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The Association of Fetuin-A With Cardiovascular Disease Mortality in Older Community-Dwelling Adults

The Rancho Bernardo Study

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Objectives The goal of this study was to evaluate the prospective association of fetuin-A levels with cardiovascular disease (CVD) mortality.

Background Fetuin-A is a circulating inhibitor of calcium deposition in the vasculature and of insulin action in muscle and fat, and may be involved in the pathogenesis of CVD.

Methods This is a population-based prospective study of 633 men and 1,025 women (median age = 73 years) who had fetuin-A levels and CVD risk factors evaluated in 1992 to 1996 and were followed for vital status through 2010.

Results Plasma fetuin-A (g/l ± SD) was highest in women using oral estrogens (0.55 ± 0.12), intermediate for women not using oral estrogens (0.51 ± 0.10), and lowest for men (0.50 ± 0.10), $p < 0.001$. Lower fetuin-A levels were associated with older age, but with lower levels of other CVD risk factors including adiposity, blood pressure, lipids, triglycerides, and insulin resistance (all $p < 0.01$). During the median 12-year follow-up, 273 deaths were attributed to CVD. The association of fetuin-A with CVD mortality differed by diabetes status (p for interaction = 0.003). Adjusting for age, sex, oral estrogens, and lifestyle, the hazard ratio for CVD mortality comparing the lowest fetuin-A quartile with all higher values was 1.76 (95% confidence interval [CI]: 1.34 to 2.31; $p < 0.001$) for participants without diabetes and 0.43 (95% CI: 0.19 to 0.98; $p = 0.046$) for participants with diabetes.

Conclusions Low fetuin-A levels predicted greater risk for CVD mortality in older adults without diabetes, but were associated with reduced risk of CVD death in those with diabetes. Fetuin-A may provide novel insight into mechanisms leading to CVD death in those with versus without diabetes. (J Am Coll Cardiol 2012;59:1688–96) © 2012 by the American College of Cardiology Foundation

Fetuin-A is a multifunctional liver-derived protein found in high concentrations in human serum (1). Two of the primary physiological functions of fetuin-A may be critically important to cardiovascular health. First, fetuin-A acts as an inhibitor of calcification by increasing the blood solubility of calcium and phosphorus, and preventing spontaneous mineral precipitation in the vasculature (2,3). In end-stage renal disease populations, lower plasma fetuin-A levels are associated with greater prevalence and severity of vascular calcification (4,5) and increased risk of cardiovascular disease (CVD) events and mortality (5–8), independent of traditional CVD and kidney disease risk factors. Recent evidence suggests that fetuin-A may also inhibit vascular calcification in individuals with normal kidney function. We and others demonstrated that lower fetuin-A levels are independently correlated with coronary artery calcification in older adults with normal kidney function and no known CVD (9) and with cardiac valvular calcification in a cohort with normal kidney function and prevalent CVD (10,11).

Fetuin-A also regulates insulin signaling. Only 2 proteins are known to bind directly to the extracellular domain of the insulin receptor—insulin and fetuin-A. Experimental evidence indicates that fetuin-A binding inhibits the insulin receptor tyrosine kinase (12) and induces insulin

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resistance in muscle and fat (13). In epidemiological studies, higher fetuin-A levels are associated with insulin resistance among individuals without diabetes (14–16) and predict incident type 2 diabetes mellitus, independent of other markers of insulin resistance (17,18).

These dual physiological roles of fetuin-A are evident in fetuin-A knockout mice who are characterized by ectopic calcification, but also display greater insulin sensitivity and resistance to weight gain compared with their wild-type littermates (19–21). Thus, fetuin-A sufficiency may be necessary to prevent vascular calcification, but fetuin-A excess may lead to insulin resistance and metabolic dysregulation. In this study, we examined the prospective association of plasma fetuin-A levels with CVD mortality among community-dwelling older adults from the Rancho Bernardo study. On the basis of the existing literature, we hypothesized that lower fetuin-A levels would be associated with increased CVD mortality risk in individuals without diabetes mellitus, and hypothesized no association of fetuin-A with CVD death in diabetics due to competing influences of insulin resistance and calcification in this subset.

Methods

Study population. Between 1972 and 1974, community-dwelling residents living in Rancho Bernardo, California, aged 30 to 79 years were invited to participate in a study of heart disease risk factors, and 82% (n = 5,052) enrolled. Nearly all were middle to upper-middle class, and relatively well educated. Since then, sequential study visits have been conducted at approximately 4-year intervals. The present analysis included individuals who participated in the 1992 to 1996 clinic visit. The study 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 evaluated at the 1992 to 1996 visit; 2) availability of stored sera; 3) post-menopausal status for women; and 4) follow-up for vital status. Of the 1,781 participants who attended the 1992 to 1996 clinic visit, 49 were excluded for age <50 years, 6 women for pre-menopausal status, 39 for insufficient stored plasma for fetuin-A determination, and 8 for no follow-up after their 1992 to 1996 visit. The final sample consisted of 663 men and 1,025 women.

During the 1992 to 1996 visit, information regarding medical history, medication use, physical activity, alcohol consumption, and current smoking was obtained using standard questionnaires. Current medication use was validated by examination of pills and prescriptions brought to the clinic for that purpose, and participants were asked to rate their overall health on a 5-point scale (excellent, very good, good, fair, or poor).

Clinical measurements. Height, weight, and waist and hip girth were measured in the clinic with participants wearing light clothing and no shoes. Body mass index (BMI) (kg/m^2) and waist-to-hip ratio were used as estimates of overall and central adiposity, respectively. Blood pressures were measured twice in seated resting subjects using the Hypertension Detection and Follow-Up Program protocol (22); the mean of the 2 measures was used in analyses.

Abbreviations and Acronyms

BMI = body mass index
CI = confidence interval
CVD = cardiovascular disease
eGFR = estimated glomerular filtration rate
HDL = high-density lipoprotein
HOMA-IR = homeostasis model assessment for insulin resistance
HR = hazard ratio
LDL = low-density lipoprotein

Blood samples were obtained by venipuncture between 0730 h and 1100 h after a requested 12-h fast; serum and plasma were separated and frozen at −70°C. Fetuin-A levels were measured in duplicate in 2010 on EDTA plasma samples using a human enzyme-linked immunoadsorbent assay kit (Epitope Diagnostics, San Diego, California). This assay uses a 2-site "sandwich" technique with polyclonal antibodies that bind different epitopes of human fetuin-A. Intra-assay and interassay coefficients of variation were 2.4% to 4.7% and 9.5% to 9.9%, respectively, for the set of assays used for the present sample. Plasma total and high-density lipoprotein (HDL) cholesterol and triglycerides were measured in a Centers for Disease Control Certified Lipid Research Clinic laboratory using established methods (23). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation. Serum creatinine was measured using the Jaffe reaction; liver enzymes by spectrophotometry; and serum phosphorus and calcium by a standard clinical automated analyzer.

Prevalent conditions and mortality assessment. Prevalent CVD was defined as physician-diagnosed myocardial infarction, coronary artery revascularization, congestive heart failure, stroke or transient ischemic attack, carotid surgery, peripheral arterial surgery, or physician-diagnosed intermittent claudication. Validation of self-reported heart attack (by chest pain, enzyme elevation, and electrocardiography) was achieved for 72% of a subset for whom hospital records could be obtained. Diabetes was defined by physician diagnosis, fasting plasma glucose ≥126 mg/dl, 2-h post-challenge glucose ≥200 mg/dl, or use of diabetes medications. The metabolic syndrome was defined according to the 2002 Adult Treatment Panel III criteria (24). Hypertension was defined as blood pressure ≥140/90 mm Hg or use of antihypertensive medication. Estimated glomerular filtration rate (eGFR) was calculated by the MDRD (Modification of Diet in Renal Disease study) equation (25); participants with eGFR <60 ml/min/1.73 m^2 were classified as having moderate chronic kidney disease (26). Homeostasis model assessment for insulin resistance (HOMA-IR) was used to estimate insulin resistance (27). Comorbidities recorded included thyroid, liver, kidney, and heart

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Table 1 Baseline Characteristics of the Study Population by Sex and Oral Estrogen–Specific Quartile of Fetuin-A

	Total (N = 1,688)	Fetuin-A Quartile Q1 (n = 423)	Q2 (n = 422)	Q3 (n = 423)	Q4 (n = 422)	p Trend*
Fetuin, g/l†						
Men (n = 663)	0.50 (0.10)	0.26, 0.44	0.44, 0.50	0.50, 0.56	0.56, 1.24	—
Women—no oral ET (n = 623)	0.51 (0.10)	0.25, 0.44	0.44, 0.51	0.51, 0.57	0.57, 0.86	—
Women—using oral ET (n = 402)	0.55 (0.12)	0.22, 0.47	0.47, 0.54	0.54, 0.63	0.63, 1.04	—
Demographic and anthropomorphic factors						
Age, yrs	72.2 (10.5)	75 (10)	72 (10)	71 (11)	70 (10)	<0.001
Body mass index, kg/m^2	25.4 (4.0)	24.5 (3.7)	25.4 (4.2)	25.7 (3.9)	26.1 (4.1)	<0.001
Waist circumference, cm	85.3 (12.8)	83.5 (12.3)	85.0 (12.9)	85.4 (12.4)	87.5 (13.4)	<0.001
Waist-to-hip ratio	0.84 (0.09)	0.84 (0.09)	0.84 (0.09)	0.84 (0.09)	0.85 (0.10)	0.03
CVD risk factors						
Systolic blood pressure, mm Hg	136.8 (22.1)	137 (22)	136 (22)	136 (23)	138 (22)	0.60
Diastolic blood pressure, mm Hg	75.7 (9.5)	75 (10)	75 (9)	76 (9)	77 (9)	<0.001
Total cholesterol, mg/dl	209.6 (36.6)	202 (35)	209 (37)	212.2 (35)	216 (38)	<0.001
LDL cholesterol, mg/dl	126.9 (32.5)	121 (32)	127 (33)	129 (31)	131 (33)	<0.001
HDL cholesterol, mg/dl‡	55.7 (45, 69)	57 (46, 72)	57 (47, 70)	55 (45, 68)	53 (42, 66)	<0.001
Triglycerides, mg/dl‡	105.6 (74, 148)	92 (66, 120)	100 (68, 142)	112 (79, 159)	124 (85, 184)	<0.001
Fasting plasma glucose, mg/dl	98.7 (23.0)	95.9 (17.3)	99.2 (20.8)	99.7 (26.7)	100.2 (25.9)	0.007
HOMA-IR	2.7 (2.3)	2.4 (2.3)	2.5 (1.9)	2.7 (2.3)	3.2 (2.6)	<0.001
eGFR, ml/min/1.73 m^2	67.5 (16.1)	67 (17)	68 (15)	68 (17)	68 (15)	0.47
Health status markers						
Number of comorbidities	1.5 (1.2)	1.6 (1.2)	1.5 (1.2)	1.3 (1.1)	1.5 (1.2)	0.02
Number of medications	1.2 (0.6)	1.2 (0.6)	1.2 (0.5)	1.2 (0.6)	1.2 (0.5)	0.85
Fair/poor self-assessed health, %	9.1	11.4	9.7	6.6	8.8	0.08
Lifestyle parameters, %						
Current smoker, yes	6.9	7.6	8.3	5.0	6.7	0.27
Exercise, 3+ times/week	70.8	69.3	73.2	71.6	69.0	0.82
Daily alcohol (vs. less or none)	33.3	40.5	34.7	32.1	25.8	<0.001

*Test for linear trend; †mean (SD) for total and minimum, maximum for quartiles; ‡geometric mean (quartile 1 [Q1], quartile 3 [Q3]).
CVD = cardiovascular disease; eGFR = estimated glomerular filtration rate; ET = estrogen therapy; HDL = high-density lipoprotein; HOMA-IR = homeostasis model insulin resistance; LDL = low-density lipoprotein.

disease, diabetes, cancer (non-skin), emphysema, arthritis, hip fracture, and hypertension.

Participants were followed through 2010, with 98% ascertainment of vital status. Death certificates were classified for underlying cause of death by a certified nosologist using the International Classification of Diseases, Ninth Revision. CVD deaths included codes 401 to 448.

Statistical analysis. On the basis of a priori hypotheses of nonlinear associations, we evaluated quartiles of fetuin-A. Preliminary analysis demonstrated substantial differences in fetuin-A levels by sex and by use of oral estrogens in women; therefore, participants were categorized into sex- and oral estrogen–specific quartiles of fetuin-A levels. Trends in baseline characteristics by fetuin-A quartiles were evaluated using analysis of variance with linear trend for continuous variables and Cochran-Armitage test for trend for nominal variables. HDL cholesterol and triglyceride levels were not normally distributed and were log-transformed for analyses; reported values are geometric means and interquartile ranges. Skew was −0.09 and 0.26, and kurtosis 2.67 and 3.29, after log-transformation for HDL cholesterol and triglycerides, respectively.

Single-predictor associations between the variables listed in Table 1 and fetuin-A levels were determined by linear regression analysis. Multivariable regression analysis was used to determine which covariates were independently associated with fetuin-A levels.

The association between fetuin-A and CVD mortality was determined using Cox proportional hazards regressions; model assumptions were tested by applying the time-dependent covariate test (28), by Schoenfeld residual visualizations (29), and by visualization of log-log survival plots and Kaplan-Meier versus Cox estimated survivor functions (30). All models presented met the proportional hazards assumption. Three separate regression models were assessed: the first adjusted for age, sex and use of oral estrogens; the second added adjustment for lifestyle characteristics including physical activity (3+ times per week, yes/no), alcohol use (1+ drinks/day vs. less or none), and current smoking habit (yes/no); and the third added adjustment for traditional CVD risk factors (BMI, waist-to-hip ratio, systolic blood pressure, triglycerides, LDL cholesterol, fasting plasma glucose, HOMA-IR, and eGFR). There was no significant multicollinearity (variance inflation factor

Table 2 Beta-Coefficients for Individual and Multivariable Regressions on Fetuin-A Levels

	Individual		Multivariable†	
Independent Variable*	β-Coefficient	p Value	β-Coefficient	p Value
Oral estrogen therapy	0.0491	<0.001	0.0331	<0.001
Demographics				
Sex (1 = male)	−0.0274	<0.001		
Age (10.5 yrs)	−0.0232	<0.001	−0.0227	<0.001
Anthropomorphics				
Body mass index (4.0 kg/m^2)	0.0112	<0.001		
Waist-to-hip ratio (0.09)	−0.0056	0.03		
CVD risk factors				
Systolic blood pressure (22.1 mm Hg)	−0.0002	0.24	0.0095	0.003
Diastolic blood pressure (9.5 mm Hg)	0.0069	0.01		
HDL cholesterol (17.4 mg/dl)	−0.0035	0.23		
LDL cholesterol (23.5 mg/dl)	0.0098	<0.001	0.0064	0.012
Triglycerides (77.0 mg/dl)	0.0273	<0.001	0.0192	<0.001
Fasting plasma glucose (23.0 mg/dl)	0.0028	0.27		
HOMA-IR (2.3)	0.0089	<0.001		
eGFR (16.1 ml/min/1.73 m^2)	0.0020	0.43		
Lifestyle parameters				
Daily alcohol (vs. less or none)	−0.0261	<0.001	−0.0203	<0.001
Current smoker (yes vs. no)	−0.0070	0.47		
Exercise (3+ times/week)	−0.0046	0.41		

*Continuous explanatory variables were standardized prior to analysis, values in parentheses are 1 SD. †Model included all variables, only significant betas presented. Adjusted $R^2 = 0.14$.
Abbreviations as in Table 1.

>2) between the independent variables. Separate secondary Cox models were performed to test the influence of specific comorbidities and of a set of health status markers. Biologically plausible effect modifiers were tested by interaction terms on a multiplicative scale.

All p values presented are 2-tailed; $p < 0.05$ was considered statistically significant for all analyses, including interaction terms. Data were analyzed using STATA (v11.1, Stata Corp., College Station, Texas) and SPSS (v155, SPSS Inc., Chicago, Illinois).

Results

Baseline characteristics. Baseline characteristics are presented in Table 1. The mean age of the 1,688 participants was 72 years (range: 50 to 98); 61% (n = 1,025) were female, of whom 402 (39%) reported current use of oral estrogens. Fetuin-A levels (median, interquartile range in grams/liter) were highest in women using oral estrogens (0.54, 0.47 to 0.63), intermediate for women not using oral estrogens (0.51, 0.44 to 0.57), and lowest for men (0.50, 0.44 to 0.56) ($p < 0.001$ for all). Use of non-oral estrogens (n = 37 women) did not influence fetuin-A levels (data not shown); these women were included in the no oral estrogen group.

Fetuin covariates. The linear associations of individual variables with fetuin-A levels are shown in Table 2. Oral estrogen therapy, female sex, and triglycerides showed the strongest individual positive associations with fetuin-A levels with age and daily alcohol consumption showing the strongest negative associations. In multivariate analyses, oral estrogen therapy, systolic blood pressure, LDL cholesterol, and triglycerides were independently associated with higher levels of fetuin-A, whereas older age and daily alcohol consumption associated with lower fetuin-A levels (adjusted $R^2 = 0.14$).

Overall, 18% of participants met criteria for the metabolic syndrome, 14% had diabetes, and 20% had prevalent CVD. The prevalence of the metabolic syndrome and diabetes increased in a stepwise fashion across fetuin-A quartiles, whereas the prevalence of CVD decreased as fetuin-A levels increased (Fig. 1) (all $p < 0.001$ for linear trend).

CVD mortality. During the 16-year follow-up (median 12 years), 273 deaths were attributed to CVD (153 women, 120 men). Results of Cox proportional hazards models for CVD mortality by fetuin-A quartile are presented in Table 3. The age-, sex-, and oral estrogen therapy–adjusted hazard ratio (HR) for the lowest quartile of fetuin-A versus the highest was 1.30 (95% confidence interval [CI]: 0.93 to 1.78, p = 0.12) for CVD mortality. HRs for the 2nd and 3rd quartiles were not significantly different than 1.0, suggesting a low threshold (a significant test for quadratic trend [p = 0.018] supported the nonlinear association of fetuin quartiles with CVD risk). Further adjustment for lifestyle factors (Model 2) had minimal influence on the low fetuin-A association. Adding adjustment for traditional CVD risk factors increased the HR for the lowest versus the highest quartile to 1.42 (95% CI: 1.01 to 1.99, p = 0.041).

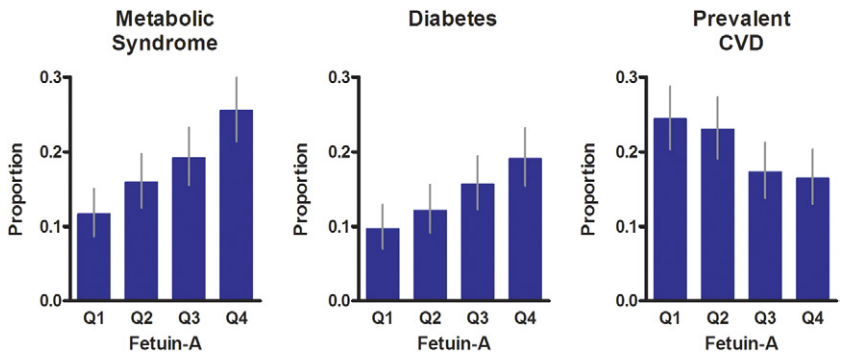

Figure 1 Prevalence of Metabolic Syndrome, Diabetes, and Prevalent CVD by Fetuin-A Quartile

p <0.001 for linear trend for all. **Lines within the bars** indicate binomial exact confidence intervals.

Modifiers of the low fetuin–CVD mortality association. Next, we examined whether the association of low fetuin-A with CVD mortality differed across strata of selected risk factors comparing low fetuin-A levels (quartile 1) with all higher (quartiles 2 to 4) and adjusting for age, sex, oral estrogen therapy, alcohol intake, regular exercise, and current smoking as appropriate (Table 4). There was no statistical evidence of effect modification by age, sex, overweight (BMI ≥25 kg/m^2 vs. lower), daily alcohol consumption, HOMA-IR, hypertension, hypercholesterolemia, hypertriglyceridemia, metabolic syndrome, prevalent CVD, or by death in the first 10 years of follow-up versus after 10 years (p interactions all >0.29). Use of oral estrogens did not modify the association of low fetuin-A with CVD mortality in women (p interaction = 0.63) (data not shown).

A strong interaction was observed for diabetes (p interaction = 0.003), such that low fetuin-A levels were associated with 76% higher risk of CVD death in individuals without diabetes (p < 0.001), but with 57% lower risk of CVD death in those with diabetes (p = 0.046) (Fig. 2). These differences persisted after adjustment for additional CVD risk factors including BMI, waist-to-hip ratio, triglycerides, LDL cholesterol, systolic blood pressure, fasting plasma glucose, HOMA-IR, and eGFR. In this multiply adjusted model, the HR for CVD mortality for low fetuin-A levels versus higher was 1.90 (95% CI: 1.43 to 2.50; p < 0.001) for nondiabetic patients and 0.48 (95% CI: 0.20 to 1.14; p = 0.097) for those with diabetes.

Secondary analyses by diabetes status. The influence of known and unknown comorbidities and overall health status on the diabetes-specific association of low fetuin-A with CVD mortality was examined in secondary analyses comparing low fetuin-A levels to all higher; adjusted for age, sex, oral estrogen therapy, alcohol intake, regular exercise, and current smoking (Table 5). In sequential analyses, adding adjustment for metabolic syndrome, prevalent CVD, or a set of health status markers had minimal effect on low fetuin-A risk estimates in either the diabetes or no diabetes group. Excluding participants with the metabolic syndrome or prevalent CVD modestly attenuated the low fetuin-A association in the diabetic group, but had minimal influence on risk estimates for the no diabetes group. Excluding CVD deaths that occurred within the first 2 years of follow-up did not alter results.

In the diabetes group, adding adjustment for liver function markers to the base model strengthened the association of low fetuin-A with CVD mortality, whereas adjusting for serum phosphorus and calcium reduced it; neither adjustment influenced results for the nondiabetic group (Table 5). Adjusting for HOMA-IR, or sequential adjustment for the most commonly used medications (aspirin, calcium supplements, and antihypertensive agents used by 34%, 34%, and 30% of participants, respectively) (data not shown) also

Table 3 Multivariable Cox Proportional Hazards Models for the Association of Quartile of Fetuin-A With CVD Mortality

Fetuin Quartile	(Range, g/l)	Mortality Rate*	Model 1†		Model 2‡		Model 3§	
			HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value
Q1	(0.22–0.47)	20	1.30 (0.93, 1.78)	0.12	1.30 (0.94, 1.80)	0.12	1.41 (1.01, 1.99)	0.043
Q2	(0.44–0.54)	15	0.89 (0.62, 1.28)	0.54	0.90 (0.62, 1.29)	0.56	0.93 (0.65, 1.36)	0.70
Q3	(0.50–0.63)	13	0.76 (0.52, 1.11)	0.16	0.78 (0.53, 1.14)	0.20	0.72 (0.48, 1.06)	0.13
Q4	(0.56–1.24)	16	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	

*Indirect standardization for age, deaths per 100. †Model 1, adjusted for age, sex, and oral estrogen therapy. ‡Model 2, model 1 + alcohol use, current smoking, and regular exercise. §Model 3, model 2 + body mass index, waist-to-hip ratio, triglycerides, LDL cholesterol, systolic blood pressure, fasting glucose, eGFR, and HOMA-IR.
CI = confidence interval; HR = hazards ratio; Q = quartile; Ref = reference category; other abbreviations as in Table 1.

Table 4 Multivariable Hazard Ratios for CVD Mortality by Low Fetuin-A (Quartile 1) Versus Higher (Quartiles 2 to 4) Stratified by Potential Moderators

Group	Events/N	Person-Years	HR (95% CI)*		p Value for interaction
All	273/1,679	17,417	1.46 (1.14–1.87)		—
Sex					
Male	120/659	6,590	1.27 (0.87–1.85)		0.29
Female	153/1,020	10,826	1.68 (1.21–2.34)	†	
Age					
<72 yrs	27/776	9,450	0.88 (0.33–2.36)		0.32
≥72 yrs	246/903	7,957	1.54 (1.19–2.00)	‡	
Overweight					
BMI <25 kg/m^2	152/845	8,455	1.52 (1.10–2.12)	§	0.62
BMI ≥25 kg/m^2	116/822	8,889	1.29 (0.86–1.93)		
HOMA-IR					
<2.2 (median)	113/820	8,577	1.41 (0.96–2.06)		0.93
≥2.2	157/851	8,778	1.57 (1.12–2.19)	‡	
Daily alcohol drinker					
No	183/1,120	11,595	1.45 (1.07–1.98)	§	0.85
Yes	90/559	5,822	1.54 (1.01–2.36)	§	
Metabolic syndrome					
No	218/1,378	14,391	1.48 (1.13–1.95)	‡	0.88
Yes	55/301	3,026	2.09 (1.05–4.18)	§	
Diabetes					
No	221/1,443	15,186	1.76 (1.34–2.31)	†	0.003
Yes	53/236	2,231	0.43 (0.19–0.98)	§	
High blood pressure					
No	49/535	6,071	0.95 (0.52–1.74)		0.24
Yes	224/1,144	11,346	1.63 (1.24–2.15)	*	
High triglycerides					
<150 mg/dl	210/1,266	13,034	1.49 (1.13–1.98)	‡	0.93
≥150 mg/dl	63/411	4,360	1.62 (0.83–3.16)		
High cholesterol					
<200 mg/dl	126/679	6,730	1.57 (1.09–2.27)	§	0.46
≥200 mg/dl	147/999	10,673	1.35 (0.95–1.92)		
Prevalent CVD					
No	168/1,339	14,629	1.56 (1.13–2.14)	‡	0.35
Yes	105/340	2,788	1.23 (0.82–1.85)		
Time to CVD death					
After 10 yrs	82/1,108	10,385	1.29 (0.81–2.06)		0.90
First 10 yrs	191/571	7,032	1.64 (1.22–2.23)	‡	

*Adjusted for age, sex, oral estrogen therapy, alcohol use, current smoking, and regular exercise. †$p < 0.001$; ‡$p < 0.01$; §$p < 0.05$.
BMI = body mass index; other abbreviations as in Tables 1 and 3.

failed to influence results for either group. Finally, in tests for effect modification within the nondiabetic group, the association of low fetuin-A with increased risk of CVD death was not modified by HOMA-IR, by overweight (BMI >25 kg/m^2), or by obesity (BMI >30 kg/m^2) (p values for interaction >0.30).

Discussion

This study evaluated the prospective association of plasma fetuin-A levels with CVD mortality in a large population of older, community-dwelling men and women. We observed a striking difference in the association of fetuin-A with CVD mortality risk by diabetes status. Low fetuin-A levels were associated with 76% higher risk of CVD death in individuals without diabetes, but with 57% lower risk of CVD mortality in those with diabetes. Both associations were statistically significant, and both were independent of traditional CVD risk factors, insulin resistance, and measures of liver and kidney function. These findings are consistent with the hypothesis that fetuin-A protects against vascular calcification, but promotes insulin resistance and metabolic dysregulation, and suggest that the balance between these 2 functions may depend on the metabolic milieu or prior disease processes.

The association of low fetuin-A with increased CVD risk in individuals without diabetes is somewhat paradoxical given that low fetuin-A is also associated with beneficial levels of most traditional CVD risk factors including lower blood pressure, better lipids, lower adiposity, and reduced likelihood of metabolic syndrome. Only older age and a

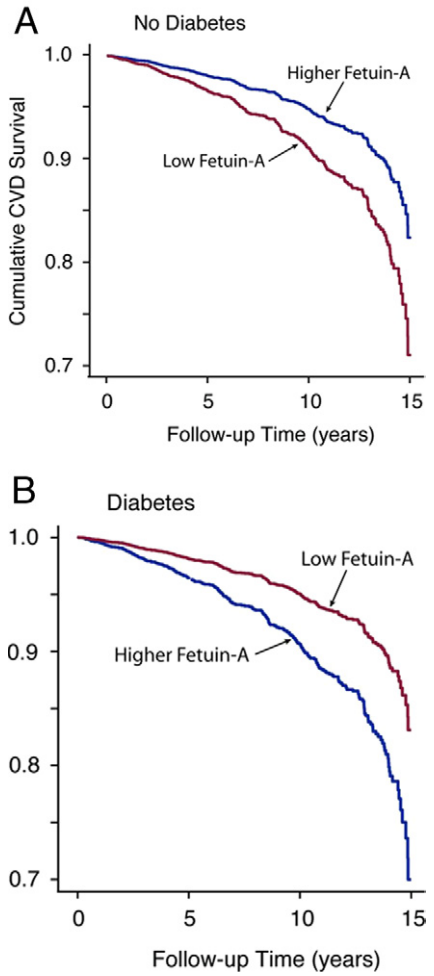

Figure 2 **Survival Plots Showing the Differential Association of Low Fetuin-A With CVD Mortality by Diabetes Status**

Adjusted hazards ratios = 1.76 (95% confidence interval [CI]: 1.34, 2.31, $p < 0.001$) for participants without diabetes (n = 1,443) **(A)** and 0.43 (95% CI: 0.19, 0.98, $p = 0.046$) for those with diabetes (n = 236) **(B)**. Models are adjusted for age, sex, oral estrogen therapy, current smoking, alcohol use, and regular exercise.

higher prevalence of pre-existing CVD associate with low fetuin-A, and in general, adjusting for these, as well as the other CVD risk factors, strengthened the risk estimate for fatal CVD among nondiabetic individuals with low fetuin-A. Thus, mechanisms other than conventional pathogenic pathways are likely to be involved in the biology underlying the low fetuin-A association. Circulating fetuin-A is a well-described inhibitor of vascular calcification in patients with end-stage renal disease (4,5), an association that has been extended to the general population (10,11). We recently demonstrated that low fetuin-A levels were also associated with coronary artery calcification in the Rancho Bernardo participants (9). Whether vascular calcification accounts for all, or most, of the CVD risk associated with low fetuin-A should be evaluated in future studies of populations with baseline ectopic calcification measurements and long-term follow-up.

Few prior studies have examined the association of fetuin-A with CVD events in the general population, and existing data are mixed. In a case-cohort study nested within the EPIC-Potsdam study (European Prospective Investigation into Cancer and Nutrition–Potsdam study), high plasma fetuin-A levels were associated with greater risk of incident myocardial infarction and ischemic stroke (31); results that are opposite in direction to our findings in nondiabetic individuals and to observations in patients with end-stage renal disease. Age differences may be important. Our participants were 20 years older on average than the EPIC-Potsdam cohort, and were probably more likely to have prevalent arterial calcification at baseline than the EPIC-Potsdam participants. If the link between low fetuin-A and CVD events is mediated through accelerated arterial calcification, then this difference may have made an association of low fetuin-A with CVD risk more likely among our older nondiabetic participants. It is also possible that fetuin-A associations with *incident* CVD events in a younger population (EPIC-Potsdam) differs from that with *fatal* CVD events in an older population (Rancho Bernardo).

In contrast to the nondiabetic group, low fetuin-A was associated with significantly lower, not higher, risk of CVD death in Rancho Bernardo participants with diabetes. In our previous investigation in the Heart and Soul Study (a cohort of 1,024 individuals, all of whom had prevalent CVD and a spectrum of kidney function similar to that in the Rancho Bernardo study), low fetuin-A levels were associated with aortic stenosis among individuals without diabetes, whereas no association was observed in those with diabetes (10). This finding, as well as the dual actions of fetuin-A, led us to the a priori hypothesis that the relationship of fetuin-A with other measures of vascular calcification and CVD would also differ by diabetes status. The present study extends the link between low fetuin-A levels and vascular disease to a prospective association with CVD mortality in community-dwelling adults, and demonstrates that this association is not dependent on pre-existing CVD. Importantly, we confirmed the observation that diabetes modifies the fetuin-A association, using a distinct but related outcome and in a different population. Diabetes was the only 1 of several potential effect modifiers that was statistically significant, and the significance of the interaction was strong ($p = 0.003$). This new evidence supports the reproducibility of our original findings and may lead to important new insights with respect to fetuin-A biology.

Effect modification by diabetes was also examined in the EPIC-Potsdam study, and none was found; however, diabetes cases were identified by self-report and examination of medical records, and did not include fasting and post-challenge glucose measurements as in the present study, thus undiagnosed cases might have been missed. Most other studies of fetuin-A and CVD have been in kidney disease populations, and almost all have identified increased risk of

Table 5 Multivariable Hazard Ratios for CVD Mortality by Low Fetuin-A (Quartile 1) Versus Higher (Quartiles 2 to 4) Adjusting for, or Excluding, Potential Covariates and Effect Modifiers

	No Diabetes HR (95% CI)	p Value	Diabetes HR (95% CI)	p Value
Events/N	220/1,450		53/238	
Base model*	1.76 (1.34–2.31)	<0.001	0.43 (0.19–0.98)	0.046
Base model* plus				
Liver function (AST, ALT, albumin)	1.79 (1.36–2.36)	<0.001	0.36 (0.15–0.85)	0.020
Serum phosphorus, calcium	1.80 (1.37–2.36)	<0.001	0.52 (0.22–1.22)	0.132
Insulin resistance (HOMA)	1.78 (1.36–2.34)	<0.001	0.43 (0.18–0.99)	0.048
Metabolic syndrome	1.83 (1.39–2.40)	<0.001	0.43 (0.18–0.99)	0.047
Prevalent CVD	1.69 (1.29–2.22)	<0.001	0.49 (0.21–1.12)	0.089
Health status markers†	1.72 (1.31–2.26)	<0.001	0.48 (0.21–1.12)	0.090
Base model* excluding				
Metabolic syndrome‡	1.66 (1.25–2.22)	0.001	0.49 (0.16–1.57)	0.23
Prevalent CVD§	1.80 (1.28–2.54)	0.001	0.58 (0.18–1.89)	0.37
CVD death within 2 yrs‖	1.66 (1.24–2.21)	0.001	0.43 (0.17–1.05)	0.06

*Base model adjusted for age, sex, oral ET, alcohol use, current smoking, regular exercise. †Health status markers: number medications, number comorbidities, self-assessed health. ‡Events/N = 191/1,267 for no diabetes, 27/117 for diabetes. §Events/N = 138/1,175 for no diabetes, 30/169 for diabetes. ‖Events/N = 196/1,425 for no diabetes, 47/223 for diabetes.

ALT = alanine aminotransferase; AST = aspartate aminotransferase; other abbreviations as in Tables 1 and 3.

vascular calcification, CVD events, and mortality in individuals with low fetuin-A levels (4–8). One notable exception is a study reporting a direct correlation of fetuin-A levels with the extent of coronary artery calcification among diabetic patients in the pre-dialysis stages of chronic kidney disease (32). This result is consonant with our finding of a protective association of low fetuin-A with CVD mortality among diabetic individuals and may be related to the role of fetuin-A in mediating insulin resistance. Fetuin-A inhibits the insulin receptor tyrosine kinase, preventing insulin-mediated autophosphorylation of the insulin receptor (12) and inducing insulin resistance (13). Fetuin-A knockout mice demonstrate improved insulin sensitivity and resistance to weight gain and fat accumulation when fed a high-fat diet (19), and intraperitoneal delivery of fetuin-A to wild-type mice acutely induces peripheral insulin resistance (33). Thus, low fetuin-A may slow the development or severity of insulin resistance and its consequences in diabetic individuals.

We observed a direct association between plasma fetuin-A and HOMA-IR, triglyceride levels, LDL cholesterol, measures of adiposity, and diabetes prevalence; all of which are consistent with a possible pathogenic role for fetuin-A in exacerbating the insulin resistance and the proatherogenic milieu associated with type 2 diabetes mellitus. This possibility is supported by prior studies by our group and others showing that higher plasma fetuin-A levels both predict incident diabetes and characterize those with established diabetes (17,18). Low fetuin-A in individuals with diabetes may represent a successful adaptive response that reduces CVD risk in a subgroup of diabetes patients. Many of the diabetic participants in the present study were captured only by high fasting or post-challenge glucose, and may therefore have been cases earlier in the disease course with a lower prevalence of vascular disease. Whether fetuin-A's role in the pathogenesis of diabetic CVD depends on the stage and severity of disease should be addressed in future studies.

Strengths of this study are its prospective design, the relatively large sample size, inclusion of both sexes, and the availability of a wide spectrum of potential confounding variables.

Study limitations. Associations were based on fetuin-A values measured at a single time point; nonetheless, we identified a strong signal for CVD mortality that was robust to statistical adjustment for multiple covariates. Almost half of the diabetes cases were identified based on fasting or post-challenge glucose levels, not on physician diagnosis, thus information on diabetes severity or duration was not available. Participants were predominantly Caucasian and middle to upper middle class. This limits generalizability, but is a strength to the extent that confounding by ethnicity, socioeconomic status, and access to health care is minimized. Finally, the majority of participants were elderly men and women, and our results may not generalize to younger adults.

Conclusions

In summary, in community-dwelling individuals, low plasma fetuin-A levels are independently associated with increased risk of CVD mortality among men and women without diabetes, but with reduced risk of CVD death in those with diabetes. These results suggest the relationship of fetuin-A to cardiovascular health is more complex than previously thought. Future studies, with larger populations, are required to determine whether measurement of plasma fetuin-A will be useful as a CVD risk stratification tool and whether prediction criteria will differ for those with and without diabetes.

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