

ORIGINAL ARTICLE

Inflammatory Markers and the Risk of Coronary Heart Disease in Men and Women

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ABSTRACT

BACKGROUND

Few studies have simultaneously investigated the role of soluble tumor necrosis factor α (TNF- α) receptors types 1 and 2 (sTNF-R1 and sTNF-R2), C-reactive protein, and interleukin-6 as predictors of cardiovascular events. The value of these inflammatory markers as independent predictors remains controversial.

METHODS

We examined plasma levels of sTNF-R1, sTNF-R2, interleukin-6, and C-reactive protein as markers of risk for coronary heart disease among women participating in the Nurses' Health Study and men participating in the Health Professionals Follow-up Study in nested case-control analyses. Among participants who provided a blood sample and who were free of cardiovascular disease at baseline, 239 women and 265 men had a nonfatal myocardial infarction or fatal coronary heart disease during eight years and six years of follow-up, respectively. Using risk-set sampling, we selected controls in a 2:1 ratio with matching for age, smoking status, and date of blood sampling.

RESULTS

After adjustment for matching factors, high levels of interleukin-6 and C-reactive protein were significantly related to an increased risk of coronary heart disease in both sexes, whereas high levels of soluble TNF- α receptors were significant only among women. Further adjustment for lipid and nonlipid factors attenuated all associations; only C-reactive protein levels remained significant. The relative risk among all participants was 1.79 for those with C-reactive protein levels of at least 3.0 mg per liter, as compared with those with levels of less than 1.0 mg per liter (95 percent confidence interval, 1.27 to 2.51; P for trend <0.001). Additional adjustment for the presence or absence of diabetes and hypertension moderately attenuated the relative risk to 1.68 (95 percent confidence interval, 1.18 to 2.38; P for trend = 0.008).

CONCLUSIONS

Elevated levels of inflammatory markers, particularly C-reactive protein, indicate an increased risk of coronary heart disease. Although plasma lipid levels were more strongly associated with an increased risk than were inflammatory markers, the level of C-reactive protein remained a significant contributor to the prediction of coronary heart disease.

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INFLAMMATION PLAYS AN ESSENTIAL ROLE in the development of insulin resistance and type 2 diabetes mellitus, the initiation and progression of atherosclerotic lesions, and plaque disruption.^{1,2} Interleukin-6 and tumor necrosis factor α (TNF- α) are inflammatory cytokines and the main inducers of the secretion of C-reactive protein in the liver.³ C-reactive protein is a marker of low-grade inflammation, and recent studies suggest that this protein has a role in the pathogenesis of atherosclerotic lesions in humans.⁴ The effects of TNF- α are mediated by two receptors, type 1 and type 2 (TNF-R1 and TNF-R2), which circulate in soluble forms (sTNF-R1 and sTNF-R2, respectively) and can be measured with greater sensitivity and reliability than can TNF- α itself.⁵ The soluble receptors may attenuate the bioactivity of TNF- α but may also serve as slow-release reservoirs and promote inflammation in the absence of free TNF ligand.⁶

Nonetheless, only a few studies have examined the relationship between levels of sTNF-R1, sTNF-R2, and interleukin-6 and the risk of coronary heart disease.⁷⁻¹⁰ The predictive value of C-reactive protein for screening and its causal relationship to coronary heart disease remain matters of controversy.¹¹⁻¹⁷ We prospectively examined the association between inflammatory markers and the risk of coronary heart disease and the role of potential mediators among men and women in a nested case-control analysis.

METHODS

STUDY POPULATION

The Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS) are prospective cohort investigations respectively involving 121,700 female U.S. registered nurses who were 30 to 55 years old at baseline in 1976 and 51,529 U.S. male health professionals who were 40 to 75 years old at baseline in 1986. Information about health and disease is assessed biennially, and information about diet is obtained every four years by means of self-administered questionnaires.^{18,19} From 1989 through 1990, a blood sample was requested from all participants in the NHS, and 32,826 women provided one. Similarly, between 1993 and 1995, a blood sample was provided as requested by 18,225 men in the HPFS. Participants who provided blood samples were similar to those who did not, albeit the men who provided samples were somewhat younger than those who did not. In the NHS, among wom-

en without cardiovascular disease or cancer before 1990, we identified 249 women who had a nonfatal myocardial infarction or fatal coronary heart disease between the date of blood drawing and June 1998. In the HPFS, we identified 266 men who had a nonfatal myocardial infarction or fatal coronary heart disease between the date of blood drawing and the return of the 2000 questionnaire. Using risk-set sampling,²⁰ we randomly selected controls in a 2:1 ratio who were matched for age, smoking status, and date of blood sampling from the subgroup of participants who were free of cardiovascular disease at the time coronary disease was diagnosed in the case patients. Within the NHS cohort, an additional matching criterion was fasting status at the time of blood sampling.

ASSESSMENT OF CORONARY HEART DISEASE

Study physicians who were unaware of the participant's exposure status confirmed the diagnosis of myocardial infarction on the basis of the criteria of the World Health Organization (symptoms plus either diagnostic electrocardiographic changes or elevated levels of cardiac enzymes). Deaths were identified from state vital records and the National Death Index or reported by the participant's next of kin or the postal system. Fatal coronary heart disease was confirmed by an examination of hospital or autopsy records, by the listing of coronary heart disease as the cause of death on the death certificate, if coronary heart disease was the underlying and most plausible cause, and if evidence of previous coronary heart disease was available.

ASSESSMENT OF OTHER FACTORS

Anthropometric, lifestyle, and dietary data were derived from the questionnaire administered in 1990 to women and 1994 to men, with missing information substituted from previous questionnaires. Body-mass index was calculated as the weight in kilograms divided by the square of the height in meters. Average nutrient intake was computed with the use of a semiquantitative food-frequency questionnaire. Physical activity was expressed in terms of metabolic equivalent (MET)-hours. The questionnaires and the validity and reproducibility of measurements have been described previously.^{18,21}

MEASUREMENT OF BIOCHEMICAL VARIABLES

Blood samples from women were collected in tubes treated with liquid sodium heparin, and those from men were collected in EDTA-treated tubes. The

tubes were then placed on ice packs, stored in Styrofoam containers, returned to our laboratory by overnight courier, centrifuged, and divided into aliquots for storage in liquid-nitrogen freezers (-130°C or colder).

The levels of C-reactive protein were determined by means of a highly sensitive immunoturbidimetric assay with the use of reagents and calibrators from Denka Seiken; this assay has a day-to-day variability of 1 to 2 percent. Levels of sTNF-R1, sTNF-R2, and interleukin-6 were measured by means of enzyme-linked immunosorbent assays (R&D Systems), which have a day-to-day variability of 3.5 to 9.0 percent. Levels of inflammatory markers were largely unaffected by transport conditions and reproducible within subjects over time.^{22,23} Total, high-density lipoprotein (HDL), and directly obtained low-density lipoprotein (LDL) cholesterol and triglycerides were measured according to standard methods with the use of reagents from Roche Diagnostics and Genzyme. Study samples were sent to the laboratory for analysis in randomly ordered batches, and the laboratory personnel were unaware of a sample's case-control status.

The study protocol was approved by the institutional review board of the Brigham and Women's Hospital and the Human Subjects Committee Review Board of Harvard School of Public Health.

EXCLUSIONS

After the exclusion of participants with missing data on biomarker levels, our data sets consisted of 708 women (239 patients and 469 controls) and 794 men (265 patients and 529 controls). The assay for interleukin-6 required slightly more plasma than we originally reserved for this assay among women. Therefore, analyses involving interleukin-6 were restricted to the subgroup of 676 women for whom interleukin-6 levels were available.

STATISTICAL ANALYSIS

We analyzed the two cohorts separately. Inflammatory markers were divided into quintiles, from the lowest to highest levels, on the basis of the sex-specific distributions among the controls. With risk-set sampling, the odds ratio derived from the logistic regression directly estimates the hazard ratio and, thus, the relative risk.²⁰ We analyzed the association between biomarker levels and the risk of coronary heart disease using both conditional and unconditional logistic regression, with adjustment for matching factors. Because both analyses provided

essentially the same results, we present the results of unconditional logistic regression, which parallel the results in the subgroup analyses.

In our multivariable model, we further adjusted for parental history of coronary heart disease before the age of 60 years (yes vs. no), alcohol intake (non-drinker, 0.1 to 4.9 g per day, 5.0 to 14.9 g per day, 15.0 to 29.9 g per day, or at least 30.0 g per day), body-mass index (less than 20, 20 to 24, 25 to 29, 30 to 34, or 35 or more), physical activity (in quintiles from lowest to highest level), ratio of total to HDL cholesterol (in quintiles from lowest to highest ratio), and use of postmenopausal hormone therapy (yes vs. no — for women only). Finally, we also added a history of diabetes (yes vs. no) and hypertension (yes vs. no) at baseline to the model to assess the effect of these potential mediators. Baseline was defined as the year blood was drawn.

Correlation coefficients were calculated with the use of age-adjusted Spearman partial-correlation coefficients. To test for linear trend, we used the median levels of inflammatory markers in the control categories as a continuous variable. To pool the estimates of relative risk for men and women, we used the weighted average of estimates according to the random-effects model of DerSimonian and Laird.²⁴

All P values are two-tailed, and P values below 0.05 were considered to indicate statistical significance. All analyses were performed with the use of SAS software, version 8.2 (SAS Institute).

RESULTS

BASELINE CHARACTERISTICS

Women in whom coronary heart disease developed during follow-up had significantly higher baseline levels of sTNF-R1 and sTNF-R2 than did control women; however, the levels did not differ significantly between men in whom coronary heart disease developed during follow-up and men in the control group (Table 1). In the case of both men and women, patients had significantly higher baseline levels of interleukin-6 and C-reactive protein than controls.

The levels of sTNF-R1 and sTNF-R2 showed a high degree of correlation with each other (Table 2). The correlation with and between the other inflammatory markers was moderate and ranged from 0.27 for sTNF-R1 and C-reactive protein to 0.45 for interleukin-6 and C-reactive protein. The levels of inflammatory markers were moderately inversely associated with HDL cholesterol levels.

Table 1. Baseline Characteristics of Women and Men in Whom Coronary Heart Disease Developed during Follow-up and Matched Controls.*

Characteristic	Women			Men		
	Patients (N=239)	Controls (N=469)	P Value†	Patients (N=265)	Controls (N=529)	P Value†
Age (yr)	60.4±6.5	60.2±6.5	—	65.2±8.3	65.1±8.3	—
Current smoker (%)	31.4	31.8	—	12.4	11.5	—
Body-mass index	26.9±5.7	25.3±4.3	<0.001	26.2±3.5	25.7±3.5	0.05
Parental history of CHD before 60 yr of age (%)	21.3	12.4	0.002	15.1	11.0	0.10
Postmenopausal (%)	89.9	87.3	0.31	—	—	—
Postmenopausal hormone therapy among postmenopausal women (%)	31.7	41.0	0.03	—	—	—
Medications (%)						
Aspirin‡	15.1	21.3	0.05	39.1	34.9	0.25
Cholesterol-lowering drug	4.2	2.6	0.24	8.8	6.9	0.32
History of hypertension (%)	57.7	28.8	<0.001	42.3	30.6	0.001
History of diabetes (%)	19.7	6.4	<0.001	9.4	4.4	0.005
Metabolic syndrome (%)§	43.9	18.3	<0.001	40.4	26.1	<0.001
Total fat intake (% of energy)	31.8±5.8	31.7±6.1	0.82	31.0±6.7	30.3±7.0	0.23
Saturated fat intake (% of energy)	10.8±2.5	10.7±2.7	0.84	10.4±2.7	10.1±2.9	0.12
Alcohol consumption (g/day)						
Median	0.9	1.8	<0.001	5.5	7.0	0.11
Interquartile range	0.0–3.7	0.0–8.6		0.9–15.4	0.9–18.3	
Physical activity (MET-hr/wk)						
Median	11.0	11.5	0.26	22.8	27.3	0.06
Interquartile range	3.9–22.7	5.1–23.0		8.5–44.7	11.8–48.9	
sTNF-R1 (pg/ml)	1438±585	1267±354	<0.001	1513±502	1506±541	0.86
sTNF-R2 (pg/ml)	2777±987	2489±710	<0.001	2991±869	2945±870	0.48
Interleukin-6 (pg/ml)¶						
Median	1.99	1.65	0.001	1.86	1.53	0.01
Interquartile range	1.30–3.05	1.15–2.65		1.10–3.07	0.98–2.88	
C-reactive protein (mg/liter)						
Median	3.10	2.20	<0.001	1.68	1.08	<0.001
Interquartile range	1.30–7.50	1.00–5.10		0.76–3.15	0.52–2.38	
Cholesterol (mg/dl)						
Total	235.4±40.1	225.7±38.7	0.002	214.7±39.9	204.7±36.7	<0.001
LDL	142.9±34.1	132.2±36.4	<0.001	135.6±36.4	127.0±31.1	0.001
HDL	51.5±14.7	60.5±17.4	<0.001	42.1±11.3	45.9±12.5	<0.001
Total-to-HDL cholesterol ratio	4.91±1.55	4.02±1.31	<0.001	5.37±1.41	4.74±1.40	<0.001
Triglycerides (mg/dl)	157.6±96.7	126.3±76.3	<0.001	181.8±116.7	153.8±121.1	0.002

* Data on women are from the Nurses' Health Study and include eight years of follow-up, and data on men are from the Health Professionals Follow-up Study and include six years of follow-up. Matching criteria were age, smoking status, and date of blood sampling; among women, additional matching criteria included fasting status at the time of blood sampling. Plus–minus values are means ±SD. To convert values for cholesterol to millimoles per liter, multiply by 0.02586. To convert values for triglycerides to millimoles per liter, multiply by 0.01129. sTNF-R1 and sTNF-R2 denote soluble tumor necrosis factor receptor types 1 and 2, CHD coronary heart disease, and MET-hr metabolic equivalent–hours. The body-mass index is the weight in kilograms divided by the square of the height in meters.

† P values for the difference between patients and controls (unadjusted) were determined by Student's t-test for variables expressed as means ±SD, by Wilcoxon's rank-sum test for variables expressed as medians, and by the chi-square test for variables expressed as percentages.

‡ Current aspirin use was defined as every one to four days for women and as two or more times per week for men.

§ The metabolic syndrome is defined by the presence of at least three of the following five abnormalities: a body-mass index of at least 25, a triglyceride level of at least 150 mg per deciliter (1.7 mmol per liter), an HDL cholesterol level of less than 50 mg per deciliter for women or less than 40 mg per deciliter for men, a history of hypertension or a history of diabetes or the development of diabetes during follow-up, or a glycosylated hemoglobin level of at least 7 percent at baseline.

¶ Data on interleukin-6 levels were missing for 32 women.

Table 2. Age-Adjusted Spearman Partial-Correlation Coefficients between Selected Cardiovascular Risk Factors among 469 Control Women and 529 Control Men.*

Sex and Risk Factor	Risk Factor								
	sTNF-R1	sTNF-R2	Interleukin-6†	CRP	TC	LDL	HDL	TC:HDL	BMI
Women									
sTNF-R1	—								
sTNF-R2	0.77‡	—							
Interleukin-6	0.31‡	0.28‡	—						
CRP	0.29‡	0.28‡	0.44‡	—					
TC	-0.07	-0.09§	-0.05	0.03	—				
LDL	0.02	<0.01	-0.03	0.04	0.87‡	—			
HDL	-0.30‡	-0.36‡	-0.15¶	-0.17‡	0.08	-0.22‡	—		
TC:HDL	0.22‡	0.27‡	0.09	0.15‡	0.45‡	0.67‡	-0.83‡	—	
BMI	0.30‡	0.27‡	0.26‡	0.37‡	0.12§	0.18‡	-0.33‡	0.37‡	—
Men									
sTNF-R1	—								
sTNF-R2	0.67‡	—							
Interleukin-6	0.32‡	0.28‡	—						
CRP	0.27‡	0.28‡	0.45‡	—					
TC	-0.16‡	-0.13‡	-0.17‡	0.03	—				
LDL	-0.16‡	-0.11§	-0.16‡	-0.003	0.86‡	—			
HDL	-0.25‡	-0.21‡	-0.20‡	-0.24‡	0.20‡	0.13¶	—		
TC:HDL	0.15‡	0.12¶	0.10§	0.25‡	0.39‡	0.39‡	-0.80‡	—	
BMI	0.16‡	0.14‡	0.23‡	0.40‡	0.04	0.01	-0.28‡	0.31‡	—

* sTNF-R1 and sTNF-R2 denote soluble tumor necrosis factor receptor types 1 and 2, CRP C-reactive protein, TC total cholesterol, LDL low-density lipoprotein cholesterol, HDL high-density lipoprotein cholesterol, and BMI body-mass index.

† Seventeen women were excluded from the analysis of interleukin-6 because they had missing values.

‡ P<0.001.

§ P<0.05.

¶ P<0.01.

MAIN EFFECTS

After adjustment for matching factors, women in the highest quintile of each inflammatory marker, as compared with women in the lowest quintile, had a significantly increased risk of coronary heart disease — by a factor of 1.95 to 2.57 — with significant trends across quintiles (Table 3). After additional adjustment for the presence or absence of a parental history of coronary heart disease before the age of 60 years, alcohol intake, level of physical activity, the ratio of total to HDL cholesterol, body-mass index, and the use or nonuse of postmenopausal hormone therapy, these associations were attenuated and no longer significant, except for C-reactive protein (model 2 in Table 3). Additional adjustment for the presence or absence of diabe-

tes and hypertension, which are potentially in the causal pathway, further reduced the association for all inflammatory markers.

Among men, we did not find an association between the levels of soluble TNF- α receptors and the risk of coronary heart disease (Table 3). Men in the highest quintile of interleukin-6 had a 57 percent increase in the risk of coronary heart disease, as compared with men in the lowest quintile, after adjustment for matching factors, although this association was not significant and was further attenuated after multivariable adjustment. However, we found a significant association between C-reactive protein levels and the risk of coronary heart disease. Multivariable adjustment and adjustment for the presence or absence of hypertension and diabetes

Table 3. Relative Risks of Coronary Heart Disease during Follow-up, According to the Quintile of Plasma Levels of Inflammatory Markers at Baseline.*

Variable†	Quintile of Plasma Level					P for Trend‡
	1	2	3	4	5	
	<i>relative risk (95 percent confidence interval)</i>					
Women						
sTNF-R1						
Median — pg/ml	880	1083	1221	1379	1744	
Quintile value — pg/ml	<928	928–1146	1147–1296	1297–1508	≥1509	
Model 1 (matching factors)	1.0	1.21 (0.69–2.11)	1.20 (0.68–2.09)	1.56 (0.90–2.70)	2.57 (1.50–4.39)	<0.001
Model 2 (multivariable)	1.0	1.08 (0.60–1.97)	0.91 (0.50–1.67)	1.14 (0.63–2.08)	1.50 (0.82–2.74)	0.12
Model 3 (model 2+diabetes and hypertension)	1.0	1.06 (0.57–1.97)	0.90 (0.48–1.69)	1.02 (0.54–1.90)	1.24 (0.66–2.34)	0.43
sTNF-R2						
Median — pg/ml	1718	2060	2365	2724	3405	
Quintile value — pg/ml	<1892	1892–2223	2224–2549	2550–3019	≥3020	
Model 1 (matching factors)	1.0	1.72 (0.97–3.04)	1.92 (1.09–3.39)	2.19 (1.24–3.88)	2.51 (1.41–4.45)	0.003
Model 2 (multivariable)	1.0	1.39 (0.75–2.56)	1.48 (0.80–2.74)	1.41 (0.76–2.60)	1.36 (0.72–2.58)	0.59
Model 3 (model 2+diabetes and hypertension)	1.0	1.40 (0.74–2.65)	1.38 (0.73–2.62)	1.30 (0.69–2.46)	1.20 (0.62–2.33)	0.96
Interleukin-6§						
Median — pg/ml	0.82	1.23	1.65	2.37	4.15	
Quintile value — pg/ml	<1.08	1.08–1.44	1.45–1.91	1.92–2.91	≥2.92	
Model 1 (matching factors)	1.0	1.42 (0.81–2.51)	1.15 (0.65–2.05)	1.98 (1.16–3.40)	1.92 (1.11–3.31)	0.01
Model 2 (multivariable)	1.0	1.16 (0.63–2.13)	0.96 (0.51–1.79)	1.32 (0.72–2.40)	1.33 (0.73–2.43)	0.30
Model 3 (model 2+diabetes and hypertension)	1.0	1.08 (0.58–2.03)	0.81 (0.42–1.55)	1.01 (0.54–1.89)	1.05 (0.56–1.97)	0.79
C-reactive protein						
Median — mg/liter	0.50	1.18	2.20	4.02	9.14	
Quintile value — mg/liter	<0.80	0.80–1.70	1.71–2.91	2.92–5.96	≥5.97	
Model 1 (matching factors)	1.0	1.28 (0.74–2.23)	1.03 (0.59–1.81)	1.54 (0.91–2.63)	2.18 (1.30–3.64)	<0.001
Model 2 (multivariable)	1.0	1.17 (0.64–2.14)	0.81 (0.43–1.52)	1.17 (0.64–2.14)	1.86 (1.00–3.46)	0.008
Model 3 (model 2+diabetes and hypertension)	1.0	1.23 (0.66–2.32)	0.89 (0.46–1.72)	1.22 (0.65–2.30)	1.61 (0.84–3.07)	0.08

moderately attenuated this relationship; after accounting for these variables, men in the highest quintile of C-reactive protein, as compared with those in the lowest quintile, had a relative risk of coronary heart disease of 2.55 (95 percent confidence interval, 1.40 to 4.65; P for trend=0.02).

For comparison, in the final multivariable-adjusted model (including the presence or absence of diabetes and hypertension and C-reactive protein levels), the relative risk of coronary heart disease for the highest quintile of the ratio of total to HDL cholesterol, as compared with the lowest quintile, was

4.33 (95 percent confidence interval, 2.11 to 8.90; P for trend <0.001) in women and 3.29 (95 percent confidence interval, 1.84 to 5.90; P for trend <0.001) in men.

SUBGROUP ANALYSES

Overall, we found no significant interactions between various low and high cardiovascular risk groups and the association of biomarkers with the risk of coronary heart disease, although the association of C-reactive protein was generally stronger in low-risk subgroups. For example, in the multivari-

Table 3. (Continued.)

Variable†	Quintile of Plasma Level					P for Trend‡
	1	2	3	4	5	
	<i>relative risk (95 percent confidence interval)</i>					
Men						
sTNF-R1						
Median — pg/ml	1005	1205	1391	1627	2124	
Quintile value — pg/ml	<1111	1111–1301	1302–1510	1511–1793	≥1794	
Model 1 (matching factors)	1.0	1.01 (0.63–1.63)	1.13 (0.70–1.82)	0.96 (0.58–1.57)	1.06 (0.64–1.77)	0.90
Model 2 (multivariable)	1.0	0.95 (0.57–1.58)	1.00 (0.60–1.65)	0.84 (0.49–1.42)	0.85 (0.49–1.46)	0.48
Model 3 (model 2+diabetes and hypertension)	1.0	0.94 (0.56–1.56)	0.99 (0.60–1.65)	0.82 (0.48–1.40)	0.78 (0.45–1.36)	0.32
sTNF-R2						
Median — pg/ml	1969	2421	2812	3209	4090	
Quintile value — pg/ml	<2242	2242–2614	2615–2966	2967–3564	≥3565	
Model 1 (matching factors)	1.0	0.80 (0.49–1.31)	0.90 (0.55–1.47)	1.12 (0.69–1.82)	1.12 (0.68–1.86)	0.33
Model 2 (multivariable)	1.0	0.68 (0.40–1.15)	0.81 (0.48–1.36)	0.94 (0.56–1.57)	0.91 (0.54–1.56)	0.78
Model 3 (model 2+diabetes and hypertension)	1.0	0.72 (0.42–1.21)	0.81 (0.48–1.37)	0.98 (0.59–1.65)	0.92 (0.53–1.58)	0.80
Interleukin-6						
Median — pg/ml	0.69	1.09	1.53	2.43	5.73	
Quintile value — pg/ml	<0.88	0.88–1.29	1.30–1.89	1.90–3.15	≥3.16	
Model 1 (matching factors)	1.0	1.09 (0.66–1.81)	1.19 (0.72–1.98)	1.52 (0.93–2.48)	1.57 (0.95–2.57)	0.06
Model 2 (multivariable)	1.0	0.94 (0.55–1.60)	0.99 (0.59–1.69)	1.25 (0.74–2.10)	1.31 (0.78–2.21)	0.17
Model 3 (model 2+diabetes and hypertension)	1.0	0.97 (0.57–1.65)	0.98 (0.58–1.68)	1.24 (0.73–2.09)	1.31 (0.77–2.22)	0.19
C-reactive protein						
Median — mg/liter	0.27	0.60	1.08	2.05	5.24	
Quintile value — mg/liter	<0.44	0.44–0.80	0.81–1.49	1.50–2.78	≥2.79	
Model 1 (matching factors)	1.0	1.81 (1.04–3.17)	2.00 (1.15–3.50)	2.74 (1.59–4.71)	3.29 (1.91–5.65)	<0.001
Model 2 (multivariable)	1.0	1.75 (0.97–3.14)	1.83 (1.02–3.30)	2.27 (1.26–4.09)	2.73 (1.51–4.96)	0.007
Model 3 (model 2+diabetes and hypertension)	1.0	1.75 (0.97–3.16)	1.74 (0.96–3.15)	2.14 (1.18–3.88)	2.55 (1.40–4.65)	0.02

* The group of women included 239 patients and 469 controls with eight years of follow-up. The group of men included 265 patients and 529 controls with six years of follow-up. sTNF denotes soluble tumor necrosis factor receptor. Quintiles and median values of plasma inflammatory markers are based on values in controls. For each relative risk, quintile 1 served as the reference group.

† Model 1 was adjusted for matching factors (age, smoking status, and the month of blood sampling). Among women, data were also adjusted for fasting status at the time of blood sampling. Model 2 was adjusted for matching factors, presence or absence of a parental history of coronary heart disease before the age of 60 years, alcohol intake, level of physical activity, ratio of total cholesterol to HDL cholesterol, and body-mass index. Among women, the multivariable model was also adjusted for the use or nonuse of postmenopausal hormone therapy.

‡ P values for trend are based on the median levels of inflammatory markers in quintiles of the controls.

§ A total of 32 women were excluded from the analyses for interleukin-6 owing to missing values for interleukin; 224 patients and 452 controls were analyzed.

able-adjusted model (excluding the presence or absence of hypertension and diabetes), the relative risk in the highest as compared with the lowest quintile of C-reactive protein was 2.53 among women with a body-mass index of less than 25 (95 percent confidence interval, 1.04 to 6.18; P for trend=0.02) and 6.25 among men with a body-mass index of less than 25 (95 percent confidence interval, 2.28 to 17.1; P for trend=0.005). Similarly, among participants with LDL cholesterol levels of less than 130 mg per

deciliter (3.4 mmol per liter), the corresponding relative risks were 3.54 (95 percent confidence interval, 1.19 to 10.5; *P* for trend=0.01) for women and 2.52 (95 percent confidence interval, 1.09 to 5.83; *P* for trend= 0.04) for men. Among participants without hypertension, the corresponding relative risks were 1.87 (95 percent confidence interval, 0.77 to 4.56; *P* for trend=0.02) for women and 3.01 (95 percent confidence interval, 1.41 to 6.44; *P* for trend=0.02) for men.

CLINICAL CUTOFF POINTS FOR C-REACTIVE PROTEIN

We further categorized the study participants, on the basis of recently proposed cutoff points for C-reactive protein, as having low levels (less than 1.0 mg per liter), moderate levels (1.0 to 2.9 mg per liter), and high levels (at least 3.0 mg per liter).²⁵ In these analyses, participants with high levels of C-reactive protein, as compared with those with low levels, had a relative risk of coronary heart disease of approximately 1.8 after adjustment for covariates (including body-mass index and lipid levels) (Table 4). When we pooled the risk estimates for men and women, the final multivariable-adjusted relative risk (including adjustment for the presence or absence of diabetes and hypertension) was 1.68 in the group with high levels of C-reactive protein, as compared with the group with low levels (95 percent confidence interval, 1.18 to 2.38; *P* for trend=0.008) (Table 4). This is similar to the pooled estimate (relative risk, 1.48; 95 percent confidence interval, 1.08 to 2.04; *P* for trend=0.03) after we controlled for covariates from the Framingham risk score,²⁶ including age, presence or absence of hypertension and diabetes, ratio of total to HDL cholesterol, and smoking status.

We found a gradient of risk of coronary heart disease within each increasing category of C-reactive protein and ratio of total to HDL cholesterol (Fig. 1). This finding supports the hypothesis that the levels of C-reactive protein may predict risk beyond the information afforded by lipid levels. However, despite the independent associations, the gradient of risk associated with lipid levels was greater than that for C-reactive protein levels.

ADDITIONAL ANALYSES

When we stratified our analysis according to the time to an event in two-year intervals, the relative risk of coronary heart disease associated with C-reactive protein levels remained relatively stable

over time (data not shown). When we repeated our main analyses after excluding participants with C-reactive protein levels of at least 10.0 mg per liter, we found essentially the same results. C-reactive protein levels may be affected by hormone therapy.¹⁰ However, results were similar when we used quintiles of C-reactive protein based on levels in women in the control group who reported never using hormones.

DISCUSSION

In these two nested case-control studies, we found that high plasma levels of C-reactive protein were associated with an increased risk of coronary heart disease among women and men without previous cardiovascular disease. Elevated plasma levels of sTNF-R1 and sTNF-R2 were related to an increased risk among women, but not men. We found only a moderate suggestion of increased risk associated with elevated levels of interleukin-6. For all markers, associations were substantially attenuated and — with the exception of C-reactive protein — no longer significant after adjustment for cardiovascular risk factors, particularly body-mass index and the presence or absence of diabetes and hypertension. These findings are consistent with a role of these inflammatory markers in the elevated risk of cardiovascular events that is associated with type 2 diabetes and hypertension.

TNF- α and interleukin-6 are the main inducers of hepatic production of acute-phase proteins, including C-reactive protein.³ These inflammatory markers are associated with biologic and environmental risk factors for cardiovascular events, including components of the metabolic syndrome (obesity, insulin resistance, diabetes, hypertension, and low HDL cholesterol levels), and lifestyle factors, such as smoking, abstinence from alcohol, and physical inactivity.²⁷⁻²⁹

Compelling evidence suggests that inflammation causally contributes to several precursors of cardiovascular disease. TNF- α and interleukin-6 can cause insulin resistance in animal models, and plasma levels of C-reactive protein and interleukin-6 have been shown to predict type 2 diabetes in humans.^{30,31} The increased cytokine synthesis in obesity may promote insulin resistance and impaired glucose uptake, type 2 diabetes, and ultimately, coronary heart disease.³⁰ In line with these hypotheses, we found that plasma levels of interleukin-6 and C-reactive protein, in particular, were related to

Table 4. Relative Risks of Coronary Heart Disease during Follow-up According to the Baseline Level of C-Reactive Protein.*

Variable†	CRP <1.0 mg/liter	CRP 1.0–2.9 mg/liter	CRP ≥3.0 mg/liter	P for Trend‡
	<i>relative risk (95 percent confidence interval)</i>			
Women				
No. of patients	41	73	125	
No. of controls	114	170	185	
Model 1 (matching factors)	1.0	1.22 (0.77–1.93)	1.93 (1.25–2.99)	<0.001
Model 2 (multivariable)	1.0	1.21 (0.75–1.96)	1.94 (1.21–3.10)	0.002
Model 3 (model 2+body-mass index)	1.0	1.16 (0.71–1.90)	1.71 (1.04–2.80)	0.02
Model 4 (model 3+TC:HDL)	1.0	1.09 (0.66–1.82)	1.64 (0.98–2.75)	0.02
Model 5 (model 4+diabetes and hypertension)	1.0	1.17 (0.69–2.00)	1.53 (0.89–2.62)	0.09
Men				
No. of patients	86	108	71	
No. of controls	254	175	100	
Model 1 (matching factors)	1.0	1.90 (1.34–2.71)	2.20 (1.46–3.32)	<0.001
Model 2 (multivariable)	1.0	1.88 (1.31–2.69)	2.17 (1.43–3.31)	0.002
Model 3 (model 2+body-mass index)	1.0	1.85 (1.28–2.68)	2.08 (1.34–3.23)	0.006
Model 4 (model 3+TC:HDL)	1.0	1.71 (1.17–2.49)	1.91 (1.22–3.00)	0.02
Model 5 (model 4+diabetes and hypertension)	1.0	1.60 (1.09–2.34)	1.79 (1.14–2.83)	0.03
Men and Women				
Model 1 (matching factors)	1.0	1.61 (1.22–2.14)	2.07 (1.54–2.79)	<0.001
Model 2 (multivariable)	1.0	1.61 (1.20–2.14)	2.06 (1.51–2.82)	<0.001
Model 3 (model 2+body-mass index)	1.0	1.57 (1.17–2.11)	1.90 (1.37–2.65)	<0.001
Model 4 (model 3+TC:HDL)	1.0	1.46 (1.08–1.98)	1.79 (1.27–2.51)	<0.001
Model 5 (model 4+diabetes and hypertension)	1.0	1.44 (1.05–1.96)	1.68 (1.18–2.38)	0.008

* Data on women are from the Nurses' Health Study and include eight years of follow-up, and data on men are from the Health Professionals Follow-up Study and include six years of follow-up. The subjects with the lowest level of C-reactive protein (CRP) served as the reference group. TC:HDL denotes the ratio of total cholesterol to high-density lipoprotein cholesterol.

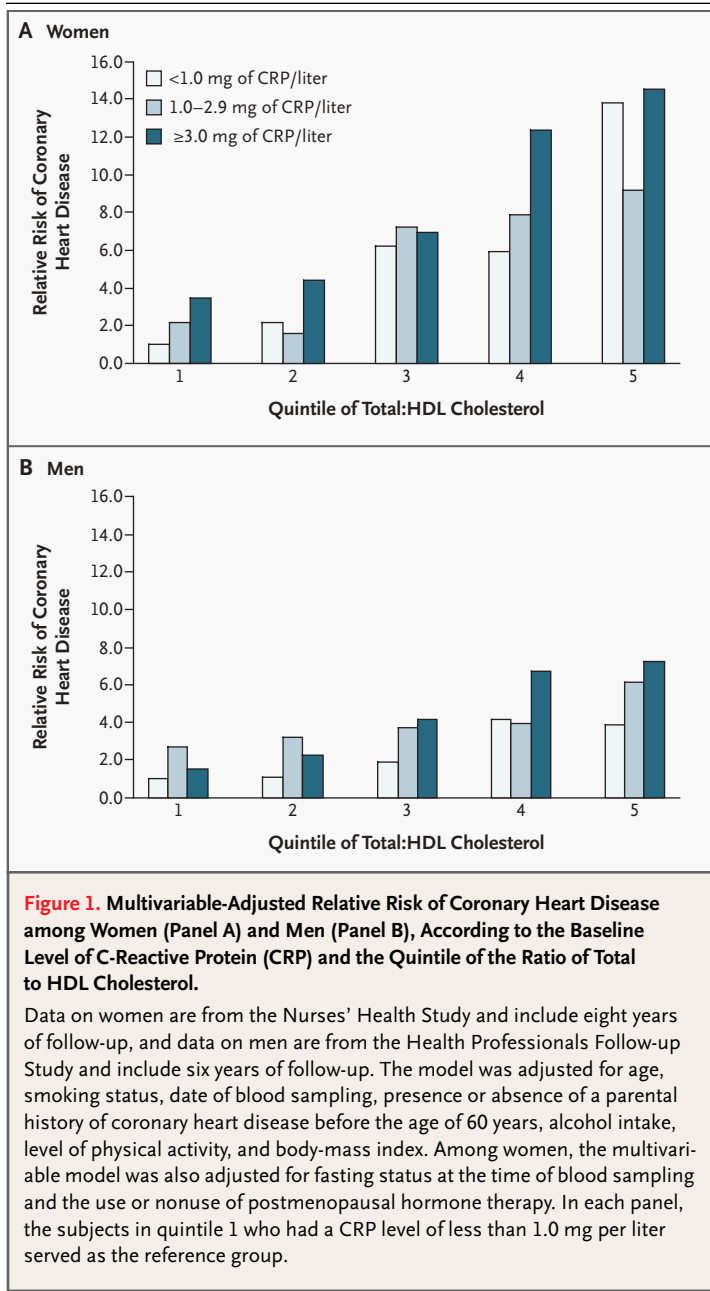
† Model 1 was adjusted for matching factors (age, smoking status, and month of blood sampling); data for women were also adjusted for fasting status at the time of blood sampling. Model 2 was adjusted for matching factors, as well as the presence or absence of a parental history of coronary heart disease before the age of 60 years, alcohol intake, level of physical activity, and use or nonuse of hormone therapy among postmenopausal women. Model 5 was adjusted for everything listed in model 4 as well as the presence or absence of diabetes and hypertension.

‡ P values for trend are based on median levels in the three C-reactive protein groups in the controls.

the risk of coronary heart disease and that the risks were attenuated after adjustment for the presence or absence of diabetes and hypertension.

TNF- α has a limited half-life and is difficult to measure in large-scale epidemiologic studies.^{5,6} In a nested case-control study, Ridker et al. reported a multivariable-adjusted relative risk of recurrent coronary events of 2.5 (95 percent confidence interval,

1.3 to 5.1) among men whose TNF- α levels exceeded the 95th percentile, as compared with men with lower levels.³² Cesari et al. reported a relative risk of of coronary events of 1.79 (95 percent confidence interval, 1.18 to 2.71) among elderly participants without cardiovascular disease who had the highest of three levels of TNF- α , as compared with those who had the lowest levels.⁸ The value of as-



sessing circulating levels of TNF- α is unknown, since such levels can be very low and unstable. The levels of soluble TNF- α receptors may be more stable and may better reflect longer-term average circulating levels of TNF- α , although data on the role of soluble TNF- α receptors in coronary heart disease are scarce.^{7,33} It is unclear why we found a difference in risk between men and women associated with elevated levels of soluble TNF- α receptors;

however, others also have found differences between women and men with respect to lipids³⁴ and in the overall prediction of risk.³⁵ Similarly, mechanisms of insulin sensitivity, rather than inflammation, may contribute more to the risk of coronary heart disease in women than men.

Findings of an association between interleukin-6 levels and the risk of coronary heart disease have been inconsistent.^{8,10,36} In our study, this association was substantially reduced and no longer significant after multivariable adjustment.

C-reactive protein is the most extensively studied inflammatory marker in prospective settings. In an early meta-analysis of 11 prospective studies, the relative risk of coronary heart disease in subjects with the highest of three C-reactive protein levels, as compared with those with the lowest levels, was 2.0 (95 percent confidence interval, 1.6 to 2.5) among population-based studies.³⁷ Eleven other prospective studies have since been published. In an updated meta-analysis, Danesh et al. reported an overall odds ratio of 1.58 (95 percent confidence interval, 1.48 to 1.68) among subjects with the highest of three levels of C-reactive protein, as compared with subjects with the lowest level.¹⁶ This risk estimate is similar to that in our comparisons of C-reactive protein levels of at least 3.0 mg per liter with those of less than 1.0 mg per liter. However, the degree of adjustment for traditional cardiovascular risk factors differed markedly among the studies included in the meta-analysis.

An important question is whether knowing the level of C-reactive protein adds materially to risk prediction. In the Women's Health Study, Ridker et al. reported that the level of C-reactive protein was a stronger predictor than the LDL cholesterol level and that it added to the information provided by the Framingham risk score.^{12,38} Comparing C-reactive protein levels of at least 3.0 mg per liter with those of less than 1.0 mg per liter, they reported a relative risk of 1.5 (95 percent confidence interval, 1.2 to 1.9) after adjustment for the Framingham risk score and the presence or absence of diabetes.³⁸

In the Atherosclerosis Risk in Communities Study, Ballantyne et al. reported a relative risk of coronary heart disease of 1.72 (95 percent confidence interval, 1.24 to 2.39) among subjects with a C-reactive protein level of at least 3.0 mg per liter, as compared with subjects with a level of less than 1.0 mg per liter (adjusted for components of the Framingham risk score, including the presence or ab-

sence of diabetes).¹⁴ In the Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) study, comparing C-reactive protein levels of at least 3.0 mg per liter with those of less than 1.0 mg per liter, Koenig et al. reported a hazard ratio of 2.21 (95 percent confidence interval, 1.49 to 3.27), adjusted for the Framingham risk score.¹³ In contrast, in the Rotterdam Study, measuring the level of C-reactive protein did not improve the prediction of coronary events beyond that afforded by the Framingham risk score, with an odds ratio of 1.2 (95 percent confidence interval, 0.6 to 2.2) among participants in the highest quartile of C-reactive protein, as compared with those in the lowest quartile.³⁹

In our analysis, the pooled relative risk among men and women classified according to clinical cutoff points for the levels of C-reactive protein was 1.48 (95 percent confidence interval, 1.08 to 2.04; *P* for trend=0.03) after we accounted for covariates in the Framingham risk score, including the presence or absence of diabetes. Our results are similar to those of Ridker et al.³⁸ and Ballantyne et al.,¹⁴ as well as those of the recent meta-analysis by Danesh et al.,¹⁶ a fact that suggests that after adjustment for the Framingham risk score, the relative risk associated with a clinical cutoff point of at least 3.0 mg per liter, as compared with a cutoff of less than 1.0 mg per liter, is probably moderately less than previously suggested in the guidelines for the clinical assessment of inflammatory markers issued by the American Heart Association and the Centers for Disease Control and Prevention (relative risk, 1.5 vs. approximately 2.0).²⁵ Nevertheless, our findings support the theory that the level of C-reactive protein provides an additional measure of the risk of coronary heart disease beyond that afforded by the Framingham risk score.

Our study has some limitations. As with any observational study design, there is the possibility of unmeasured confounding. However, we controlled for most known cardiovascular risk factors. Though we obtained only a single blood sample at baseline, previous studies have shown the levels of biomarkers to be relatively stable over time.^{22,23} Since the ranges of anthropometric variables in our cohorts were quite broad, the biologic relationships found should be widely generalizable. Though we excluded men and women with missing data on blood levels, generalizability should be minimally

affected because the participants were similar to those who did not provide blood samples.

Although the Framingham risk score is a tool for estimating the 10-year risk of coronary heart disease among healthy subjects,²⁶ it does not include other well-established risk factors, such as body-mass index, alcohol intake, level of physical activity, or the presence or absence of a parental history of coronary heart disease.⁴⁰ Therefore, to examine the role of inflammatory markers in coronary heart disease, we used an etiologic approach in our main analyses, to take into account the pathophysiology of coronary heart disease and include the major cardiovascular risk factors, beyond those included in the Framingham risk score, for comparison.

Our questionnaires did not include questions on the use of hydroxymethylglutarylcoenzyme A reductase inhibitors (statins) because these drugs were not widely used at time of blood sampling. However, the reported use of cholesterol-lowering drugs was generally low in both cohorts.

In conclusion, our findings suggest that high levels of C-reactive protein are associated with an increased risk of coronary heart disease among men and women and that the level of C-reactive protein is a significant marker of the risk of coronary heart disease, even after careful multivariable adjustment. Though all other associations were attenuated after multivariable adjustment, high levels of sTNF-R1 and sTNF-R2 may be also associated with an increased risk and deserve further exploration in other populations. From a clinical standpoint, although the ratio of total to HDL cholesterol was more strongly associated with the risk of coronary heart disease than were the levels of inflammatory markers, the level of C-reactive protein was still a significant contributor to the prediction of coronary heart disease.

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