Association of Lower Plasma Fetuin-A Levels With Peripheral Arterial Disease in Type 2 Diabetes

Luis H. Eraso, MD[1]
Naeema Ginwala, BA[1]
Atif N. Qasim, MD[1]
Nehal N. Mehta, MD[1]
Rachel Dlugash, BA[1]
Shiv Kapoor, PHD[1]
Stanley Schwartz, MD[2]
Mark Schutta, MD[2]
Nayyar Iqbal, MD[2]
Emile R. Mohler III, MD[1,3]
Muredach P. Reilly, MB[1,2,3]

OBJECTIVE — Fetuin-A is an inhibitor of vascular calcification and a mediator of insulin resistance. This study evaluated the association of plasma fetuin-A and peripheral arterial disease (PAD).

RESEARCH DESIGN AND METHODS — A total of 738 individuals with type 2 diabetes (mean age 58.7 years, 37.1% female) without known cardiovascular or kidney disease were included in this cross-sectional analysis.

RESULTS — Subjects with PAD had a significantly lower fetuin-A (264.3 vs. 293.4 ng/dl, $P < 0.001$). In multivariable analysis, a 1-SD decrease in fetuin-A increased the odds of PAD (odds ratio 1.6, $P = 0.02$). Subgroup analysis revealed an increased odds even in subjects with glomerular filtration rate >80 (odds ratio 1.9, $P = 0.05$) or high-sensitivity C-reactive protein <3 mg/dl (odds ratio 2.7, $P = 0.002$).

CONCLUSIONS — Lower circulating fetuin-A is associated with PAD in type 2 diabetes beyond traditional and novel cardiovascular risk factors. Our findings suggest a potentially unique role for fetuin-A deficiency as a biomarker of PAD in patients with type 2 diabetes.

Diabetes Care **33:408–410, 2010**

Individuals with type 2 diabetes have an increased risk for infra-inguinal arterial involvement and microvascular disease and a higher tendency to develop intimal atherosclerotic calcification as well as medial arterial calcification (1). Despite advances in the understanding of the pathophysiology of atherosclerosis, however, limited data are available on the determinants of the distinct patterns of peripheral arterial disease (PAD) observed in type 2 diabetes.

Based on the relationship of fetuin-A deficiency with peripheral vascular calcification in experimental models (2,3), we hypothesized that low fetuin-A levels in type 2 diabetes are associated with PAD independent of the presence of kidney disease or cardiovascular disease (CVD).

RESEARCH DESIGN AND METHODS

— The Penn Diabetes Heart Study (PDHS) is an ongoing, cross-sectional study of individuals with type 2 diabetes (aged 35–75 years) without clinical evidence of CVD (myocardial infarction, coronary revascularization or angiographic disease, positive stress test, clinical peripheral arterial disease or peripheral arterial revascularization, stroke, or transient ischemic attack). Subjects with type 1 diabetes and creatinine >2.5 mg/dl are excluded. The University of Pennsylvania Institutional Review Board approved the study protocol, and all subjects gave written informed consent (4).

Evaluated parameters

The ankle-brachial index (ABI) was calculated by dividing the average of the ankle pressures by the highest brachial pressure. An ABI <0.9 in either leg was used to define PAD. Participants with an ABI >1.4 ($n = 13$) reflecting noncompressible arteries were excluded (5). Each subject had the estimated glomerular filtration rate (eGFR) calculated based on the abbreviated Modification of Diet in Renal Disease Study equation (6). Plasma levels of fetuin-A (4.3 and 10.2%, respectively, intra-assay and inter-assay coefficient of variation) were measured by enzyme-linked immunosorbent assay (Biovendor Laboratory Medicine, Modrice, Czech Republic). High-sensitivity C-reactive protein (hsCRP) was measured by immunoturbidimetric assay (Wako, Osaka, Japan).

Statistical analysis

Categorical variables were compared with an χ^2 test and/or Fisher's exact test. Student's t test was used for normal continuous variables and the Kruskal-Wallis for nonnormal distributed variables. All nonnormal distributed variables were log-transformed for the analysis. The association between a 1-SD change in fetuin-A and PAD was examined using unadjusted and multivariable adjusted logistic regression models as described in Table 1. A $P < 0.05$ was considered statistically significant. Analyses were performed on Stata 10.0 software (Stata, College Station, TX).

RESULTS — Among 738 subjects in the study sample, PAD prevalence was 5.1%, the mean age was 58.7 ± 9.3 years, 37.1% were female, 63.2% were Caucasians, and 32.1% were African Americans. The median fetuin-A level was 292.4 ng/ml (interquartile range 115.5). Individuals with PAD had a significantly lower fetuin-A level compared with individuals without PAD (269.3 vs. 293.4, $P < 0.001$). PAD subjects were older and

From the [1]Cardiovascular Institute, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania; the [2]Institute of Diabetes Obesity and Metabolism, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania; and the [3]Institute for Translational Medicine and Therapeutics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania.
Corresponding author: Muredach P. Reilly, muredach@mail.med.upenn.edu.
Received 17 August 2009 and accepted 28 October 2009. Published ahead of print at http://care.diabetesjournals.org on 12 November 2009. DOI: 10.2337/dc09-1541.
© 2010 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See http://creativecommons.org/licenses/by-nc-nd/3.0/ for details.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Downloaded from http://ada.silverchair.com/care/article-pdf/33/2/408/605851/zdc00210000408.pdf by guest on 11 April 2024

Table 1—*Association between fetuin-A and PAD*

	Odds of PAD	*P*
Unadjusted	1.6 (1.1–2.3)	0.01
Model 1	1.6 (1.08–2.2)	0.02
Model 2	1.8 (1.1–2.6)	0.007
Model 3	1.6 (1.05–2.5)	0.03

Data are odds ratios (95% CI) and were obtained by logistic regression model. Model 1: adjusted for age, sex, race, and eGFR. Model 2: model 1 + smoking, hypertension, hypercholesterolemia, metabolic syndrome, A1C, Framingham risk score (%), and medications (ACE inhibitors, aspirin, statins, insulin, metformin, sulfonylureas, and thiazolidinediones). Model 3: model 2 + hsCRP.

had a higher proportion of African Americans. No significant differences were noted in other cardiovascular risk factors, metabolic syndrome, kidney function, inflammation, BMI, serum albumin (the only surrogate marker of liver function collected on the PDHS), or medication use. Fetuin-A levels decreased consistently (*P* for trend <0.02) across ABI clinically relevant cut points (<0.7, 0.7–0.9, 0.9–1.1, and 1.1–1.4) (7) (see Supplemental Fig. 1 in the online appendix [available at http://care.diabetesjournals.org/cgi/content/full/dc09-1541/DC1]).

The odds of PAD were significantly increased for each SD decrease in fetuin-A (odds ratio 1.6 [95% CI 1.1–2.3], $P = 0.001$) and the association persisted in incremental models that adjusted fully for age, sex, race, kidney function, cardiovascular risk factors, medication use, and hsCRP (1.6 [1.05–82.5], $P = 0.03$) (Table 1). A similar trend was noted among subjects with eGFR >80 ml · min^{-1} · 1.73 m^{-1} (1.9 [1.0–3.4], $P = 0.05$), suggesting that our findings were not confounded by the presence of moderate kidney disease. Finally, in a fully adjusted subgroup analysis, participants with hsCRP levels <3 mg/dl ($n = 460$) had higher odds of PAD (2.6 [1.4–5.0], $P = 0.002$), whereas subjects with high hsCRP levels (≥3 mg/dl; $n = 234$) did not (0.82 [0.4–1.6], $P = 0.59$).

CONCLUSIONS — Low levels of fetuin-A have been linked to medial arterial calcification and flow limiting aortic stenosis in humans (8,9). In this study, we demonstrate that lower levels of fetuin-A are associated with PAD in subjects with type 2 diabetes. To our knowledge, this is the first study to report such a relationship in the absence of advanced kidney disease or prevalent CVD. Notably, in analysis stratified by the Centers for Disease Control and Prevention/American Heart Association–defined hsCRP risk strata (10), fetuin-A conferred increased odds of PAD in subjects with hsCRP <3 mg/dl and also in participants with interleukin (IL)-6 levels below the median (data not shown). This is consistent with studies reporting an increased CVD risk mortality with fetuin-A deficiency independent of hsCRP and IL-6 (11) and points to a unique role for this negative acute-phase protein as a biomarker of PAD beyond traditional and novel cardiovascular risk factors.

Remarkably, animal models appear to track with our clinical observations. In mice lacking the fetuin-A gene, the aorta was found to be spared of calcification and fibrosis, whereas peripheral vessels in the skin and kidney showed evidence of extensive calcification (2), and the small artery involvement preceded the renal impairment (3). However, in the absence of direct imaging data, we are unable to define the type of vascular phenotype (intimal calcification or medial calcification) that account for the observed association. An ABI of <0.9 is 95% sensitive and 99% specific for a stenotic lesion (>50%) (12). Therefore, we assume that some degree of eccentric atherosclerotic calcification contributes to the association observed while acknowledging that multiple types of vascular calcification may coexist in type 2 diabetes (13).

Nonalcoholic liver disease and other phenotypes related to insulin resistance, including type 2 diabetes (14), are associated with higher levels of fetuin-A (15). We controlled for most potential confounding factors and found no attenuation of the inverse association of fetuin-A with PAD. In particular, because of this inverse relationship, a significant confounding effect was not expected by any condition associated with high fetuin-A levels. Finally, our study is limited by cross-sectional design, which limits causal inferences.

In summary, low plasma fetuin-A levels are associated with PAD in type 2 diabetes independent of traditional and contemporary risk factors. Our findings suggest a unique role for fetuin-A deficiency as a biomarker of PAD in the setting of type 2 diabetes.

Acknowledgments— This work was supported by a Clinical and Translational Science Award (UL1-RR024134), a Diabetes and Endocrinology Research Center Award (P20 DK019525), and a RO1 HL-073278 and a P50 HL-083799-SCCOR (to M.P.R.). L.H.E. was supported by a National Heart, Lung, and Blood Institute K12 Career Development Award (K12-HL083772).

No potential conflicts of interest relevant to this article were reported.

References

1. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. JAMA 2002; 287:2570–2581
2. Merx MW, Schafer C, Westenfeld R, Brandenburg V, Hidajat S, Weber C, Ketteler M, Jahnen-Dechent W. Myocardial stiffness, cardiac remodeling, and diastolic dysfunction in calcification-prone fetuin-A-deficient mice. J Am Soc Nephrol 2005; 16:3357–3364
3. Schafer C, Heiss A, Schwarz A, Westenfeld R, Ketteler M, Floege J, Muller-Esterl W, Schinke T, Jahnen-Dechent W. 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
4. Reilly MP, Iqbal N, Schutta M, Wolfe ML, Scally M, Localio AR, Rader DJ, Kimmel SE. Plasma leptin levels are associated with coronary atherosclerosis in type 2 diabetes. J Clin Endocrinol Metab 2004;89: 3872–3878
5. Resnick HE, Lindsay RS, McDermott MM, Devereux RB, Jones KL, Fabsitz RR, Howard BV. Relationship of high and low ankle brachial index to all-cause and cardiovascular disease mortality: the Strong Heart Study. Circulation 2004;109:733–739
6. Levey AS, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW, Hogg RJ, Perrone RD, Lau J, Eknoyan G. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Ann Intern Med 2003;139:137–147
7. Criqui MH, Ninomiya JK, Wingard DL, Ji M, Fronek A. Progression of peripheral arterial disease predicts cardiovascular disease morbidity and mortality. J Am Coll Cardiol 2008;52:1736–1742
8. 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
9. Kaden JJ, Reinohl JO, Blesch B, Brueckmann M, Haghi D, Borggrefe M, Schmitz F, Klomfass S, Pillich M, Ortlepp JR. Systemic and local levels of fetuin-A in calcific aortic valve stenosis. Int J Mol Med 2007;20:193–197

Downloaded from http://ada.silverchair.com/care/article-pdf/33/2/408/605851/zdc00210000408.pdf by guest on 11 April 2024

10. Myers GL, Rifai N, Tracy RP, Roberts WL, Alexander RW, Biasucci LM, Catravas JD, Cole TG, Cooper GR, Khan BV, Kimberly MM, Stein EA, Taubert KA, Warnick GR, Waymack PP. CDC/AHA workshop on markers of inflammation and cardiovascular disease: application to clinical and public health practice: report from the laboratory science discussion group. Circulation 2004;110: e545–e549
11. Hermans MMH, Brandenburg V, Ketteler M, Kooman JP, van der Sande FM, Boeschoten EW, Leunissen KML, Krediet RT, Dekker FW. Association of serum fetuin-A levels with mortality in dialysis patients. Kidney Int 2007;72:202–207
12. McDermott MM. Ankle brachial index as a predictor of outcomes in peripheral arterial disease. J Lab Clin Med 1999;133: 33–40
13. Luscher TF, Creager MA, Beckman JA, Cosentino F. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part II. Circulation 2003;108:1655–1661
14. Stefan N, Fritsche A, Weikert C, Boeing H, Joost HG, Haring HU, Schulze MB. Plasma fetuin-A levels and the risk of type 2 diabetes. Diabetes 2008;57: 2762–2767
15. Reinehr T, Roth CL. Fetuin-A and its relation to metabolic syndrome and fatty liver disease in obese children before and after weight loss. J Clin Endocrinol Metab 2008;93:4479–4485

Downloaded from http://ada.silverchair.com/care/article-pdf/33/2/408/605851/zdc00210000408.pdf by guest on 11 April 2024