Fetuin-A, Type 2 Diabetes, and Risk of Cardiovascular Disease in Older Adults

The Cardiovascular Health Study

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OBJECTIVE—Fetuin-A, a hepatic secretory protein that simultaneously inhibits arterial calcification and insulin action, is associated with type 2 diabetes, but its association with cardiovascular disease (CVD) is uncertain. Preliminary studies suggest that the association of fetuin-A with CVD might differ among individuals with or without type 2 diabetes.

RESEARCH DESIGN AND METHODS—This was a prospective study of 3,810 community-living individuals older than 65 years (511 with type 2 diabetes) and free of CVD in 1992 when fetuin-A levels were measured. Participants were followed-up for incident CVD through June 2008.

RESULTS—Mean age was 75 years, and 61% were women; 1,456 participants had an incident CVD event (248 among individuals with type 2 diabetes). The association of fetuin-A with CVD was modified by type 2 diabetes (*P* interaction = 0.02). Higher fetuin-A was associated with lower CVD risk among persons without type 2 diabetes [hazard ratio per SD 0.1 g/L higher fetuin-A, 0.93 (95% CI, 0.88–0.99)], whereas a trend in the opposite direction was observed among individuals with type 2 diabetes, although it was not statistically significant [1.07 (0.93–1.22)]. Among individuals without type 2 diabetes, similar effect modification was observed by obesity and insulin resistance. Consistently, higher fetuin-A was associated with lower CVD risk only in the subgroups without obesity or with HOMA-IR below the median [0.91 (0.85–0.97) and 0.87 (0.79–0.95), respectively].

CONCLUSIONS—The association of fetuin-A with risk of CVD differs among elderly individuals with and without insulin resistance or type 2 diabetes.

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Fetuin-A (α-Heremans-Schmid glycoprotein) is a liver-synthesized protein that is secreted into serum. Fetuin-A can bind the insulin receptor and thereby inhibit insulin signaling (1–3). In humans, higher levels are associated with higher triglycerides, LDL cholesterol, BMI, and insulin resistance (4,5), in addition to incident diabetes (6–8). However, fetuin-A also complexes with circulating calcium and phosphorus and increases the solubility of these minerals (9), thereby inhibiting arterial calcium deposition. Fetuin-A knockout mice are characterized by both improved insulin sensitivity and ectopic calcification (9–11). Thus far, little is known about the relationship of fetuin-A with cardiovascular disease (CVD) in human populations. Most existing studies have evaluated populations with end-stage renal disease, a condition characterized by increased cardiovascular calcification. Inverse associations of fetuin-A levels with risk of CVD events and all-cause mortality are consistently observed in end-stage renal disease patients (12–15). To our knowledge, only two previous studies have evaluated the association of fetuin-A with CVD in community-living populations free of kidney disease. In the EPIC-Potsdam study, higher fetuin-A levels were associated with incident myocardial infarction and stroke, and thus the direction of association was opposite to that reported in end-stage renal disease patients (16). More recently, we reported that the association of fetuin-A with risk of CVD death was modified by type 2 diabetes status (*P* interaction = 0.003). In community-living older persons who participated in the Rancho Bernardo Study, higher fetuin-A levels were associated with CVD death only in individuals with type 2 diabetes, whereas fetuin-A was inversely associated with CVD death in those without type 2 diabetes (17). The finding of effect modification by type 2 diabetes confirmed our preliminary observations from a cross-sectional study of aortic stenosis in which fetuin-A levels were inversely associated with aortic stenosis only in individuals without type 2 diabetes (18).

Whether underlying population characteristics that render the specific study population more or less susceptible to either of the important and contrasting roles of fetuin-A in insulin resistance and arterial calcification may explain the discrepant observations between the EPIC-Potsdam study and our previous studies

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remains unknown. Because both abnormalities increase with age, we sought to evaluate the association of fetuin-A with incident CVD and possible effect modification by type 2 diabetes in the Cardiovascular Health Study (CHS), a community-living sample of older adults with long-term follow-up and adjudicated CVD events.

RESEARCH DESIGN AND METHODS

Study design

The CHS is a longitudinal study of adults aged 65 years or older at recruitment. Eligible participants were noninstitutionalized and were expected to remain in the area for at least 3 years, had capacity to give informed consent without requiring a proxy, and were not receiving active treatment for cancer. An original sample of 5,201 adults was recruited from 1989 to 1990, from Medicare files in Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Pittsburgh, Pennsylvania. Participants were predominantly white (95%). In 1992–1993, a second cohort of 687 African American participants was recruited in three of the four communities (19). The 1992–1993 study visit was considered baseline for the current study, which 5,265 individuals attended. We excluded individuals with prevalent cardiovascular disease [myocardial infarction (MI) and stroke] at baseline ($n = 797$), as well as those with insufficient blood specimens for fetuin-A measurement ($n = 438$) and missing covariate data ($n = 220$), resulting in a final analytic sample of 3,810 participants. All participants provided written informed consent, and the study was approved by the Investigational Review Boards of the four clinical sites, the Data Coordinating Center at the University of Washington, and the University of California San Diego.

Fetuin-A measures

Samples were collected at the 1992–1993 study visit and stored at −70°C until 2010, when they were thawed and fetuin-A levels were measured in plasma using an ELISA kit (Epitope Diagnostics, San Diego, CA). The measurements were conducted at the CHS Central Blood Analysis Laboratory at the University of Vermont (Burlington, VT). The assay uses a two-site "sandwich" technique with polyclonal antibodies that bind different epitopes of human fetuin-A. Serum samples were measured twice in each participant, and results were averaged. The coefficients of variation ranged between 3% and 9%, with a mean coefficient of variation of 6%.

Other characteristics

Age, sex, and race were obtained by self-report. Other self-reported information obtained at the 1992–1993 visit included smoking status (current, former, never), alcohol intake, use of hormone therapy among women, and medical history. Leisure physical activity was calculated as a weighted sum of kilocalories consumed in specific physical tasks (20). Anthropometric measures and seated systolic blood pressure and diastolic blood pressure were measured in standardized fashion by trained personnel. Height was measured in centimeters using a stadiometer, and weight was measured using a balance beam scale in pounds while subjects were wearing examination gowns and no shoes. Waist and hip circumferences were measured on standing subjects at the level of the umbilicus and maximal protrusion of the gluteal muscles, respectively. BMI was computed as weight (kg) divided by height (m^2). Missing smoking and BMI values were replaced with data from previous years when available. Fasting laboratory measurements included LDL cholesterol and HDL cholesterol, triglycerides, C-reactive protein, cystatin C (21), insulin (22), and glucose. Type 2 diabetes was defined as use of a hypoglycemic agent or insulin, fasting glucose ≥126 mg/dL, or nonfasting glucose ≥200 mg/dL. Hypertension was defined as systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg, or medical treatment for hypertension. Estimated glomerular filtration rate (eGFR) was calculated using the equation eGFR = 76.7 × cystatin C $(mg/L)^{-1.19}$ (23). Insulin resistance was assessed by the homeostasis model assessment for insulin resistance (HOMA-IR) method in nondiabetic participants (24). Quality-control procedures, laboratory methods, and procedures for blood pressure measurement have been published previously (22).

Outcomes

Follow-up visits were conducted in persons annually and by telephone every 6 months through 1998–1999; subsequently, telephone calls every 6 months continued through 2005–2006. We evaluated a composite incident CVD event outcome, defined as time to first MI, stroke, or CVD death. Hospital records of all potential events were obtained, and all events were adjudicated by a CHS Events Committee. MI was indicated by a clinical history of cardiac symptoms, elevated cardiac enzyme levels, and serial electrocardiographic changes (25). Stroke was adjudicated by a committee of neurologists, neuroradiologists, and internists on the basis of interviews with patients, medical records, and brain imaging studies (26). Deaths were identified by a review of obituaries, medical records, death certificates, and the Centers for Medicare and Medicaid Services health care utilization database for hospitalizations and from household contacts; 100% complete follow-up for ascertainment of mortality status was achieved. Death from cardiovascular causes included deaths caused by coronary heart disease, heart failure, peripheral vascular disease, or cerebrovascular disease (27).

Statistical analysis

We evaluated the distribution of the baseline characteristics by quartiles of fetuin-A concentrations. Cox proportional hazards models were used to examine the association between fetuin-A and risk of the composite CVD event end point and each individual end point separately (28). Follow-up was continued through 30 June 2008. Because our previous work suggested potential effect modification of the association of fetuin-A with ectopic calcification or CVD events by type 2 diabetes status (17,18), we specified a priori to test whether type 2 diabetes status modified the association between fetuin-A and incident CVD. To confirm this interaction, we also tested for effect modification by obesity (BMI ≥30 vs. less) and by HOMA-IR [above vs. below the median (2.2 units)] among the subgroup of participants without type 2 diabetes. We tested secondarily for effect modification by age, sex, race, and eGFR in the entire sample. Statistical interaction was assessed on the multiplicative scale by deviance tests based on comparisons of likelihoods in nested models with and without cross-product terms.

A sequence of Cox models was evaluated. An initial model adjusted for age (continuous), race (African American or other), sex, and CHS clinic (four sites). A subsequent model added the following lifestyle factors: physical activity (log-transformed total kcal); smoking status

(never, former, current); use of hormone therapy (yes or no in women); alcohol intake (0, ≤7, 7–14, >14 drinks per week); and use of insulin or hypoglycemic drugs (yes or no among subset with type 2 diabetes). A final model additionally adjusted for hypertension, eGFR (continuous), C-reactive protein (continuous log-transformed), BMI (continuous), triglycerides (continuous log-transformed), HDL cholesterol (continuous), and LDL cholesterol (continuous).

We also assessed the potential for a nonlinear association of fetuin-A with CVD by evaluating penalized cubic spline functions with 3 degrees of freedom. These plots excluded the highest and lowest 2.5% of the distribution of fetuin-A to avoid implausible extrapolation based on the extremes. Proportional hazards assumptions were tested in models using Schoenfeld residuals and by including time-by-covariate interactions; there were no meaningful violations. Competing risk regression models also were used to address the influence of the high mortality risk in this elderly study population. A proportional hazards model for the subdistribution was fit to compare CVD events with non-CVD death as first event (29). This model produces subhazard ratios. All analyses were conducted in STATA version 11.2 (StataCorp LP, College Station, TX).

RESULTS—At baseline, the mean age of the study sample with 3,810 persons was 75 years, 61% were female, 17% were African American, and 511 (13%) had type 2 diabetes. The mean fetuin-A level was 0.47 ± 0.10 g/L. Compared with participants in the lowest fetuin-A quartile, those with higher concentrations were more likely to be female and Caucasian, to have type 2 diabetes, and to have had higher BMI, waist circumference, HOMA-IR, C-reactive protein, triglycerides, and LDL cholesterol (Table 1). Individuals with higher fetuin-A levels were less likely to have a high school education or to be current smokers, consumed less alcohol, and had lower eGFR.

During a median follow-up of 10.9 years (interquartile range, 5.9–15.3), 1,456 participants experienced incident CVD events: 599 had an MI; 593 had a stroke; and 848 died of CVD (522 had more than one event). The association between fetuin-A and occurrence of first CVD event differed between participants with or without type 2 diabetes (*P* for interaction = 0.02). Among individuals without type 2 diabetes (*n* = 3,299), there were 1,208 incident CVD events. Compared with quartile 1, the risk of incident CVD among nondiabetic individuals was 18% lower (95% CI, 4–30%) among persons in the highest quartile in unadjusted analysis (Table 2). This association was attenuated with adjustment for age, sex, race, and field center, and remained fairly consistent when additionally adjusted for lifestyle factors and traditional CVD risk factors. In the final adjusted model, each SD higher fetuin-A level was associated with a hazard ratio (HR) of 0.93 (0.88–0.99) for incident CVD events. Supplementary Fig. 1*A* shows the nature of the association of fetuin-A with incident CVD events among nondiabetic individuals in the fully adjusted model.

Table 1—*Baseline characteristics by quartiles of fetuin-A in older adults at baseline (1992): the CHS*

Characteristics	Total *N* = 3,810	Quartile 1 *N* = 954	Quartile 2 *N* = 955	Quartile 3 *N* = 959	Quartile 4 *N* = 942
Fetuin-A range, g/L		<0.41	0.41–<0.47	0.47–0.53	>0.53
Fetuin-A median, g/L (SD)	0.47 (0.10)	0.36 (0.05)	0.44 (0.02)	0.50 (0.02)	0.60 (0.06)
Mean age, years (SD)	75 (5)	75 (6)	75 (5)	74 (5)	74 (5)
Male, *N* (%)	1,473 (39)	432 (45)	397 (42)	339 (35)	305 (32)
African American, *N* (%)	634 (17)	253 (27)	183 (19)	126 (13)	72 (8)
Less than high school education, *N* (%)	976 (26)	236 (25)	247 (26)	228 (24)	265 (28)
Mean BMI, kg/m^2 (SD)	26.9 (4.8)	26.2 (5.1)	26.6 (4.7)	27.1 (4.6)	27.5 (4.6)
Mean WC, cm (SD)	97 (13)	95 (14)	97 (13)	98 (13)	98 (13)
Median physical activity, kcal/day (IQR)	870 (278–1,920)	819 (270–1,800)	810 (270–2,025)	893 (300–1,905)	945 (345–1,995)
Current smoker, *N* (%)	394 (10)	104 (11)	105 (11)	95 (10)	90 (10)
No alcohol intake, *N* (%)	2,068 (54)	460 (48)	527 (55)	514 (54)	567 (60)
Low self-reported health, *N* (%)	688 (18)	189 (20)	185 (19)	140 (15)	174 (19)
Estrogen use (women), *N* (%)	327 (14)	55 (11)	51 (9)	97 (16)	124 (19)
Prevalent type 2 diabetes, *N* (%)	511 (13)	116 (12)	124 (13)	119 (12)	152 (16)
Use of insulin,* *N* (%)	25 (22)	27 (22)	7 (6)	16 (11)	75 (15)
Use of oral hypoglycemic,* *N* (%)	47 (41)	63 (51)	64 (54)	60 (40)	234 (46)
Family history of MI, *N* (%)	1,064 (31)	229 (27)	275 (31)	277 (32)	283 (33)
Prevalent hypertension, *N* (%)	2,117 (56)	518 (54)	520 (55)	540 (56)	539 (57)
Mean eGFR, mL/min/1.73m^2 (SD)	74 (18)	75 (19)	75 (18)	74 (18)	71 (18)
Median HOMA-IR (IQR)	2.4 (1.7–3.7)	2.0 (1.5–3.0)	2.3 (1.6–3.5)	2.6 (1.8–3.8)	2.9 (1.9–4.7)
Median fasting glucose, mg/dL (IQR)	99 (92–109)	97 (91–106)	98 (92–108)	100 (92–109)	100 (93–112)
Median C-reactive protein, g/L (IQR)	2.5 (1.2–5.7)	2.3 (1.1–5.7)	2.5 (1.2–6.0)	2.4 (1.1–5.6)	2.8 (1.3–5.8)
Median triglycerides, mg/dL (IQR)	121 (88–168)	102 (77–136)	115 (85–155)	129 (94–177)	146 (106–204)
Mean HDL cholesterol, mg/dL (SD)	54 (14)	56 (15)	54 (14)	54 (14)	54 (14)
Mean LDL cholesterol, mg/dL (SD)	127 (34)	122 (34)	127 (33)	130 (32)	131 (34)
Mean albumin, g/L (SD)	3.9 (0.3)	3.9 (0.3)	3.9 (0.3)	3.9 (0.3)	4.0 (0.3)

IQR, interquartile range; WC, waist circumference. *Among participants with prevalent type 2 diabetes at baseline

Table 2—*Association of fetuin-A with incident cardiovascular disease events in older adults with and without type 2 diabetes at baseline (1992): the CHS*

	Fetuin-A quartiles				
	Q1 (<0.41 g/L)	Q2 (0.41–<0.47 g/L)	Q3 (0.47–0.53 g/L)	Q4 (>0.53)	Continuous*
Nondiabetic participants					
Cases, *N* (total *N*)	325 (838)	299 (831)	304 (840)	280 (790)	
Incidence (rate per 1,000 person-years)	39.7	34.5	33.8	33	
Unadjusted	1 (ref)	0.86 (0.74–1.01)	0.84 (0.72–0.98)	0.82 (0.70–0.96)	0.91 (0.86–0.96)
Demographic adjusted†	1 (ref)	0.88 (0.75–1.03)	0.91 (0.78–1.07)	0.93 (0.79–1.10)	0.95 (0.90–1.01)
Lifestyle adjusted‡	1 (ref)	0.86 (0.73–1.00)	0.90 (0.77–1.06)	0.92 (0.78–1.08)	0.95 (0.89–1.01)
Fully adjusted§	1 (ref)	0.87 (0.74–1.02)	0.88 (0.75–1.04)	0.88 (0.74–1.04)	0.93 (0.88–0.99)
Participants with type 2 diabetes					
Cases, *N* (total *N*)	45 (116)	61 (124)	58 (119)	84 (152)	
Incidence (rate per 1,000 person-years)	45.6	58.4	55.7	59.6	
Unadjusted	1 (ref)	1.28 (0.87–1.89)	1.20 (0.81–1.77)	1.28 (0.89–1.85)	1.06 (0.94–1.20)
Demographic adjusted†	1 (ref)	1.29 (0.88–1.91)	1.22 (0.81–1.83)	1.32 (0.90–1.94)	1.08 (0.95–1.23)
Lifestyle adjusted‡	1 (ref)	1.29 (0.87–1.92)	1.17 (0.78–1.78)	1.29 (0.87–1.90)	1.07 (0.94–1.23)
Fully adjusted§	1 (ref)	1.34 (0.90–1.99)	1.18 (0.77–1.80)	1.35 (0.90–2.02)	1.08 (0.94–1.24)

Data are HR (95% CI). Q, quartile. *Per SD = 0.097 g/L greater. †Age, race, sex, and CHS clinic. ‡Age, race, sex, CHS clinic, physical activity, smoking status, use of hormone therapy (women), alcohol, and use of insulin or oral hypoglycemic (participants with type 2 diabetes). §Age, race, sex, CHS clinic, physical activity, smoking status, use of hormone therapy (women), alcohol, use of insulin or oral hypoglycemic (participants with type 2 diabetes), hypertension, eGFR, C-reactive protein, BMI, triglycerides, HDL cholesterol, and LDL cholesterol.

Among 511 participants with type 2 diabetes at baseline, 248 experienced incident CVD events during follow-up. In contrast to the nondiabetic strata, individuals with type 2 diabetes who were within the lowest quartile of fetuin-A had the lowest risk of CVD. Although there was a trend toward higher CVD risk with higher fetuin-A levels, this association was not statistically significant among the diabetic strata (Supplementary Data). The HR for each SD higher fetuin-A level was 1.07 (95% CI, 0.93–1.22) for incident CVD (Table 2). Additional adjustment for abdominal obesity (waist or waist-to-hip ratio) did not change the results (data not shown).

The inverse association between fetuin-A and CVD in the participants without type 2 diabetes was very similar when using competing risk regression models to account for competing mortality risk (Table 3), whereas the competing risk model showed a slightly stronger association between fetuin-A and CVD in the participants with type 2 diabetes, suggesting a statistically significantly elevated risk with high levels of fetuin-A in this secondary analysis [subhazard ratio for each SD higher fetuin-A level was 1.14 (95% CI, 1.00–1.31)]. In secondary analyses, we evaluated each component of the composite end point separately among those with and without diabetes. The associations of fetuin-A with MI, stroke, and CVD death were similar to those observed for the combined end point (Supplementary Data).

Next, we limited the study sample to individuals without type 2 diabetes at baseline and evaluated whether the association of fetuin-A with CVD differed among those with obesity or high HOMA-IR. Among nondiabetic individuals who were not obese (BMI <30 kg/m^2), the HR for incident CVD was 0.91 (95% CI, 0.85–0.97) per SD greater fetuin-A level, whereas no association was observed among individuals with obesity [1.01 (0.88–1.16)] (Table 4). The test for interaction with obesity did not reach statistical significance (*P* interaction = 0.15). Similarly, among nondiabetic participants with a HOMA-IR below the population median (2.22 units), fetuin-A was inversely associated with risk of CVD [HR per SD greater fetuin-A level, 0.87 (0.79–0.95)], whereas no association was observed among nondiabetic participants with HOMA-IR above the median [0.99 (0.91–1.08)]. These associations differed significantly from one another (*P* interaction = 0.03). Associations of fetuin-A with CVD in strata of waist-to-hip ratio (median cut-point = 96) showed similar divergent trends [HR per SD greater fetuin-A level, 0.89 (0.82–0.97) for less than the median, and 0.98 (0.90–1.07) for equal to or higher than the median, respectively].

In a post hoc analysis, we combined individuals with type 2 diabetes, obesity, or HOMA-IR above the median (*n* = 2,243) versus individuals without any of these (*n* = 1,567). Fetuin-A was inversely associated with risk of CVD in individuals without type 2 diabetes, obesity, and high HOMA-IR [HR per SD, 0.85 (0.77–0.93)]. Among individuals with at least one factor, the HR was 1.03 (0.96–1.10; *P* interaction = 0.001). We observed no evidence of effect modification by age, sex, race, or eGFR in the overall study sample (all *P* interaction >0.1).

CONCLUSIONS—True biological interactions are rare and often difficult to detect or confirm in epidemiologic studies. In this community-living population of older persons with long-term follow-up, we confirmed that the association of fetuin-A with risk of incident CVD differed between individuals with or without type 2 diabetes, and that an inverse association of fetuin-A with CVD is limited to persons without type 2 diabetes. Moreover, the presence of interactions with BMI and HOMA-IR among those free of type 2 diabetes extends our results to populations who are generally healthy but with early signs of underlying pathophysiology along the same disease trajectory. In the context of previous studies demonstrating a dual function of fetuin-A to influence arterial calcification and insulin

Table 3—*Subhazard ratios from competing risk models for the association of fetuin-A with incident cardiovascular disease events in older adults with and without type 2 diabetes at baseline (1992): the CHS*

	Fetuin-A quartiles				
	Q1 (<0.41 g/L)	Q2 (0.41–<0.47 g/L)	Q3 (0.47–0.53 g/L)	Q4 (>0.53)	Continuous*
Nondiabetic participants					
Cases, *N* (total N)	325 (838)	299 (831)	304 (840)	280 (790)	
SHR (95% CI)	1 (ref)	0.92 (0.78–1.09)	0.94 (0.80–1.11)	0.93 (0.77–1.11)	0.95 (0.89–1.01)
Participants with type 2 diabetes					
Cases, *N* (total *N*)	45 (116)	61 (124)	58 (119)	84 (152)	
SHR (95% CI)	1 (ref)	1.33 (0.89–1.98)	1.24 (0.81–1.89)	1.57 (1.05–2.33)	1.14 (1.00–1.31)

Q, quartile; SHR, subhazard ratio. Data are SHR (95% CI). All models adjusted for age, race, sex, CHS clinic, physical activity, smoking status, use of hormone therapy (women), alcohol, hypertension, eGFR, C-reactive protein, BMI, triglycerides, HDL cholesterol, and LDL cholesterol. *Per SD = 0.097 g/L greater

resistance in in vitro and animal studies, these findings suggest that similar biology may be occurring in humans and may predispose to risk of CVD differentially in persons with or without type 2 diabetes or insulin resistance.

Few previous studies have evaluated the association of fetuin-A with CVD in the general population, and results from existing studies are conflicting (16,17). In the EPIC-Potsdam study among middle-aged community-living Europeans, higher fetuin-A concentrations were associated with higher risk of MI and stroke and, thus, were in the opposite direction of our findings. The investigators did not observe effect modification by type 2 diabetes status (16). In the Rancho Bernardo Study, among approximately 1,700 community-living Caucasians with long-term follow-up, we previously reported that low fetuin-A was associated with CVD death in persons without type 2 diabetes, whereas higher fetuin-A was associated with CVD death in those with type 2 diabetes (17). These findings are confirmed here in the CHS and are extended in several important ways. First, this study extends the finding to include adjudicated nonfatal CVD events (MI and stroke). Second, the availability of HOMA-IR at baseline allowed us to conduct a secondary analysis in individuals without type 2 diabetes. Within this subset, we observed that the association of low fetuin-A with CVD was also stronger in persons who were lean and with HOMA-IR levels below the median.

Although the reasons for the discrepancy between this study and our previous study in the Rancho Bernardo study versus the EPIC-Potsdam study are uncertain, one of several possibilities may be responsible. First, we had a larger study sample, longer follow-up time, greater number of individuals with type 2 diabetes, and a much larger number of incident CVD events during follow-up, which provided greater statistical power to detect effect modification by type 2 diabetes or insulin resistance. Second, the mean age was 49 years in the EPIC-Potsdam study, 73 years in the Rancho Bernardo cohort, and 75 years in our cohort. We applied competing risk models to investigate whether the high competing risk of mortality in this elderly population affected our findings, which had little impact on the results. Because arterial calcification is strongly related to age (30), individuals in our study were more likely to have prevalent arterial calcification at baseline. If the link between low fetuin-A and CVD events is mediated through accelerated arterial calcification, then a higher prevalence of vascular calcification may have rendered it easier to detect an association of low fetuin-A with CVD among the older nondiabetic, nonobese, or noninsulin-resistant subgroups. Finally, the EPIC Potsdam study measured fetuin-A with a different ELISA platform than the one used in the current study. Reports have suggested differences in the specificity of these ELISAs for the detection of glycosylated plasma fetuin-A also might contribute to between-study heterogeneity (31).

Beyond its role as an inhibitor of calcium deposition in arterial walls, fetuin-A also binds to the insulin receptor and

Table 4—*Association of fetuin-A levels* with incident cardiovascular events among older adults without type 2 diabetes stratified by obesity and HOMA-IR at baseline (1992): the CHS*

	BMI <30 kg/m^2	BMI ≥30 kg/m^2	*P* for interaction	HOMA-IR less than the median†	HOMA-IR at the median or greater than the median†	*P* for interaction
Cases, *N* (total *N*)	979 (2,697)	229 (602)		585 (1,643)	612 (1,641)	
Incidence (rate per 1,000 person-years)	35	35.9		34.2	35.8	
Unadjusted	0.88 (0.83–0.94)	1.02 (0.89–1.16)	0.06	0.84 (0.77–0.92)	0.96 (0.88–1.04)	0.03
Demographic-adjusted‡	0.93 (0.87–0.99)	1.06 (0.93–1.21)	0.08	0.88 (0.81–0.97)	1.00 (0.92–1.09)	0.04
Lifestyle-adjusted§	0.92 (0.86–0.99)	1.06 (0.93–1.22)	0.06	0.88 (0.81–0.96)	1.00 (0.92–1.09)	0.04
Fully adjusted¶	0.91 (0.85–0.97)	1.01 (0.88–1.16)	0.15	0.87 (0.79–0.95)	0.99 (0.91–1.08)	0.03

*Per SD (0.097 g/L) difference. †Median used as cut-point: 2.22. ‡Age, race, sex, and CHS clinic. §Age, race, sex, CHS clinic, physical activity, smoking status, use of hormone therapy (women), and alcohol. ¶Age, race, sex, CHS clinic, physical activity, smoking status, use of hormone therapy (women), alcohol, hypertension, eGFR, C-reactive protein, BMI, triglycerides, HDL cholesterol, and LDL cholesterol.

impairs insulin signaling, thereby inducing peripheral insulin resistance (2). Fetuin-A knockout mice have improved insulin sensitivity and are resistant to weight gain when fed high-fat diets when compared with wild-type controls (11). Conversely, wild-type mice treated with exogenous fetuin-A had acute development of insulin resistance (32). Previous cross-sectional studies in humans have shown strong positive correlations between fetuin-A and several metabolic factors, such as body weight, triglycerides, LDL cholesterol, and insulin resistance, both by HOMA and insulin clamps (4,5). In addition, individuals with higher fetuin-A concentrations are at higher risk for incident type 2 diabetes, independent of other metabolic factors (6,7). Thus, whereas mechanisms responsible for different directions of association of fetuin-A with CVD among individuals with or without type 2 diabetes or insulin resistance are not definitively known, we propose that the beneficial effects of fetuin-A to lower arterial calcification could be counterbalanced by insulin resistance, free fatty acid efflux from adipose tissue, and cytokine production in persons with type 2 diabetes or obesity, resulting in no association or even a direct association between fetuin-A and incident CVD in individuals with type 2 diabetes. In contrast, it is possible that the impact of worsened insulin resistance induced by fetuin-A may be more aptly tolerated among persons without type 2 diabetes, obesity, or insulin resistance. In this setting, low fetuin-A levels may predispose to greater arterial calcification, which may affect the risk of CVD events through mechanisms distinct from insulin resistance. Further prospective investigations may shed light on this hypothesis by evaluating the complex interplay between insulin and fetuin-A in relation to future cardiometabolic risk.

Strengths of this study include its prospective design, relatively large sample size, long-term follow-up, large number and adjudication of CVD events, complete follow-up, and the ability to evaluate many potential confounders. Moreover, the availability of insulin levels at baseline allowed evaluation of effect modification by HOMA among the nondiabetic participants. The study also has limitations. We studied older persons of African American and Caucasian races. Results may not generalize to other populations. Studying an older population also may lead to bias if diabetic individuals with low fetuin-A levels were more likely to die or to be too ill to participate in this study at baseline. Although we observed statistically significant interactions by type 2 diabetes and insulin resistance, the confidence intervals in each stratum were relatively wide. Larger samples of individuals with type 2 diabetes will be required to determine if a direct association of fetuin-A with CVD is statistically significant in such individuals. Finally, a single measurement of fetuin-A may be susceptible to random measurement error, which could have biased our results toward the null. A single measurement also precludes analysis of whether longitudinal trajectories in fetuin-A would provide additional information on CVD risk above and beyond the baseline level.

In a population of older community-living persons, the association of fetuin-A concentrations with risk of incident CVD is modified by insulin resistance and type 2 diabetes. Future studies should investigate disparate mechanisms linking the dual functions of fetuin-A with health outcomes in older community-living individuals.

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M.K.J. wrote the manuscript. M.K.J., K.J.M., and J.H.I. assisted in study design. M.K.J., T.M.B., K.J.M., L.D., J.R.K., R.P.T., S.J.Z., E.B.R., D.S.S., M.S., and J.H.I. interpreted the data. T.M.B. conducted statistical analysis. T.M.B., K.J.M., L.D., J.R.K., R.P.T., S.J.Z., E.B.R., D.S.S., M.S., and J.H.I. critically edited the manuscript. K.J.M., L.D., J.R.K., S.J.Z., and J.H.I. obtained funding for the project. R.P.T. and D.S.S. were responsible for CHS study implementation, including quality-control procedures, directed the central laboratory, and conducted the fetuin-A laboratory measurements. T.M.B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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References

1. Srinivas PR, Wagner AS, Reddy LV, et al. Serum alpha 2-HS-glycoprotein is an inhibitor of the human insulin receptor at the tyrosine kinase level. Mol Endocrinol 1993;7:1445–1455
2. Auberger P, Falquerho L, Contreres JO, et al. Characterization of a natural inhibitor of the insulin receptor tyrosine kinase: cDNA cloning, purification, and anti-mitogenic activity. Cell 1989;58:631–640
3. Mathews ST, Srinivas PR, Leon MA, Grunberger G. Bovine fetuin is an inhibitor of insulin receptor tyrosine kinase. Life Sci 1997;61:1583–1592
4. Stefan N, Hennige AM, Staiger H, et al. Alpha2-Heremans-Schmid glycoprotein/fetuin-A is associated with insulin resistance and fat accumulation in the liver in humans. Diabetes Care 2006;29:853–857
5. Ix JH, Shlipak MG, Brandenburg VM, Ali S, Ketteler M, Whooley MA. Association between human fetuin-A and the metabolic syndrome: data from the Heart and Soul Study. Circulation 2006;113:1760–1767
6. Stefan N, Fritsche A, Weikert C, et al. Plasma fetuin-A levels and the risk of type 2 diabetes. Diabetes 2008;57:2762–2767
7. Ix JH, Wassel CL, Kanaya AM, et al; Health ABC Study. Fetuin-A and incident diabetes mellitus in older persons. JAMA 2008;300:182–188
8. Ix JH, Biggs ML, Mukamal KJ, et al. Association of fetuin-a with incident diabetes mellitus in community-living older adults: the cardiovascular health study. Circulation 2012;125:2316–2322
9. Schafer C, Heiss A, Schwarz A, et al. The serum protein alpha 2-Heremans-Schmid glycoprotein/fetuin-A is a systemically acting inhibitor of ectopic calcification. J Clin Invest 2003;112:357–366
10. Mathews ST, Rakhade S, Zhou X, Parker GC, Coscina DV, Grunberger G. Fetuin-null mice are protected against obesity and insulin resistance associated with aging. Biochem Biophys Res Commun 2006;350:437–443
11. Mathews ST, Singh GP, Ranalletta M, et al. Improved insulin sensitivity and resistance to weight gain in mice null for the Ahsg gene. Diabetes 2002;51:2450–2458
12. Ketteler M, Bongartz P, Westenfeld R, et al. Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: a cross-sectional study. Lancet 2003;361:827–833
13. Stenvinkel P, Wang K, Qureshi AR, et al. Low fetuin-A levels are associated with cardiovascular death: Impact of variations in the gene encoding fetuin. Kidney Int 2005;67:2383–2392

14. Hermans MM, Brandenburg V, Ketteler M, et al.; Netherlands cooperative study on the adequacy of Dialysis (NECOSAD). Association of serum fetuin-A levels with mortality in dialysis patients. Kidney Int 2007;72:202–207
15. Moe SM, Reslerova M, Ketteler M, et al. Role of calcification inhibitors in the pathogenesis of vascular calcification in chronic kidney disease (CKD). Kidney Int 2005;67:2295–2304
16. Weikert C, Stefan N, Schulze MB, et al. Plasma fetuin-a levels and the risk of myocardial infarction and ischemic stroke. Circulation 2008;118:2555–2562
17. Laughlin GA, Cummins KM, Wassel CL, Daniels LB, Ix JH. The association of fetuin-A with cardiovascular disease mortality in older community-dwelling adults: the Rancho Bernardo study. J Am Coll Cardiol 2012;59:1688–1696
18. Ix JH, Chertow GM, Shlipak MG, Brandenburg VM, Ketteler M, Whooley MA. Association of fetuin-A with mitral annular calcification and aortic stenosis among persons with coronary heart disease: data from the Heart and Soul Study. Circulation 2007;115:2533–2539
19. Fried LP, Borhani NO, Enright P, et al. The Cardiovascular Health Study: design and rationale. Ann Epidemiol 1991;1: 263–276
20. Taylor HL, Jacobs DR Jr, Schucker B, Knudsen J, Leon AS, Debacker G. A questionnaire for the assessment of leisure time physical activities. J Chronic Dis 1978;31:741–755
21. Shlipak MG, Sarnak MJ, Katz R, et al. Cystatin C and the risk of death and cardiovascular events among elderly persons. N Engl J Med 2005;352:2049–2060
22. Cushman M, Cornell ES, Howard PR, Bovill EG, Tracy RP. Laboratory methods and quality assurance in the Cardiovascular Health Study. Clin Chem 1995;41:264–270
23. Stevens LA, Coresh J, Schmid CH, et al. Estimating GFR using serum cystatin C alone and in combination with serum creatinine: a pooled analysis of 3,418 individuals with CKD. Am J Kidney Dis 2008;51:395–406
24. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–419
25. Ives DG, Fitzpatrick AL, Bild DE, et al. Surveillance and ascertainment of cardiovascular events. The Cardiovascular Health Study. Ann Epidemiol 1995;5:278–285
26. Price TR, Psaty B, O'Leary D, Burke G, Gardin J. Assessment of cerebrovascular disease in the Cardiovascular Health Study. Ann Epidemiol 1993;3:504–507
27. Psaty BM, Kuller LH, Bild D, et al. Methods of assessing prevalent cardiovascular disease in the Cardiovascular Health Study. Ann Epidemiol 1995;5:270–277
28. Cox DR, Oakes D. *Analysis of survival data*. London, Chapman and Hall, 1984
29. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc 1999;94:496–509
30. Detrano R, Guerci AD, Carr JJ, et al. Coronary calcium as a predictor of coronary events in four racial or ethnic groups. N Engl J Med 2008;358:1336–1345
31. Smith ER, Ford ML, Tomlinson LA, Rocks BF, Rajkumar C, Holt SG. Poor agreement between commercial ELISAs for plasma fetuin-A: An effect of protein glycosylation? Clin Chim Acta 2010;411:1367–1370
32. Hennige AM, Staiger H, Wicke C, Machicao F, Fritsche A, Haring HU, Stefan N. Fetuin-A induces cytokine expression and suppresses adiponectin production. PLoS ONE 2008;3:e1765.