Fetuin-A Levels Are Increased in Patients With Type 2 Diabetes and Peripheral Arterial Disease

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OBJECTIVE — Low levels of fetuin-A, a systemic calcification inhibitor, are linked to mortality in patients on dialysis. In contrast, elevated fetuin-A is associated with cardiovascular events in non-renal patients. We investigated fetuin-A in patients with type 2 diabetes and peripheral arterial disease (PAD).

RESEARCH DESIGN AND METHODS — We studied fetuin-A in 76 patients with PAD and normal glucose metabolism (NGM-PAD) and in 129 patients with PAD and type 2 diabetes (type 2 diabetes–PAD). Additionally, 40 patients with diabetes without any complications (type 2 diabetes–non-PAD) were examined.

RESULTS — Type 2 diabetes–PAD subjects (399 ± 155 μg/ml) had significantly higher fetuin-A levels than type 2 diabetes–non-PAD subjects (247 ± 42; $P < 0.001$). In NGM-PAD subjects (376 ± 144), fetuin-A was significantly higher than in type 2 diabetes–non-PAD subjects ($P < 0.001$). Type 2 diabetes–PAD patients with mediasclerosis had lower fetuin-A than subjects without ($P < 0.03$). Regression analysis in type 2 diabetes–PAD subjects revealed that glycated A1C ($P < 0.001$) and mediasclerosis ($P = 0.004$) were the strongest predictors of fetuin-A. Multivariate regression revealed that a 1-SD increase in fetuin-A was associated with an odds ratio (OR) of 2.1 (95% CI 1.1–3.3; $P < 0.001$) for the prevalence of PAD and an OR of 1.4 (1.0–1.7, $P = 0.039$) for the prevalence of myocardial infarction.

CONCLUSIONS — In contrast to previous findings, fetuin-A was higher in type 2 diabetes–PAD patients than in type 2 diabetes–non-PAD patients. In NGM-PAD patients, fetuin-A was also higher than in type 2 diabetes–non-PAD patients. In type 2 diabetes–PAD patients, fetuin-A was inversely associated with mediasclerosis—the calcification process pathognomonic for diabetic PAD. This association persisted in multivariate regression, which is in line with the calcification inhibition in coronary heart or renal disease.

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Patients suffering from type 2 diabetes and peripheral artery disease (PAD) (type 2 diabetes–PAD) have a five times higher risk for cardiovascular mortality than patients with one disease alone (1–3). Furthermore, the risk of lower-extremity amputation is higher than in patients without diabetes (3).

Fetuin-A, also known as α2-Schmid Heremans glycoprotein (ASHG), is a potent systemic calcification inhibitor (4). Fetuin-A knockout mice develop severe calcification of various organs (4). In a cross-sectional study, low levels of fetuin-A were associated with cardiovascular mortality in patients on dialysis (5). In addition, low fetuin-A has been linked to vascular calcification (6) and flow-limiting aortic stenosis (7).

Fetuin-A interacts with the insulin receptor tyrosine kinase and induces insulin resistance in rodents (8,9). Stefan et al. (10) demonstrated in a prospective case-cohort study that elevated fetuin-A is an independent risk factor for developing diabetes. Contrariwise to renal (dialysis) patients, several studies showed that high levels of fetuin-A were associated with atherosclerosis and its manifestations in non-renal patients (11–13). Likewise, high levels of fetuin-A were linked to myocardial infarction and ischemic stroke (12). This possible involvement of fetuin-A in the pathogenesis of cardiovascular disease has been confirmed by a recent trans-European cohort study with 2,520 patients (13). Thus, it seems that high levels of fetuin-A are associated with atherosclerosis and its manifestations in non-renal patients.

In contrast to the latter findings, a recent article (14) suggested that fetuin-A levels in a non-dialysis condition are lower in type 2 diabetes–PAD patients ($n = 38$) than in patients with diabetes alone.

However, the role of fetuin-A and its involvement in atherosclerosis seems to be very complex and yet not understood. The situation is even more complex in patients with type 2 diabetes–PAD, who generally suffer from advanced/systemic atherosclerosis (1–3,15). In those high-risk patients, up to 30% show mediasclerosis (2,15). The aim of this study was to investigate fetuin-A levels in patients with type 2 diabetes with or without PAD in comparison with PAD patients with diabetes.

RESEARCH DESIGN AND METHODS

— The study was approved by the institutional ethics committee and complies with the Declaration of Helsinki and Good Clinical Practice. After giving written informed consent, patients were enlisted at the Angiology Outpatients' Division, Department of Medicine II, Medical University and General Hospital of Vienna, and the Diabetes Outpatients' Division, Department of Medicine I, Rudolfstiftung Hospital, Vienna.

In this cross-sectional study, we present 205 patients with PAD. Blood samples were collected after an overnight fast (12 h) from the cubital vein, immediately centrifuged, and deep-frozen at

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Table 1—*Baseline characteristics of different study groups*

	Type 2 diabetes–non-PAD (group 1)	NGM-PAD (group 2)	Type 2 diabetes-PAD (group 3)	*P* (all groups)	*P* (1 vs. 2)	*P* (1 vs. 3)	*P* (2 vs. 3)
n	40	76	129				
Age (years)	61.1 ± 10.0	68.3 ± 10.5	70.8 ± 9.2	<0.001*	<0.001*	<0.001*	0.081
Male sex (%)	21 (53)	42 (55)	93 (72)	0.015*	0.776	0.021*	0.014*
Systolic blood pressure (mmHg)	145 ± 22	139 ± 20	145 ± 23	0.115	0.146	0.894	0.044*
Diastolic blood pressure (mmHg)	90 ± 11	78 ± 14	78 ± 11	<0.001*	<0.001*	0.004*	0.970
BMI (kg/m^2)	31.9 ± 5.4	25.7 ± 3.6	28.6 ± 4.5	<0.001*	<0.001*	<0.001*	<0.001*
eGFR (ml/min/1.73 m^2)	88.0 ± 40.5	71.8 ± 16.5	66.4 ± 19.1	<0.001*	0.032*	0.005*	0.043*
Triglyceride (mmol/l)	2.2 ± 1.4	1.8 ± 0.9	2.0 ± 1.2	0.101	0.084	0.416	0.087
HDL (mmol/l)	1.3 ± 0.4	1.5 ± 0.3	1.3 ± 0.3	0.001*	0.158	0.240	<0.001*
Total cholesterol (mmol/l)	5.1 ± 0.9	5.3 ± 1.2	4.7 ± 1.2	0.002*	0.338	0.108	0.001*
LDL (mmol/l)	2.8 ± 0.8	3.1 ± 1.0	2.6 ± 1.0	0.001*	0.196	0.182	<0.001*
Bilirubin (μmol/l)	11.3 ± 6.8	10.9 ± 3.6	11.6 ± 4.8	0.738	0.828	0.851	0.381
Alkaline phosphatase (U/l)	82.3 ± 29.2	79.2 ± 23.8	85.7 ± 49.3	0.542	0.560	0.711	0.29
Aspartate transaminase (U/l)	27.1 ± 8.9	25.2 ± 6.2	25.7 ± 9.3	0.566	0.283	0.434	0.686
Alanine transaminase (U/l)	27.6 ± 12.5	24.1 ± 10.1	28.0 ± 12.8	0.079	0.129	0.880	0.026
γ-Glutamyl transferase (U/l)	54.5 ± 41.7	41.2 ± 34.8	52.7 ± 100.4	0.554	0.089	0.920	0.341
C-reactive protein (mg/l)	NA	2.6 (1.5, 4.4)	3.2 (1.4, 6.1)	—	—	—	0.092
A1C (relative %)	7.7 ± 1.3	5.7 ± 0.4	6.9 ± 1.1	<0.001*	<0.001*	<0.001*	<0.001*

Data are means ± SD, median (25, 75 percentile), or *n* (%). *$P < 0.05$ is considered statistical significant. NA, not applicable.

−86°C. Similar to the ADA 2009 guidelines (16), patients were classified according to a standardized oral glucose tolerance test as overt type 2 diabetes or normal glucose metabolism (NGM). NGM was defined as having fasting plasma glucose <5.6 mmol/l and a 2-h postload plasma glucose <7.8 mmol/l. The NGM group did not contain patients, with impaired fasting glucose or impaired glucose tolerance.

As manifestations of atherosclerosis, we defined PAD, upper-extremity arterial disease, renal arterial disease, thoracic or abdominal aortic disease, coronary heart disease, cerebrovascular disease (CVD), and associated events such as myocardial infarction or stroke.

PAD was classified after Fontaine by the self-reported pain-free walking distance. Blood pressures to calculate ankle brachial index (ABI) and toe brachial index (TBI) were measured by trained personnel (2,15). Asymptomatic PAD (stage I Fontaine) was defined by ABI <0.9 and/or TBI <0.7. Digital oscillography was performed. In the case of noncomparable wave forms, stress oscillograms (17) were performed to detect occult stenosis. If noncompressible arteries or an ABI >1.4 and typical wave form deviations by oscillography were observed, patients were diagnosed as having Mönckeberg's mediasclerosis (17). Estimated glomerular filtration rate (eGFR) (18) was calculated. Exclusion criteria were PAD Fontaine stage III or IV, type 1 diabetes, serum creatinine >3 mg/dl, dialysis, connective tissue disease, critical illness within the last 6 months, hormone replacement therapy, and known cancer.

As reference cohorts, we enrolled 40 patients with diabetes without any micro- or macrovascular complications (type 2 diabetes–non-PAD) (Rudolfstiftung Hospital). To ensure that the type 2 diabetes–non-PAD group has no clinical evidence of vascular complications, inclusion criteria were as following: duration of type 2 diabetes <5 years, at maximum one oral antidiabetic drug, no insulin intake, A1C ≤8%, creatinine <1.4 and no albuminuria (albuminuria ≤30 mg/day/24-h urine), ABI ≥0.90, and/or TBI ≥0.70. In addition, the patients underwent a stress ergometer test, and a fundal inspection of the eye was performed.

Serum concentrations of fetuin-A were assessed by a commercially available ELISA (BioVendor Laboratory Medicine, Modrice, Czech Republic). Measured intra-assay and interassay variability coefficients were 10.2 and 10.0%, respectively.

All data are presented as means ± SD or medians (25th, 75th percentile). Statistical analyses were performed using SPSS 15.0 (SPSS, Chicago, IL) and included an independent-sample Student *t* test, χ^2 test, ANOVA, Kruskal-Wallis, Mann-Whitney *U* test, and univariate and multivariate, linear, logistic, and multi-nominal regression analysis as appropriate. Confounding in multivariate analysis was identified by a change in β of >10% (19). A two-sided α level <0.05 was considered statistically significant. To investigate differences of fetuin-A in diabetes and PAD independent of age and sex, a cutoff of 69 years of age was used to obtain patient groups that did not differ in age and sex.

RESULTS

Baseline characteristics

Baseline characteristics are presented in Table 1. Patient groups differed for age, BMI, eGFR, lipids, and A1C.Thus, age and sex matching was applied. However, in the matched groups, differences in lipids, BMI, and A1C persisted (Table 2).

Fetuin-A levels

Fetuin-A in different groups is shown in Fig. 1: The highest fetuin-A concentration was found in type 2 diabetes–PAD subjects (399 ± 155 μg/ml) followed by patients with PAD and normal glucose metabolism (NGM-PAD) (376 ± 144 μg/ml). Furthermore, fetuin-A levels in type 2 diabetes–PAD subjects were higher than in type 2 diabetes–non-PAD subjects (247 ± 42 μg/ml; $P < 0.001$). In patients with PAD alone (NGM-PAD), fetuin-A was higher compared with patients with type 2 diabetes–

Table 2—*Baseline characteristics of age- and sex-matched patients in the different study groups*

	Type 2 diabetes–non-PAD (group 1)	NGM-PAD (group 2)	Type 2 diabetes–PAD (group 3)	*P* (all groups)	*P* (1 vs. 2)	*P* (1 vs. 3)	*P* (2 vs. 3)
n	40	44	55				
Age (years)	61.1 ± 10.0	61.1 ± 6.6	62.2 ± 4.9	0.685	0.995	0.525	0.367
Male sex (%)	21 (53)	28 (64)	38 (69)	0.253	0.301	0.100	0.567
Systolic blood pressure (mmHg)	145 ± 22	139 ± 21	141 ± 17	0.440	0.252	0.351	0.696
Diastolic blood pressure (mmHg)	90 ± 11	82 ± 14	78 ± 11	0.001*	0.024*	0.001*	0.124
BMI (kg/m^2)	31.9 ± 5.4	26.1 ± 3.4	29.6 ± 4.7	<0.001*	<0.001*	0.031*	<0.001*
eGFR (ml/min/1.73 m^2)	88.0 ± 40.5	76.9 ± 15.1	74.6 ± 19.2	0.050	0.141	0.082	0.525
Triglyceride (mmol/l)	2.2 ± 1.4	1.8 ± 1.0	2.3 ± 1.2	0.182	0.155	0.922	0.070
HDL (mmol/l)	1.3 ± 0.4	1.4 ± 0.3	1.2 ± 0.3	0.002*	0.309	0.064	<0.001*
Total cholesterol (mmol/l)	5.1 ± 0.9	5.3 ± 1.4	4.7 ± 1.3	0.033*	0.382	0.112	0.018*
LDL (mmol/l)	2.8 ± 0.8	3.1 ± 1.2	2.5 ± 1.1	0.026*	0.260	0.167	0.012*
Bilirubin (μmol/l)	11.3 ± 6.8	10.7 ± 3.4	11.0 ± 3.6	0.838	0.635	0.829	0.636
Alkaline phosphatase (U/l)	82.3 ± 29.2	78.6 ± 23.6	90.7 ± 69.7	0.459	0.534	0.515	0.272
Aspartate transaminase (U/l)	27.1 ± 8.9	26.3 ± 6.0	25.4 ± 11.2	0.683	0.689	0.456	0.597
Alanine transaminase (U/l)	27.6 ± 12.5	27.0 ± 11.4	30.2 ± 14.2	0.417	0.834	0.376	0.222
γ-Glutamyl transferase (U/l)	54.5 ± 41.7	51.6 ± 40.8	64.8 ± 145.7	0.787	0.765	0.693	0.563
C-reactive protein (mg/l)	NA	2.7 (1.5, 4.5)	3.5 (1.6, 7.0)	—	—	—	0.095
A1C (relative %)	7.7 ± 1.3	5.7 ± 0.4	7.2 ± 1.2	<0.001*	<0.001*	0.034*	<0.001*
Fetuin-A (μg/ml)	247.19 ± 41.82	356.45 ± 125.59	411.19 ± 163.02	<0.001*	<0.001*	<0.001*	0.070

Data are means ± SD, medians (25, 75 percentile), or *n* (%). *$P < 0.05$ is considered statistically significant. NA, not applicable.

non-PAD ($P < 0.001$). Differences of fetuin-A levels in the age- and sex-matched subjects persisted (Table 2).

To investigate a possible association of fetuin-A with atherosclerotic burden, we defined subgroups of type 2 diabetes–PAD: patients who had atherosclerosis beside PAD such as coronary heart disease, CVD, stroke, or myocardial infarction ($n = 62$) and patients with PAD as only manifestation of atherosclerosis ($n = 67$); fetuin-A was not different (408 ± 171 vs. 391 ± 139 μg/ml; $P = 0.54$). In addition, in all patients ($n = 245$), the association of number of sites of atherosclerosis and fetuin-A levels were significant in univariate regression ($\beta = 0.336$, $P < 0.001$). In the matched subjects (Table 2), the association strengthened ($\beta = 0.424$, $P < 0.001$).

Because fetuin-A is a calcification inhibitor, we analyzed whether fetuin-A is associated with mediasclerosis in type 2 diabetes–PAD. Type 2 diabetes–PAD patients with mediasclerosis ($n = 49$) had lower fetuin-A levels than patients without ($n = 53$) (370 ± 135 vs. 434 ± 157 μg/ml, $P < 0.03$), but still had higher levels than patients with type 2 diabetes–non-PAD ($P < 0.001$).

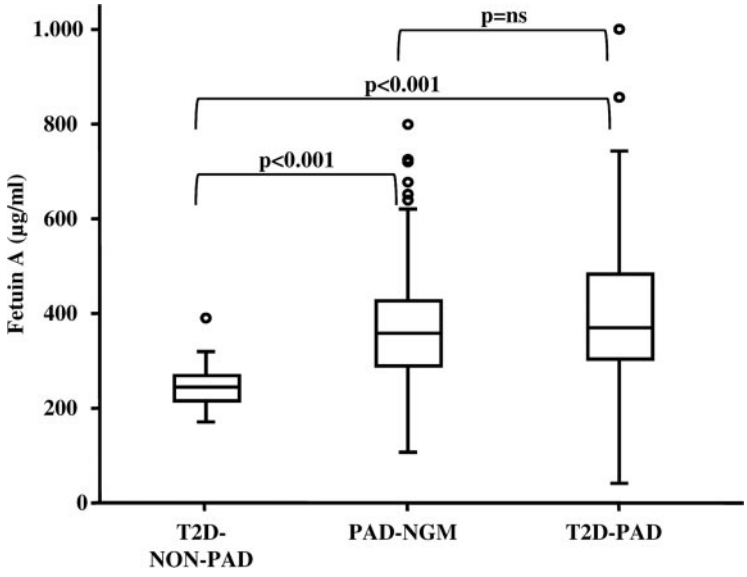

Figure 1—*The levels of fetuin-A in micrograms per milliliter in all three patient groups. T2D, type 2 diabetes. In-between group differences were analyzed by an unpaired Student* t *test.*

Tertiles of fetuin-A

The patients were divided into tertiles to investigate associations of quantitative and qualitative parameters with high fetuin-A levels (Table 3). Patients in the lowest and highest tertile of fetuin-A differed for coronary heart disease ($P = 0.006$) and myocardial infarction ($P = 0.020$).

As expected from the baseline characteristics (Table 1) and as shown in Fig. 1, fetuin-A in tertiles was also associated with the assigned patient cohorts: the lowest-risk group, type 2 diabetes–non-PAD, was >40% of the patients in the first tertile of fetuin-A ($P < 0.001$).

To date, a direct association of fetuin-A levels with clinical stages of PAD is not known. We found a significant increase of more pronounced stages of PAD in the highest versus the lowest or the intermediate tertile of fetuin-A levels ($P < 0.001$, Table 3).

Table 3—*Baseline characteristics of patients in fetuin-A tertiles*

	Tertile 1	Tertile 2	Tertile 3	*P* (all three groups)	*P* (1 vs. 3)
Fetuin-A (μg/ml)	≤286.69	286.70–389.97	≥389.98		
n	82	82	81		
Age (years)	66.7 ± 11.2	69.5 ± 9.6	69.2 ± 9.9	0.161	0.135
Male sex (%)	53 (65)	51 (62)	52 (64)	0.942	0.954
Systolic blood pressure (mmHg)	140 ± 21	147 ± 23	142 ± 22	0.146	0.583
Diastolic blood pressure (mmHg)	81 ± 13	79 ± 11	80 ± 14	0.512	0.578
BMI (kg/m^2)	29.0 ± 5.0	27.9 ± 5.1	27.8 ± 4.3	0.190	0.096
eGFR (ml/min/1.73 m^2)	77.1 ± 30.9	67.4 ± 21.2	69.4 ± 17.5	0.028*	0.063
Triglyceride (mmol/l)	1.9 ± 1.2	2.0 ± 1.2	2.1 ± 1.0	0.474	0.217
HDL (mmol/l)	1.4 ± 0.4	1.3 ± 0.3	1.3 ± 0.3	0.571	0.316
Total cholesterol (mmol/l)	5.1 ± 1.5	4.8 ± 1.0	5.0 ± 1.1	0.460	0.777
LDL (mmol/l)	2.8 ± 1.2	2.6 ± 0.9	2.8 ± 1.0	0.391	0.903
Bilirubin (μmol/l)	11.5 ± 5.4	11.4 ± 4.6	11.1 ± 3.5	0.805	0.542
Alkaline phosphatase (U/l)	88.8 ± 56.2	81.7 ± 35.0	79.4 ± 23.9	0.325	0.175
Aspartate transaminase (U/l)	26.9 ± 9.1	25.3 ± 9.4	25.0 ± 6.1	0.320	0.132
Alanine transaminase (U/l)	27.0 ± 12.8	26.0 ± 11.8	27.0 ± 11.6	0.844	0.979
γ-Glutamyl transferase (U/l)	62.7 ± 126.9	44.6 ± 39.3	41.5 ± 36.1	0.193	0.153
C-reactive protein (mg/l)	2.9 (1.0, 5.7)	2.3 (1.0, 4.5)	3.0 (1.6, 5.6)	0.256	0.888
A1C (relative %)	6.9 ± 1.3	6.5 ± 1.2	6.7 ± 1.1	0.082	0.422
Coronary heart disease	13 (16)	25 (31)	28 (35)	0.018*	0.006*
Myocardial infarction	6 (7)	16 (20)	16 (20)	0.043*	0.020*
Carotid artery disease	10 (12)	12 (15)	14 (17)	0.656	0.359
Stroke	6 (7)	8 (10)	5 (6)	0.682	0.771
PAD					
Non-PAD	35 (43)	4 (5)	1 (1)		
Stage I	21 (25)	38 (46)	34 (42)	<0.001*	<0.001*
Stage II	26 (32)	40 (49)	46 (57)		
Group					
Type 2 diabetes–non-PAD	35 (43)	4 (5)	1 (1)		
NGM-PAD	28 (34)	46 (56)	55 (68)	<0.001*	<0.001*
Type 2 diabetes–PAD	19 (23)	32 (39)	25 (31)		

Data are means ± SD, medians (25, 75 percentile), or *n* (%). *$P < 0.05$ is considered statistically significant.

Predictors of fetuin-A in univariate and multivariate regression

Univariate regression for fetuin-A levels in all patients included all available quantitative parameters (Table 1): only age ($\beta = 0.115$, $P = 0.073$) showed a trend toward significant association. After reduction of confounding (19), multivariate regression did not change the prediction of fetuin-A. Next, age- and sex-matched subjects (Table 2) (our groups differed for age) were introduced to multivariate regression: we failed to obtain any significant model for the explanation of fetuin-A.

In patients with PAD (NGM-PAD and type 2 diabetes–PAD), two variables were associated with fetuin-A in univariate fashion: A1C ($\beta = 0.196$, $P = 0.005$) and pack-years of smoking ($\beta = -0.166$, $P = 0.033$). Both variables remained independent from each other predictive for fetuin-A in multivariate fashion.

Multivariate regression in type 2 diabetes–PAD revealed that the two variables that were associated with fetuin-A levels in univariate fashion remained predictive in multivariate analysis: A1C ($\beta = 0.348$, $P < 0.001$) and mediasclerosis ($\beta = -0.281$, $P = 0.004$) were the strongest predictors for fetuin-A in type 2 diabetes–PAD.

Prediction of PAD

Because the prevalence of PAD was associated with tertiles of fetuin-A, we tested whether linear fetuin-A predicted PAD in a univariate or multivariate fashion. In a univariate fashion, each 10 μg/ml increase in fetuin-A resulted in a 13% increase in odds ratio (OR) (95% CI 8.0–18, $P < 0.001$) having advanced PAD (increase from no PAD over PAD Fontaine stage I to PAD Fontaine stage II). A multivariate model adjusted for age, eGFR, LDL, HDL, A1C, BMI, and sex revealed that the predictive power of fetuin-A improved by 30%: each 10 μg/ml increase of fetuin-A now resulted in a 17% increase in OR (95% CI 9–25, $P < 0.001$). In this model, apart from fetuin-A, age was identified as a significant positive predictor: an increase of age in 1 unit in the latter variables resulted in a 9% likelihood of advancement of PAD (95% CI 1–17, $P = 0.031$). To compare the power of those factors, OR per 1-SD change was calculated: 1-SD change of fetuin-A resulted in an OR of 3.5 (95% CI 2.3–4.7, $P < 0.001$); 1-SD change in age resulted in an OR of 1.9 for advancement of PAD (95% CI 1.1–2.8, $P < 0.001$).

Because Eraso et al. (14) showed an unadjusted and adjusted OR of 1.6 per decrease of 1 SD fetuin-A for the prevalence (no versus yes) of PAD in their study cohort, we applied a similar model in our cohort. Unadjusted, the OR per increase of 1 SD fetuin-A for the prevalence of PAD

was 2.9 (95% CI 2.2–3.7, $P < 0.001$). Adjustment for age, sex, eGFR, HDL, LDL, triglycerides, total cholesterol, A1C, systolic blood pressure, bilirubin, alkaline phosphatase, aspartate transaminase, alanine transaminase, and γ-glutamyl transferase strengthened the association: OR per 1-SD increase of fetuin: 3.8 (95% CI 2.5–5.0).

CONCLUSIONS — In this study, we demonstrated that patients with type 2 diabetes who additionally suffer from PAD have significantly higher fetuin-A levels than patients with diabetes but without any atherosclerotic burden.

Our data are in opposition to the results from Eraso et al. (14), who assumed that patients with type 2 diabetes and PAD have lower plasma concentrations of fetuin-A than patients with type 2 diabetes alone. In the latter study, 738 patients with type 2 diabetes but without clinical evidence of cardiovascular disease were enrolled. From these study participants, only 5.1% ($n = 38$) had PAD, defined by an ABI <0.9. Although PAD patients show the highest co-burden of manifestations of atherosclerosis (15), which is even higher in patients with PAD and type 2 diabetes (1–3,15), the 38 patients in the latter article did not show any other manifestation of atherosclerosis and must thus be regarded as highly selected and not representative (20).

In contrast to Eraso et al. (14), a decrease of fetuin-A did not result in an increase of PAD. In our study, an increase of 1 SD fetuin-A resulted in an increase of PAD: OR 2.9 (95% CI 2.2–3.7, $P < 0.001$). Adjustment for multiple variables did not attenuate this association, but increased its strength: OR 3.8 (95% CI 2.5–5.0, $P < 0.001$).

Recent studies showed a strong association between fetuin-A levels and events of CVD (11,12). Weikert et al. (12) obtained a significantly increased risk for myocardial infarction (relative risk [RR] 3.3) and ischemic stroke (RR 3.8) for individuals in the highest compared with the lowest quintile of fetuin-A in a model adjusted for cardiovascular risk factors including age, sex, diabetes, BMI, HDL, and high sensitive C-reactive protein. The change per category of fetuin-A quintiles in the study of Weikert et al. (12) of an RR of 1.7 for myocardial infarction was similar to our OR of 1.6 per categorical fetuin-A change (95 CI 1.1–2.5, $P = 0.030$). In addition, cases with coronary heart disease in the fetuin-A tertiles increased from 13 (16%), over 25 (31%), to 28 (35%) ($P = 0.018$, Table 3). The findings by Fisher et al. (13) suggest the suspicion of a causal relationship of fetuin-A levels, ASHG (fetuin-A) gene polymorphisms, and atherosclerotic events. Likewise, the ASHG rs4917 C → T polymorphism showed a significant association with myocardial infarction (adjusted hazard rate 1.34, 95% CI 1.05–1.70, $P < 0.02$), although it explained only 21.2% of the phenotypic variance of fetuin-A plasma levels independent of potential confounding factors. Results of the latter studies (11–13) nourish the theory that fetuin-A may play a causal role in the pathophysiology of atherosclerosis leading to CVD, not only in rodents but also in humans. Prevalence of myocardial infarction was limited to 16% in our cross-sectional study. Nevertheless, the power was sufficient to obtain significant associations: a categorical change in fetuin-A levels resulted in an OR of 1.7 (95% CI 1.0–2.7, $P = 0.041$) after multivariate adjustment for age, LDL, eGFR, A1C, and sex. Our results underline the findings that higher fetuin-A levels are associated with atherosclerosis (11–13,21), although due to the cross-sectional design of our study, we cannot answer yet whether fetuin-A has predictive power in patients with type 2 diabetes with and without PAD.

Stefan et al. (10) have shown that fetuin-A levels predict type 2 diabetes. In our cohort, patients with advanced atherosclerosis (type 2 diabetes–PAD and NGM-PAD) had higher levels of fetuin-A regardless of diabetes status, suggesting that fetuin-A might be independently (of diabetes) associated with CVD.

In agreement with Ix et al. (22), we found that higher fetuin-A was associated with increased odds for the metabolic syndrome (MetS) in our patients with PAD. An association of features of MetS and fetuin-A was also described by Reinehr and Roth (23) in obese children. In our patients with PAD, the association of fetuin-A and MetS was restricted to the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) 3 definition and did not result in significance in the World Health Organization and International Diabetes Federation classification of MetS. A 1-SD change in fetuin-A resulted in an OR for MetS of 1.3 (95% CI 1–1.6, $P = 0.044$). Vice versa, only MetS defined by NCEP ATP-3 predicted fetuin-A at a level of significance ($\beta = 0.142$, $P = 0.042$). In summary, high fetuin-A levels seem to be associated with MetS, insulin resistance, diabetes, myocardial infarction, and PAD in patients with and without diabetes.

In contrast, low levels of fetuin-A have been associated with CVD in patients on dialysis (5). In the last years, many biological parameters in humans have been shown to follow not a simple linear association, but a U-shaped relationship, e.g., BMI, hemoglobin, and A1C (the latter in diabetes). Ix et al. (23) suggested a U-shaped relationship also for fetuin-A values with CVD. They considered either high or low levels of fetuin-A to predict cardiovascular events. High levels by associations with MetS and atherogenic lipids result in CVD; low levels by associations with vascular calcification also result in CVD.

Mehrotra et al. (24) and Mori et al. (25) showed a positive association between fetuin-A and coronary artery calcification and arterial stiffness in subjects without dialysis. Because those studies were cross-sectional, it is not impossible that fetuin-A had detrimental effects on vasculature or that fetuin-A is upregulated to protect against calcification processes in atherosclerosis. Low fetuin-A levels were associated with mitral annular calcification and aortic stenosis in patients with coronary heart disease (6,7). Therefore, it is possible that fetuin-A is upregulated to protect against calcification but deteriorates atherosclerosis (6).

Nevertheless, in our study, patients with media artery sclerosis showed significantly lower levels of fetuin-A. Thus, our study is in line with the calcification inhibition competence of fetuin-A, but levels of fetuin-A were still significantly higher compared with patients with diabetes alone (type 2 diabetes–non-PAD).

Supporting the idea that low fetuin-A causes vascular calcification, we observed in patients with PAD (NGM-PAD and type 2 diabetes–PAD) that the total smoking dosage showed a negative association ($\beta = -0.166$, $P = 0.033$) with fetuin-A. The observation that long term smoking results in severe clinical vessel calcification is not understood and is now the focus of scientific interest. Because our study is cross-sectional, we cannot state if smoking decreases fetuin-A levels and thus affects vascular calcification.

In summary, we demonstrate that fetuin-A levels are not decreased in patients with diabetes and atherosclerosis, but are

elevated. We show that PAD and myocardial infarction are associated with higher fetuin-A values. In addition, we support the hypothesis that low fetuin-A might result in vascular calcification. Finally, we are first to report that low fetuin-A is associated with mediasclerosis in patients with diabetes and PAD.

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D.P.L. researched data and wrote the manuscript. M.G. researched data. C.H. and J.-M.B. researched data and contributed to discussion. F.H. researched data. G.S. contributed to discussion and reviewed the manuscript. R.K. reviewed the manuscript. G.-H.S. researched data, contributed to discussion, and wrote and reviewed the manuscript.

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