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Leptin and Coronary Heart Disease

Prospective Study and Systematic Review

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Objectives This study sought to better determine the link between leptin and coronary heart disease (CHD).

Background Circulating leptin is considered a risk factor for CHD but larger studies are needed.

Methods Leptin levels were measured in 550 men with fatal CHD or nonfatal myocardial infarction and in 1,184 controls nested within a prospective study of 5,661 British men and set in context with a meta-analysis.

Results Baseline leptin correlated with body mass index (BMI), blood pressure, total cholesterol, triglyceride, and inflammatory markers; correlations persisted after BMI adjustment. The within-person consistency of leptin values over 4 years (correlation coefficient: 0.79; 95% confidence interval [CI]: 0.73 to 0.83) was higher than those of some established cardiovascular risk factors. In a comparison of individuals in the top third with those in the bottom third of baseline leptin, the age- and town-adjusted odds ratio for CHD was 1.25 (95% CI: 0.96 to 1.62), decreasing to 0.98 (95% CI: 0.72 to 1.34) after adjustment for BMI. A systematic review identified 7 prospective reports with heterogeneous findings (I^2 = 60%, 13% to 82%). The combined adjusted risk ratio across all studies was 1.44 (95% CI: 0.95 to 2.16) in a comparison of extreme thirds of leptin levels. The inconsistency between studies was partially explained by sample size, with combined estimates from studies involving >100 CHD cases (1.28, 95% CI: 0.80 to 2.04) being somewhat weaker than those from smaller studies (1.81, 95% CI: 0.76 to 4.31).

Conclusions Previous studies appear to have overestimated associations of leptin and CHD risk. Our results suggest a moderate association that is largely dependent on BMI. (J Am Coll Cardiol 2009;53:167–75) © 2009 by the American College of Cardiology Foundation

There has been considerable interest in the potential relevance of leptin to human metabolism and disease following the discovery that the adipocyte *ob* gene in mice encodes this secreted protein to help regulate body weight (1). Human leptin concentrations increase in obesity and correlate strongly with percentage body fat in both men and women (2), encouraging suggestions that leptin levels are adiposity signals for the long-term regulation of body weight by the brain. The possibility that leptin levels might also be relevant to vascular disease has been raised by animal experiments suggesting that leptin promotes atherosclerosis and thrombosis in apolipoprotein E-deficient mice (3) and by clinical studies reporting correlations of leptin levels with established vascular risk factors (such as lipid concentrations and blood pressure) (4–7), or with markers of impaired fibrinolysis (8), vascular dysfunction (9), and inflammation (10–12).

Prospective data on circulating leptin levels and incident coronary heart disease (CHD) risk are, however, sparse. By mid-2007, 7 prospective studies (13–19) had reported on this association. The largest such study, the WOSCOPS (West of Scotland Coronary Prevention Study) nested case-control study of 377 male cases and 783 controls, reported about a 20% increase in CHD per 1 standard

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Abbreviations and Acronyms

BMI = body mass index

CHD = coronary heart disease

deviation increase in leptin levels after adjustment for several established cardiovascular risk factors (15). We measured circulating leptin concentrations in stored serum samples of 550 men who suffered nonfatal myocardial infarction or coronary death and 1,184 controls who remained disease free during a mean of 16 years of follow-up in the prospective BRHS (British Regional Heart Study) of 5,661 men ages 40 to 59 years from 18 British towns. In addition to its larger number of incident CHD cases than in previous reports, the present study also recorded paired leptin measurements made 4 years apart in 219 men, enabling quantification of the within-person fluctuations in leptin values. To help place our new data in context, we conducted a systematic review and meta-analysis of previous prospective reports of leptin and CHD (13–19), involving a total of 1,335 CHD cases and 3,407 controls.

Methods

Participants. From 1978 to 1980, 7,735 men were randomly selected from general practices in each of 24 British towns and invited to take part in the BRHS (response rate: 78%). Nurses administered questionnaires, made physical measurements, recorded an electrocardiograph, and in 5,661 men in 18 of the towns, collected nonfasting venous blood samples from which serum was stored at −20°C for subsequent analyses. Evidence of coronary disease at entry to the study was defined by the presence of either recall of any doctor diagnosis of CHD and/or electrocardiographic evidence of possible or definite myocardial infarction or ischemia. All men were followed up for all cause mortality and cardiovascular morbidity, and follow-up has been achieved for 99% of the cohort (20). A "nested" case-control study was established within the cohort. Eligible cases were 279 men who died from CHD and 364 men who had nonfatal myocardial infarction before 1996 (21). Fatal cases were ascertained through National Health Service Central Registers on the basis of a death certificate with International Classification of Diseases-Ninth Revision codes 410 to 414. Nonfatal myocardial infarction was based on reports from general practitioners, supplemented with hospital records confirming the diagnosis in accordance with World Health Organization criteria (22). Cases were frequency matched with 1,278 controls, on town of residence and age in 5-year bands, randomly selected from among men who did not record incident CHD by 1996. Due to limited blood sample availability, leptin measurements were available for 550 (86%) CHD cases and 1,184 controls (93%). However, the characteristics of cases and controls with available blood samples did not differ from those without blood samples.

Laboratory methods. Laboratory measurements were made blind to participants' disease status, with samples from patients and controls randomly distributed among assay plates. Serum leptin was measured by an in-house radioimmunoassay validated thoroughly against the commercially available Linco assay (St. Charles, Missouri) (2). The intra-assay and interassay coefficients of variation were <7% and <10%, respectively, over the sample concentration range. The detection limit of the assay was 0.5 ng/ml, which is substantially lower than detection limits typically observed with commercially available assays (2). Because of fluctuations of leptin levels over time, case-control comparisons of single measurements made at baseline can underestimate the magnitude of any associations with CHD risk. Hence, leptin measurements were made in pairs of samples collected at an interval of 4 years apart in 219 healthy control individuals to help quantify the within-individual variation (23). Methods for the measurement of other risk factors in the BRHS and their association with CHD risk have been reported previously (24–26).

Statistical analyses. We pre-specified that case-control analysis would be based on comparison of extreme thirds of leptin values in controls. Distributions of leptin, triglyceride, C-reactive protein, fibrin D-dimer, and adiponectin were highly positively skewed and geometric means of these measures were used in descriptive analyses and natural logarithmic transformations in regression analyses. Hypotheses of equal cases' and controls' means, proportions, and distributions were examined by the *t*, likelihood ratio, and Kruskal-Wallis tests, respectively (Table 1). Analysis of covariance was used to obtain mean levels of continuous established risk factors by thirds of leptin distribution in controls, adjusted for age and then age and body mass index (BMI) (Table 2); for dichotomous variables, logistic regression was used to provide tests for trend across the 3 leptin groups adjusted for age and BMI. Unconditional logistic regression models were used to calculate odds ratios for the association of leptin with CHD, with progressive adjustment for possible confounding factors (Table 3). Smoking status was grouped into 4 categories: never smoked, ex-smokers, ≤20 cigarettes/day, and >20 cigarettes/day; physical activity into 3 categories; alcohol intake into 6 categories: never drank or monthly/special, weekends 1 to 2 drinks, weekends 3 to 6 drinks, weekends 6+ drinks, daily 1 to 2 drinks, daily 3 to 6 drinks, and daily 6+ drinks; and social class into 3 categories: manual, nonmanual, and special forces; age, BMI, and blood measurements were fitted as continuous values if not stated otherwise. Finally, "town" was fitted as a categorical variable in the fixed-effect model.

A systematic review of studies published before May 2007 with over 1 year's follow-up was conducted using search, abstraction, and data synthesis methods previously described (27) using (where possible) nonfatal

Table 1 Baseline Characteristics of Participants in BRHS

Characteristic	CHD Cases (n = 550)	Controls (n = 1,184)	p Value
Questionnaire-based			
Age, yrs	52.6 (5.2)	52.5 (5.3)	Matched
Current cigarette smoker, n (%)	285 (52.0%)	502 (42.5%)	<0.001
Evidence of coronary disease at baseline, n (%)	180 (32.8%)	205 (17.4%)	<0.001
History of diabetes at baseline,* n (%)	15 (2.7%)	19 (1.6%)	0.124
Regular daily consumption of alcoholic drinks,† n (%)	106 (19.2%)	268 (22.8%)	0.090
Nonactive, n (%)	313 (67.0%)	637 (60.8%)	0.021
Nonmanual occupation, n (%)	190 (34.6%)	475 (40.0%)	0.031
Biophysical			
Body mass index, kg/m^2	25.9 (3.4)	25.4 (3.3)	0.001
Systolic blood pressure, mm Hg	151.9 (22.0)	146.7 (21.1)	<0.001
Diastolic blood pressure, mm Hg	85.7 (13.6)	82.8 (13.3)	<0.001
Blood-based			
Total cholesterol, mmol/l	6.6 (1.05)	6.2 (0.99)	<0.001
HDL cholesterol, mmol/l	1.09 (0.27)	1.15 (0.27)	<0.001
Triglyceride,‡ mmol/l	1.94 (1.37–2.78)	1.68 (1.14–2.42)	<0.001
C-reactive protein,‡ mg/l	2.4 (1.2–5.3)	1.4 (0.5–3.4)	<0.001
White cell count, 10^9 cells/l	7.6 (1.8)	7.2 (1.8)	<0.001
Albumin, g/l	44.4 (2.6)	44.5 (2.5)	0.486
Fibrin D-dimer,‡ ng/ml	92 (49–145)	77 (42–121)	<0.001
tPA, ng/ml	13.1 (6.0)	11.5 (5.6)	<0.001
von Willebrand factor, IU/dl	118.0 (41.6)	112.4 (43.5)	0.011
Adiponectin,‡ ng/ml	10.2 (7.3–13.9)	10.3 (7.3–14.9)	0.626
Leptin,‡ ng/ml	4.7 (3.1–8.8)	4.3 (2.6–8.3)	0.067

Data are presented as mean (SD) unless otherwise stated. *Based on self-report. †Regular daily consumption of alcoholic drinks was defined as >2 drinks per day. ‡Geometric mean (interquartile range).

BRHS = British Regional Heart Study; CHD = coronary heart disease; HDL = high-density lipoprotein; tPA = tissue plasminogen activator.

myocardial infarction or CHD death as end points. Corresponding investigators of all identified studies were contacted to provide supplementary tabular data (6 of the 7 contacted investigators responded). Analyses involved only within-study comparisons to limit possible biases. Summary risk ratios were calculated using a random effects model. Heterogeneity was assessed by standard chi-square tests and the I^2 statistic, with its 95% confidence interval (CI), which describes the percentage of variation in the logarithmic odds ratios that is attributable to genuine differences across studies rather than random error (28). Evidence of publication bias was assessed using the Egger test and by comparing pooled results from studies involving at least 100 CHD cases with those from smaller studies. Odds ratios are given with 95% CIs, and 2-sided probability values are used. All analyses were performed using Stata Statistical Software, Release 10 (StataCorp LP, College Station, Texas).

Results

Associations of leptin levels with baseline characteristics. As expected, established cardiovascular risk factors showed highly statistically significant differences between cases and controls (Table 1) (24,29,30). Leptin levels were slightly higher in cases than in controls, but the difference was of borderline statistical significance (p = 0.067). Among controls, there were statistically significant positive associations of leptin levels with BMI, total cholesterol, systolic and diastolic blood pressure, triglyceride and several inflammatory markers, and inverse associations with smoking, after adjustment for age alone (Table 2). Leptin levels were not associated with alcohol intake or baseline history of CHD or diabetes. Further adjustment for BMI attenuated associations of leptin levels with systolic blood pressure and high-density lipoprotein cholesterol, but associations with smoking, social class, total cholesterol, triglyceride, diastolic blood pressure, and C-reactive protein remained highly statistically significant. Among inflammatory markers, leptin levels were positively associated with levels of C-reactive protein, serum amyloid A, albumin, tissue-plasminogen activator antigen, and von Willebrand factor; no appreciable adjusted associations were observed with white cell count, D-dimer, or adiponectin levels. Further adjustment for smoking did not materially alter these cross-sectional associations (data available upon request).

Within-person variation in leptin levels. In an analysis of paired leptin measurements made in 219 participants approximately 4 years apart, the within-person correlation coefficient was 0.79 (95% CI: 0.73 to 0.83): somewhat

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Table 2 Baseline Characteristics Among Controls Across Thirds of Leptin Levels

	Bottom Third (n = 395)	Middle Third (n = 396)	Top Third (n = 393)	p Value for Trend (Adjusted for Age)	p Value for Trend (Further Adjusted for BMI)
Leptin range, ng/ml	≤3.24	3.24–7.03	>7.03		
Questionnaire-based					
Age, yrs	52.3 (0.3)	52.2 (0.3)	53.0 (0.3)	0.061	0.150
Current cigarette smoker, n (%)	213 (53.9%)	158 (40.0%)	131 (33.3%)	<0.001	<0.001
Evidence of coronary disease at baseline, n (%)	69 (17.5%)	62 (15.7%)	74 (18.9%)	0.882	0.216
History of diabetes at baseline, n (%)	4 (1.0%)	11 (2.8%)	4 (1.1%)	0.957	0.378
Consumption of >2 alcoholic drinks/day, n (%)	83 (21.0%)	88 (22.3%)	97 (24.6%)	0.174	0.530
Nonmanual occupation, n (%)	101 (25.6%)	167 (42.3%)	175 (44.4%)	<0.001	<0.001
Nonactive, n (%)	237 (31.3%)	247 (32.6)	273 (36.1%)	0.012	0.004
Biophysical					
Body mass index, kg/m^2	23.1 (0.1)	25.4 (0.1)	27.7 (0.1)	<0.001	NA
Systolic blood pressure, mm Hg	143.1 (1.0)	145.5 (1.0)	151.5 (1.0)	<0.001	0.312
Diastolic blood pressure, mm Hg	77.8 (0.6)	83.1 (0.6)	87.6 (0.6)	<0.001	<0.001
Blood-based					
Total cholesterol, mmol/l	5.87 (0.05)	6.27 (0.05)	6.43 (0.05)	<0.001	<0.001
HDL cholesterol, mmol/l	1.20 (0.01)	1.14 (0.01)	1.12 (0.01)	<0.001	0.557
Log$_e$ triglyceride, mmol/l	0.29 (0.03)	0.57 (0.03)	0.70 (0.03)	<0.001	<0.001
Log$_e$ C-reactive protein, mg/l	0.03 (0.07)	0.35 (0.07)	0.67 (0.07)	<0.001	0.003
Log$_e$ serum amyloid A, mg/l	1.78 (0.8)	1.96 (0.67)	2.07 (0.56)	<0.001	0.002
White cell count, 10^9 cells/l	7.3 (1.9)	7.1 (1.7)	7.2 (1.7)	0.584	0.516
Albumin, g/l	44.1 (2.5)	44.6 (2.5)	44.8 (2.4)	<0.001	0.009
Log$_e$ fibrin D-dimer, ng/ml	4.37 (0.86)	4.31 (0.91)	4.35 (0.90)	0.616	0.615
tPA, ng/ml	9.1 (4.8)	11.3 (4.7)	14.3 (6.0)	<0.001	<0.001
von Willebrand factor, IU/dl	108.8 (42.1)	109.9 (42.8)	118.5 (45.1)	<0.001	<0.001
Log$_e$ adiponectin, ng/ml	2.39 (0.52)	2.32 (0.56)	2.28 (0.54)	0.001	0.236

Data are presented as mean (SD) unless otherwise stated.
BMI = body mass index; NA = not available; other abbreviations as in Table 1.

higher than corresponding coefficients observed in these men over the same interval for levels of total cholesterol (0.71; 95% CI: 0.58 to 0.81) and systolic blood pressure (0.59; 95% CI: 0.48 to 0.70) (31).

Leptin levels and CHD risk. In a comparison of men in the top third of measured baseline control leptin values with those in the bottom third, the age and town adjusted odds ratio of CHD was 1.25 (95% CI: 0.96 to 1.62) (Table 3). Further adjustment for BMI attenuated the odds ratio to 0.98 (95% CI: 0.72 to 1.34), and after additional adjustment for other established cardiovascular risk factors the odds ratio was 0.95 (95% CI: 0.65 to 1.37). When fitted as a linear continuous variable, the logarithm of leptin levels was not significantly associated with CHD risk, even in the age- and town-adjusted model (p = 0.112). Exclusion of men with pre-existing CHD or diabetes made no material difference to the findings (Table 3), and there was no important difference in the magnitude of the association in

Table 3 Relative Odds of CHD Among Participants Who Had Leptin Levels in the Top Third of the Distribution of Values for Controls Versus Those Who Had Values in the Bottom Third of the Same Distribution*

	CHD Cases			Controls			Odds Ratio (95% CI) p Value for Trend Across Thirds		
	Top Third	Middle Third	Bottom Third	Top Third	Middle Third	Bottom Third	Adjusted for Age and Town	Adjusted for Age, Town, and BMI	Adjusted for Age, Town, BMI, and Other Potential Confounders†
All men‡	188	211	151	393	396	393	1.25 (0.96–1.62) 0.11	0.98 (0.72–1.34) 0.85	0.95 (0.65–1.37) 0.73
Excluding men with evidence of prevalent CHD at baseline	114	142	112	317	333	326	1.04 (0.76–1.42) 0.80	0.91 (0.63–1.32) 0.61	0.79 (0.51–1.23) 0.29
Excluding men with evidence of prevalent diabetes at baseline	181	208	146	389	385	390	1.24 (0.95–1.62) 0.13	0.94 (0.69–1.30) 0.65	0.93 (0.64–1.36) 0.65

*Cut points for bottom and top thirds leptin were 3.24 and 7.03 ng/ml, respectively. †Other potential confounders included total cholesterol, HDL cholesterol, triglyceride, smoking status, systolic blood pressure, alcohol consumption, physical activity, and social class. ‡The adjusted odds ratios for CHD in the British Regional Heart Study with the use of alternative comparisons were as follows: 1.18 (95% CI: 0.86 to 1.62) for the top third as compared with the middle third of the distribution, and 0.98 (95% CI: 0.92 to 1.03) for a 50% increase in baseline leptin levels.
CI = confidence interval; other abbreviations as in Table 1.

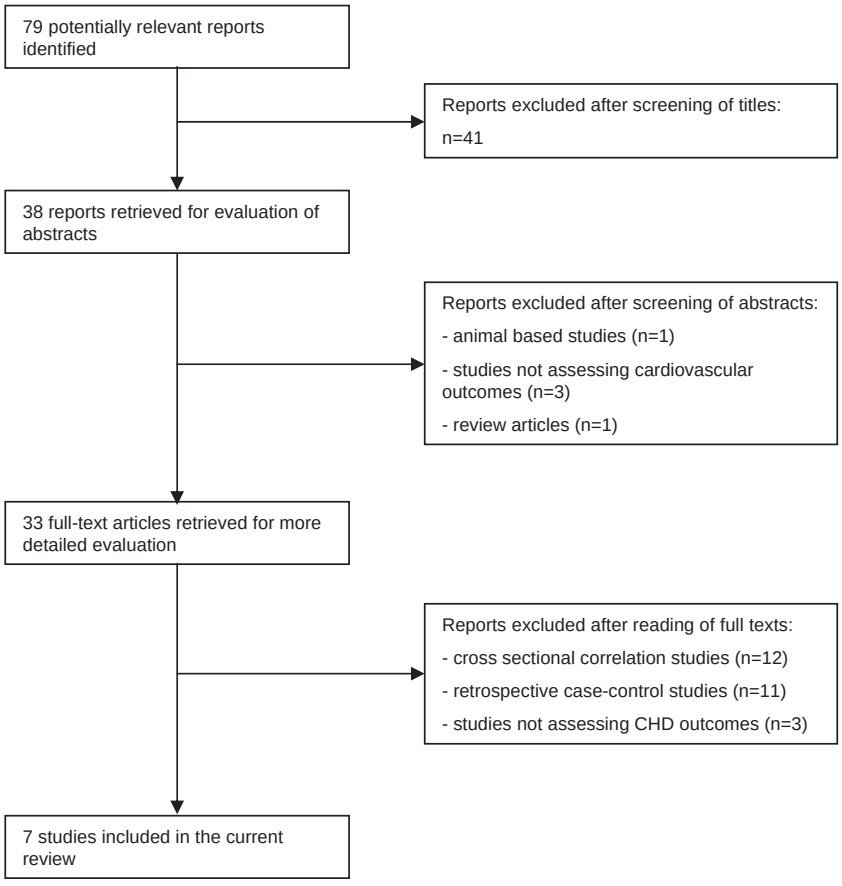

Figure 1 **Quality of Reporting of Meta-Analyses Flow Diagram**

The diagram summarizes the search strategy used to identify prospective studies of circulating lipid levels and coronary heart disease (CHD) outcomes.

smokers and nonsmokers ($p = 0.14$ in a test for interaction). Results were similar in analyses of extreme fifths of leptin levels.

Systematic review and meta-analysis of available prospective studies. We identified 8 relevant published prospective studies (including the current study) that reported on circulating leptin levels and CHD risk (Fig. 1), involving a total of 1,335 cases and 3,407 controls (weighted mean age at entry: 55 years; weighted mean follow-up: 10 years) (Table 4). One study did not report risk estimates for CHD that had been adjusted for age and sex only. There was evidence of substantial heterogeneity among these studies of risk estimates adjusted for age and sex only (chi-square test with 6 degrees of freedom for heterogeneity = 30.38, $p < 0.001$; $I^2 = 80\%$ [60% to 90%]), and of risk estimates further adjusted for established cardiovascular risk factors (chi-square test with 7 degrees of freedom = 17.52, $p = 0.014$; $I^2 = 60\%$ [13% to 82%]). In a comparison of extreme thirds of leptin levels, the combined risk ratio across all studies was 2.28 (95% CI: 1.42 to 3.68) in analyses adjusted for age and sex only, and it was 1.44 (95% CI: 0.95 to 2.16) in analyses further adjusted (Fig. 2). Although formal statistical tests did not detect strong evidence of significant publication bias (Egger test: $p = 0.15$), studies involving at least 100 CHD cases yielded weaker risk ratios compared with smaller studies (1.62 [95% CI: 1.17 to 2.24] vs. 3.55 [95% CI: 1.08 to 11.74] for analyses adjusted for age and sex only; 1.28 [95% CI: 0.80 to 2.04] vs. 1.81 [95% CI: 0.76 to 4.31] for analyses further adjusted), which might reflect preferential publication of striking findings from smaller studies.

Discussion

The present report is the largest single study thus far on leptin levels and incident CHD. It found a moderate and statistically nonsignificant association between leptin levels and CHD risk that attenuated to the null following adjustment for BMI. But, even after adjustment for measured BMI values, leptin levels remained significantly associated with baseline values of smoking, lipids, blood pressure, and several circulating markers of inflammation. In addition, this study found a high degree of within-individual consistency in leptin levels over several years, somewhat higher

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Table 4 Characteristics of Prospective Studies of Leptin and CHD

Study	Location	Year of Baseline Survey	Mean Duration of Follow-Up (yrs)	No. of CHD Cases	No. of Controls	Age Range (yrs)	Male (%)	Leptin Levels Median (IQR) Cases/Controls (ng/ml)	Assay		
									Source	Type	Sample State at Analysis
Studies based in essentially general populations											
BRHS	United Kingdom	1978–1980	16	550	1,184	40–59	100	5.2 (3.2–8.8)/5.0 (2.6–8.2)	In-house	RIA	Frozen (−20°C)
WOSCOPS	Scotland	1989–1991	5	377	783	45–64	100	5.9 (2.04)/5.0 (2.1)*	In-house	RIA	Frozen (−70°C)
Quebec	Canada	1985	5	86‡	95	47–76	100	5.6 (3.12)/5.4 (2.9)†	Linco	RIA	Frozen (−80°C)
Northern Sweden	Sweden	1985	NS	62	124	25–64	100	6.1 (5.0–7.4)/4.4 (4.0–4.9)§	Linco	RIA	Frozen (−80°C)
BWHHS	United Kingdom	1999–2001	4	55	335	60–79	0	22.8 (14.6–34.2)/26.6 (13.0–35.2)	In-house	RIA	Frozen (−80°C)
Studies based in populations with pre-existing disease											
CAPPP	Sweden	1998	6	171	342	50–64	74	25.1 (20.0)/20.0 (16.6)†	DSL	ELISA	Frozen (−70°C)
Mayo	U.S.	1998	4	19	317	52–72	70	NS/9.9 (5.6–19.1)	Linco	RIA	NS
Zoccali et al. (18)	Italy	1997–1998	2.5	15	227	45–75	55	NS/NS	Linco	RIA	NS

*Geometric mean (SD); †mean (SD); ‡included individuals with angina and coronary insufficiency, as results were not reported separately from cases of nonfatal myocardial infarction or CHD death; §geometric mean (95% confidence interval).
BWHHS = British Women's Heart Health Study; CAPPP = Captopril Prevention Project; ELISA = enzyme-linked immunoadsorbent assay; IQR = interquartile range; NS = not stated; RIA = radioimmunoassay; WOSCOPS = West of Scotland Coronary Outcomes Prevention Study; other abbreviations as in Table 1.

than the corresponding values in the same participants for established cardiovascular risk factors such as total cholesterol and systolic blood pressure.

On the basis of the present study and systematic review, it is likely that the strength of any independent association of leptin levels with CHD is, at most, moderate. Given the strong relationship between leptin, body weight and BMI, this would be consistent with the moderate strength of association between BMI and CHD risk in middle age. In the present study, the age-adjusted relative risk of CHD in the top third of the control BMI distribution was 1.35 (95% CI: 1.06 to 1.71) compared with the lowest third, with an increase in CHD risk of 5% (95% CI: 2% to 8%) for each kilogram per square meter increase in BMI. The moderate strength of this association is consistent with that observed in other prospective studies (32).

Obese individuals are characterized by hyperleptinemia and potentially leptin "resistance." There is extensive basic experimental evidence linking elevations in leptin, or loss of leptin function via resistance, to atherogenesis and CHD (33–36). For example, it has been suggested that normally functioning leptin, via its effects to up-regulate fatty acid beta-oxidation, helps to prevent toxic lipid accumulation in critical organs so that the peripheral resistance is the critical pathogenic feature, rather than leptin excess itself (36). Equally complex is the potential interaction between leptin and the endothelium. For example, although elevated leptin levels have been associated with poor vascular compliance in adolescents (9) and impaired coronary vasoreactivity in otherwise healthy young obese individuals (37), evidence from cellular work suggests that leptin may have both vasoconstrictor and vasodilator effects through the endothelium-dependent mechanism (38). Leptin induces vasoconstriction via the potent vasoconstrictor, endothelin-1, but can also promote vasodilation via release of nitric oxide (39). It is conceivable that, in parallel with insulin-mediated vascular effects, leptin-induced vasodilation is blunted in obese subjects by other pathways (e.g., perivascular adipocyte release of cytokines) (40), also linked to obesity, enabling its vasoconstrictor actions to dominate.

The strengths and potential limitations of the present study merit careful consideration. The present study almost doubles the number of incident CHD cases studied in relation to leptin levels. Data were derived from analyses in the BRHS, a population-based prospective study with high response and follow-up rates and robust ascertainment of incident major CHD outcomes (20,21). As many established cardiovascular risk factors, emerging markers, and other characteristics were recorded at baseline in the BRHS, this information has enabled a detailed assessment of the baseline correlates of leptin levels and careful adjustment for potential confounding factors. Data from paired measurements in 219 participants in this study provide the most reliable evidence thus far on the long-term variability of leptin levels, suggesting that leptin is a comparatively stable

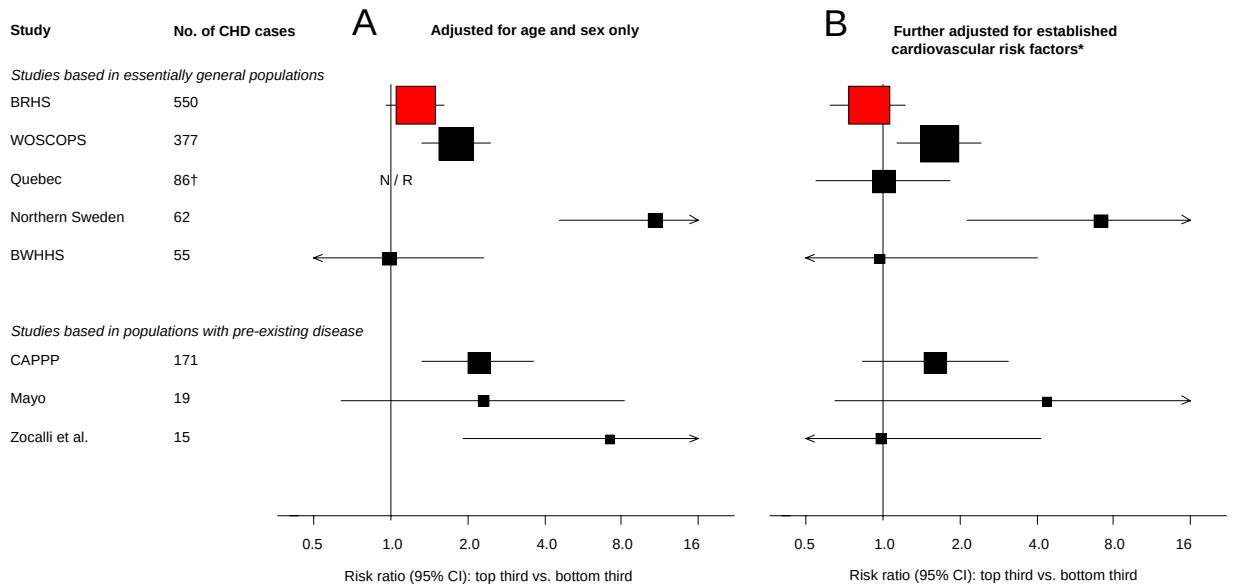

Figure 2 **Prospective Studies of Circulating Leptin Levels and CHD**

There was evidence of substantial heterogeneity among the studies presented in plot **(A)** (chi-square test with 6 degrees of freedom for heterogeneity = 30.38, $p < 0.001$; $I^2 = 80\%$ [60% to 90%]), and plot **(B)** (chi-square test with 7 degrees of freedom = 17.52, $p = 0.014$; $I^2 = 60\%$ [13% to 82%]). The combined risk ratio across all studies was 2.28 (95% confidence interval [CI]: 1.42 to 3.68) in analyses adjusted for age and sex only, and 1.44 (95% CI: 0.95 to 2.16) in analyses further adjusted for established risk factors. Studies involving at least 100 CHD cases yielded weaker risk ratios compared with smaller studies (1.62 [95% CI: 1.17 to 2.24] vs. 3.55 [95% CI: 1.08 to 11.74] for analyses adjusted for age and sex only; 1.28 [95% CI: 0.80 to 2.04] vs. 1.81 [95% CI: 0.76 to 4.31] for analyses further adjusted). Risk ratios for CHD in a comparison of extreme thirds of leptin levels are presented for each study. Horizontal lines represent 95% confidence intervals and box sizes are proportional to the inverse of the variance. *All studies adjusted for a minimum of smoking status, lipids, systolic blood pressure, and body mass index, except the Quebec Cardiovascular Study, which did not adjust for lipids. †Included individuals with angina and coronary insufficiency. BRHS = British Regional Heart Study; BWHHS = British Women's Heart Health Study; CAPPP = Captopril Prevention Project; CHD = coronary heart disease; N/R = not reported; WOSCOPS = West of Scotland Coronary Outcomes Prevention Study.

circulating marker. As the BRHS has previously demonstrated the expected associations of CHD incidence with several established cardiovascular risk factors (24,25), it is an informative study with which to put into perspective the weaker association observed between leptin levels and CHD risk. Our systematic review of previous prospective studies of leptin levels and CHD highlights the strengths of the present report and helps to put the present data into context. Despite attempts to improve comparability between studies by contacting principal investigators to provide results in a consistent manner, there was considerable heterogeneity among the available prospective studies, which warrants further investigation. Nevertheless, available data suggest a more modest association of leptin with CHD than previously suspected, with any association largely dependent upon BMI.

Our primary study was conducted solely in men and the majority of previously published prospective studies were also either solely in men or contained a majority of men (Table 4). Only 1 previous study (BWHHS [British Women's Heart and Health Study]) was conducted solely in women and this study demonstrates that women have substantially higher circulating leptin levels than men (Table 4). Although we recently reported no statistically significant association of leptin levels with CHD risk (including fatal CHD, acute myocardial infarction, revascularization, and new angina) in 165 cases and 355 controls in BWHHS (19) (results in Table 4 are for the 55 cases with fatal CHD or acute myocardial infarction), much larger studies are needed to assess any gender-specific associations. Further studies are also needed in different ethnic populations (41,42). Although serum samples in the present study were stored at −20°C for many years, leptin does not appear to be appreciably influenced by delayed measurement, prolonged storage, or by repeated freeze-thaw cycles (43). Moreover, our demonstration of high levels of leptin reproducibility validates the sample storage and assay methods used in the present study, as do the observations that mean leptin levels in the present study were very similar to those previously reported in a prospective study of middle-aged British men involving the same laboratory personnel and using the same assay as in the current study (15). As leptin levels in the present study were, as expected, correlated with BMI, certain other established cardiovascular risk factors, and markers of inflammation, these findings further reinforce the validity of the present study.

Conclusions

Previous studies may have overestimated associations of leptin and CHD risk, as available data indicate the existence of comparatively moderate associations that are largely dependent on BMI.

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