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Serum fetuin-A in nondialyzed patients with diabetic nephropathy: Relationship with coronary artery calcification

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Background. Fetuin-A is the most potent circulating inhibitor of calcium phosphorus precipitation and, possibly, an important mediator of insulin resistance.

Methods. In order to determine the role of fetuin-A in the high coronary artery calcification (CAC) burden seen in nondialyzed individuals with diabetic nephropathy (DN), post-hoc analyses of data collected from a cross-sectional study of 88 patients with type 2 diabetes mellitus was done [age, 40–65 years; normoalbuminuria, $N = 30$ (Latinos); DN, $N = 58$ (Latinos and African Americans)].

Results. The serum levels of fetuin-A were significantly higher among Latinos with DN when compared to either African Americans with DN or Latino diabetics with normoalbuminuria. Upon adjusting the data for race/ethnicity, there was a strong, direct relationship between serum fetuin-A levels and the CAC score ($r = 0.22$, $P = 0.038$) in the study cohort; however, a strong interaction between the nephropathy status and relationship of serum fetuin-A levels with CAC score was present (DN: $r = 0.36$, $P = 0.006$; diabetic controls, $r = 0.0$, $P = 0.98$). Among individuals with DN, the significance of the association persisted even after controlling the data for other predictors of CAC (partial $r = 0.33$, $P = 0.018$). Furthermore, there was a significant direct relationship between serum fetuin-A and serum triglycerides (partial $r = 0.27$, $P = 0.01$) and albumin (partial $r = 0.30$, $P = 0.005$), and an inverse relationship with glomerular filtration rate ($r = -0.24$, $P = 0.03$).

Conclusion. This first study in early stages of diabetic chronic kidney disease shows that the role of serum fetuin-A may be far more complex than previously described. During predialysis stage of DN, there is a direct relationship between serum fetuin-A levels and CAC score. The reasons for this association in the presence of nephropathy are unclear, but may be secondary to proatherogenic insulin resistance.

In the general population, vascular calcification is invariably localized to intimal atherosclerotic plaques, and the coronary artery calcification (CAC) score, as ascertained by electron-beam computed tomography (EBCT), reliably predicts plaque burden [1–4]. The intimal calcification associated with atherosclerosis is an active, cell-mediated process, and is promoted by a variety of stimuli, such as oxidized low-density lipoprotein [5, 6]. Not only is CAC present in most individuals with chronic kidney disease (CKD), it is often substantially more severe than seen among the general population or normoalbuminuric diabetics, respectively [7–11]. Among individuals with diabetes mellitus or those with CKD, vascular calcification occurs both in the intima (in association with atherosclerosis) and the media; the relative contribution of intimal and medial calcification to the composite CAC score on EBCT remains unclear. Nevertheless, recent data suggest that medial calcification [(either in diabetes mellitus or end-stage renal disease (ESRD)] is also an active cell-mediated process, involving processes similar to those that lead to intimal calcification [12, 13].

While little is known about the mechanisms that contribute to the high calcification burden seen in the relatively early stages of DN, there is a greater understanding of some of the factors involved in the setting of ESRD. Thus, several observational studies suggest that disordered mineral metabolism (e.g., elevated serum calcium, phosphorus, or parathyroid hormone levels) or its treatment (e.g., calcium-based phosphate binders)

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may play an important role in the high severity and rapid progression of vascular and valvular calcification [9, 13–18]. However, it appears that disordered mineral metabolism is either not sufficient or not the only mechanism that contributes to the arterial calcification seen in ESRD. Several novel factors such as osteoprotegrin, α2-Heremans-Schmid protein (fetuin-A), or matrix Gla protein, are being studied in this context [17, 19, 20].

Fetuin-A (α2-Heremans Schmid glycoprotein) is synthesized in the liver and plays an important role in osteogenesis. Recent experimental evidence shows that fetuin-A may be an important mediator of insulin resistance, is a negative acute-phase reactant, participates in the recovery from acute inflammation, and is a potent circulating inhibitor of the precipitation of calcium and phosphorus [21–23]. Fetuin-A levels are significantly lower among individuals with ESRD, and consistent with its status as a negative acute-phase reactant, there is a significant, inverse association between serum fetuin-A levels and mortality [23]. Several lines of evidence suggest that a deficiency of fetuin-A may contribute to the vascular calcification seen in patients with ESRD. First, extensive vascular calcification is noted in fetuin-A knockout mice in the presence of a diet rich in mineral and vitamin D [20]. Second, the serum levels of fetuin-A are significantly lower among patients with calciphylaxis than among other patients with ESRD [20]. Third, the failure of uremic human plasma to inhibit the precipitation of calcium and phosphorus is corrected by the addition of fetuin-A [23]. There is virtually no data regarding the relationship of serum fetuin-A to the vascular calcification burden that is apparent even in early stages of DN.

We undertook this post-hoc analysis of the data from a cross-sectional study of CAC in nondialyzed patients with type 2 diabetes mellitus to determine the relationship of serum fetuin-A levels with vascular calcification, as ascertained by the CAC score on EBCT.

METHODS

Patient selection and data collection

This is a cross-sectional study of CAC in a cohort of middle-aged, nondialyzed individuals with type 2 diabetes mellitus. Three groups of 30 individuals between the ages of 40 and 65 years were studied ($N = 90$): Latinos with diabetes mellitus and normoalbuminuria ("diabetic controls"), Latinos with DN, and African Americans with DN. The details regarding this study cohort have been previously described [11]. Stored sera (−80°C) were available for the measurement of serum fetuin-A in all but two African Americans with DN ($N = 88$). In this study, DN was deemed to be present using the criteria established by the NIDDK-sponsored Family Investigation of Nephropathy in Diabetes (FIND) study, and was defined as the presence of one of the following: (*1*) renal biopsy evidence of DN with overt proteinuria (urine protein >0.5 g/day or urine protein/creatinine ratio >0.5); (*2*) diabetes duration ≥10 years and urine protein excretion >2.5 g/day or protein creatinine ratio >2.5; or (*3*) diabetes duration ≥5 years, presence of diabetic retinopathy, and urine protein excretion >1.0 g/day or protein creatinine ratio >1.0. The highest documented urine protein excretion in the patient's medical chart was used to determine patient eligibility.

All eligible patients who consented to participate in the study were scheduled for an outpatient clinic visit at the General Clinical Research Center located at the Los Angeles Biomedical Institute at Harbor-UCLA Medical Center. All the data thus collected were used to determine the prevalence and/or severity of traditional (nonmodifiable: age, gender, family history of premature cardiovascular disease; modifiable: hypertension, dyslipidemia, current smoking, and obesity), renal-related [serum creatinine, estimated glomerular filtration rate (GFR), serum calcium, phosphorus, intact parathyroid hormone (iPTH), and 1,25 di-hydroxy vitamin D levels] and diabetes-related [glycosylated hemoglobin (HbA1c) and duration of type 2 diabetes mellitus] risk factors. Clinical evidence of coronary artery disease (CAD) was defined as the presence of one of the following: angina on the Rose questionnaire, history of myocardial infarction, or previous revascularization. GFR was estimated using the abbreviated Modification of Diet in Renal Disease (MDRD) equation [GFR (mL/min/1.73m^2) = 186 × (age, years)$^{-0.203}$ × (serum creatinine, mg/dL)$^{-1.154}$ × 0.742, if female, × 1.21, if African American] [24, 25]. Serum phosphorus, iPTH, and 1,25 dihydroxy vitamin D levels were measured on the day of the study visit only after the first nine patients had been enrolled; thus, these data were available for 24 Latinos with DN, 25 African Americans with DN, and 30 diabetic control patients. Of note, none of the patients enrolled in the study were being treated with phosphate binders, vitamin D, or its analogs at the time of the study visit.

Serum fetuin-A levels were measured on serum samples that had been stored at −80°C. The measurements were made using an indirect enzyme-linked immunosorbent assay (ELISA) technique using polyclonal antihuman fetuin-A antiserum, secondary peroxidase-labeled antibody, and the chromogenic substrate ABTS (2,2-azinobis-3[ethylbenz-thiazoline-6-sulphonic acid]; Roche, Mannheim, Germany) as described previously [23]. Briefly, serum was prediluted 10,000-fold and coated (100μL) onto microtiter plates (MaxiSorp, Nunc, Wiesbaden, Germany) in a 2-fold dilution series. After incubation with the above antibodies, extinction was measured with an automated plate reader at 405 nm (MR5000; Dynatech, Chantilly, VA, USA). Results were compared with an appropriate standard curve of human fetuin-A. All samples were measured in parallel and in

duplicates. Intra-assay and interassay coefficients of variations were below 10%.

Electron beam computed tomography

EBCT studies were performed with a C-150 XL Ultrafast computed tomographic scanner (Imatron, South San Francisco, CA, USA). The protocol used for data acquisition has been described previously [26]. In order to minimize interscan variability, particularly at low scores, scans were acquired at end-systole using electrocardiographic triggering [27]. The scans of all individuals were retrieved, and the CAC was scored by a single, highly experienced technician, using both the Agatston as well as volumetric method [28, 29]. The Agatston and volumetric scores were highly correlated in each of the three groups studied ($r = 0.99$). Since most of the published data are based upon Agatston scores, only those data are reported.

Statistical analyses

Data are expressed as mean along with 95% confidence interval (CI) for the mean; geometric means are reported for data that are not normally distributed. The mean fetuin-A levels were compared among the three subgroups in the cohort (diabetic controls, Latino DN, and African American DN) using analysis of variance (ANOVA). The CAC score was log transformed as log (CAC score+1) to test these associations. Partial correlation coefficient was computed to test the association of serum fetuin-A levels with CAC score, after controlling the data for race/ethnicity and nephropathy status. Since a significant interaction for the fetuin-A-CAC association with nephropathy status was identified, the correlation of serum fetuin-A with CAC score was tested in each of the three subgroups. Finally, among individuals with DN, partial correlation coefficient between serum fetuin-A levels and CAC score were computed after controlling the data for five predictors that were identified in our previous study to be related to the CAC score in this cohort (age, gender, history of CAD, number of antihypertensive medications, and duration of diabetes mellitus) [11].

Exploratory analyses were performed to test the association of the following variables with serum fetuin-A levels: traditional cardiovascular risk factors [age, gender, measures of hypertension (presence/absence of hypertension, number of antihypertensive medications, systolic and diastolic blood pressure), dyslipidemia (presence/absence of dyslipidemia, serum total, high-density lipoprotein, and low-density lipoprotein cholesterol and serum triglycerides), obesity (BMI), family history of premature cardiovascular disease, and current smoking], history of manifest CAD, diabetes-related risk factors (HbA1c, duration of diabetes), and renal-related risk factors (serum creatinine, Ca, P, iPTH, and 1,25 dihydroxy vitamin D, creatinine, and GFR). The data on serum low-density lipoprotein and high-density lipoprotein cholesterol, serum triglycerides, creatinine, phosphorus, and iPTH and GFR were log-transformed to achieve approximate normal distributions. Since mean fetuin-A levels differed among the three subgroups, these associations were examined in each of the subgroups separately. Pearson correlations and *t* tests, respectively, were used to examine associations between fetuin-A and a continuous or categorical subject characteristic. Partial correlations were then used to identify characteristics independently associated with fetuin-A in each subgroup, after adjusting for other characteristics. Additionally, characteristics with (possibly nonsignificant) associations with fetuin-A in the same direction for all three subgroups were examined in the entire cohort with partial correlations controlling for subgroup. Such characteristics that were significantly independently associated with fetuin-A, after adjusting for other characteristics, were identified. These two sets of identified independently associated characteristics—those significant in a particular subgroup or those in the entire cohort and consistent in all subgroups—were considered as potential associated characteristics. To determine if the relationships thus determined were independent of other correlations the analyses were repeated after adjusting the data for such factors.

All statistical analyses were performed using SAS, version 8.2 (SAS Institute, Cary, NC, USA) and SPSS, version 11.5 (SPSS, Inc., Chicago, IL, USA) software. A significance level of 0.05 was used for all analyses. No formal correction was made for multiple statistical tests.

RESULTS

Patient characteristics

The key clinical characteristics of the 88 patients (42 men) are summarized in Table 1. Among individuals with DN, 15 (25%) had clinical evidence of CAD (6 Latinos, 9 African Americans)—angina, 6; history of myocardial infarction, 9; and previous revascularization, 5. Among diabetic control patients, 7 (23%) individuals had clinical CAD—angina, 4; history of myocardial infarction, 5; and previous revascularization, 3.

The serum fetuin-A levels among Latino individuals with DN (0.80 mg/mL; 95% CI: 0.72-0.88) were significantly higher than among either African Americans with DN (0.64 mg/mL; 95% CI: 0.58-0.71, $P = 0.003$) or Latino diabetic control patients (0.63 mg/mL; 95% CI: 0.56-0.71, $P = 0.002$) (Table 1). Furthermore, despite a similar severity of renal failure, Latinos with DN had significantly higher serum phosphorus and serum triglycerides than the African Americans (Table 1). The significance of difference in serum fetuin-A levels between Latinos and

Table 1. Clinical and laboratory characteristics of the study cohort

	Diabetic control patients	Diabetic nephropathy	
	Latinos ($N = 30$)	Latinos ($N = 30$)	African Americans ($N = 28$)
Age *years*	54 (51, 56)	55 (52, 57)	56 (53, 59)
Gender *M/F*	13/17	14/16	15/13
Urinary protein/albumin excretion			
24-hour protein *g/day*		7.0 (5.3, 8.7)	7.4 (5.4, 9.4)
Albumin-creatinine ratio *mg/mg*	0.012 (0.005, 0.20)		
Serum fetuin-A *mg/mL* [b,e]	0.63 (0.56, 0.71)	0.80 (0.72, 0.88)	0.64 (05.8, 0.71)
Hypertension			
Prevalence% [c]	73%	97%	96%
Systolic arterial pressure *mm Hg* [a]	140 (132, 148)	159 (148, 170)	158 (148, 168)
Antihypertensive medications, *number* [c]	1.2 (0.7, 1.6)	2.9 (2.4, 3.4)	3.1 (2.6, 3.5)
Dyslipidemia			
Prevalence%	80%	87%	86%
LDL cholesterol *mg/dL*[d]	110 (94, 128)	116 (99, 135)	108 (91, 126)
HDL cholesterol *mg/dL*[d]	41 (36, 46)	42 (37, 46)	40 (36, 46)
Triglycerides *mg/dL*[d]	150 (116, 193)	195 (142, 268)	124 (100, 154)
Body mass index kg/m^2	31.6 (29.1, 34.2)	31.3 (28.4, 34.2)	31.0 (28.0, 34.0)
Renal function			
Serum creatinine *mg/dL*[c,d]	0.7 (0.6, 0.8)	2.1 (1.6, 2.8)	2.5 (2.0, 3.2)
Estimated GFR $mL/min/1.73m^2$[c,d]	104 (94, 114)	30 (23, 39)	30 (23, 40)
Divalent ion metabolism			
Serum calcium *mg/dL*	9.6 (9.5, 9.7)	9.6 (9.5, 9.8)	9.7 (9.4, 9.9)
Serum phosphorus *mg/dL*[c,e]	3.6 (3.5, 3.8)	4.7 (4.2, 5.1)	3.9 (3.5, 4.3)
Serum parathyroid hormone *pg/mL*[a,c]	44 (39, 50)	106 (72, 155)	164 (106, 255)
Serum 1,25 vitamin D *pg/mL*[c]	49 (42, 55)	34 (29, 39)	33 (28, 37)
Serum albumin *g/dL*[c]	3.7 (3.6, 3.8)	3.2 (3.0, 3.3)	3.1 (2.9, 3.4)
Diabetes-related factors			
Glycosylated hemoglobin %	8.6 (8.0, 9.3)	7.8 (6.8, 8.8)	7.9 (7.0, 8.8)
Duration of diabetes mellitus *years*	15.9 (12, 17)	16 (14, 18)	19 (15, 22)
Coronary artery calcification			
Prevalence%[c]	63%	93%	96%
Agatston Score[c,d]	11 (4, 28)	56 (22, 140)	38 (15, 95)

Data are expressed as mean with 95% confidence intervals; ageometric means are reported for variables that are not normally distributed.
Latino diabetic control patients vs. Latinos with diabetic nephropathy: [a]$P \le 0.05$; [b]$P \le 0.01$; [c]$P \le 0.001$.
Latino vs. African Americans with diabetic nephropathy: [d]$P \le 0.05$; [e]$P \le 0.01$.

African Americans with DN persisted, even after individually adjusting the data for either of these two variables.

Latinos with DN had significantly higher serum creatinine, phosphorus, and iPTH levels, and lower GFR, serum albumin, and 1,25 dihydroxy vitamin D levels when compared to diabetic control patients (Table 1). The significance of difference in serum fetuin-A levels between Latinos with and without nephropathy persisted even after controlling for each of the individual characteristics.

Serum fetuin-A and CAC

CAC was significantly more prevalent and severe among individuals with DN than among diabetic control patients (Table 1). Upon controlling the data for nephropathy and ethnicity, there was a significant relationship between CAC score and serum fetuin-A levels ($r = 0.22$, $P = 0.038$); however, there was a significant interaction of the CAC score-serum fetuin-A relationship with the nephropathy status. Thus, while there was a positive relationship between the CAC score and serum fetuin-A levels among each of the two subgroups with DN (Latinos with DN, $r = 0.26$, $P = 0.17$, and African Americans with DN, $r = 0.51$, $P = 0.006$), no such correlation was demonstrable among Latino diabetic control patients ($r = 0.0$, $P = 0.98$) (Fig. 1). Upon adjusting the data for race/ethnicity, there was a significant correlation between serum-fetuin-A levels and CAC (partial correlation = 0.36, $P = 0.006$) among individuals with DN. The five factors previously identified as significantly associated with CAC in this population (age, gender, history of CAD, number of antihypertensive medications, and duration of diabetes) [11] remained so even after adjusting the data for serum fetuin-A levels. Furthermore, among individuals with DN, the relationship between serum fetuin-A levels and CAC score remained significant (partial $r = 0.33$, $P = 0.018$) even after controlling the data for these five variables (age, gender, history of CAD, number of antihypertensive medications, and duration of diabetes mellitus).

Associations between serum fetuin-A and other subject characteristics

Because the mean serum fetuin-A levels were significantly greater for the Latino DN subgroup than for

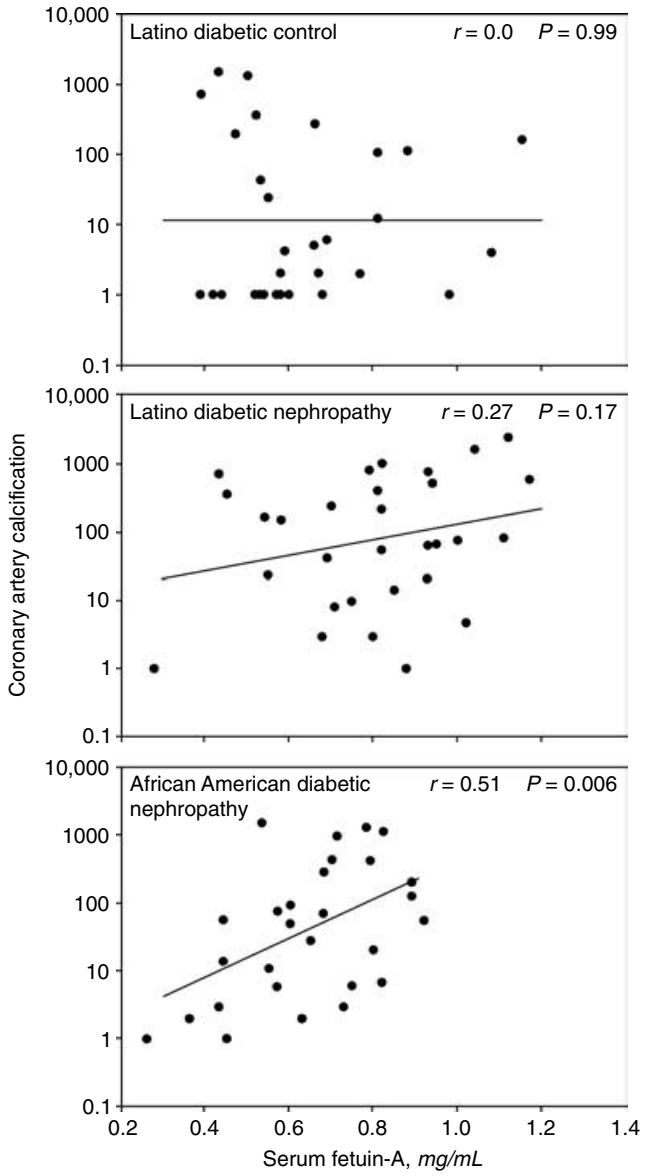

Fig. 1. Scatter plot showing the relationship between serum fetuin-A levels and coronary artery calcification score. Among Latino diabetic control patients, there was no significant relationship between serum fetuin-A and coronary artery calcification score ($r = 0.0$, $P = 0.98$). There was a direct relationship between serum fetuin-A levels and CAC between the Latino ($r = 0.27$, $P = 0.17$) and African Americans ($r = 0.51$, $P = 0.006$) with DN. Upon adjusting the data for race/ethnicity, there was a significant, direct relationship between serum fetuin-A levels and coronary artery calcification score (partial $r = 0.36$, $P = 0.006$).

Latino diabetic control patients and African Americans with DN, associations between serum fetuin-A and other subject characteristics were examined separately in the three subgroups (Table 2). Serum triglycerides and glomerular filtration rate were nonsignificantly positively correlated with serum fetuin-A in all three subgroups, and this consistency over subgroups provided independent significant correlation (partial r: triglycerides, 0.27, $P = 0.01$, and GFR, 0.22, $P = 0.04$; Table 2) in the entire cohort after controlling for the subgroups. There was a significant association between serum albumin and fetuin-A levels in Latinos with and without nephropathy (Latinos with DN, $r = 0.53$; $P = 0.003$) and Latino diabetic control patients ($r = 0.36$, $P = 0.05$); no significant association was detectable in African Americans ($r = 0.13$, $P = 0.52$) (Table 2). The significant association between serum albumin and fetuin-A persisted even when the data were controlled for GFR. On the other hand, the correlation between fetuin-A and GFR was reduced to a nonsignificant level, after adjusting the data for serum albumin.

There was an inverse association between systolic blood pressure and serum fetuin-A levels in each of the three subgroups—the relationship was significant only among the African Americans ($r = -0.52$, $P = 0.005$), but yielded a significant relationship for the entire study cohort (partial $r = -0.22$, $P = 0.039$) (Table 2). Since systolic blood pressure and GFR were significantly related, the relationship between systolic blood pressure and serum fetuin-A was reanalyzed after controlling for GFR. This reduced the association to a nonsignificant level. Among Latinos with DN, both HbA_{1C} ($r = 0.43$) and 1,25 dihydroxy vitamin D ($r = 0.41$) were individually significantly correlated with serum fetuin-A. However, this association was reduced to a nonsignificant level after adjusting the data for serum albumin and duration of diabetes. Moreover, the associations of HbA1c and serum 1,25 dihydroxy vitamin D were not discernible in other subgroups.

DISCUSSION

This study is the first evaluation of the relationship of serum fetuin-A in a cohort of diabetics with and without nephropathy. Data derived from animal models and patients undergoing maintenance dialysis have suggested that CKD is associated with significantly lower serum fetuin levels and this, in turn, may contribute to the progression of vascular and soft tissue calcification. This study demonstrates that the relationship between serum fetuin-A levels and CKD and its contribution to vascular calcification in the setting of chronic kidney disease is far more complex than previously thought.

Our study shows that Latinos with diabetic nephropathy not undergoing maintenance dialysis have significantly higher serum fetuin-A levels when compared to diabetic control patients (in contrast to significantly lower levels among patients with ESRD). Not withstanding the relatively higher levels among individuals with DN, the serum fetuin-A levels seem to decline with decrease in GFR. Since our study did not include African American diabetic control patients, it is unclear if these differences are seen in individuals belonging to racial/ethnic groups other than the Latinos. However, hepatic synthesis of

Table 2. Summary of significant correlations for serum fetuin-A levels in patient subgroups and pooled data, as appropriate

	Diabetic control patients	Diabetic nephropathy		Pooled data
	Latinos ($N = 30$)	Latinos ($N = 30$)	African Americans ($N = 28$)	$N = 88$
Serum fetuin-A *mg/mL*[a]	0.63 ± 0.19	0.80 ± 0.21	0.64 ± 0.17	
Serum triglycerides *mg/dL*				
Correlation, r	0.28	0.36	0.12	0.27
P value	0.14	0.06	0.54	0.01
GFR *mL/min/1.73m*^{2}				
Correlation, r	0.24	0.35	0.07	0.22
P value	0.20	0.06	0.73	0.04
Serum albumin *g/dL*				
Correlation, r	0.36	0.53	0.13	0.30
P value	0.05	0.003	0.53	0.005
Systolic blood pressure *mm Hg*				
Correlation, r	−0.02	−0.18	−0.52	−0.22
P value	0.94	0.34	0.005	0.04
Diabetes duration *years*				
Correlation, r	−0.06	0.45	−0.04	
P value	0.77	0.01	0.83	

[a]Mean ± standard deviation.

several proteins (like lipoprotein(a)) is increased in patients with proteinuric renal diseases; it is possible that the same mechanisms may lead to relatively higher serum fetuin-A levels among individuals with DN. Furthermore, our data showing the direct relationship between serum fetuin-A levels and GFR are consistent with the previous reports of low serum fetuin-A levels among individuals with ESRD. Putting our data in context to previous reports suggests that serum fetuin-A levels probably decline to subnormal levels only late during the course of progression of diabetic CKD (i.e., after approaching ESRD).

Another unexpected finding in this biracial cohort of diabetics with DN was a direct relationship between serum fetuin-A levels and the CAC score. This relationship is the opposite of what would be expected from the animal studies or the studies in humans with ESRD [23]. However, these differences may indicate a different pathogenetic role of fetuin-A in different stages of CKD. With respect to vascular calcification, there appear to be two relevant effects of fetuin-A: (*1*) it prevents the precipitation of calcium and phosphorus; and (*2*) it is an important mediator of insulin resistance. Experimental evidence suggests that fetuin-A inhibits the insulin receptor tyrosine kinase and, thus, prevents insulin-mediated autophosphorylation of the insulin receptor [30]. This, in turn, would result in insulin resistance [31]. The human fetuin-A gene is localized to chromosome 3q27, a region that has been linked to susceptibility for type 2 diabetes as well as the metabolic syndrome [32, 33]. Furthermore, fetuin-A knockout mice demonstrate improved insulin sensitivity and resistance to weight gain and fat accumulation when fed a high-fat diet [22]. Finally, serum fetuin-A levels are significantly higher among women with gestational diabetes mellitus when compared to otherwise healthy pregnant women or nonpregnant control patients [34]. Our observation of a direct association between serum fetuin-A and triglyceride levels is consistent with a possible pathogenetic role for fetuin-A in exacerbating the insulin resistance associated with diabetic chronic kidney disease.

Thus, it is possible that in the presence of nephropathy, fetuin-A exacerbates insulin resistance and, thus, worsens the proatherogenic milieu among individuals with type 2 diabetes mellitus. This may, in turn, promote atherosclerosis and lead to intimal calcification of the coronary arteries. With progressive loss of GFR, serum levels of fetuin-A seem to decline. In patients with stage 4 or 5 CKD and hyperphosphatemia, this fetuin-A deficiency may lead to the precipitation of calcium and phosphorus and worsening of coronary calcification.

It is possible, however, that rather than fetuin-A leading to greater CAC, the converse may be true. In this context, an alternative explanation of the positive relationship between serum fetuin-A and CAC scores could be that hepatic fetuin-A up-regulation may be a feedback defense mechanism protecting against excessive vascular calcifications in early stages of diabetic nephropathy. This view is supported by immunohistochemical findings showing strongly increased fetuin-A deposition, but not expression, in areas of vascular calcification [abstract; Moe et al, *J Am Soc Nephrol* 14:692A, 2003]. While in ESRD patients CAC scores of >400 are frequently found, the mean scores in our cohort were 11 (diabetic control patients), 56 (Latinos with DN), and 38 (AA with DN), respectively. The more extreme calcification burden in ESRD patients may thus exhaust the fetuin-A system, causing deficiency of the circulating fetuin-A, and consequently, starting a vicious cycle of even more progressive extraosseous calcification. In contrast, fetuin-A levels

may be a marker of a physiologic attempt to counteract early vascular calcification. If this interpretation was correct, future research identifying circulating fetuin-A regulatory factors and an understanding of the hepatocellular capacity of hepatic fetuin-A synthesis might implicate new anti-atherogenic therapies.

Our data do not permit us to provide explanations for some of the other findings reported in this study. First, because the direct relationship between fetuin-A and CAC was restricted to individuals with DN, it appears that factors that covary with the appearance of proteinuria may facilitate the actions or feedback mechanisms associated with fetuin-A. Second, African Americans with DN had significantly lower fetuin-A levels than Latinos. Furthermore, African Americans with DN had significantly lower serum triglyceride levels than Latinos—a finding that has been well documented by other investigators [35, 36]. The observation of lower fetuin-A levels in African Americans warrants further investigation in larger cohorts because lower CAC scores were previously and consistently reported in this racial/ethnic group [37, 38]. In this study, the trend toward lower CAC scores among African Americans with DN did not reach statistical significance, probably because of relatively small numbers of patients in each group. Finally, our study shows a direct relationship between serum fetuin-A and albumin levels. Both fetuin-A and albumin are negative acute-phase reactants [23]. It is likely, then, that the direct association between the levels of these two proteins is confounded by microinflammation. However, this possibility was not tested directly in our study. Larger studies enrolling multiethnic populations are needed to further corroborate and clarify all the findings listed above.

There are several limitations to this study. First, no formal assessments of the degree of insulin resistance were made. However, the presence of CKD is known to be associated with an increase in insulin resistance at the postreceptor level, similar to what has been described as an effect of fetuin-A. Second, there was no control group of nondiabetic individuals with/without renal disease. Third, an indirect estimation of GFR was used to determine renal function. Some recent studies have raised the concern that the relationship of cardiovascular risk factors (e.g., age, body weight, and body mass index) and renal function may be affected by the method selected to estimate renal function [39]. However, the equation used in this study has been validated in a biracial population, and the direction of change reported in this study is consistent with significantly lower serum fetuin-A levels in patients with ESRD [23, 24, 40]. Fourth, we did not make any assessments of the functional activity of fetuin-A in this cohort. The in vitro studies suggest that only the phosphorylated form of fetuin-A is functionally active, and in normal humans, it is estimated that 20% of the circulating fetuin-A is phosphorylated [30, 41–43]. It is unclear if the presence of diabetes mellitus or CKD modifies the proportion of circulating fetuin-A that is phosphorylated. Furthermore, in the setting of type 2 diabetes, the protein may be hyperglycosylated; this, in turn, may modify the functional activity of fetuin-A. Finally, because these analyses were exploratory in nature, multiple comparisons were made, predisposing to the possibility of false-positive observations. The latter is less likely, given that our findings are consistent with the biologic understanding of fetuin-A. Nevertheless, these observations require confirmation in larger study populations.

CONCLUSION

This study shows that the relationship of serum fetuin-A levels to the presence of CKD and its role in vascular calcification is far more complex than suggested by previous studies. In conjunction with the previously published data, the direct association between serum fetuin-A levels and CAC in nondialyzed patients with DN would suggest that the high fetuin-A levels may be proatherogenic, presumably by inducing and/or enhancing insulin resistance. Thus, the role of fetuin-A in inducing CAC may differ at different stages of chronic kidney disease.

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