



## Original Contribution

# Association of Adiponectin with Coronary Heart Disease and Mortality

## The Rancho Bernardo Study

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Hypoadiponectinemia has been implicated in the development of obesity-related conditions, including dyslipidemia and coronary heart disease (CHD). In this study, the authors examined the association of adiponectin with CHD prevalence, incidence, and mortality among 1,513 community-dwelling men and women aged 50–91 years who were followed from 1984–1987 through 2004. In cross-sectional analyses, adiponectin concentrations were positively related to female sex, age, and high density lipoprotein cholesterol level and inversely related to waist girth, triglyceride level, and fasting plasma glucose level (all  $p$ 's < 0.001). Adiponectin levels in the highest sex-specific quintile, as compared with the lowest, were associated with 44% decreased odds of prevalent CHD ( $p$  for trend = 0.03); adjustment for high density lipoprotein cholesterol and/or triglycerides eliminated this association. In 20-year prospective analyses, higher adiponectin concentrations predicted reduced risk of nonfatal myocardial infarction in men only; adiponectin was not associated with fatal incident CHD events or 20-year CHD mortality ( $n = 215$  deaths) in either sex. Adiponectin levels in the highest sex-specific quintile, as compared with lower levels, were associated with almost 40% increased risks of cardiovascular disease death ( $n = 441$ ) and death from all causes ( $n = 925$ ), independent of age, sex, waist girth, lipid levels, and glucose level (both  $p$ 's < 0.001). These results suggest that use of adiponectin for cardiovascular disease risk stratification is premature.

adiponectin; cardiovascular diseases; coronary disease; mortality

Abbreviations: CHD, coronary heart disease; HDL, high density lipoprotein.

Adiponectin, the most abundant circulating adipocyte-derived protein identified thus far, appears to play an important role in glucose and lipid metabolism, vascular biology, and energy homeostasis (1, 2). In animal studies, recombinant adiponectin improves insulin sensitivity, inhibits inflammatory responses, and reverses diet-induced lipid abnormalities (3). Cross-sectional population-based studies in humans show that low levels of adiponectin are associated with an adverse cardiovascular disease risk profile, even among relatively healthy persons (4–7). Hypoadiponectinemia is strongly linked to central adiposity, a low level of high density lipoprotein (HDL) cholesterol, high triglyceride lev-

els, insulin resistance, and high blood pressure—all characteristics of the metabolic syndrome (4–8). The identification of low adiponectin levels in men and women with prevalent coronary heart disease (CHD) (9–11), coupled with adiponectin's known antiatherogenic effects (3, 12), has generated enthusiasm for the idea that hypoadiponectinemia might be a pathogenic element in the development of CHD (3, 13).

There have been few prospective studies of adiponectin and CHD in healthy populations, and results have been mixed. The first, a nested case-control study of men in the Health Professionals Follow-up Study, supported the thesis

that adiponectin is cardioprotective; a doubling of adiponectin was associated with a 30 percent decreased risk of incident myocardial infarction over 6 years (14). This study was followed by two nested case-control studies with negative findings, one from the Strong Heart Study (15) and the other from the British Women's Heart and Health Study (16); neither observed an association of adiponectin with incident CHD events during 4 years of follow-up. More recently, in older Dutch men and women, high baseline levels of adiponectin were associated with a favorable cardiovascular disease risk profile but increased risk of 15-year mortality (17). Thus, the nature of the adiponectin link to the pathogenesis of CHD and its long-term sequelae is not clear.

We report here the association of adiponectin with CHD prevalence, incidence, and mortality among 1,513 community-dwelling older men and women from the Rancho Bernardo Study who were followed for 20 years. Sex differences were evaluated, and analyses were based on sex-specific adiponectin distributions. Associations of adiponectin with cardiovascular disease death and all-cause mortality are also reported.

## MATERIALS AND METHODS

### Study population

The Rancho Bernardo Study is a population-based study of healthy aging in Caucasian residents of a Southern California community. Between 1984 and 1987, 82 percent ( $n = 2,480$ ) of surviving community-dwelling older participants attended a research clinic visit. During this visit, information regarding medical history, date of the last menstrual cycle (for women), medication use, physical activity (exercising three or more times per week; yes/no), alcohol consumption (one or more drinks per day vs. fewer or none), and current smoking (yes/no) was obtained using standard questionnaires. Current medication use was validated by examination of pills and prescriptions brought to the clinic for that purpose. The study protocol was approved by the institutional review board of the University of California, San Diego; all participants gave written informed consent.

Eligibility criteria for the present analysis included 1) age 50 years or older when the participant was evaluated at the 1984–1987 visit; 2) availability of stored serum; 3) postmenopausal status (for women); and 4) no estrogen or insulin use at the time of the clinic visit. Of the 2,480 participants who attended the 1984–1987 clinic visit, 332 women were excluded for current estrogen use; 1,588 (891 men and 697 women) of the remaining participants had sufficient amounts of stored sera for measurement of adiponectin. Of these persons, 28 were excluded for being under 50 years of age, eight were excluded for being premenopausal, 11 were excluded because of insulin use, and 28 were excluded because they had sex hormone levels outside the normal physiologic range (18). The remaining 1,513 participants (835 men and 678 postmenopausal, non-estrogen-using women) were the subjects of this report.

Height, weight, and waist girth were measured in 1984–1987, and 99 percent of the participants also had their

height and weight recorded in 1972–1974. Compared with those without adiponectin assays, participants included in this study were slightly older and more likely to be male, but they did not differ in terms of weight, body mass index, lifestyle characteristics, prevalent heart disease, or weight loss of  $\geq 10$  pounds in the previous 10 years.

### Measurements

Height, weight, and waist girth were measured in the clinic with participants wearing light clothing and no shoes. Body mass index (weight (kg)/height (m)<sup>2</sup>) and waist girth (cm) were used as estimates of overall and central adiposity. Weight change was determined by subtracting the participant's weight at the 1972–1974 visit from that at the 1984–1987 visit. Weight loss was defined as a loss of 10 or more pounds ( $\geq 4.5$  kg), based on a previous report from the Rancho Bernardo cohort showing a significant association of this degree of weight loss with survival (19). Systolic blood pressure was measured twice in seated, resting subjects using the Hypertension Detection and Follow-up Program protocol (20); the mean of two readings was used in analyses. Pulse pressure was calculated as the difference between systolic blood pressure and diastolic blood pressure.

Blood samples were obtained by venipuncture between 7:30 a.m. and 11:00 a.m. after a requested 12-hour fast; serum and plasma were separated and frozen at  $-70^{\circ}\text{C}$ . In 2004, adiponectin levels were measured by radioimmunoassay in twice-thawed serum samples at Linco Diagnostics Laboratory, St. Louis, Missouri. The Linco adiponectin assay measures total adiponectin, that is, all molecular forms. The sensitivity and the intra- and interassay coefficients of variation were 0.8 mg/liter, 6 percent, and 7 percent, respectively. Linco reports reproducible results for the adiponectin assay after two freeze-thaw cycles, and adiponectin levels did not vary by years of frozen sample storage or hour of sample collection.

Fasting levels of plasma total cholesterol, HDL cholesterol, low density lipoprotein cholesterol, and triglycerides were determined in a Lipid Research Clinic Laboratory certified by the Centers for Disease Control and Prevention. Total cholesterol and triglyceride levels were measured by enzymatic techniques using an ABA-200 biochromatic analyzer (Abbott Laboratories, Irving, Texas). HDL cholesterol level was measured after precipitation of the other lipoproteins with heparin and manganese chloride. Low density lipoprotein cholesterol level was estimated using the Friedewald formula (21). Plasma glucose levels were measured by the glucose oxidase method and serum creatinine by the Jaffe reaction method.

### Assessment of outcomes

Medical history and information on incident CHD was obtained using standardized questionnaires at baseline, at clinic visits approximately every 4 years thereafter, and from periodic mailings. Follow-up continued through 2004, a 20-year follow-up period. Prevalent CHD was defined as doctor-diagnosed myocardial infarction or cardiac revascularization. Incident CHD was defined as the first occurrence

of a nonfatal myocardial infarction or fatal CHD. Vital status was known for 96 percent of participants. Death certificates, obtained for 91 percent of decedents, were classified for underlying cause of death by a certified nosologist using the *International Classification of Diseases*, Ninth Revision. CHD deaths included codes 410–414; cardiovascular disease deaths included codes 401–414, 426–438, and 440–448; and deaths from all causes encompassed codes 0–999.

Diabetes mellitus was defined by a physician's diagnosis, a fasting plasma glucose level of  $\geq 7.0$  mmol/liter (126 mg/dl), a 2-hour postchallenge glucose level of  $\geq 11.1$  mmol/liter (200 mg/dl), or use of diabetes medications (22). The metabolic syndrome was defined according to the 2002 Adult Treatment Panel III criteria (23). Hypertension was defined as blood pressure  $\geq 130/85$  mmHg or use of antihypertensive medication.

### Statistical analysis

Adiponectin, HDL cholesterol, and triglyceride levels were not normally distributed and were  $\log_{10}$ -transformed for analyses; reported values are geometric means or medians and interquartile ranges. Baseline characteristics were compared by prevalent CHD status using general linear models adjusted for age and sex for continuous variables and chi-squared analysis for categorical variables. Age- and sex-adjusted associations between adiponectin levels and selected CHD risk factors were examined using partial correlations for continuous variables and general linear models for categorical variables. The association between adiponectin and prevalent CHD was assessed using logistic regression analyses; goodness of fit was confirmed by the Hosmer and Lemeshow method (24). Associations between baseline adiponectin levels and incident CHD and all-cause, CHD, and cardiovascular disease mortality were investigated using Cox proportional hazards regression; goodness of fit was confirmed by the May and Hosmer method (25). All models presented met the proportional hazards assumption. None of the regression results were significantly influenced by outliers.

For regression analyses, adiponectin was examined as a continuous variable ( $\log_{10}$  adiponectin), as sex-specific standard-deviation increases in  $\log_{10}$  adiponectin, and as sex-specific quintiles based on the entire population. Three separate models were evaluated: The first adjusted for age and sex, the second added adjustment for waist girth, and the third added further adjustment for potential adiponectin covariates, including levels of HDL cholesterol, triglycerides, and fasting plasma glucose. There was no significant multicollinearity between the independent variables. No significant interactions were found between adiponectin and age and sex or any other risk factor; nevertheless, sex-specific analyses are presented for incident CHD to allow comparisons with other findings in the literature. Incident CHD events were further analyzed on the basis of whether the first event was fatal or nonfatal. These event-specific analyses included all participants. When fatal CHD was being considered as the first event, participants with nonfatal incident CHD were censored at the time of the nonfatal event, and vice versa for the nonfatal analysis. The same approach

was used for cause-specific mortality analyses; that is, participants whose deaths were attributed to causes other than the one of interest were censored at the time of death.

All  $p$  values presented are two-tailed;  $p \leq 0.05$  was considered statistically significant. Data were analyzed using SAS, version 9.1 (SAS Institute, Inc., Cary, North Carolina); SPSS, version 11.5 (SPSS, Inc., Chicago, Illinois); and STATA, version 9 (Stata Corporation, College Station, Texas).

## RESULTS

### Baseline characteristics

The mean age of the population at baseline was 74.0 years (standard deviation, 8.1) for women and 71.0 years (standard deviation, 9.7) for men (range, 50–91 years); 55 percent of participants were male. Median adiponectin levels were 50 percent higher in women (15.5 mg/liter) than in men (9.8 mg/liter) ( $p < 0.001$ ). Age- and sex-adjusted comparisons of characteristics for participants with and without prevalent CHD are shown in table 1. Adiponectin levels were lower in persons with prevalent CHD than in those without CHD, although this difference was of borderline statistical significance ( $p = 0.056$ ).

### Adiponectin and CHD risk factors

Age- and sex-adjusted associations of adiponectin with CHD risk factors were similar for persons with and without prevalent CHD (table 2). Adiponectin levels were negatively correlated with body mass index, waist girth, triglycerides, and fasting plasma glucose and positively correlated with age and HDL cholesterol. Adiponectin concentrations were higher in persons who reported drinking one or more alcohol beverages daily compared with those who drank less or not at all. Current smoking and exercise were not related to adiponectin. Adiponectin levels were higher in persons who had lost 10 or more pounds in the preceding 10 years than in those who had not, irrespective of CHD status and independent of current weight (data not shown). The metabolic syndrome was associated with lower adiponectin levels, independent of CHD status, whereas diabetes was associated with significantly lower adiponectin in participants without prevalent CHD but not in those with CHD ( $p$  for interaction = 0.15).

### Adiponectin and prevalent CHD

The age-adjusted prevalence of CHD was threefold higher ( $p < 0.001$ ) in men (15.2 percent) than in women (5.0 percent). Table 3 shows age- and sex-adjusted odds ratios for prevalent CHD by sex-specific adiponectin quintiles (quintile cutpoints were 10.6, 13.9, 17.5, and 22.4 mg/liter for women and 6.2, 8.5, 11.4, and 15.8 mg/liter for men). The odds of CHD were 44 percent lower among participants with adiponectin values in the highest quintile as compared with the lowest quintile ( $p_{\text{trend}} = 0.03$ ); adjustment for waist girth strengthened this association to a 55 percent reduction in odds. Adjusting for other CHD risk factors, including lifestyle variables (exercise, current smoking,

**TABLE 1. Age-, sex-, and age- and sex-adjusted baseline characteristics of 1,513 older men and postmenopausal women according to prevalent and incident coronary heart disease, Rancho Bernardo Study, 1984–1987 through 2004†**

	No prevalent CHD‡ (n = 1,352)		Prevalent CHD (n = 161)		No incident§ CHD (n = 1,100)		Incident§ CHD (n = 252)	
	Mean or %	95% CI‡	Mean or %	95% CI	Mean or %	95% CI	Mean or %	95% CI
Demographic and anthropomorphic parameters (mean values, except for sex)								
Sex (% male)	52.7		75.8***		52.5		53.6	
Age (years)	71.9	71.5, 72.4	75.6	74.2, 77.0***	71.2	70.7, 71.8	75.4	74.2, 76.5***
Body mass index¶ (kg/m <sup>2</sup> )	25.1	24.9, 25.3	24.9	24.2, 25.5	25.0	24.8, 25.2	25.6	25.1, 26.0*
Waist girth (cm)	87.3	86.8, 87.8	86.0	84.6, 87.5	86.6	86.0, 87.1	88.9	97.7, 90.0***
Metabolic parameters (mean values)								
Systolic blood pressure (mmHg)	141.6	140.5, 142.6	139.9	136.8, 143.0	140.8	139.6, 141.9	143.6	141.1, 146.0*
Diastolic blood pressure (mmHg)	77.3	76.8, 77.8	74.6	73.2, 76.1***	77.2	76.6, 77.8	77.3	76.1, 78.4
Pulse pressure (mmHg)	64.3	63.4, 65.1	65.2	62.7, 67.7	63.5	62.6, 64.4	66.2	64.3, 68.2*
Total cholesterol level (mmol/liter)	5.69	5.63, 5.74	5.79	5.62, 5.95	5.65	5.59, 5.71	5.91	5.79, 6.04***
Low density lipoprotein cholesterol level (mmol/liter)	3.55	3.50, 3.60	3.63	3.48, 3.78	3.51	3.46, 3.57	3.78	3.66, 3.89***
High density lipoprotein cholesterol level (mmol/liter)#	1.48	1.46–1.50	1.35	1.30–1.41***	1.51	1.48–1.53	1.40	1.35–1.45***
Triglyceride level (mmol/liter)#	1.15	1.12–1.19	1.38	1.27–1.51***	1.12	1.09–1.16	1.31	1.23–1.41***
Fasting plasma glucose level (mmol/liter)	5.65	5.58, 5.71	5.85	5.66, 6.04	5.60	5.53, 5.68	5.82	5.67, 5.97***
Creatinine level (μmol/liter)	102.1	99.9, 103.4	109.6	105.2, 114.0**	100.8	99.1, 102.5	103.2	99.6, 106.8
Adiponectin level (mg/liter)#	11.9	11.6–12.3	11.0	10.1–11.9	12.1	11.8–12.5	11.2	10.5–11.9*
Lifestyle parameters (%)								
Alcohol consumption (≥1 drink/day)	44.3		45.3		44.2		42.7	
Current smoker	12.4		10.9		12.4		13.9	
Physical exercise (≥3 times/week)	81.3		77.2		82.3		75.6*	
Prevalent conditions (%)								
Diabetes mellitus	14.3		23.0**		13		19.2*	
Metabolic syndrome	17.3		23.0**		15.4		25.5***	
Hypertension	77.5		70.5*		44.0		48.4	
Weight loss of ≥10 pounds (≥4.5 kg) (%)	21.7		24.4		21.5		19.9	
Family history of myocardial infarction (%)	4.3		5.7		4.1		5.0	
Aspirin use (%)	20.7		45.1***		21.3		17.0	

\*  $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$  (prevalent CHD vs. no prevalent CHD or incident CHD vs. no incident CHD).

† Values shown are adjusted means (with 95% CIs) for continuous variables and percentages for categorical variables.

‡ CHD, coronary heart disease; CI, confidence interval.

§ Incident CHD excludes prevalent CHD cases.

¶ Weight (kg)/height (m)<sup>2</sup>.

# Data were log<sub>10</sub>-transformed for analysis; values shown are geometric means and interquartile ranges.

**TABLE 2. Age- and sex-adjusted associations of adiponectin with selected cardiovascular disease risk factors in persons with and without prevalent coronary heart disease, Rancho Bernardo Study, 1984–1987 through 2004†**

Cardiovascular disease risk factor	No CHD‡ (n = 1,352)		CHD (n = 161)	
	Correlation (R)			
Age	0.32***		0.32***	
Body mass index	-0.29***		-0.23**	
Waist girth	-0.30***		-0.26***	
Waist:hip ratio	-0.26***		-0.21**	
Systolic blood pressure	-0.02		-0.11	
Diastolic blood pressure	-0.03		-0.09	
Pulse pressure	-0.01		-0.08	
Total cholesterol	0.01		0.02	
Low density lipoprotein cholesterol	-0.05		0.02	
High density lipoprotein cholesterol§	0.43***		0.52***	
Triglycerides§	-0.35***		-0.47***	
Fasting plasma glucose	-0.17***		-0.18*	
Creatinine	-0.03		-0.06	
	Geometric mean			
	Yes	No	Yes	No
Lifestyle variables				
Current smoking	12.2	11.9	12.1	10.4
Alcohol consumption (≥1 drink/day)	12.7***	11.4	11.7*	9.6
Physical exercise (≥3 times/week)	11.9	12.1	10.7	10.2
Concomitant conditions				
Diabetes mellitus	9.6***	12.4	9.7	10.9
Metabolic syndrome	8.5***	12.9	7.1***	11.9
Hypertension	11.8	12.5	10.3	11.3
Weight loss ≥10 pounds (≥4.5 kg)	13.9***	11.5	13.8***	9.6
Family history of myocardial infarction	12.0	11.9	11.8	10.5
Aspirin use	12.4	11.9	10.7	10.4

\*  $p \leq 0.05$ ; \*\* $p \leq 0.01$ ; \*\*\* $p \leq 0.001$ .

† Values shown are Pearson partial correlation coefficients for continuous variables and adjusted geometric means for categorical variables.

‡ CHD, coronary heart disease.

§ Data were  $\log_{10}$ -transformed for analysis.

and alcohol intake), family history of myocardial infarction, aspirin use, serum creatinine, weight loss, and prevalent diabetes and hypertension, did not materially alter these results (data not shown). However, the addition of HDL cholesterol, triglycerides, and fasting plasma glucose together (model 3), HDL cholesterol alone, or triglycerides alone (data not shown) eliminated the association between adiponectin and prevalent CHD. Results were similar in separate analyses excluding 230 participants with diabetes; adiponectin was not significantly related to prevalent CHD in analyses excluding 271 persons with the metabolic syndrome.

#### Adiponectin and incident CHD

After exclusion of the 161 participants with prevalent CHD at baseline, 252 (18.6 percent) of the remaining 1,352 par-

ticipants had a first CHD event during the 20-year follow-up period; 144 (57.1 percent) of the events were fatal. The age-adjusted event rates did not differ significantly ( $p = 0.14$ ) between men (20.1 percent) and women (17.0 percent); the median time to event was 7.5 years. In comparisons adjusted for age and sex, participants who experienced an incident CHD event differed significantly from those who did not with regard to almost every attribute (table 1). They were older and had greater body mass index and waist girth, more adverse blood pressure and lipid profiles, and higher fasting plasma glucose levels. They also tended to exercise less and were more likely to have diabetes and the metabolic syndrome. Baseline age- and sex-adjusted adiponectin levels were significantly lower among persons with incident CHD than among those without it (mean values: 11.99 mg/liter and 12.22 mg/liter, respectively;  $p = 0.03$ ); this difference was

**TABLE 3. Age- and sex-adjusted odds ratios for prevalent coronary heart disease ( $n = 161$ ) according to adiponectin level in 1,513 older men and postmenopausal women, Rancho Bernardo Study, 1984–1987 through 2004**

	Model 1†		Model 2‡		Model 3§	
	OR¶	95% CI¶	OR	95% CI	OR	95% CI
Per quintile						
1 (38 cases)	1		1		1	
2 (29 cases)	0.64	0.38, 1.08	0.58	0.34, 1.00	0.72	0.42, 1.26
3 (33 cases)	0.66	0.40, 1.11	0.64	0.38, 1.07	0.87	0.50, 1.49
4 (27 cases)	0.53	0.31, 0.90	0.47	0.27, 0.82	0.71	0.40, 1.28
5 (34 cases)	0.56	0.33, 0.94	0.45	0.26, 0.79	0.83	0.45, 1.55
$p$ for trend	0.025		0.006		0.56	
Per unit increase in log	0.49*	0.24, 0.98	0.39*	0.19, 0.81	0.96	0.41, 2.25
Per standard-deviation log increase	0.84*	0.71, 0.99	0.80*	0.67, 0.95	0.98	0.80, 1.21
Excluding diabetes#	0.81*	0.67, 0.99	0.78*	0.64, 0.95	0.92	0.73, 1.15
Excluding metabolic syndrome**	0.9	0.73, 1.10	0.86	0.69, 1.06	1	0.78, 1.28

\*  $p \leq 0.05$ .

† Adjusted for age and sex.

‡ Adjusted for age, sex, and waist girth.

§ Adjusted for model 2 variables plus high density lipoprotein cholesterol, triglycerides, and fasting plasma glucose.

¶ OR, odds ratios; CI, confidence interval.

# Excludes 230 participants with diabetes and 38 participants with coronary heart disease.

\*\* Excludes 271 participants with metabolic syndrome and 37 participants with coronary heart disease.

significant for nonfatal CHD (means: 10.54 mg/liter and 11.99 mg/liter, respectively;  $p = 0.01$ ) but not fatal CHD (means: 11.72 mg/liter and 12.16 mg/liter, respectively;  $p = 0.41$ ).

In Cox regression analyses (table 4), there was a marginally significant ( $p = 0.056$ ) trend toward reduced risk of incident CHD at higher adiponectin concentrations for the population as a whole. Sex-specific analyses were also conducted, first because sex differences have been reported in the literature (14, 16) and second because the  $p$  value for the sex  $\times$  log<sub>10</sub> adiponectin interaction term was 0.10, which in our opinion justifies further exploration. Age-adjusted adiponectin levels were lower for men with an incident CHD event than for those without one (means: 8.66 mg/liter and 9.93 mg/liter, respectively;  $p = 0.01$ ), and a higher adiponectin level predicted reduced risk of incident CHD in age-adjusted analysis. This association was not independent of waist girth. Age-adjusted adiponectin levels did not differ at baseline for women with and without incident CHD (means: 15.03 mg/liter and 15.20 mg/liter, respectively;  $p = 0.84$ ), and adiponectin was not associated with future CHD risk in women.

We investigated whether the association of adiponectin levels with incident CHD differed for fatal and nonfatal events. For men, adiponectin was protective against future nonfatal myocardial infarction but not fatal CHD, independent of waist girth (table 5). This association became borderline significant ( $p = 0.07$ ) after additional adjustment for HDL cholesterol, triglycerides, and fasting plasma glucose. For women, adiponectin was not associated with incident CHD in analyses using either a first nonfatal myocardial

infarction or fatal CHD as the first medical presentation (data not shown). Age-adjusted adiponectin levels did not differ for persons with a first fatal CHD event as compared with those with a nonfatal CHD event in either sex (data not shown).

### Adiponectin and mortality

Among the 1,361 participants with complete follow-up and death certificate data, the age-adjusted proportion of deaths was 70.9 percent for men and 64.0 percent for women during the 20-year follow-up period. The age-adjusted mortality rates per 1,000 person-years were 61.9 and 46.4, respectively. Of 925 total deaths, 441 (48 percent) were attributed to cardiovascular disease; 215 (49 percent) of these were due to CHD.

The age-adjusted proportion of CHD deaths was higher among men than among women (17.5 percent vs. 13.5 percent;  $p = 0.04$ ). Age-adjusted CHD mortality rates per 1,000 person-years were 16.5 and 9.9, respectively. Age- and sex-adjusted baseline adiponectin levels did not differ for persons with CHD death and persons without CHD death (means: 11.32 mg/liter and 11.83 mg/liter, respectively;  $p = 0.27$ ). Adiponectin concentrations were not significantly related to CHD mortality before or after adjustment for covariates (table 6), and there was no evidence for a trend across adiponectin quintiles (figure 1, part A).

We also examined the associations of adiponectin with cardiovascular disease and all-cause mortality. Age- and sex-adjusted baseline adiponectin levels did not differ on

**TABLE 4. Age-adjusted hazard ratios for incident fatal and nonfatal coronary heart disease according to adiponectin level in 1,352 older men and postmenopausal women, Rancho Bernardo Study, 1984–1987 through 2004**

	Total population (n = 1,352; 252 events)		Men (n = 713; 135 events)		Women (n = 639; 117 events)	
	HR†	95% CI†	HR	95% CI	HR	95% CI
Per quintile‡						
1 (63 cases)	1		1		1	
2 (70 cases)	0.88	0.59, 1.27	0.64	0.38, 1.09	1.25	0.70, 2.23
3 (61 cases)	0.70	0.47, 1.04	0.59	0.35, 0.99	0.84	0.45, 1.56
4 (58 cases)	0.70	0.48, 1.04	0.53	0.32, 0.90	0.99	0.55, 1.79
5 (56 cases)	0.69	0.48, 1.01	0.55	0.32, 0.94	1	0.56, 1.81
p for trend	0.056		0.02		0.74	
Per log increase‡	0.62	0.34, 0.94	0.46*	0.23, 0.93	1.02	0.41, 2.54
Per standard-deviation log increase						
Model 1‡	0.90	0.79, 0.99	0.83*	0.69, 0.98	0.99	0.86, 1.21
Model 2§	0.96	0.82, 1.05	0.87	0.72, 1.05	1.09	0.88, 1.33
Model 3¶	1.10	0.89, 1.17	0.97	0.79, 1.18	1.30*	1.03, 1.65
Model 1						
Excluding diabetes#	0.95	0.82, 1.10	0.90	0.73, 1.09	1.02	0.82, 1.28
Excluding metabolic syndrome**	1.06	0.90, 1.25	0.95	0.76, 1.19	1.20	0.94, 1.53

\*  $p \leq 0.05$ .

† HR, hazard ratio; CI, confidence interval.

‡ Adjusted for age and sex.

§ Adjusted for age, sex, and waist girth.

¶ Adjusted for model 2 variables plus high density lipoprotein cholesterol, triglycerides, and fasting plasma glucose.

# Excludes 192 participants with diabetes and 54 participants with incident CHD.

\*\* Excludes 234 participants with metabolic syndrome and 75 participants with incident CHD.

the basis of future cardiovascular disease death or death from all causes (data not shown). In age- and sex-adjusted analyses, there was a trend toward increased risk of mortality from all causes across adiponectin quintiles ( $p = 0.036$ ), which increased in strength for all-cause mortality and became significant for cardiovascular disease mortality after additional adjustment for waist girth and other adiponectin covariates (HDL cholesterol, triglycerides, and fasting plasma glucose). As is shown in figure 1, parts B and C, the risks of death due to cardiovascular disease or all causes were similar for the first four quintiles of adiponectin, and markedly elevated for the top quintile. Participants with adiponectin levels above the 80th percentile, compared with persons with lower levels, had almost 40 percent increased risks of fatal cardiovascular disease and all-cause mortality after adjustment for major adiponectin covariates (table 6).

Separate analyses adjusting for additional risk factors, including lifestyle variables (exercise, current smoking, and alcohol intake), family history of myocardial infarction, aspirin use, serum creatinine level, and history of diabetes, hypertension, or the metabolic syndrome yielded similar results (data not shown). Although weight loss during the 10 years prior to baseline was a strong independent predictor of mortality (hazard ratio = 1.54, 95 percent confidence interval: 1.32, 1.80), adjustment for weight loss

(table 6) or stratification by weight loss (data not shown) did not alter the associations. Sequential exclusion of persons with diabetes, the metabolic syndrome, or CHD at baseline had a negligible effect on the mortality results (table 6).

**TABLE 5. Hazard ratios for incident fatal coronary heart disease and nonfatal myocardial infarction per standard-deviation log increase in adiponectin level among 713 older men, Rancho Bernardo Study, 1984–1987 through 2004**

	Fatal coronary heart disease (74 events)		Nonfatal myocardial infarction (61 events)	
	HR†	95% CI†	HR	95% CI
Model 1‡	0.95	0.74, 1.20	0.67*	0.52, 0.86
Model 2§	1.04	0.80, 1.35	0.69*	0.53, 0.90
Model 3¶	1.18	0.88, 1.58	0.76	0.57, 1.08

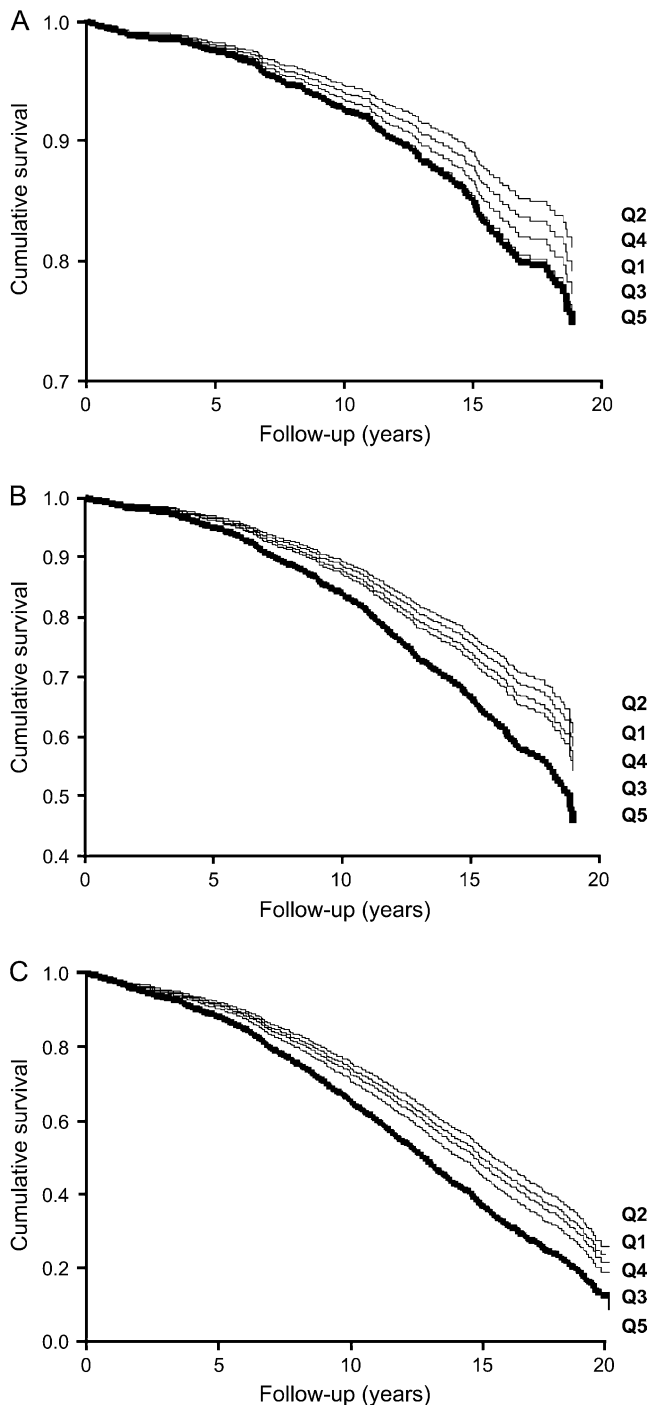
\*  $p \leq 0.01$ .

† HR, hazard ratio; CI, confidence interval.

‡ Adjusted for age.

§ Adjusted for age and waist girth.

¶ Adjusted for model 2 variables plus high density lipoprotein cholesterol, triglycerides, and fasting plasma glucose.



**FIGURE 1.** Survival according to quintile (Q) of adiponectin level for (A) coronary heart disease mortality, (B) cardiovascular disease mortality, and (C) all-cause mortality, after adjustment for age, sex, waist girth, and levels of high density lipoprotein cholesterol, triglycerides, and fasting plasma glucose, Rancho Bernardo Study, 1984–1987 through 2004. (Note differences in the y-axis scales in the three sections.) The heavy line represents the highest quintile. Hazard ratios for the highest quintile versus the lowest were 1.13 (95% confidence interval (CI): 0.69, 1.85) for coronary heart disease mortality, 1.46 (95% CI: 1.04, 2.07) for cardiovascular disease mortality, and 1.43 (95% CI: 1.13, 1.82) for all-cause mortality. *P* values for trend across the adiponectin quintiles were 0.51, 0.015, and 0.002, respectively.

## DISCUSSION

To our knowledge, this is the first long-term prospective study to investigate the association of adiponectin with multiple CHD outcomes in men and women from the same population. We confirm that a high serum adiponectin level is associated with a favorable CHD risk profile and reduced odds of prevalent CHD in both sexes. Higher adiponectin concentrations also showed a protective association with risk of nonfatal myocardial infarction over the following 20 years in men without diagnosed cardiovascular disease. However, adiponectin concentrations did not predict future CHD events in women and were not associated with 20-year fatal CHD in either sex. These observations, together with the unfavorable association of high adiponectin with mortality, suggest that use of serum adiponectin for cardiovascular disease risk stratification is premature.

The inverse, dose-dependent association between serum adiponectin level and prevalent CHD was independent of adiposity, diabetes, hypertension, and several other CHD risk factors but was not independent of HDL cholesterol and triglyceride levels. These results suggest that a significant proportion of any protective effect of high concentrations of adiponectin may be mediated by lipid metabolism, specifically promotion of HDL cholesterol and reduction of circulating triglycerides. Although the exact mechanism by which adiponectin modulates lipid metabolism is unknown, adiponectin treatment reverses dyslipidemia in adiponectin-deficient mice (26, 27), and hypoadiponectinemia in humans is associated with increased hepatic lipase activity (28), a major determinant of HDL and triglyceride concentrations; both suggest a causal association.

Only longitudinal studies can distinguish cause from effect. In our prospective analysis, baseline levels of adiponectin were lower in men but not in women who had a first CHD event during the 20-year follow-up period, and higher adiponectin was associated with reduced risk of future CHD for men only. These results agree with two single-sex studies reporting protective associations of adiponectin with incident CHD in men (14) but not women (16). Further analyses showed that the inverse association of adiponectin with incident CHD in men was specific to a first nonfatal myocardial infarction; adiponectin was not associated with incident CHD events that resulted in death. Although the number of events in our subgroup analysis was small, Pischon et al. (14) reported the same result with a larger Health Professionals Follow-up Study data set in which data were stratified by type of event.

Why would adiponectin protect against the development of nonfatal myocardial infarction but not fatal myocardial infarction, and why only in men? The lack of association of adiponectin with fatal CHD potentially reflects misclassification of CHD as a cause of death in the elderly, whereas biologic interactions between sex hormones, adipocytokines, and CHD may account for the sex differences. Alternatively, adiponectin has been shown to influence thrombus formation and platelet aggregation in mouse models (29); thus, adiponectin may influence myocardial infarction through thrombotic processes as well as atherosclerotic processes. Larger prospective studies comparing the sexes and

**TABLE 6. Age- and sex-adjusted hazard ratios for mortality due to coronary heart disease, cardiovascular disease, and all causes during 20 years of follow-up, according to baseline serum adiponectin level, among 1,361 older men and postmenopausal women, Rancho Bernardo Study, 1984–1987 through 2004**

	CHD† death (215 events)		CVD† death (441 events)		All causes of death (925 events)	
	HR†	95% CI†	HR	95% CI	HR	95% CI
Per quintile‡,§						
1	1		1		1	
2	0.74	0.47, 1.18	0.87	0.63, 1.22	0.89	0.71, 1.11
3	0.94	0.62, 1.43	1.01	0.74, 1.39	1.08	0.87, 1.33
4	0.72	0.46, 1.11	0.92	0.67, 1.25	0.96	0.77, 1.18
5	0.79	0.51, 1.20	1.15	0.84, 1.53	1.22	0.99, 1.51
<i>p</i> for linear trend	0.28		0.34		0.036	
Per log increase§	0.88	0.49, 1.58	1.36	0.89, 2.09	1.38*	1.03, 1.85
Quintile 5 vs. lower quintiles						
Model 1§	0.92	0.67, 1.27	1.19	0.96, 1.48	1.24**	1.07, 1.45
Model 2¶	0.98	0.70, 1.37	1.21	0.96, 1.50	1.26**	1.08, 1.48
Model 3#	1.18	0.83, 1.69	1.38**	1.09, 1.76	1.36***	1.15, 1.60
Plus weight loss††	1.17	0.81, 1.69	1.38**	1.08, 1.76	1.35***	1.14, 1.60
Excluding diabetes mellitus‡‡	1.13	0.79, 1.62	1.30*	1.01, 1.67	1.30**	1.09, 1.54
Excluding metabolic syndrome§§	1.11	0.75, 1.62	1.30*	1.01, 1.66	1.35***	1.13, 1.60
Excluding CHD¶¶	1.14	0.76, 1.72	1.38**	1.07, 1.79	1.36***	1.14, 1.63

\*  $p \leq 0.05$ ; \*\* $p \leq 0.01$ ; \*\*\* $p \leq 0.001$ .

† CHD, coronary heart disease; CVD, cardiovascular disease; HR, hazard ratio; CI, confidence interval.

‡ Numbers of deaths in quintiles 1–5, respectively—CHD: 40, 34, 50, 42, and 49; CVD: 70, 70, 92, 92, and 117; all causes: 151, 153, 192, 194, and 235.

§ Adjusted for age and sex.

¶ Adjusted for age, sex, and waist girth.

# Adjusted for model 2 variables plus high density lipoprotein cholesterol, triglycerides, and fasting plasma glucose.

†† Weight loss of  $\geq 10$  pounds ( $\geq 4.5$  kg) in the 10 years prior to baseline.

‡‡ No prevalent diabetes (total  $n = 1,155$ ; 172 CHD deaths, 360 CVD deaths, and 765 all-cause deaths).

§§ No prevalent metabolic syndrome (total  $n = 1,122$ ; 170 CHD deaths, 357 CVD deaths, and 742 all-cause deaths).

¶¶ No prevalent CHD (total  $n = 1,210$ ; 166 CHD deaths, 363 CVD deaths, and 793 all-cause deaths).

comparing fatal disease with nonfatal disease are needed to resolve these issues.

Participants in the present study were older than in any of the previous longitudinal studies of incident CHD, and follow-up was longer (20 years vs. 4–6 years). However, restriction of our analysis to the first 5 years of follow-up did not alter the results for incident disease. The presence of unrecognized CHD in the group identified as CHD-free at baseline could have led to overestimation of the association between adiponectin and incident events, since these persons may have been more likely to have low levels of adiponectin as well as increased event risk. However, differences in baseline characteristics between persons who did and did not experience CHD events were strong and were consistent with established CHD risk profiles, and this bias is unlikely to have preferentially influenced results for women.

Despite the contradictory results obtained in prospective studies, a large body of experimental evidence and several cross-sectional studies in humans support the thesis that

adiponectin is a protective factor for the cardiovascular system. The association of hypo adiponectinemia with increased cancer risk (30–33) suggests that this protein could also have beneficial effects on other physiologic systems. Thus, the absence of an inverse association of adiponectin with CHD mortality seems counterintuitive, and the association of high adiponectin levels with increased risk of death from any cause over 20 years is surprising. However, we are not the first investigators to make this observation. Higher adiponectin levels were associated with increased risk of death in a 15-year population-based study of more than 2,000 Dutch men and women aged 50–75 years (17), in a 4-year study of 195 patients with congestive heart failure (34), and in a 9-year follow-up of 1,025 Danish patients with stable CHD (35). Not all mortality studies have agreed. In a study of 227 patients with end-stage renal failure by Zoccali et al. (36), adiponectin did not predict overall mortality over a mean follow-up period of 2.5 years, and in a study by Efstathiou et al. (37), low, not high, levels were related to increased risk of 5-year mortality after a first ischemic stroke.

The physiology underlying increased mortality risk at higher adiponectin concentrations is likely to be complex, particularly in the elderly, who have multiple causes of death. Adiponectin receptors have been identified in the brain, and both peripheral and central adiponectin administration decreases weight by increasing energy expenditure and fatty acid oxidation (38, 39). As Kistorp et al. (34) have proposed, unusually high adiponectin levels may be a marker for catabolic processes leading to wasting, which often presages death. In the present study, weight loss of 10 or more pounds during the 10 years prior to baseline was associated with higher adiponectin levels, independent of current weight, and weight loss was associated with a marked increase in mortality risk, as previously reported for this cohort (19). However, weight loss did not account for the association of high adiponectin levels with mortality in multivariate analyses or in analyses stratified by weight-loss status. The positive association of adiponectin with mortality may be due to confounding by some other unidentified factor and not to direct detrimental effects of adiponectin.

Some limitations of this study should be noted. Our results were based on a predominantly White middle- to upper-middle-class community and may not apply to other ethnic and socioeconomic groups. Nonfatal CHD events were based on self-report, which could have resulted in misclassification and biased results toward the null. However, at an earlier Rancho Bernardo study visit, 30 percent of the cohort had their medical records searched for validation of self-reported CHD, and 85 percent of the events were confirmed. The number of events in several of the stratified analyses in this study was small and may have resulted in missed associations. Serum adiponectin levels were measured in a single sample, which may have limited the observed associations. However, adiponectin levels show minimal diurnal variation and are stable during the morning hours, when these samples were collected, and single measurements have been shown to accurately reflect levels over a 1-year period (40–43). It is not known whether long-term storage affects adiponectin levels; in this cohort, adiponectin concentrations were not significantly related to storage time, and levels were similar to published values for persons of comparable age and adiposity (4, 15, 16).

In conclusion, we found divergent associations of circulating adiponectin levels with prevalent and incident CHD and with mortality. Although higher adiponectin concentrations were associated with decreased odds of prevalent CHD for both sexes, adiponectin had only a weak association with future nonfatal CHD events, and only in men. These results, together with those of other investigators, bring into question whether serum adiponectin level is an independent risk factor for CHD or is simply a risk marker. Proposals for pharmacologic and lifestyle interventions aimed at enhancing adiponectin levels may be premature.

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