Cardiovascular Death and the Metabolic Syndrome

Role of adiposity-signaling hormones and inflammatory markers

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OBJECTIVE — Levels of adiposity-signaling hormones and inflammatory markers are less favorable in individuals with the metabolic syndrome; their role in the association between the metabolic syndrome and cardiovascular mortality remains unclear.

RESEARCH DESIGN AND METHODS — We conducted a prospective study of 977 men and 1,141 women aged 40–94 years in 1984–1987, followed for mortality for a maximum of 20 years. Adiponectin, leptin, ghrelin, interleukin-6 (IL-6), C-reactive protein (CRP), and Adult Treatment Panel III–defined metabolic syndrome components were measured in fasting blood samples obtained in 1984–1987. Cox-proportional hazards models were used in survival analyses.

RESULTS — The age- and sex-adjusted hazard ratio (HR) (95% CI) for coronary heart disease (CHD) mortality associated with the metabolic syndrome was 1.65 (1.25–2.18) ($P < 0.001$); this association did not differ significantly by sex, age, or diabetic status ($P > 0.2$ for each interaction). The association between the metabolic syndrome and CHD mortality was not materially changed after adjustment for adiponectin, leptin, and ghrelin; it was attenuated by 25% after adjustment for IL-6 and 35% after adjustment for CRP. CHD mortality increased linearly with greater levels of IL-6 and CRP ($P_{trend} < 0.001$ for each); the age- and sex-adjusted HRs comparing highest versus lowest quarter were 3.0 (1.87–4.89) for IL-6 and 2.1 (1.41–3.21) for CRP. IL-6, but not CRP, remained a significant predictor of CHD mortality in models including both inflammatory markers and the metabolic syndrome.

CONCLUSIONS — Adiposity-signaling hormones and inflammatory markers explain little to some of the association between the metabolic syndrome and CHD mortality. IL-6 levels predict CHD mortality independently of CRP.

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The prevalence of cardiovascular risk factor clustering termed the metabolic syndrome has risen rapidly with the background of the global obesity epidemic (1). Adiponectin, leptin, and ghrelin are hormonal signals that reflect current body size and are implicated in weight homeostasis; adiponectin and leptin are adipocytokines, synthesized and secreted by adipose tissue, whereas ghrelin is primarily produced by the stomach. Low levels of adiponectin and ghrelin and high levels of leptin are generally observed in the obese state and have also been associated with insulin resistance and the metabolic syndrome (2–10). Preliminary evidence has linked these adiposity-signaling hormones to measures of atherosclerosis and cardiovascular risk (11–15); prospective studies suggested their importance for the development of future cardiovascular events (16–19). This raises the question whether adiponectin, leptin, and ghrelin play a role for the increased risk of cardiovascular mortality in people with the metabolic syndrome, but this has not been investigated. A chronic low-grade inflammatory state is associated with the presence of the metabolic syndrome (20), potentially reflecting enhanced production of proinflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-α by adipocytes and monocyte-derived macrophages of the greater adipose tissue mass associated with the syndrome, and also C-reactive protein (CRP) in the liver (1). Inflammatory mechanisms are implicated in the atherosclerotic process underlying coronary heart disease (CHD) (21,22), and both CRP and IL-6 levels have been shown to predict future coronary events (23–32). This suggests that the consequences of low-grade inflammation may underlie the link between the metabolic syndrome and cardiovascular mortality, but it has not been investigated.

To identify potential mechanisms of the association between the metabolic syndrome and cardiovascular mortality, we studied the role of adiposity-signaling hormones (adiponectin, leptin, and ghrelin) and proinflammatory markers (IL-6 and CRP) in older adults with or without the metabolic syndrome and the contribution of IL-6 and CRP, separately and combined, for the prediction of cardiovascular death.

RESEARCH DESIGN AND METHODS

— The Rancho Bernardo Study comprises a cohort of 82% of all adult residents of a southern California residential community, established in 1972–1974 (33). Between 1984 and 1987, 82% (1,093 men and 1,396 women) of surviving community-dwelling members attended a follow-up clinic visit when blood samples were ob-

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Abbreviations: CHD, coronary heart disease; CRP, C-reactive protein; CVD, cardiovascular disease; HRT, hormone replacement therapy; IL-6, interleukin-6.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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tained. This visit included a standardized questionnaire on medical and medication history, smoking status (current vs. previous or never), alcohol consumption (<3 vs. ≥3 times/week), physical activity (<3 vs. ≥3 times/week), and women's menopausal status and hormone replacement therapy (HRT) use (current vs. previous or never users).

Measurements

Waist circumference was measured at the participant's natural waist, and hip measurements were taken at the iliac crest. Systolic and diastolic blood pressures were measured using the Hypertension Detection and Follow–Up Program protocol. Blood samples were obtained by venipuncture between 0730 and 1100 after a requested 12-h fast. Lipid and lipoprotein levels were measured in a Centers for Disease Control and Prevention-certified Lipid Research Clinics laboratory (10). LDL cholesterol was estimated using the Friedewald formula. Fasting and 2-h glucose after a 75-g oral glucose tolerance test were measured by the glucose oxidase method.

Blood samples for measurement of CRP, IL-6, adiponectin, ghrelin and leptin were obtained by venipuncture between 0730 and 1100 after a requested 12-h fast; samples (sera and plasma) were frozen at −70°C until January through April 2000, when IL-6 and CRP were measured in previously unthawed plasma. CRP was measured in a university laboratory by an automated, high-sensitivity method (N Latex CRP mono, sensitivity: 0.2 mg/l; Dade Behring). IL-6 was measured in an endocrinology research laboratory using a high sensitivity (0.094 pg/ml) commercial enzyme-linked immunosorption assay with an alkaline phosphatase signal amplification system (Quantikine HS, human IL-6 immunoassay; R&D Systems, Minneapolis, MN). Adiponectin, ghrelin, and leptin were measured by radioimmunoassay (Linco Diagnostics Laboratory, St. Louis, MO) from previously thawed sera in 2004; women using HRT were excluded from these measurements (10). Log transformation of IL-6 CRP, adiponectin, ghrelin, and leptin was performed, and sex-specific standardized deviation scores were calculated for comparison purposes.

The prevalence of the metabolic syndrome and its components was calculated using Adult Treatment Panel III recommended cut points (34); use of antihypertensive medication was included as high blood pressure in this analysis. Persons with diabetes at baseline were included in this study. Impaired fasting glucose was defined as fasting glucose ≥6.1 mmol/l (110 mg/dl) and type 2 diabetes (13.8% of participants) as fasting glucose ≥7 mmol/l (126 mg/dl), 2-h glucose ≥11.1 mmol/l (200 mg/dl), or use of diabetes medication.

History of cardiovascular disease (CVD) in 1984–1987 (13.2% of participants) was defined as abnormalities from a 12-lead resting electrocardiogram (Minnesota codes 1.1–1.3, 4.1–4.3, 5.1–5.3, and 7.1.1), a positive Rose questionnaire for prolonged chest pain, hospitalization for coronary revascularization procedures, or reported myocardial infarction or stroke.

Vital status

Vital status was determined annually by mailed questionnaires or telephone calls to previously identified informants through 2003; vital status was known for 95% of participants and death certificates were obtained for 93% of decedents. Cause of death was classified by a certified nosologist (ICD-9), including CHD (ICD-9 codes 410–414), stroke (ICD-9 codes 430–436), cardiovascular disease (ICD-9 codes 390–459), and all causes.

Statistical analyses

Age- and sex-adjusted geometric means of adiposity-signaling hormones and inflammatory markers by metabolic syndrome status were calculated and differences were assessed using *t* tests of log-transformed values. Cox proportional hazards models were used to investigate associations between the metabolic syndrome and mortality, statistical differences were tested using χ^2 tests adjusting for age, sex, smoking, and LDL cholesterol. Differences in associations of the metabolic syndrome and mortality by sex, age, baseline diabetes, or CVD history were assessed by including interaction terms, each tested in a separate model. The follow-up time was divided into three periods including an approximately equal number of cardiovascular deaths (<6, 6–11, and >11 years) to test the proportionality assumption.

Age- and sex-adjusted hazard ratios (HRs) were calculated before and after additional adjustments for adipose-signaling hormones and inflammatory markers. Relative changes in HRs were calculated by dividing the difference between the adjusted and unadjusted excess risk by the unadjusted excess risk. Likelihood ratio tests were used to test metabolic syndrome by exposure interaction terms, adjusting for age and sex.

χ^2 tests for trend of HRs across four equally sized groups of IL-6 and CRP were carried out including and excluding participants with CRP levels >15 mg/l. HRs were calculated per unit increase in IL-6 and CRP; sex- and age-adjusted models were compared with those including the metabolic syndrome and both inflammatory markers as well as fully adjusted models additionally including LDL cholesterol, smoking status, exercise, alcohol consumption, menopausal status, and HRT use for women. Potential interactions were assessed as described above. All analyses were performed using SAS (version 9.1, SAS Institute, Inc., Cary, NC).

RESULTS — There were 977 men and 1,141 women (mean ages 71.1 and 70.0 years, respectively; range 40.1–93.9 years) who had complete information on all metabolic syndrome components, known vital status, and if deceased, had a known time and assigned cause of death (85% of all participants attending the 1984–1987 clinic visit). The age-adjusted prevalence of the metabolic syndrome was 16.9% (14.6–19.2) in men and 15.1% (13.0–17.2) in women ($P = 0.26$). Inflammatory markers were available in 1,610 of participants (76% of the sample); ghrelin, adiponectin, and leptin were available in 1,398 men and non–HRT-using women (66% of the sample). The overall prevalence of the metabolic syndrome in these subgroups was similar, being 16.5 and 17.5%, respectively.

In age- and sex-adjusted comparisons, levels of adiponectin and ghrelin were significantly lower and levels of leptin, IL-6, and CRP were significantly higher in those with the metabolic syndrome, compared with those without (Table 1). Investigation of individual components revealed a similar pattern of associations (Table 1).

The age-adjusted CHD mortality rate per 1,000 person-years was 14.0 [95% CI 11.9–16.3] in men and 8.5 [7.2–10.1] in women; rates increased linearly with the number of metabolic syndrome components present (Table 2) ($P_{trend} \leq 0.0001$ in both sexes). The age- and sex-adjusted HR for CHD mortality associated with the metabolic syndrome (presence of ≥3 components) was 1.65 ([1.25–2.18], *P* value ≤ 0.001); this changed little after

Table 1—*Age- and sex-adjusted geometric mean levels (95% CI) of IL-6 and CRP (1,610 men and women) and ghrelin, leptin, and adiponectin (1,398 men and women) according to the presence or absence of the metabolic syndrome and components*

Metabolic syndrome component	*n*	Present	Absent	*P* value*
IL-6 (pg/ml)				
Metabolic syndrome	266	3.14 (2.91–3.38)	2.31 (2.23–2.40)	<0.0001
Hyperglycemia	300	2.59 (2.40–2.79)	2.41 (2.32–2.50)	0.095
High waist girth	252	3.03 (2.80–3.28)	2.35 (2.27–2.43)	<0.0001
Hypertriglyceridemia	382	2.78 (2.60–2.97)	2.35 (2.26–2.43)	<0.0001
Low HDL cholesterol	248	2.98 (2.75–3.23)	2.36 (2.28–2.44)	<0.0001
Hypertension	1,113	2.60 (2.50–2.71)	2.12 (1.99–2.25)	<0.0001
CRP (mg/l)				
Metabolic syndrome	266	3.36 (2.98–3.78)	1.62 (1.53–1.72)	<0.0001
Hyperglycemia	300	2.26 (2.00–2.56)	1.73 (1.63–1.84)	0.002
High waist girth	252	3.10 (2.72–3.54)	1.65 (1.56–1.75)	<0.0001
Hypertriglyceridemia	382	2.70 (2.42–3.00)	1.61 (1.52–1.71)	<0.0001
Low HDL cholesterol	248	2.71 (2.37–3.10)	1.70 (1.60–1.80)	<0.0001
Hypertension	1,113	2.09 (1.95–2.23)	1.34 (1.21–1.49)	<0.0001
Ghrelin (pg/ml)				
Metabolic syndrome	245	1,255.5 (1,200.4–1,313.0)	1,363.9 (1,331.2–1,397.4)	0.004
Hyperglycemia	297	1,332.0 (1,270.4–1,397.0)	1,343.1 (1,310.7–1,376.3)	0.77
High waist girth	225	1,247.1 (1,182.0–1,315.8)	1,359.9 (1,327.9–1,392.6)	0.004
Hypertriglyceridemia	331	1,281.9 (1,226.2–1,340.2)	1,359.6 (1,326.2–1,393.8)	0.023
Low HDL cholesterol	227	1,212.5 (1,149.7–1,278.8)	1,367.5 (1,335.4–1,400.3)	<0.0001
Hypertension	1,048	1,332.6 (1,298.7–1,367.3)	1,365.5 (1,304.4–1,429.4)	0.37
Leptin (ng/ml)				
Metabolic syndrome	245	13.77 (12.62–15.04)	7.60 (7.30–7.91)	<0.0001
Hyperglycemia	297	11.23 (10.45–12.07)	8.45 (8.14–8.77)	<0.0001
High waist girth	225	17.84 (16.57–19.19)	7.84 (7.59–8.10)	<0.0001
Hypertriglyceridemia	331	12.04 (11.26–12.86)	8.18 (7.88–8.49)	<0.0001
Low HDL cholesterol	227	11.29 (10.40–12.25)	8.57 (8.26–8.88)	<0.0001
Hypertension	1,048	9.54 (9.17–9.92)	7.46 (6.96–8.00)	<0.0001
Adiponectin (mg/l)				
Metabolic syndrome	245	8.15 (7.59–8.74)	12.57 (12.17–12.98)	<0.0001
Hyperglycemia	297	10.25 (9.66–10.87)	12.54 (12.16–12.93)	<0.0001
High waist girth	225	9.74 (9.11–10.41)	12.53 (12.17–12.91)	<0.0001
Hypertriglyceridemia	331	8.82 (8.36–9.30)	13.24 (12.85–13.64)	<0.0001
Low HDL cholesterol	227	7.91 (7.43–8.43)	13.06 (12.69–13.43)	<0.0001
Hypertension	1,048	11.78 (11.41–12.17)	12.78 (12.07–13.54)	0.026

**P* values are based on a *t* test of log-transformed values.

further adjustment for smoking and LDL cholesterol (1.63 [1.23–2.16]) (Table 2). Estimates did not differ significantly by sex, age, or follow-up period (all interaction *P* values > 0.2). The association appeared slightly stronger in the 293 participants with diabetes (age- and sex-adjusted HR 1.81 [1.09–3.02]), compared with those without (1.49 [1.04–2.11]) and slightly weaker in the 279 participants with a history of CHD or stroke (1.26 [0.72–2.20]), compared with those without (1.79 [1.29–2.47]); however, none of these differences were statistically significant (interaction *P* values = 0.5 and 0.2, respectively). Adjustment for menopausal status (32 premenopausal women) and HRT use (349 current users) in women had little effect on the effect estimate (1.78 [1.20–2.65]); furthermore, the influence of the metabolic syndrome did not differ by either menopausal status or HRT use (both interaction *P* values > 0.2). Associations were weaker or absent for mortality from stroke, CVD, or all causes (Table 2).

Adjustments for adiponectin, leptin, or ghrelin had little influence on the association between the metabolic syndrome and CHD mortality (Table 3); the maximum change in HR was observed after adjustment for adiponectin, which strengthened the effect estimate by 15.4%. The association (HR [95% CI]) between the metabolic syndrome and CHD mortality (1.65 [1.19–2.28]) was attenuated to 1.49 (1.07–2.06), or by 24.6%, after adjustment for IL-6 and to 1.42 (1.02–1.98), or by 35.4%, after adjustment for CRP (Table 3). No evidence was found that the influence of the metabolic syndrome differed according to levels of adipose-signaling hormones or inflammatory markers (all tests for interaction > 0.1). IL-6 and CRP each showed a linear and significant relationship with CHD mortality in age- and sex-adjusted analyses ($P_{trend} < 0.001$ for both; Table 4). These associations did not differ significantly by sex, age, or follow-up period (all interaction *P* values > 0.2). Weaker associations were present for CVD mortality, but did not reach statistical significance for stroke.

The age- and sex-adjusted HRs (95% CI) for CHD mortality comparing top versus bottom quarter were 3.0 (1.87–4.89)

Table 2—*HR (95% CI) for mortality associated with the number of metabolic syndrome components in 1,141 men and 977 women of the Rancho Bernardo Study*

Outcomes	Number of components	*n*	Deaths (women/men)	HR (95%CI)*		
				Total population	Women	Men
CHD	0	405	29 (12/17)	1	1	1
	1	875	127 (58/69)	1.19 (0.79–1.79)	1.13 (0.60–2.12)	1.26 (0.74–2.15)
	2	501	72 (29/43)	1.17 (0.75–1.80)	1.25 (0.63–2.46)	1.11 (0.63–1.95)
	3	213	33 (17/16)	1.55 (0.93–2.57)	1.83 (0.86–3.91)	1.35 (0.68–2.69)
	4	98	21 (13/8)	2.00 (1.13–3.64)	2.21 (0.97–5.02)	1.96 (0.84–4.59)
	5	26	11 (4/7)	4.12 (2.04–8.32)	3.18 (1.00–10.1)	5.08 (2.09–12.4)
				$P_{trend} = 0.0002$	$P_{trend} = 0.004$	$P_{trend} = 0.02$
CHD	<3	1,781	228 (99/129)	1	1	1
	≥3	337	65 (34/31)	1.63 (1.23–2.16)	1.80 (1.18–2.73)	1.54 (1.04–2.29)
Stroke	<3	1,781	110 (55/55)	1	1	1
	≥3	337	22 (13/9)	1.28 (0.80–2.05)	1.84 (0.98–3.47)	1.04 (0.51–2.12)
CVD	<3	1,781	519 (245/274)	1	1	1
	≥3	337	128 (74/54)	1.50 (1.23–1.83)	1.18 (1.38–2.39)	1.28 (0.95–1.71)
All causes	<3	1,781	1,048 (514/534)	1	1	1
	≥3	337	241 (118/123)	1.43 (1.24–1.65)	1.46 (1.19–1.80)	1.44 (1.18–1.76)

*Adjusted for age and sex, as appropriate, smoking, and LDL cholesterol; sex interaction *P* values = 0.6, 0.6, 0.2, and 0.9 for mortality from CHD, stroke, CVD, and all causes, respectively.

for IL-6 and 2.1 (1.41–3.21) for CRP. These estimates remained essentially unchanged after excluding participants (n = 53) with CRP levels >15 mg/l (2.9 [1.77–4.69] for IL-6 and 2.0 [1.32–3.08] for CRP, $P_{trend} < 0.001$ for both). The age- and sex-adjusted HR per SD increase in log-transformed IL-6 was 1.58 (1.37–1.83); this estimate remained essentially unchanged after adjustment for the metabolic syndrome (1.56 [1.35–1.81]) and was reduced slightly after additional adjustment for CRP (1.46 [1.23–1.74]). In contrast, the analogous HR for CRP was 1.40 (1.22–1.61); this changed to 1.37 (1.18–1.58) after adjustment for the metabolic syndrome, but was attenuated further and to nonsignificance after additional inclusion of IL-6 in the model (1.13 [0.96–1.33]). Similar results were obtained in fully adjusted analyses (Table 4). Stratified analyses showed that associations between the inflammatory markers and CHD mortality were stronger for participants without, compared with those with a history of CHD or stroke at baseline (interaction *P* values 0.05 and 0.02 for IL-6 and CRP, respectively). Further analyses restricted to participants without prevalent CVD confirmed that IL-6 (1.51 [1.24–1.85]) but not CRP (1.18 [0.97–1.43]) was a significant predictor of CHD mortality in models including both inflammatory markers and the metabolic syndrome.

Table 3—*Age- and sex-adjusted HR (95% CI) for CHD mortality associated with the presence of the metabolic syndrome before and after additional adjustments*

Adjustments	*n*	HR (95%CI)*
Adiposity hormones	1,398	
Unadjusted*		1.52 (1.10–2.09)
Ghrelin		1.51 (1.10–2.09)
Leptin		1.50 (1.05–2.13)
Adiponectin		1.60 (1.13–2.26)
All above		1.56 (1.08–2.25)
Inflammatory markers	1,610	
Unadjusted*		1.65 (1.19–2.28)
IL-6		1.49 (1.07–2.06)
CRP		1.42 (1.02–1.98)
All above		1.43 (1.03–1.99)

*All HRs are adjusted for age and sex.

CONCLUSIONS — In this prospective cohort followed for up to 20 years, levels of adiposity-signaling and proinflammatory cytokines differed significantly by the presence or absence of the metabolic syndrome and explained little to some of the association between the metabolic syndrome and CHD mortality. Adipose tissue is now recognized as an active endocrine organ producing a variety of bioactive substances. This has led to the speculation that metabolic and circulatory diseases associated with fat accumulation may develop as a consequence of adipocyte dysfunction and altered levels or receptor expression of adipocytokines, including leptin and adiponectin. In line with this hypothesis, previous studies have linked adiposity-signaling hormones to measures of atherosclerosis and cardiovascular risk (11–15). Prospective studies have shown a detrimental effect of high leptin levels for cardiovascular events in some (16,17) but not all cases (35,36). Adiponectin levels were inversely related to the risk of myocardial infarction in men without prior heart disease (18) and among patients with end-stage renal disease (19). The suggestion of adiposity-signaling hormones as cardiovascular risk factors on the one hand and their close link with obesity, insulin resistance, and diabetes on the other raises the possibility of adiposity-signaling hormones being one mechanism for the increased CHD risk associated with the metabolic syndrome. Results of the present study provide little support for this hypothesis; adjustment for adiponectin, leptin, and ghrelin separately or combined did not materially change the association between the metabolic syndrome and coronary mortality. Likewise, the metabolic syndrome has been linked to a chronic low-grade inflammatory state (20). In this study, levels of IL-6 and CRP explained approximately one-third of the association between the metabolic syndrome and CHD mortality. IL-6 showed a strong and linear association with CHD mortality, independent of the metabolic syndrome and CRP levels. In contrast, the association between CRP and CHD mor-

Table 4—*Age- and sex-adjusted HRs (95% CI) for mortality from CHD, stroke, and CVD, according to levels of IL-6 and CRP in 1,610 men and women*

	CHD deaths	Stroke deaths	CVD deaths
n	209	84	450
IL-6			
Quartile 1 (<1.51 pg/ml)	1	1	1
Quartile 2 (1.51–2.27 pg/ml)	1.41 (0.85–2.36)	1.28 (0.63–2.61)	1.30 (0.95–1.80)
Quartile 3 (2.27–3.64 pg/ml)	2.00 (1.23–3.26)	1.57 (0.79–3.14)	1.60 (1.18–2.19)
Quartile 4 (≥3.64 pg/ml)	3.03 (1.87–4.89)	1.87 (0.93–3.77)	2.32 (1.70–3.15)
P_{trend}	<0.0001	0.06	<0.0001
Per log increase	1.94 (1.57–2.39)	1.34 (0.95–1.89)	1.62 (1.40–1.88)
Per SD in log increase*	1.58 (1.37–1.83)	1.22 (0.97–1.55)	1.40 (1.26–1.54)
Adjusted for metabolic syndrome	1.56 (1.35–1.81)	1.19 (0.94–1.52)	1.38 (1.25–1.53)
Additionally adjusted for CRP	1.46 (1.23–1.74)	1.27 (0.96–1.67)	1.38 (1.23–1.56)
Fully adjusted†	1.47 (1.24–1.76)	1.20 (0.90–1.58)	1.35 (1.19–1.52)
No history of CHD or stroke‡	1.67 (1.42–1.97)	1.15 (0.89–1.49)	1.43 (1.28–1.60)
History of CHD or stroke‡	1.18 (0.85–1.64)	1.73 (0.91–3.30)	1.13 (0.88–1.45)
P value for interaction	0.05	0.29	0.06
CRP			
Quartile 1 (<0.84 mg/l)	1	1	1
Quartile 2 (0.84–1.73 mg/l)	1.32 (0.85–2.04)	0.79 (0.43–1.44)	1.03 (0.78–1.35)
Quartile 3 (1.73–3.90 mg/l)	1.47 (0.96–2.26)	0.79 (0.43–1.47)	1.15 (0.88–1.51)
Quartile 4 (≥3.90 mg/l)	2.13 (1.41–3.21)	1.08 (0.60–1.94)	1.40 (1.07–1.83)
P_{trend}	0.0002	0.80	0.008
Per log increase	1.36 (1.20–1.54)	1.04 (0.84–1.28)	1.17 (1.07–1.28)
Per SD in log increase*	1.40 (1.22–1.61)	1.04 (0.83–1.31)	1.19 (1.08–1.32)
Adjusted for metabolic syndrome	1.37 (1.18–1.58)	0.99 (0.78–1.26)	1.16 (1.05–1.28)
Additionally adjusted for IL-6	1.13 (0.96–1.33)	0.89 (0.68–1.16)	0.99 (0.89–1.11)
Fully adjusted†	1.12 (0.95–1.32)	0.92 (0.70–1.20)	1.00 (0.90–1.12)
No history of CHD or stroke‡	1.51 (1.28–1.77)	1.01 (0.79–1.31)	1.24 (1.11–1.38)
History of CHD or stroke‡	1.03 (0.78–1.36)	1.08 (0.62–1.88)	0.96 (0.78–1.19)
P value for interaction	0.02	0.89	0.03

*SD in log increase (IL-6 2.81 in women and 2.61 in men; CRP 5.24 in women and 5.15 in men). †Fully adjusted model includes age, sex, metabolic syndrome, CRP, IL-6, LDL cholesterol, smoking, exercise, alcohol consumption, menopausal status, and HRT use. ‡1,399 participants without prevalent disease (deaths from CHD = 152, stroke = 69, and CVD = 345); 211 participants with prevalent disease (deaths from CHD = 57, stroke = 16, and CVD = 105).

tality was attenuated to nonsignificance after adjustment for IL-6. Although the association between CRP and cardiovascular events has been extensively studied, including a recent meta-analysis (23), fewer investigators have examined the role of IL-6 (24–32). The correlation between IL-6 and CRP is high (age- and sex-adjusted partial correlation coefficient 0.5 [$P < 0.0001$] in this study), because IL-6 is the main stimulant of hepatic production of CRP (30). Whether the CRP-CVD association largely reflects the underlying effect of IL-6 or is an IL-6-independent mechanism is uncertain: some (26–28,30), but not all, studies (25,31) reported an effect of IL-6 that is either independent of or stronger than that of CRP. From an etiological and interventional perspective, certainty is required regarding which factor is the primary cause or mediator.

To our knowledge, this is the first long-term prospective study to investigate the role of adiposity-signaling hormones and proinflammatory markers for the association between the metabolic syndrome and cardiovascular mortality; however, some limitations should be noted. Participants are adult residents of a predominantly white, upper-middle class community; results may not apply to other social classes or ethnic groups. The prevalence of the metabolic syndrome is difficult to compare across populations due to differences in the definition used and characteristics of the sample, particularly social and ethnic composition (1). In this study, its prevalence was lower than that reported for white men and women from the National Health and Nutrition Examination Survey III using the same diagnostic criteria (37), and lower levels of obesity, particularly in the women of this study, may have contributed to this difference. This lower prevalence may affect the generalizability of our results if associations were expected to differ significantly among populations with varying metabolic syndrome prevalences; however, findings of associations between the metabolic syndrome and CHD mortality in a variety of settings provide little support for this theory.

Ghrelin, adiponectin, and leptin were measured from previously thawed serum of blood samples taken and stored 20 years earlier; however, our levels are similar to those reported in the literature using the same assays (10), and no significant alterations have been reported for freezing and thawing (38–40). Values obtained from a single morning sample may inadequately reflect levels throughout the day or over a longer period. Although ghrelin levels are highly variable during the day, fasting morning levels accurately reflect daily ghrelin exposure (41). This has also been reported for adiponectin and leptin (42,43). Single measurements of IL-6 and CRP have been

shown to adequately represent usual levels over a prolonged period (44,45).

A number of statistical tests were performed, and 5% of comparisons are expected to be significant by chance alone with $\alpha = 0.05$. Adjustment for multiple comparisons was not performed for several reasons. All analyses were based on a priori hypotheses, and many of the tests were performed to obtain adjusted and unadjusted estimates rather than to add new comparisons. Further, adjustment for multiple comparisons may obscure true associations (46), and in this study emphasis was given to the discussion of estimates and their 95% CIs rather than to levels of significance alone.

In summary, adiposity-signaling hormones and inflammatory markers differ significantly among persons with and without the metabolic syndrome and explain little to some of the association between the metabolic syndrome and CHD mortality in older adults. IL-6 predicts CHD mortality independent of CRP.

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