On follow-up, 133 participants died of CVD and 258 of non-CVD. In multivariable-adjusted models including BMI, log-leptin squared was not related to CVD death (*P* = 0.70) but was associated with non-CVD death (*P* = 0.006).

Table 2—Clinical determinants of log-leptin in elderly individuals

Variable	β	SE	Partial R ²	<i>P</i> value
Age	−0.007	0.004	0.002	0.071
Male sex	−0.918	0.037	0.282	<0.0001
BMI	0.094	0.004	0.257	<0.0001
Total cholesterol-to-HDL ratio	0.042	0.012	0.005	0.001
History of CVD	0.070	0.038	0.002	0.067
Antihypertensive treatment	0.064	0.036	0.001	0.080
Current smoking	−0.119	0.065	0.002	0.067

The regression coefficient indicates the increase in log-leptin per 1-unit increment in the predictor variable, meaning that, e.g., a 1-unit increment in BMI leads to a 0.094-unit increase in log-leptin (1.10 in nanograms per milliliter).

Table 3—Association of standardized log-leptin with incident outcome events: multivariable Cox regression models without and with adjustment for BMI

Model	HR per SD increment in log-leptin (95% CI)	P value
I. Incidence of CHF (<i>n</i> = 775 at risk)		
A. Adjusting for established risk factors*	1.26 (1.03–1.55)	0.024
B. Model A and BMI	0.97 (0.75–1.24)	0.79
II. Incidence of CVD (<i>n</i> = 532 at risk)		
A. Adjusting for established risk factors†	1.28 (1.09–1.50)	0.003
B. Model A and BMI	1.23 (1.00–1.51)	0.052

SD of log-leptin in men = 0.67 and in women = 0.70. *Models adjusted for age, sex, systolic and diastolic blood pressure, hypertension treatment, ratio of total-to-HDL cholesterol, diabetes, smoking, history of atrial fibrillation, and prevalent CVD (including myocardial infarction). †Models adjusted for age, sex, systolic and diastolic blood pressure, hypertension treatment, ratio of total-to-HDL cholesterol, diabetes, smoking, and history of atrial fibrillation.

Secondary analyses

In exploratory analyses, associations of leptin with CHF and CVD were similar in participants with (*n* = 270 [33%]) and without (data not shown) a history of obesity. Likewise, a U-shaped association of leptin with mortality was observed in both subgroups.

CONCLUSIONS

Principal findings

First, higher circulating leptin levels were associated with female sex and BMI. Second, leptin levels were positively associated with incident CHF and CVD in multivariable-adjusted models that did not adjust for BMI. Upon additional adjustment for BMI, the association with CHF was rendered nonsignificant, and the association with CVD was borderline significant (*P* = 0.052). Third, we observed a U-shaped relation of leptin with total mortality driven primarily by an association of leptin with non-CVD mortality. Finally, although leptin displayed strong positive correlations with recent and remote BMI, current BMI displayed stronger correlations with past BMI (relative to leptin). These data indicate that current BMI provides reasonable information about the weight history of individuals, and leptin may not provide incremental information over BMI.

The attenuation of the relations of leptin to the incidence of CHF and CVD upon adjustment for BMI can be interpreted in several ways. Leptin may not offer incremental prognostic information because of its strong correlation with BMI. It is also conceivable that leptin is along the causal pathway from excess adiposity to CVD/CHF. Indeed, an extensive body of literature supports a pathogenic role for leptin in

cardiovascular remodeling. Thus, the attenuation of the relations of leptin to CVD upon adjustment for BMI may not exclude an important pathogenic role for this adipokine. Another possible interpretation is that covariates in the models (total-to-HDL cholesterol ratio, diabetes, and blood pressure) may mediate some of the effects of leptin on CVD risk.

The weaker association of leptin with CHF and CVD (relative to BMI) merits discussion. Most adults in developed countries gain weight until their 60s and then begin to lose weight. In humans, obesity occurs despite higher levels of leptin because of relative leptin resistance that prevents the anorexic effects of this hormone. Leptin resistance persists after weight loss, so that levels may remain relatively elevated compared with those in weight-stable control subjects. Nevertheless, when we measured leptin in elderly adults who are already past their peak adult weight, it is likely that the plasma leptin in many of them is spuriously elevated compared with their “true” biological state. In contrast, BMI reflects current weight and is thus less likely to misclassify elderly adults in terms of their physiological obesity state. Also, our results underscore the fact that BMI remains an important predictor of CVD, notwithstanding the pathogenic importance of adipokines, including leptin.

Clinical correlates of leptin: comparison with the published literature

Our observations that BMI and female sex are principal determinants of leptin are consistent with findings in the literature (15,16). Women have greater body fat than men, and adipose tissue is a major source of leptin, which might explain the

higher leptin levels in women. It has been suggested that leptin captures the weight history of individuals (12). We observed that leptin correlated with the mean BMI averaged over 0–10 and 10–20 years before the baseline examination. However, leptin was not as strongly correlated with past weight history as current BMI.

Association of leptin levels with CHF

In previous cross-sectional studies, increased levels of leptin and its receptor were reported in patients with CHF (17,18). Furthermore, increasing evidence indicates that leptin may modulate cardiac remodeling (3). In experimental studies, leptin exhibited a dose-dependent inhibition of myocyte shortening in ventricular myocytes (19). Likewise, leptin had a direct hypertrophic effect on cultured myocytes (6). However, some investigators have highlighted an antihypertrophic effect of leptin (7,20). These findings suggest that leptin has a cardioprotective role and that increased levels in patients with cardiac disease might be a response to cardiac injury or failure rather than being directly causal.

It is difficult to conclude from the positive relations of leptin to CHF incidence that leptin is an important mediator of disease (1) or whether higher levels are a compensatory response to underlying cardiac remodeling. In our analysis, leptin did not add incremental predictive information beyond current BMI.

Association of leptin levels with CVD and mortality

Clinical data on the association of leptin with CVD are inconsistent. In some studies, leptin was associated with incident myocardial infarction or coronary artery disease (10,21), whereas other investigators did not find an association between leptin and CHD (11).

Experimental data on leptin and vascular remodeling are likewise controversial, with some studies suggesting an atherogenic effect (4,5) and other reports indicating a vasculoprotective effect (9,22). In our analyses, leptin was positively associated with CVD only in models that did not include adjustment for BMI. These data are consistent with the concept that leptin may be an important mediator of obesity-associated CVD risk, but it offers no incremental prognostic information over BMI.

We observed a U-shaped relation between leptin levels and total mortality, with low and high levels being associated

with greater mortality. Such a U-shaped relation has been described for BMI and mortality (23,24). Additional analyses confirmed that the U-shaped relation was largely driven by the association of lower and higher leptin levels with noncardiovascular mortality. It is conceivable that low and high leptin levels are a marker of greater risk of death due to noncardiovascular causes (such as cancer). Such an association would parallel the known association of both low and high BMI with noncardiovascular mortality, including that due to cancer (23,25). However, the association of leptin with mortality remained U-shaped after the analyses was restricted to individuals who survived the first year of follow-up, suggesting that the results are not confounded by greater mortality risk in individuals with comorbidities and low leptin levels.

Limitations

We must acknowledge the limitations of our approach. Leptin levels were determined on nonfasting blood samples, and diurnal variations in concentrations may result in random misclassification, which may have biased our results toward the null hypothesis of no association between leptin and the outcomes evaluated. Our data were obtained in an elderly sample of white individuals of European ancestry. Thus, the generalizability of our data to other age-groups or ethnicities is unknown.

In summary, we observed that leptin is associated positively with incident CHF and CVD. However, adjustment for BMI attenuated these associations, suggesting that leptin does not offer incremental prognostic information over BMI. The relationship of leptin with mortality was U-shaped. Additional studies are warranted to confirm our findings.

Acknowledgments—This work was supported through National Institutes of Health/National Heart, Lung, and Blood Institute Contracts N01-HC25195, N01-HV28178, K24-HL04334, and R01-DK080739. Contract N01-HC25195 funded the performance of examinations at which leptin levels were obtained. The other grants supported the interpretation of data and preparation of the manuscript.

No potential conflicts of interest relevant to this article were reported.

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