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Fetuin-A and CVD risk by glycemia

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Abstract

Fetuin-A is a hepatic secretory protein that both promotes insulin resistance and inhibits arterial calcification. Previous studies have suggested that the association of fetuin-A with cardiovascular disease (CVD) might depend on glycemic status. We conducted a case-cohort study of fetuin-A and incident non-fatal CVD nested in the Multi-Ethnic Study of Atherosclerosis with follow-up from 2000 - 2007. Fetuin-A concentrations were measured from baseline serum samples among 2,505 randomly selected subcohort members and 142 incident cases (68 in the subcohort). In weighted multivariable Cox regression models, no association was observed between fetuin-A and incident CVD in the total study population (HR per SD = 1.01; 95% CI: 0.55, 1.47). Although differences by baseline glycemic status were not significant (p = 0.09), our results tended to support the interaction with glycemic status observed in other studies, with a positive trend restricted to participants with elevated fasting blood glucose (HR for participants with impaired fasting glucose = 1.56 [95% CI: 0.84, 2.89]; HR for participants with diabetes = 1.27 [95% CI: 0.85, 1.90]). Our results suggest that fetuin-A is not associated with an overall risk of CVD, but support prior evidence indicating that the association may be modified by glycemic status.

Introduction

Fetuin-A is a hepatic secretory glycoprotein with divergent biological activities related to cardiovascular disease (CVD) risk. On the one hand, fetuin-A forms stable colloidal complexes with calcium and phosphorus, potentially reducing arterial calcification[1]. On the other hand, fetuin-A promotes insulin resistance by inhibiting the insulin receptor tyrosine kinase[2], and by mediating free fatty acid inflammatory signaling through toll-like receptor 4[3].

Observational studies of the association between fetuin-A concentrations and CVD risk have generated mixed results. Among participants with advanced kidney disease, a condition prone to arterial calcification, lower fetuin-A concentrations were associated with risk of stroke[4], and cardiovascular mortality[5]. However, in populations with preserved kidney function, higher fetuin-A concentrations were associated with greater CVD risk and CVD mortality in two studies[6,7], but were associated with lower risk of coronary heart disease in another study[8]. Further investigations in general population samples have detected significant effect modification of the association of fetuin-A concentrations with CVD outcomes by diabetes status[7,9] and markers of dysglycemia in participants free of diabetes[9], indicating that higher fetuin-A levels are associated with higher CVD risk only in those with signs of insulin resistance. Thus far, these findings have not been confirmed in ethnically diverse populations.

Therefore, we investigated the association of fetuin-A concentrations with risk of CVD in the Multi-Ethnic Study of Atherosclerosis (MESA), a cohort of men and women from four major racial/ethnic groups in the U.S. We specifically aimed to investigate whether glycemic status would modify the association of fetuin-A with CVD.

Methods

Study Population and Design

Participants were enrolled in MESA, an ongoing prospective cohort study designed to study risk factors for the progression of cardiovascular disease[10]. MESA began in 2000 – 2002 with the recruitment of 6,814 men and women aged 45-84 years of Caucasian, Chinese, African or Hispanic descent from six regions in the U.S. (Baltimore City and Baltimore County, MD, Chicago, Il, Forsyth County, NC, Los Angeles County, CA, New York, NY, and St. Paul, MN) who were free of clinical cardiovascular disease. Follow-up exams were conducted in 2002-2003 (Exam 2), 2004-2005 (Exam 3), 2005-2007 (Exam 4), and 2010-2012 (Exam 5). The institutional review boards of the participating institutions approved MESA, and the Human Subjects Committee Review Board of the Harvard T.H. Chan School of Public Health approved this study.

Participants were selected for fetuin-A measurement in 2007 using a case-cohort designed for the primary purpose of studying fetuin-A in relation to changes in coronary artery calcium (CAC), as measured by cardiac computed tomography (CT) scans. CT scans were performed on all MESA participants at baseline and by random assignment at either Exam 2 or 3. The original participant selection for analyses of CAC included all individuals who received their second CT scan at Exam 3, and thus participants eligible for our case-cohort study of incident CVD were required to survive until Exam 3. As our study selection criteria precluded the inclusion of fatal cases prior to Exam 3, we restricted our primary endpoint to non-fatal CVD, with cases occurring between Exam 1 and the end of follow-up in May 2007 included in our analyses (Fig. 1). In sensitivity analyses, we included 18 fatal CVD cases that occurred between Exam 3 and end of follow-up.

Our case-cohort sample included 2,774 subcohort members and 157 cases (76 of whom belonged to the subcohort) (Fig. 1). After excluding participants who had insufficient serum for fetuin-A measurement (n = 224) and missing CVD or covariate data (n = 45), 2,505 subcohort members and 142 nonfatal CVD cases (68 within the subcohort) remained for analysis. Thus, the final sample size for this analysis was 2,579 individuals. In our sensitivity analyses that additionally included fatal events after Exam 3, we had a total of 160 CVD events.

Measurement of Fetuin-A Concentrations

Baseline serum specimens were thawed in 2009, and fetuin-A concentrations were measured by the clinical laboratory at the University of Maryland using a human enzyme linked immunosorbent assay kit (Epitope Diagnostics, San Diego, CA). Samples were run in duplicate, and values were averaged. The intra- and inter-assay coefficients of variation for fetuin-A measurements were 2.1-3.4% and 5.7-6.8%, respectively.

Assessment of Cardiovascular Events

Participants were queried about hospital admissions and CVD outpatient diagnoses during follow-up examinations, and by telephone interviews completed every 9-12 months[10]. In this study, CVD events included myocardial infarction (MI), resuscitated cardiac arrest, and stroke. The diagnosis of MI was based on clinical, electrocardiographic and biochemical findings. Stroke events included any documented rapid onset focal neurologic deficit that was not related to other nonvascular causes, and lasted at least 24 hours, unless also accompanied by a clinically relevant lesion on brain imaging. Two physicians from the MESA study events

committee independently reviewed participants' medical records to identify incident cases of CVD[11].

Assessment of Covariates and Effect Modifiers

At baseline, participant information was collected through questionnaires and physical examinations. Information collected included age, sex, racial/ethnic group, field center, smoking (never, former, current), alcohol intake (never, former, current with <1 drink per day, current with 1-2 drinks per day, current with >2 drinks per day), body mass index (BMI) (normal weight: <25.0 kg/m^2, overweight: 25.0-29.9 kg/m^2, obese: ≥30.0 kg/m^2), systolic blood pressure, LDL and HDL cholesterol, triglycerides, family history of MI or stroke (yes, no, don't know), education (less than high-school, high-school up to technical or associate degree, bachelor's degree or more), annual income (<$25,000, $25-49,999, ≥$50,000, not stated), and physical activity (moderate-vigorous in MET-min/wk).

Fasting plasma glucose was measured from baseline blood samples using the Vitros analyzer (Johnson & Johnson Clinical Diagnostics). Glycemic status was defined according to the American Diabetic Association criteria as normoglycemic (fasting glucose <100 mg/dL), impaired fasting glucose (IFG: fasting glucose 100-125 mg/dL), and diabetes (fasting glucose ≥126 mg/dL or use of diabetes medications)[12].

Statistical Analyses

The distribution of baseline characteristics was examined among CVD cases and the random subcohort. Hazard ratios (HRs) and 95% confidence intervals (95% CI) for the association between fetuin-A concentrations and incident non-fatal CVD were estimated by Cox

proportional hazards regression, with Kalbfleisch and Lawless weights and the robust variance estimator[13] used to account for the case-cohort design.

We observed no deviations from linearity using restricted cubic splines to evaluate the functional form of fetuin-A, and we therefore modeled fetuin-A concentrations per standard deviation (SD) (0.10 g/L). We additionally evaluated associations according to tertiles of fetuin-A based on the distribution in the subcohort. Participants contributed person-time from baseline until the date of an event, death, or end of follow-up (May 2007), whichever occurred first. Cases were assigned a weight of 1, irrespective of whether the case was in the random subcohort, and subcohort non-cases were assigned a weight equal to the inverse of the probability of being in the subcohort[13]. A basic model included age, sex, racial/ethnic group, and field center, and the full multivariable model additionally included smoking, alcohol intake, BMI, systolic blood pressure, LDL cholesterol, HDL cholesterol and triglyceride concentrations, family history of CVD, education, income, and physical activity. Additional adjustment for waist circumference did not affect the multivariable-adjusted estimates. No violations of the proportional hazards assumption were observed via analysis of martingale residuals[14]. As associations did not appear to vary by sex and race, we performed all analyses in the total study population.

We examined whether the association of fetuin-A concentrations with CVD risk varied in the three defined strata of glycemic status (normoglycemic, IFG, and diabetes) and used the likelihood ratio test to compare models with and without multiplicative interaction terms. A post-hoc analysis subsequently pooled the IFG and diabetes group and compared the association of fetuin-A and CVD in this combined group with fasting glucose >100 mg/dL to that among normoglycemic individuals. All statistical tests were performed using SAS 9.4 (SAS Institute; Cary, NC).

Results

Participants were followed for a median of 6.0 years. Compared with subcohort members, participants who subsequently developed CVD were older and more likely to be Caucasian, male, and diabetic, and were more likely to currently smoke (Table 1).

We observed no overall association between fetuin-A and incident CVD (HR per SD = 1.01; 95% CI: 0.84, 1.23). Although associations were not statistically significantly different by baseline glycemic status (global p-value for interaction = 0.09), suggestive positive associations were only observed among those with IFG and prevalent DM (Table 2). In normoglycemic individuals, each SD (0.10 g/L) higher fetuin-A was associated with a slightly lower risk of CVD (HR=0.89, 95% CI: 0.69, 1.13), whereas the HR was 1.56 (95% CI: 0.84, 2.89) in the 20 participants with IFG and 1.27 (95% CI: 0.85, 1.90) among those with diabetes. When participants with either IFG or diabetes were combined, the HR per SD was 1.20 (95% CI: 0.89, 1.63), which was statistically significantly different from the estimate among normoglycemic individuals (p for interaction = 0.03).

Upon addition of the fatal CVD cases that occurred after Exam 3, main associations of fetuin-A with CVD risk were similar to those among non-fatal cases alone for individuals with normoglycemia (HR = 0.81; 95% CI: 0.64, 1.02) and IFG or diabetes (HR = 1.13; 95% CI: 0.84, 1.51) (p-interaction = 0.03).

Discussion

In this multi-ethnic case-cohort of U.S. men and women, we found no overall association between fetuin-A levels and incident CVD, but our results tended to support the reported interaction observed in other studies,[7,9] with a positive trend restricted to participants with elevated fasting plasma glucose.

Prior prospective studies of fetuin-A and CVD risk, conducted in predominantly Caucasian populations, have been conflicting. We have previously observed an interaction with diabetes in two studies of older adults,[7,9] where risk of CVD was elevated with higher fetuin-A only among participants with diabetes. In contrast, a case-cohort study within the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study found higher fetuin-A concentrations associated with greater CVD risk among all participants regardless of their glycemic status[6], and a case-control study nested in the Nurses' Health Study (NHS) observed no association of fetuin-A and CVD and no effect modification by diabetes status[8].

The dual role of fetuin-A in inhibiting arterial calcification as well as promoting insulin resistance may explain these seemingly contradictory findings. Although the burden of CAC has only been assessed in the MESA and Rancho Bernardo cohorts, differences in CAC prevalence across study populations may have contributed to the inconsistent results. In populations with greater arterial calcification, the effect of fetuin-A in preventing calcium crystal formation may be more pronounced than its effect on promoting insulin resistance, and thus the cardioprotective role of fetuin-A may predominate. Other explanations for discrepancies across studies may be differences in sample sizes and follow-up time, differing CVD endpoints, or differences in the assays used for the measurement of fetuin-A. Differences in diabetes case ascertainment and the prevalence of diabetes medication use may have also contributed to discrepancies in diabetes

subgroup analyses, as some studies may have captured more severe diabetes cases in whom the insulinogenic effects of fetuin-A were more pronounced.

We previously found an inverse association between fetuin-A and baseline CAC severity in MESA that appeared limited to individuals without diabetes[15]. However, as associations for CAC incidence and progression overall and by diabetes status were not significant, the influence of fetuin-A on CAC burden and its potential to differ by diabetes status in MESA remain unclear.

Our study has many notable strengths, including a comprehensive assessment of potential confounding factors for CVD risk and careful adjudication of CVD events. This is the first study of fetuin-A and CVD in an ethnically diverse population where we were also able to evaluate the role of baseline glycemia. However, several important limitations warrant consideration. As CVD case numbers were limited, it is difficult to determine whether the lack of significant associations was due to low power or true lack of effect, either overall or in subgroups. Due to our study design, we were limited to mostly non-fatal outcomes in the current study, and we had insufficient case numbers to evaluate the association for separate CVD endpoints. However, similar differences by glycemic status were observed when later fatal events were included as also reported in the Rancho Bernardo Study and across CVD endpoints in CHS, providing some reassurance that our results were not unique to our combined CVD endpoint. We also measured fetuin-A at only one point in time, although fetuin-A has previously been shown to be stable over several years[8].

In conclusion, our results lend further support to prior studies suggesting that positive associations between fetuin-A concentration and incident CVD may be limited to individuals with insulin resistance. Further study is needed to better understand the complex

interrelationships between fetuin-A, arterial calcification, and glycemic status in the development of CVD.

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Table 1. Baseline characteristics in a random sub-cohort and participants who developed cardiovascular disease (CVD) in the Multi-Ethnic Study of Atherosclerosis

Variable	Subcohort (n = 2505)	Incident cases (n = 142)*
Mean fetuin-A, g/L (SD)	0.48 (0.10)	0.47 (0.11)
Mean age, years (SD)	62 (10)	68 (10)
Male, *N* (%)	1198 (48)	91 (64)
Chinese-American, *N* (%)	315 (13)	7 (5)
African-American, *N* (%)	684 (27)	35 (25)
Hispanic, *N* (%)	512 (20)	31 (22)
Mean body mass index, kg/m^2 (SD)	28.3 (5.4)	28.5 (4.5)
Current smoker, *N* (%)	290 (12)	25 (18)
Median triglycerides, mg/dL (IQR)	110 (77, 156)	133 (96, 196)
Mean LDL-cholesterol, mg/dL (SD)	116 (31)	121 (32)
Median HDL-cholesterol, mg/dL (IQR)	48 (41, 59)	45 (36, 54)
Mean SBP, mmHg (SD)	126 (21)	137 (24)
Using medications for diabetes, *N* (%)	226 (9)	29 (20)
Median fasting glucose, mg/dL (IQR)	89 (82, 99)	91 (85, 107)

*68 cases that occurred within the subcohort are included in both case and subcohort number.

Table 2. Hazard ratio and 95% confidence intervals for cardiovascular disease risk across tertiles of fetuin-A, stratified by glycemic status

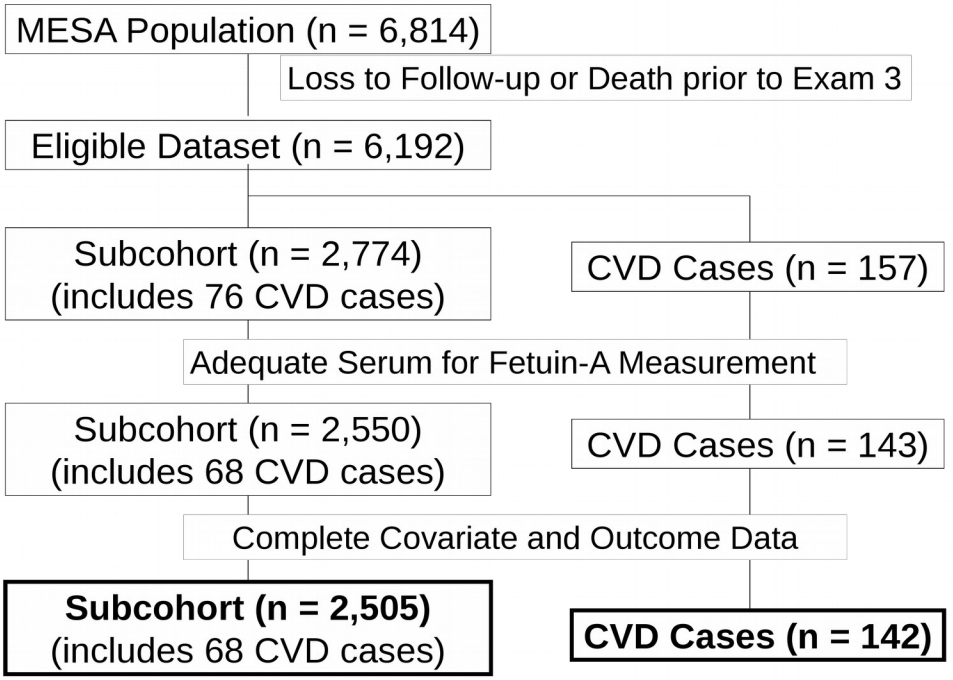
	Tertiles (g/L)			Continuous
	0.20 - 0.43	>0.43 - 0.51	>0.51 - 0.94	per SD*
Normoglycemic				
Cases	40	24	27	
HR	1.0	0.64	0.67	0.89
(95% CI)	(ref)	(0.38-1.10)	(0.38-1.18)	(0.69-1.13)
Impaired fasting glucose				
Cases	5	4	11	
HR	1.0	1.25	5.64	1.56
(95% CI)	(ref)	(0.17, 9.13)	(0.92, 34.59)	(0.84, 2.89)
Diabetes				
Cases	9	11	11	
HR	1.0	3.19	1.90	1.27
(95% CI)	(ref)	(1.00-10.14)	(0.63-5.76)	(0.85-1.90)

CI, confidence interval, HR, hazard ratio, SD, standard deviation

Normoglycemic: fasting glucose <100 mg/dL; impaired fasting glucose: fasting glucose 100 – 125 mg/dL; diabetes: fasting glucose ≥126 mg/dL or the use medications for diabetes

*SD is 0.10 g/L; p for interaction = 0.09

Figure 1. Selection of cases and subcohort for analyses of fetuin-A and incident CVD



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