The Associations of Fetuin-A With Subclinical Cardiovascular Disease in Community-Dwelling Persons
The Rancho Bernardo Study

Joachim H. Ix, MD, MAS,*†‡ Elizabeth Barrett-Connor, MD,§ Christina L. Wassel, PHD,‡ Kevin Cummins, MS,§ Jaclyn Bergstrom, MS,§ Lori B. Daniels, MD,|| Gail A. Laughlin, PHD§
San Diego, California

Objectives
The aim of this study was to determine the association of fetuin-A with subclinical cardiovascular disease (CVD) in community-living individuals.

Background
Fetuin-A is a hepatic secretory protein that inhibits arterial calcium deposition in vitro. Lower fetuin-A levels are associated with arterial calcification and death in end-stage renal disease populations. The association of fetuin-A with subclinical CVD in the general population is unknown.

Methods
Among 1,375 community-living individuals without prevalent clinical CVD, we measured plasma fetuin-A concentrations. Peripheral arterial disease (PAD) was defined by ankle brachial index <0.90, coronary artery calcification (CAC) was measured by computed tomography, and common and internal intima-media thickness (cIMT) were measured by carotid ultrasound. PAD was measured concurrent with fetuin-A, and CAC and cIMT were measured 4.6 years (mean) later.

Results
Mean age was 70 ± 11 years, and 64% were women. Fetuin-A levels were inversely associated with CAC severity. When evaluated as CAC categories (0, 1 to 100, 101 to 300, >300) with ordinal logistic regression, each SD higher fetuin-A was associated with 31% lower odds of CAC severity (proportional odds ratio: 0.69; 95% confidence interval: 0.46 to 0.92; p = 0.008) in models adjusted for demographic data, lifestyle factors, traditional CVD risk factors, and kidney function. In contrast, no association of fetuin-A was observed with PAD or high common or internal cIMT in adjusted models.

Conclusions
Lower fetuin-A levels are independently associated with greater CAC severity but not PAD or cIMT. If confirmed, fetuin-A might mark calcium deposition within the vasculature but not atherosclerosis per se. (J Am Coll Cardiol 2011;58:2372–9) © 2011 by the American College of Cardiology Foundation

Fetuin-A is a protein secreted from the liver that inhibits arterial calcium deposition in vitro (1). In serum, it interacts with calcium and phosphorus, increasing their solubility and inhibiting calcium crystal growth and precipitation, reminiscent of mechanisms by which lipoproteins solubilize lipids. Consistent with this function, fetuin-A knockout mice develop greater soft tissue calcification compared with wild-type control mice when challenged with diets enriched in vitamin D or phosphorus (2,3). As fetuin-A inhibits arterial calcification within the blood stream, it raises the possibility that blood levels might provide a useful marker of the burden of arterial calcification.

Studies in end-stage renal disease (ESRD) populations have consistently shown that lower fetuin-A levels are associated with cardiovascular disease (CVD) events and all-cause mortality (4–7). Most (8–11) but not all (12,13) studies in ESRD have also reported that low fetuin-A levels are associated with coronary or abdominal aortic calcification. However, the associations of fetuin-A with subclinical CVD events in the general population are much less clear. Prior studies in individuals with known or clinically suspected CVD have shown that lower fetuin-A levels are associated with coronary artery calcification (CAC) (14) and...
cardiac valve calcification (15), and 1 prior study in patients with type 2 diabetes reported that lower fetuin-A levels are associated with peripheral arterial disease (PAD) (16). However, 2 other small studies (n = 90 and n = 315, respectively) observed associations in the opposite direction, reporting that higher fetuin-A levels were associated with greater carotid intima-media thickness (cIMT) (17,18). Enrollment criteria required known atherosclerosis in one (18) and obesity, insulin resistance, or family history of diabetes in the other (17). Comparing these studies is difficult, not only because of seemingly conflicting directions of associations but also because they studied select populations with either prevalent CVD, diabetes, or diabetic risk factors (14–16,19,20). These conditions are marked by high CVD risk and extensive arterial calcification burden.

To our knowledge, no study has evaluated the association of fetuin-A with subclinical CVD in a single community-dwelling population not selected on the basis of prevalent disease or risk factors for disease. Therefore we sought to determine the association of fetuin-A with subclinical CVD in community-dwelling individuals without known clinical CVD. We hypothesized that lower fetuin-A levels would be associated with each marker of subclinical CVD, independent of traditional CVD risk factors or kidney function.

Methods

Study participants. The Rancho Bernardo Study is a prospective study of older community-dwelling individuals designed to investigate the epidemiology of chronic diseases in older adults. Between 1972 and 1974, all community-dwelling residents living in Rancho Bernardo—a community in Southern California—and 30 to 79 years of age were invited to participate in a study of heart disease risk factors, and 82% (n = 5,052) enrolled. Nearly all were Caucasian, middle to upper-middle class, and relatively well-educated. Since then, sequential study visits have been conducted approximately every 4 years. The present analysis included individuals who participated in the 1992 to 1996 study visit (n = 1,781). Of these, 39 had insufficient stored blood specimens for fetuin-A measurement, 349 were excluded due to known prevalent clinical CVD (history of myocardial infarction [MI], coronary artery bypass graft, or stroke), and 18 had missing covariate data, resulting in a final analytic sample of 1,375 individuals. All participants gave written informed consent; the study protocol was approved by the human research protection program at the University of California San Diego.

Fetuin-A. Fetuin-A was measured in plasma collected at the 1992 to 1996 study visit with a human enzyme-linked immunosorbent assay kit (Epitope Diagnostics, San Diego, California). Samples were stored at −70°C until assayed in 2010. The assay uses a 2-site “sandwich” technique with polyclonal antibodies that bind different epitopes of human fetuin-A. Plasma samples were measured twice in each participant, and results were averaged. Intra-assay and interassay coefficients of variation were 2.4% to 4.7% and 9.5% to 9.9%, respectively.

Subclinical CVD. CORONARY ARTERY CALCIUM. At the follow-up visit subsequent to when blood samples for fetuin-A measurements were obtained (1998 to 2002), participants were invited to have chest electron beam computed tomography scans for CAC. To be invited, participants must: 1) have been evaluated by our research team between 1997 and 1999; 2) be age 55 to 80 years; 3) be post-menopausal (more than 1 year without men- sies) if female; and 4) be free of clinically manifest congenital heart disease (no physician-diagnosed angina, knockout, or coronary artery revascularization). An Imatron C-150 ultrafast computed tomography scanner (Imatron, San Francisco, California) that produced contiguous thin-section sections was used. Scans were electrocardiographically triggered to the R-R interval, and images were obtained at end-diastole during a single breath-hold; CAC was scored according to the Agatston method (21). Of the 525 individuals who were invited, 2 were deceased, 15 were ineligible due to prevalent congenital heart disease, 57 declined the invitation, 47 had moved from the local area or were not seen due to scheduling problems, and 22 did not have fetuin-A measurements available at the preceding visit, yielding a study sample of 382 individuals for the CAC analysis.

PAD. PAD was defined on the basis of ankle brachial index (ABI) measurements made concurrent with collection of samples for fetuin-A measurements (1992 to 1996 study visit). With the participant in the supine position after 5 min of rest, a specially trained and certified nurse used a mercury sphygmomanometer to measure the blood pressure in each arm and leg. Systolic blood pressure (SBP) of the brachial artery was used for the upper extremities, whereas duplicate SBPs of the posterior tibial artery were used for the 2 lower extremities. The ABI was calculated separately for each leg by taking the higher SBP in each lower extremity and dividing by the highest upper-extremity SBP. The lowest of these 2 leg-specific ABIs was used to classify the ABI score for each participant (22). PAD was defined as an ABI <0.90. Among 1,375 individuals, 1,047 had ABI measurements available; 2 were excluded for ABI values >1.30, because such individuals frequently have medial arterial calcification and artifactualy high ABI measurements (23,24). This provided a final analytic sample of 1,063 for PAD.

Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABI</td>
<td>ankle brachial index</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>CAC</td>
<td>coronary artery calcification</td>
</tr>
<tr>
<td>cIMT</td>
<td>carotid intima-media thickness</td>
</tr>
<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
</tr>
<tr>
<td>eGFR</td>
<td>estimated glomerular filtration rate</td>
</tr>
<tr>
<td>ESRD</td>
<td>end-stage renal disease</td>
</tr>
<tr>
<td>MI</td>
<td>myocardial infarction</td>
</tr>
<tr>
<td>PAD</td>
<td>peripheral arterial disease</td>
</tr>
<tr>
<td>SBP</td>
<td>systolic blood pressure</td>
</tr>
</tbody>
</table>

Ix et al. 2011 JACC Vol. 58, No. 23 2373
November 29, 2011:2372-9
cIMT. The cIMT measurements were made at the follow-up visit subsequent to when blood samples for fetuin-A measurements were obtained (1998 to 2002). B-mode ultrasonography of the left and right common and internal carotid arteries was performed by a specially trained radiology technician. Briefly, 4 standardized images were obtained on each participant: 1 at the lateral angle of the common carotid artery, defined as the segment 1 cm proximal to the dilation of the carotid bulb; and 3 for the internal carotid artery at the site of maximal thickness in 3 distinct angles (anterior, posterior, and lateral). Ultrasound measurements were recorded on super-VHS tapes and sent to a central reading facility, where data were processed blinded to all clinical data except for the cIMT ultrasound images. The common cIMT was calculated as the mean of the left and right measurements. Similarly, the internal cIMT was determined as the mean of the 6 internal cIMT measurements (25). All results are reported in millimeters. Of the 1,375 individuals included in this study, 597 returned and participated in the cIMT testing at the next follow-up visit. All had available common cIMT measurements, whereas 14 had internal cIMT measurements that were deemed not interpretable, providing a sample size of 583 individuals for cIMT analysis.

**Other measurements.** Information on medical history, medication use, physical exercise (≥3 times/week, yes or no), alcohol consumption (≥3 drinks/week vs. less), and current smoking (yes/no) was obtained with standardized questionnaires. Current medication use was validated by examination of pills and prescriptions brought to the clinic for that purpose. Participants were asked to rate their overall health on a 5-point scale (excellent, very good, good, fair, or poor) and to report the number of hospital stays in the prior year.

Diabetes mellitus was defined as a fasting glucose ≥126 mg/dl, reported diagnosis of physician, or use of hypoglycemic medications. Systolic (SBP) and diastolic blood pressure were measured twice in the seated position after 5 min of rest, with the Hypertension Detection and Follow-up Program protocol (26). Hypertension was defined as SBP >140 mm Hg, diastolic blood pressure >90 mm Hg, or use of antihypertensive medications. Height and weight were measured with participants wearing light clothes and no shoes, and body mass index (BMI) was calculated (weight [kg]/height [m]^2). Participants with BMI >30 kg/m^2 were classified as obese. Blood samples were obtained by venipuncture between 7:30 AM and 11:00 AM after a requested 12-h fast; serum and plasma were separated and frozen at −70°C. Plasma total and high-density lipoprotein cholesterol and triglycerides were measured in a Centers for Disease Control-certified Lipid Research Clinic laboratory. Low-density lipoprotein cholesterol was calculated with the Friedewald equation (27). Serum creatinine was measured with the rate Jaffe reaction and combined with age, sex, and race in the Modification of Diet in Renal Disease equation to obtain estimated glomerular filtration rate (eGFR) (28). Participants with eGFR <60 ml/min/1.73 m^2 were classified as having moderate chronic kidney disease, according to the National Kidney Foundation criteria (29).

**Statistical analysis.** We categorized participants into quartiles of fetuin-A defined by the distribution within our study population. Differences in demographic data and baseline variables across the fetuin-A quartiles were evaluated with analysis of variance or the chi-square test for categorical variables. We visually inspected histograms of the distribution of each continuous variable in Table 1. When skewed variables were observed, we elected to use the Kruskal-Wallis test in place of analysis of variance. In these instances, we report the median and interquartile range within each quartile of fetuin-A. To maximize statistical power, we used multiple linear regression to evaluate the association of fetuin-A with each subclinical CVD measure on a continuous scale. The CAC scores were right skewed, and 25% had CAC scores of 0. Thus, we added 1 to the CAC score for each individual, natural log-transformed the resulting variable, and evaluated ln(CAC+1) as the primary outcome. The resulting transformed variable more closely approximated a normal distribution. In companion analyses, we evaluated categories of severity of CAC with cut-points that have been associated with increased risk of incident CVD in prior studies (CAC categories: 0, 1 to 100, 101 to 300, >300) (30) and used ordinal logistic regression models in analysis. The proportional odds assumption was evaluated with the Score test, and no violations were observed. We also evaluated PAD (ABI <0.90 vs. >0.90) and common and internal cIMT (>75th percentile of each) on the basis of dichotomous cut-points used in the Cardiovascular Health Study and the Multi-Ethnic Study of Atherosclerosis (31–33). Logistic regression was used to evaluate these outcomes.

For each outcome, identical models were used to facilitate comparisons. An initial model was adjusted for age and sex. A second model additionally adjusted for lifestyle factors (current smoking, alcohol use, regular physical exercise, and oral estrogen use), and a final model additionally adjusted for traditional CVD risk factors (diabetes, BMI, total cholesterol, triglycerides) and eGFR. On the basis of our prior work, we evaluated for effect modification in the associations of fetuin-A with subclinical CVD by diabetes by evaluating multiplicative interaction terms within the final adjusted models (15,34). p values <0.05 were considered statistically significant for all analyses including interaction terms, and all analyses were performed with STATA (version 11.0 SE, StataCorp LP, College Station, Texas).

**Results**

Among the 1,375-person study sample, the mean age was 70 ± 11 years, and 64% (n = 883) were women. Twelve percent had diabetes, and 30% had moderate chronic kidney disease. The median CAC score was 71 (interquartile range: 0 to 319), and the mean ABI, and common and internal
Table 1  Baseline Characteristics of Community-Living Individuals by Quartiles of Plasma Fetuin-A: The Rancho Bernardo Study

<table>
<thead>
<tr>
<th>Fetuin-A Quartiles, Fetuin-A Range (g/l)</th>
<th>I (&lt;0.45) (n = 342)</th>
<th>II 0.45–0.51 (n = 343)</th>
<th>III 0.52–0.58 (n = 345)</th>
<th>IV ≥0.59 (n = 345)</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic data</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>70 ± 11</td>
<td>73 ± 12</td>
<td>70 ± 11</td>
<td>69 ± 11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male</td>
<td>492 (36%)</td>
<td>138 (40%)</td>
<td>132 (38%)</td>
<td>133 (39%)</td>
<td>89 (26%)</td>
</tr>
<tr>
<td>Lifestyle factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoking</td>
<td>107 (8%)</td>
<td>28 (8%)</td>
<td>30 (9%)</td>
<td>21 (6%)</td>
<td>28 (8%)</td>
</tr>
<tr>
<td>Regular physical exercise</td>
<td>979 (71%)</td>
<td>243 (71%)</td>
<td>261 (76%)</td>
<td>243 (70%)</td>
<td>232 (67%)</td>
</tr>
<tr>
<td>Alcohol ≥3 drinks/week</td>
<td>626 (46%)</td>
<td>178 (52%)</td>
<td>165 (48%)</td>
<td>155 (45%)</td>
<td>128 (37%)</td>
</tr>
<tr>
<td>Health status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral estrogen use†</td>
<td>350 (25%)</td>
<td>53 (26%)</td>
<td>75 (36%)</td>
<td>81 (38%)</td>
<td>141 (55%)</td>
</tr>
<tr>
<td>Fair or poor health status</td>
<td>83 (6%)</td>
<td>27 (8%)</td>
<td>21 (6%)</td>
<td>18 (5%)</td>
<td>17 (5%)</td>
</tr>
<tr>
<td>Any hospital stays in past yr</td>
<td>106 (8%)</td>
<td>25 (8%)</td>
<td>27 (8%)</td>
<td>28 (8%)</td>
<td>26 (8%)</td>
</tr>
<tr>
<td>Comorbidities</td>
<td>1 (0–2)</td>
<td>1 (0–2)</td>
<td>1 (0–2)</td>
<td>1 (0–2)</td>
<td>1 (0–2)</td>
</tr>
<tr>
<td>Medications</td>
<td>1 (1–1)</td>
<td>1 (1–1)</td>
<td>1 (1–1)</td>
<td>1 (1–1)</td>
<td>1 (1–1)</td>
</tr>
<tr>
<td>Cardiovascular risk factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25 ± 4</td>
<td>25 ± 4</td>
<td>26 ± 4</td>
<td>26 ± 4</td>
<td>26 ± 4</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>135 ± 22</td>
<td>134 ± 21</td>
<td>134 ± 22</td>
<td>135 ± 24</td>
<td>135 ± 21</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>76 ± 9</td>
<td>75 ± 10</td>
<td>76 ± 9</td>
<td>77 ± 9</td>
<td>77 ± 9</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>211 ± 35</td>
<td>205 ± 34</td>
<td>209 ± 36</td>
<td>212 ± 35</td>
<td>217 ± 36</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>128 ± 32</td>
<td>122 ± 32</td>
<td>128 ± 32</td>
<td>130 ± 32</td>
<td>130 ± 31</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>59 ± 17</td>
<td>61 ± 17</td>
<td>59 ± 16</td>
<td>58 ± 17</td>
<td>59 ± 18</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>103 (73–146)</td>
<td>90 (67–121)</td>
<td>96 (67–139)</td>
<td>110 (76–156)</td>
<td>123 (85–187)</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>68 ± 16</td>
<td>68 ± 16</td>
<td>68 ± 14</td>
<td>69 ± 18</td>
<td>68 ± 14</td>
</tr>
<tr>
<td>eGFR &lt;60 ml/min/1.73 m²</td>
<td>413 (30%)</td>
<td>104 (30%)</td>
<td>105 (31%)</td>
<td>104 (30%)</td>
<td>1060 (29%)</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n (%), or median (interquartile range). *p value across quartiles by analysis of variance, chi-square unless otherwise indicated. †Women only. p value obtained by Kruskal-Wallis test.

cIMT were 1.06 ± 0.15, 0.95 ± 0.21 mm, and 1.47 ± 0.74 mm, respectively.

Table 1 shows the distribution of demographic data, lifestyle factors, and CVD risk factors by fetuin-A quartiles. Compared with the lowest quartile, individuals with higher fetuin-A levels were younger, more frequently female, and consumed less alcohol. Among women, use of oral estrogen was associated with higher fetuin-A levels. Individuals with higher fetuin-A levels and were also more likely to have obesity, diabetes, and an atherogenic lipid profile.

Table 2 shows the association of fetuin-A, both by quartiles and as a continuous predictor variable, with each marker of subclinical CVD evaluated as a continuous outcome measure. Fetuin-A was inversely associated with CAC. This finding remained consistent in models that adjusted for age and sex, life-style factors, traditional CVD risk factors, and kidney function. We observed no association of fetuin-A with ABI in age and sex or lifestyle adjusted models, but a statistically significant direct association emerged in the final adjusted model. This association was of modest strength (each SD higher fetuin-A associated with 0.01 higher ABI units). We observed no association of fetuin-A with common or internal cIMT across the sequence of models.

Table 3 shows the association of fetuin-A with each outcome evaluated with clinically defined cut-points. In this analysis, higher fetuin-A was also associated with less severe CAC. Quartile analysis demonstrated a dose–response relationship between fetuin-A and CAC categories (chi-square p = 0.02) (Fig. 1). Among persons without any CAC, 20% were in the lowest fetuin-A quartile, whereas 28% were in the highest quartile. Conversely, among those with CAC scores >300, 36% were in the lowest fetuin-A quartile, whereas only 13% were in the highest. In contrast, there was no association of fetuin-A with PAD or high internal cIMT defined as categorical variables across the sequence of models (Table 3). Each SD higher fetuin-A level was associated with 27% greater odds of high common cIMT in a model adjusted for lifestyle factors, but this association was attenuated and rendered no longer statistically significant when adjusted for traditional CVD risk factors and kidney function.

Last, we tested for effect modification in the association of fetuin-A with each continuous measure of subclinical CVD by diabetes. In all cases, results were similar (all p values for interaction >0.24).
Discussion

This study evaluated for the first time the associations of plasma fetuin-A levels with subclinical CVD in a population sample of community-dwelling men and women who were free of clinical CVD. We observed that fetuin-A levels were inversely associated with CAC severity, independent of traditional CVD risk factors and kidney function. In contrast, fetuin-A levels were not associated with PAD or cIMT. In vitro studies, knockout studies in rodents, and clinical studies in ESRD populations all suggest that fetuin-A inhibits arterial calcification (2,3,5,35). Here, we report that higher fetuin-A levels are also associated with lower CAC prevalence in community-living individuals, suggesting that fetuin-A might be serving a similar biological function in this setting.

We had hypothesized that lower fetuin-A levels would be associated with greater prevalence of PAD and higher cIMT, and so the inverse association of fetuin-A with CAC but not with PAD and cIMT was unexpected. However, if confirmed, these findings might provide new insights into vascular disease. Fetuin-A inhibits arterial calcium deposition, and any individual atherosclerotic lesion might be characterized by more or less calcium content. Thus, low fetuin-A might predispose to greater calcium deposition but not necessarily to the burden or progression of atherosclerosis per se.

Because low fetuin-A levels were associated with CAC but not with PAD or common or internal IMT, an alternative possibility is that fetuin-A is associated with subclinical coronary atherosclerosis but not with atherosclerosis in other vascular beds. Future studies that evaluate whether low fetuin-A levels are more strongly associated with incident MI as compared with stroke or PAD might provide additional insights for this competing hypothesis.

Individuals with fetuin-A levels in the lowest quartile were most likely to have severe CAC yet less likely to have PAD or common or internal IMT. This study evaluated for the first time the associations of plasma fetuin-A levels with subclinical CVD in a population sample of community-dwelling men and women who were free of clinical CVD. We observed that fetuin-A levels were inversely associated with CAC severity, independent of traditional CVD risk factors and kidney function. In contrast, fetuin-A levels were not associated with PAD or cIMT. In vitro studies, knockout studies in rodents, and clinical studies in ESRD populations all suggest that fetuin-A inhibits arterial calcification (2,3,5,35). Here, we report that higher fetuin-A levels are also associated with lower CAC prevalence in community-living individuals, suggesting that fetuin-A might be serving a similar biological function in this setting.

We had hypothesized that lower fetuin-A levels would be associated with greater prevalence of PAD and higher cIMT, and so the inverse association of fetuin-A with CAC but not with PAD and cIMT was unexpected. However, if confirmed, these findings might provide new insights into vascular disease. Fetuin-A inhibits arterial calcium deposition, and any individual atherosclerotic lesion might be characterized by more or less calcium content. Thus, low fetuin-A might predispose to greater calcium deposition but not necessarily to the burden or progression of atherosclerosis per se.

Because low fetuin-A levels were associated with CAC but not with PAD or common or internal IMT, an alternative possibility is that fetuin-A is associated with subclinical coronary atherosclerosis but not with atherosclerosis in other vascular beds. Future studies that evaluate whether low fetuin-A levels are more strongly associated with incident MI as compared with stroke or PAD might provide additional insights for this competing hypothesis.

Individuals with fetuin-A levels in the lowest quartile were most likely to have severe CAC yet less likely to have PAD or common or internal IMT. This study evaluated for the first time the associations of plasma fetuin-A levels with subclinical CVD in a population sample of community-dwelling men and women who were free of clinical CVD. We observed that fetuin-A levels were inversely associated with CAC severity, independent of traditional CVD risk factors and kidney function. In contrast, fetuin-A levels were not associated with PAD or cIMT. In vitro studies, knockout studies in rodents, and clinical studies in ESRD populations all suggest that fetuin-A inhibits arterial calcification (2,3,5,35). Here, we report that higher fetuin-A levels are also associated with lower CAC prevalence in community-living individuals, suggesting that fetuin-A might be serving a similar biological function in this setting.

We had hypothesized that lower fetuin-A levels would be associated with greater prevalence of PAD and higher cIMT, and so the inverse association of fetuin-A with CAC but not with PAD and cIMT was unexpected. However, if confirmed, these findings might provide new insights into vascular disease. Fetuin-A inhibits arterial calcium deposition, and any individual atherosclerotic lesion might be characterized by more or less calcium content. Thus, low fetuin-A might predispose to greater calcium deposition but not necessarily to the burden or progression of atherosclerosis per se.
low fetuin-A was associated with a greater burden of CAC, higher fetuin-A is also known to induce peripheral insulin resistance in vitro (42–45); and in humans, higher fetuin-A levels are associated with future risk of diabetes (46,47).

Diabetes is a well-established independent CVD risk factor. Higher fetuin-A levels are associated with a greater burden of CAC, and evaluation of this association should be a high priority moving forward. Few studies have evaluated this association with these outcomes was missed. We investigated each measure as a continuous variable to decrease the nature, direction, and strength of the association of fetuin-A with recurrent events or death (50). Future large-scale studies are required to confirm the nature, direction, and strength of the association of fetuin-A with incident CVD events in the general community, and to determine whether such associations might differ by diabetes status.

Study strengths and limitations. Strengths of this study include a well-characterized community-dwelling study sample without prevalent CVD and availability of 3 measures of subclinical CVD. The study also has important limitations. The CAC and IMT measurements were made only once, this study cannot determine the temporal directions of associations. Associations of fetuin-A with PAD and IMT were null, and it is possible that a true association with these outcomes was missed. We investigated each measure as a continuous variable to decrease the

Table 3: Association of Fetuin-A With Categorical Definitions of Subclinical CVD: The Rancho Bernardo Study

<table>
<thead>
<tr>
<th>Fetuin-A Quartiles, Fetuin-A Range (g/l)</th>
<th>CAC severity (0, 1–100, 101–300, &gt;300)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I ≤0.45</td>
<td>II 0.45–0.51</td>
</tr>
<tr>
<td>n with CAC =0/total (%)</td>
<td>69/86</td>
</tr>
<tr>
<td>Age- and sex-adjusted</td>
<td>1.00 (Ref)</td>
</tr>
<tr>
<td>Lifestyle-adjusted*</td>
<td>1.00 (Ref)</td>
</tr>
<tr>
<td>Fully adjusted†</td>
<td>1.00 (Ref)</td>
</tr>
<tr>
<td>PAD (ABI &lt;0.90)</td>
<td>n with PAD/total (%)</td>
</tr>
<tr>
<td>Age- and sex-adjusted</td>
<td>1.00 (Ref)</td>
</tr>
<tr>
<td>Lifestyle-adjusted*</td>
<td>1.00 (Ref)</td>
</tr>
<tr>
<td>Fully adjusted†</td>
<td>1.00 (Ref)</td>
</tr>
<tr>
<td>Common cIMT (&gt;75th percentile vs. lower)</td>
<td>n with high common cIMT/total (%)</td>
</tr>
<tr>
<td>Age- and sex-adjusted</td>
<td>1.00 (Ref)</td>
</tr>
<tr>
<td>Lifestyle-adjusted*</td>
<td>1.00 (Ref)</td>
</tr>
<tr>
<td>Fully adjusted†</td>
<td>1.00 (Ref)</td>
</tr>
<tr>
<td>Internal cIMT (&gt;75th percentile vs. lower)</td>
<td>n with high internal cIMT/total (%)</td>
</tr>
<tr>
<td>Age- and sex-adjusted</td>
<td>1.00 (Ref)</td>
</tr>
<tr>
<td>Lifestyle-adjusted*</td>
<td>1.00 (Ref)</td>
</tr>
<tr>
<td>Fully adjusted†</td>
<td>1.00 (Ref)</td>
</tr>
</tbody>
</table>

Values are odds ratio (95% confidence interval), p value. *Adjusted for age, sex, current smoking, alcohol use, regular physical exercise, and oral estrogen use. †Adjusted for lifestyle model (+) plus diabetes, body mass index, total cholesterol, triglycerides, and estimated glomerular filtration rate.

PAD = peripheral arterial disease; other abbreviations as in Table 2.

Figure 1: CAC Severity by Fetuin-A Quartiles: The Rancho Bernardo Study

Distribution of coronary artery calcification (CAC) scores by fetuin-A quartiles among 382 community-living individuals without clinically apparent cardiovascular disease: The Rancho Bernardo Study.
chance of type 2 errors. Nonetheless, results should be interpreted within the context of the 95% confidence intervals, which suggest that any missed association would likely be modest, at best. Measurements of other novel proteins that might inhibit arterial calcification were not available concurrently with fetuin-A. Participants were predominantly older and Caucasian. Results might not generalize to other settings.

Conclusions

In community-living individuals free of clinically apparent CVD, low fetuin-A levels are independently associated with CAC severity but not with PAD or cIMT. Future studies are required to determine whether blood measurements of fetuin-A are useful to assess systemic burden of arterial calcification and to determine whether fetuin-A levels might identify risk of incident CVD events in community-dwelling individuals.

Reprint requests and correspondence: Dr. Joachim H. Ix, Division of Nephrology and Hypertension, Department of Medicine, University of California-San Diego, San Diego Veterans Administration Healthcare System, 3350 La Jolla Village Drive, Mail code 111-H, San Diego, California 92161. E-mail: jix@ucsd.edu.

REFERENCES


Key Words: cardiovascular disease • coronary artery calcification • fetuin-A.