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Effect of increased leptin and C-reactive protein levels on mortality: Results from the National Health and Nutrition Examination Survey

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ABSTRACT

Objective: Leptin and C-reactive protein (CRP) have each been linked to adverse cardiovascular events, and prior cross-sectional research suggests that increased levels of both biomarkers pose an even greater risk. The effect of increased levels of both leptin and CRP on mortality has not, however, been previously assessed.

Methods: We used data from the third National Health and Nutrition Examination Survey (NHANES III) to estimate the mortality effect of high leptin and high CRP levels. Outcomes were compared with the use of inverse-probability-weighting adjustment. Among 6259 participants included in the analysis, 766 were in their sex-specific, population-weighted highest quartiles of both leptin and CRP. Median follow-up time was 14.3 years.

Results: There was no significant difference in adjusted all-cause mortality between the groups (risk ratio 1.22, 95% confidence interval [CI], 0.97–1.54). Similar results were noted with the use of several different analytic methods and in many subgroups, though high leptin and CRP levels may increase all-cause mortality in males (hazard ratio, 1.80, 95% CI, 1.32–2.46; P for interaction, 0.011). A significant difference in cardiovascular mortality was also noted (risk ratio, 1.54, 95% CI, 1.08–2.18), though that finding was not confirmed in all sensitivity analyses..

Conclusions: In this observational study, no significant difference in overall all-cause mortality rates in those with high leptin and high CRP levels was found, though high leptin and CRP levels appear associated with increased mortality in males. High leptin and CRP levels also likely increase risk for cardiovascular death..

1. Introduction

Leptin is a polypeptide hormone involved in body weight regulation and insulin homeostasis [1–3]. High leptin levels are associated with hypertension and diabetes mellitus [4–6] as well as an increased risk for endothelial dysfunction [7–9]. Leptin may adversely affect vascular wall lipid metabolism [10] and may also increase the risk for adverse cardiovascular events [11–15]. CRP is an acute-phase reactant shown to be a sensitive, non-specific marker of systemic inflammation [16] and thought to be both maker and marker of the chronic arterial inflammation underlying atherosclerosis [17,18]. CRP is associated with traditional cardiovascular risk factors including obesity [19,20]; meta-analysis confirms it independently predicts adverse cardiovascular events. [21].

Leptin and CRP may interact in an important, yet undetermined, manner that adversely influences cardiovascular and all-cause mortality risk. Prior research suggests that leptin may induce expression of CRP [22], and research supports a high correlation between the two biomarkers [23–25]. A prior study suggested that increased levels of both leptin and CRP was significantly associated with increased rates of cardiovascular disease beyond that of CRP alone [26], though its findings were limited by its cross-sectional study design.

It thus remains undetermined whether an association between increased levels in both leptin and CRP are of prognostic

Abbreviations: BMI, body-mass index; CDC, Centers for Disease Control and Prevention; CRP, C-reactive protein; ICD, International Classification of Disease; IPW, inverse probability weighting; NCHS, National Center for Health Statistics; NDI, National Death Index; NHANES, National Health and Nutrition Examination Survey.

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significance. To evaluate the potential prognostic value of high leptin and high CRP levels, we assessed prospectively collected data with long-term mortality follow-up using NHANES III.

2. Methods

2.1. Study population

From 1988 to 1994, the National Center for Health Statistics (NCHS) conducted NHANES III, a cross-sectional, stratified, multistage survey representative of the non-institutionalized U.S. population. Details of NHANES methodology have been previously described [27]. In brief, the survey includes questionnaire, physical exam, and laboratory evaluation components. Individuals' mortality status was subsequently determined through the National Death Index (NDI) [28]. The study complies with the Declaration of Helsinki. NHANES III protocols were approved by the NCHS and Centers for Disease Control and Prevention (CDC) Institutional Review Board. All participants gave written, informed consent.

There were 6415 NHANES III adult subjects ≥20 years of age with available leptin data. Of these, individuals were excluded if they had missing CRP values ($n = 48$), were pregnant ($n = 103$), or had missing mortality data ($n = 5$). The final sample consisted of 6259 participants.

2.2. Baseline measures

Fasting morning serum leptin concentrations were collected and stored at −70 °C for an average of 8 years [29]. Leptin levels were assessed by radioimmunoassay with a polyclonal antibody raised in rabbits against highly purified recombinant human leptin. The minimal detectable concentration of this assay was 0.5 mg/l and the limit of linearity was 100 mg/l. Intrassay and interassay coefficients of variation were both less than 5% [29]. CRP levels in serum were measured within 2 months of sample collection, with a modified Behring latex-enhanced CRP assay [30].

2.3. Mortality follow-up

Mortality status was based upon a NCHS search of the NDI through the year 2006. Details of the methodology are available elsewhere [28]. In this analysis, we assessed deaths due to all causes and deaths due to cardiovascular disease (i.e., ICD-10 codes I00 to I99). Follow-up duration was calculated from the date of examination to either the date of death or to 31 December 2006.

2.4. Adjustment for differences between groups

Based on individuals' baseline characteristics, propensity scores were developed with the use of logistic regression to estimate the probability individuals were in the highest sex-specific, population-weighted quartiles of both leptin and CRP levels [31], the same metric assessed in prior cross-sectional research [26]. Continuous variables were modeled as a flexible polynomial with linear and quadratic components. Included in the propensity model were patients' age, gender, race, body-mass index (BMI), waist circumference, smoking status, diabetes, hypertension, dyslipidemia, angina, chronic kidney disease (i.e., an glomerular filtration rate (GFR) < 60 ml/min/1.73 m^2 as calculated by the Modification of Diet in Renal Disease equation [32]), and a family history of coronary artery disease, as well as a self-reported personal history of cerebrovascular disease, chronic lung disease (i.e., asthma, chronic bronchitis, or emphysema), intermittent claudication, heart failure, or myocardial infarction. Diabetes mellitus was defined as a self-reported history of diabetes, a fasting plasma glucose value ≥7.0 mmol/l (≥126 mg/dl), or a hemoglobin $A_{1C} \geq 6.5$ [33]. Intermittent claudication, following prior literature [34], was defined as self-reported leg pain on walking quickly or uphill that was relieved by rest. Angina was defined using standard criteria from the Rose angina questionnaire [35] and categorized as non-severe (Grade I) and severe (Grade II). Patients were classified as having hypertension if they reported having ever been diagnosed by a physician, whether they reported having ever taken blood pressure medication, or whether the average of up to three blood pressure measurements ≥140 mmHg for systolic blood pressure or ≥90 mmHg for diastolic blood pressure. Dyslipidemia was recorded if participants reported that a doctor had ever told them they had high cholesterol, they used cholesterol-lowering medications, had HDL cholesterol levels <40 mg/dl in men or <50 mg/dl in women, or had LDL cholesterol levels above ≥160 mg/dl.

Missing data were singly imputed using an expectation-maximization algorithm [36]. Most variables had <1% missing data though 2.9% had missing waist circumference measurements and 2.7% had missing LDL data. Stabilized inverse probability weights (IPW) calculated from the propensity score were then used to adjust for differences between the two treatment groups [37]. Performance of the propensity model was verified by comparing the distribution of covariates and propensity scores between treatment groups before and after IPW.

2.5. Statistical analyses

Data were summarized as percentages in the case of categorical variables and as means with standard deviations in the case of continuous variables. χ^2 and Wald tests were used to assess differences in baseline characteristics between groups for categorical and continuous variables, respectively.

Endpoints assessed all-cause mortality and cardiovascular mortality, as defined above, separately. The Kaplan–Meier method was used to estimate unadjusted survival curves [38]. Adjusted survival curves employed IPW and represent the expected survival rate were all individuals to have had or not had both high leptin and CRP levels [39]. Risk ratios at five-year intervals were calculated using estimated rates of survival using binomial log-linear regression [40], with 95% confidence intervals calculated by bootstrapping. The comparison between those with and without both high leptin and high CRP was performed in the overall sample and in prospectively defined subgroups.

Sensitivity analyses were performed to assess the robustness of results. Survival curves were reestimated separately using Cox proportional-hazard models without propensity scores but using covariates identical to those used in the propensity model. In addition, we performed sensitivity analyses that combined IPW and model-based approaches in a "doubly robust" approach [41]. The proportional hazards assumption in the Cox models was assessed with Schoenfeld residuals. All analyses were performed using Stata 11.2.

3. Results

3.1. Characteristics of the study sample

After the exclusion criteria were applied, 6259 participants were included in the analyses. Table 1 shows baseline participant characteristics. Before adjustment with IPW, patients with high leptin and CRP levels, as compared to those without such levels, were, on average, more likely to be older and obese. Participants with high leptin and high CRP levels were also more likely to have suffered from kidney, lung, and cardiovascular diseases including CHF, MI,