Urinary Adiponectin Is an Independent Predictor of Progression to End-Stage Renal Disease in Patients With Type 1 Diabetes and Diabetic Nephropathy

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OBJECTIVE

We investigated the predictive value of urinary adiponectin (uADP) for the progression of diabetic nephropathy (DN) as well as for the principal determinants of uADP concentrations.

RESEARCH DESIGN AND METHODS

uADP was measured in 2,090 patients with type 1 diabetes followed for a median of 5.8 (4.4–6.9) years and in 111 subjects without diabetes. Progression was defined as a change in albuminuria (albumin excretion rate [AER]) to a higher stage or development of end-stage renal disease (ESRD). Various Cox regression and competing risk models were used to evaluate the predictive value of uADP for DN progression. The added predictive benefit to AER or estimated glomerular filtration rate (eGFR) was estimated by the area under the receiver operating characteristic curve, integrated discrimination improvement (IDI), continuous net reclassification improvement (NRI), and other statistical indexes. The determinants of uADP were investigated by multiple regression analyses.

RESULTS

uADP was an independent predictor of progression to ESRD (hazard ratio 1.60, $P < 0.001$) and was an even better predictor than AER ($P = 0.04$) or as good as eGFR ($P = 0.79$). Furthermore, uADP added a significant benefit when used together with AER (NRI 0.794, $P = 0.03$; IDI 0.115, $P < 0.0001$) or eGFR (NRI 0.637, $P < 0.001$; IDI 0.087, $P < 0.0001$). The common determinants of uADP were glycemic control, tubular injury, and AER.

CONCLUSIONS

uADP is a strong independent predictor of DN progression from macroalbuminuria to ESRD and adds a significant predictive benefit to current biomarkers in patients with type 1 diabetes.

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Diabetic nephropathy (DN) in type 1 diabetes is associated, already at the microalbuminuric stage, with a twofold higher mortality rate, which increases substantially toward end-stage renal disease (ESRD) (1). Prevention of DN is recommended and should be based on screening using biomarkers of progression, such as the albumin excretion rate (AER) or estimated glomerular filtration rate (eGFR). Although the molecular pathogenic mechanisms of DN are not completely understood, adiponectin (ADP) may also play a role in DN pathogenesis.

ADP is a small protein encoded by the adiponectin gene (*ADIPOQ*), which is primarily expressed in adipocytes with no apparent expression in the kidney (2). ADP is present in serum in three molecular isoforms: low molecular weight (LMW), medium molecular weight, and high molecular weight (HMW). ADP has a wide range of well-known protective effects against insulin resistance, vascular dysfunction, atherosclerosis, and inflammation (3). Animal studies suggested that ADP regulates albuminuria and podocyte function (4). In humans, serum ADP usually is inversely correlated with eGFR, whereas ADP is abundantly present in biopsy specimens of nondiabetic human kidneys (5,6). In addition, serum ADP is increased in patients with type 1 diabetes and may predict progression to ESRD (7,8). Being a protein molecule, ADP possibly passes the glomerular basement membrane and is excreted in the urine, thereby reflecting glomerular damage. Indeed, various ADP isoforms can be measured in the urine, where ADP is considered to be a marker of vascular damage (4,6). However, urinary ADP (uADP) has also been linked to renal tubular injury (9). Thus, uADP may reflect both glomerular and tubular damage in DN. In this context, studies regarding uADP level as a possible more comprehensive predictor of DN progression are warranted. Therefore, the aims of the present study were to *1*) evaluate the predictive role of uADP for progression of DN in patients with type 1 diabetes, *2*) investigate the added predictive benefit of uADP on top of AER or eGFR, and *3*) examine the principal determinants of uADP.

RESEARCH DESIGN AND METHODS

Study Subjects

Patients with type 1 diabetes included in this study were part of the Finnish Diabetic Nephropathy Study (FinnDiane) and were enrolled between January 1998 and December 2002. For this study, we also used a group of subjects without diabetes and without a family history of kidney disease or diabetes. Blood and urine samples were collected at study baseline and stored at −20°C until measured in 2008. Patients were followed for a median of 5.8 (4.4–6.9) years, and then clinical outcomes were evaluated. This study was performed with the approval of local ethics committees in accordance with the revised Declaration of Helsinki.

At baseline, we used a standardized questionnaire to assess patient clinical characteristics. The questionnaires were completed by the attending physician based on patient medical records. Blood pressure and anthropometric parameters were measured. Venous blood was collected for the assessment of common biochemical variables, which were measured using standard methods as previously described (10). During the follow-up period, all patients with type 1 diabetes were managed by their own practitioner together with his or her diabetes team. The detailed FinnDiane Study protocol has been described in detail elsewhere (11,12).

Ascertainment of Outcomes

Renal status was defined based on the AER in at least two of three consecutive timed urine collections. On the basis of the AER, patients were divided into three categories: normal AER (<20 μg/min or <30 mg/24 h), microalbuminuria (≥20 but <200 μg/min or ≥30 but <300 mg/24 h), and macroalbuminuria (≥200 μg/min or ≥300 mg/24 h). ESRD was considered present if patients were undergoing dialysis or had received a kidney transplant. Patients with ESRD at baseline were excluded. Glomerular filtration rate was estimated using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) creatinine equation (13). Progression of DN was defined as the passage from one AER stage to the next or progression to ESRD for patients with initial macroalbuminuria.

Assays

We measured uADP in a single 24-h urine collection with an ALPCO Diagnostics kit (Salem, NH) for quantitative measurement of multimeric ADP, using a modified protocol for urine samples without protease pretreatment. The uADP levels were then normalized for urinary creatinine. Urinary kidney injury molecule 1 (KIM-1), serum ADP, and urinary liver-type fatty acid–binding protein (L-FABP) values were also available. Urinary KIM-1 was measured using a cobas Elecsys 411 immunoanalyzer with a DuoSet ELISA Development Kit from R&D Systems (Abingdon, Oxon, U.K.); serum ADP and urinary L-FABP measurement methods are described elsewhere (14,15).

Statistics

Normally distributed variables are presented as mean ± SD. Nonnormally distributed variables are presented as median and interquartile range. Frequencies are given as percentages. Comparisons between groups were performed by independent samples *t* test for normally distributed variables and Mann-Whitney *U* test for nonparametric distributions. Categorical variables were compared between groups using χ^2 test.

We used Cox proportional hazards models to assess the ability of uADP to predict DN progression. The algorithm by which we constructed the basic models of DN progression in the cohort was previously published (15). First, we used simple Cox proportional hazards models to investigate uADP as a predictor for progression of nephropathy. After that, we adjusted the result with the basic models of progression for each stage to test the independence of the biomarker. Next, we included AER, KIM-1, L-FABP, or serum ADP in the models to see whether uADP predicts progression independent of these other markers. The Cox model fit was assessed by cumulative Cox-Snell residuals to (−log) Kaplan-Meier estimates. Fine and Gray regression analysis, which extends the Cox proportional hazards model to competing risk data by consideration of the subdistribution hazard, was also performed to take into account the competing event of death instead of progression to a higher stage (16,17). In the competing risk analysis, we included the same covariates as in the previous Cox regression analysis. The colinearity of the models was estimated by the variance inflation factor, tolerance, and *R* values. The cutoff values considered acceptable were <10 for variance inflation factor, >0.5 for tolerance, and <0.7 for *R* (18). The validity of the assumption for the

prediction models was tested by checking the normal distribution of the residuals using the D'Agostino-Pearson test (19).

Receiver operating characteristic (ROC) curve analysis of the Cox models was performed and the areas under the curve (AUCs) compared using the method described by DeLong et al. (20) to assess the predictive abilities of uADP for progression of DN. First, we compared the ROC curves of uADP and AER alone. Next, we compared the ROC curve of the model formed by AER and uADP together with either AER or ADP alone. The improvement of prediction given by the addition of uADP to either AER alone or basic progression models plus AER was assessed by calculating the continuous net reclassification improvement (NRI) and the integrated discrimination improvement (IDI) obtained by 10-fold cross-validation using 1,000 bootstrap repetitions of the whole data set through the Stata incrisk module (21,22). The predictive performance of the Cox models was evaluated by the explained variation (R^2) using 1,000 bootstrap repetitions of the whole data set through the Stata str2ph module (23). In addition, the mean risk increment and delta standardized total gain were calculated using the same Stata module.

For all tests, $P < 0.05$ was considered statistically significant. The data analysis was performed using MedCalc 12.1.3.0 (MedCalc Software BVBA, Mariakerke, Belgium) and Stata/MP2 version 13 (StataCorp LP, College Station, TX) software.

RESULTS

Clinical and Biochemical Characteristics of the Study Subjects

At baseline, 1,451 patients had normal AER, 319 had microalbuminuria, and 320 had macroalbuminuria. In addition, 111 subjects without diabetes were enrolled. The baseline characteristics of the cohort are shown in Table 1. Patients were followed for a median of 5.8 (4.4–6.9) years. During the follow-up, 101 patients progressed from normal AER to microalbuminuria, 42 progressed from micro- to macroalbuminuria, and 71 progressed from macroalbuminuria to ESRD. The differences in baseline clinical characteristics between the patients who progressed to a higher stage of DN and nonprogressors are detailed in Supplementary Table 1.

The uADP concentrations were higher in patients with diabetes and normal AER than in subjects without diabetes (0.56 vs. 0.34 μg/g, $P < 0.0001$). Furthermore, uADP increased with worsening DN ($P < 0.0001$) (Table 1 and Supplementary Fig. 1), and uADP was

Table 1—Clinical baseline data for patients enrolled in the study

Variable	Healthy control subjects	Patients with normoalbuminuria	Patient with microalbuminuria	Patients with macroalbuminuria
Number of patients (male/female)	111 (41/70)	1,451 (688/763)	319 (185/134)	320 (178/142)
Age (years)	39.6 ± 11.9	37.0 ± 12.3	39.1 ± 12.6	42.1 ± 10.5
Age of onset (years)	—	17.4 ± 9.4	13.7 ± 9.4	12.8 ± 8.3
Diabetes duration (years)	—	19.6 ± 11.7	25.4 ± 10.8	29.3 ± 7.8
BMI (kg/m^2)	23.8 ± 2.8	24.9 ± 0.14	25.7 ± 3.7	26.2 ± 4.1
WHR				
Men	0.94 ± 0.05	0.89 ± 0.07	0.92 ± 0.07	0.94 ± 0.07
Women	0.84 ± 0.04	0.80 ± 0.06	0.83 ± 0.07	0.84 ± 0.07
History of smoking (%)	27.0	42.5	53.7	60.6
SBP (mmHg)	126 ± 14	130 ± 16	137 ± 17	144 ± 20
DBP (mmHg)	77 ± 9	78 ± 9	81 ± 10	83 ± 10
HbA_{1c} (%)	5.6 ± 0.3	8.3 ± 1.4	8.8 ± 1.5	9.1 ± 1.6
HbA_{1c} (mmol/mol)	38 ± 0.9	67 ± 4.2	73 ± 4.5	76 ± 4.8
Total cholesterol (mmol/L)	4.82 ± 0.93	4.83 ± 0.90	4.97 ± 0.90	5.39 ± 1.10
HDL cholesterol (mmol/L)	1.56 ± 0.32	1.36 ± 0.38	1.30 ± 0.38	1.21 ± 0.37
LDL cholesterol (mmol/L)	2.80 ± 0.84	2.96 ± 0.81	3.07 ± 0.82	3.39 ± 0.87
Triglycerides (mmol/L)	0.90 (0.69–1.17)	0.94 (0.73–1.29)	1.06 (0.81–1.52)	1.37 (1.02–2.05)
AER (mg/24 h)	3 (1–4)	7 (5–12)	51 (25–100)	440 (176–1,207)
eGFR (mL/min/1.73 m^2)	92 (76–111)	87 (72–107)	81 (64–101)	46 (28–69)
C-reactive protein (mg/L)	1.04 (0.53–2.37)	1.87 (1.13–3.55)	2.16 (1.27–4.70)	2.68 (1.66–5.81)
Serum ADP (mg/L)				
All	9.69 (7.33–12.17)	10.69 (7.96–14.82)	10.78 (7.92–15.08)	14.7 (10.26–22.00)
Men	7.60 (5.26–9.65)	8.80 (6.67–11.53)	10.01 (7.24–13.19)	12.45 (9.05–15.87)
Women	10.80 (8.37–13.40)	13.14 (9.71–16.70)	12.55 (9.04–19.07)	18.68 (12.67–26.27)
uADP (μg/g)				
All	0.34 (0.21–0.66)	0.56 (0.26–1.31)	0.97 (0.39–2.42)	5.52 (1.53–22.9)
Men	0.23 (0.15–0.50)	0.43 (0.21–0.99)	0.82 (0.32–2.23)	5.03 (1.51–20.14)
Women	0.42 (0.26–0.83)	0.72 (0.32–1.54)	1.07 (0.50–2.62)	5.95 (1.77–24.67)
L-FABP (μg/μmol)	0.01 (0.00–0.04)	0.04 (0.01–0.09)	0.09 (0.03–0.18)	0.52 (0.19–1.97)
KIM-1 (ng/mmol)	37.3 (18.6–58.3)	26.2 (12.2–48.7)	34.5 (16.4–62.0)	48.5 (27.3–88.7)

Data are mean ± SD or median (interquartile range) unless otherwise indicated. DBP, diastolic blood pressure; SBP, systolic blood pressure; WHR, waist-to-hip ratio.

significantly higher in patients who progressed to a higher stage of DN compared with nonprogressors (Supplementary Table 1 and Supplementary Fig. 1).

uADP Predicts Progression of DN

Univariate Cox proportional hazards models showed that uADP predicted progression to a higher stage of DN in patients with normal AER (hazard ratio $[HR]_{normalAER}$ 1.32 [95% CI 1.13–1.54]), microalbuminuria (HR_{micro} 1.38 [95% CI 1.09–1.74]), and macroalbuminuria (HR_{macro} 2.03 [95% CI 1.76–2.34]) at baseline. uADP was still a significant predictor of progression after adjustment for the basic progression models and sex ($HR_{normalAER}$ 1.25 [95% CI 1.05–1.48], HR_{micro} 1.35 [95% CI 1.04–1.73], HR_{macro} 1.52 [95% CI 1.30–1.78]). However, after AER was added to the model, uADP independently predicted progression only to ESRD ($HR_{normalAER}$ 0.99 [95% CI 0.82–1.20], HR_{micro} 1.02 [95% CI 0.76–1.37], HR_{macro} 1.37 [95% CI 1.07–1.75]) (Table 2). The results did not change in the competing risk analysis considering death as a competing event for progression at any stage (Supplementary Table 2).

Added Predictive Benefit for the Prediction of DN Progression

Comparison of ROC AUCs for uADP and AER alone showed AER as a better predictor of progression to microalbuminuria ($\Delta AUCs_{normalAER}$ 0.180, $P < 0.0001$) and macroalbuminuria ($\Delta AUCs_{micro}$ 0.218, $P < 0.0001$) but not to ESRD ($\Delta AUCs_{macro}$ 0.057, $P = 0.04$), where uADP was superior. Comparison of the ROC curve of the model formed by AER together with uADP with AER alone showed an added predictive benefit regarding progression to ESRD in favor of the model formed by uADP together with AER ($\Delta AUCs_{macro}$ 0.056, $P = 0.02$). Finally, the ROC curve comparisons between uADP ($AUC_{ADPmacro}$ 0.842) and eGFR alone ($AUC_{eGFRmacro}$ 0.853) showed no significant differences ($\Delta AUCs_{macro}$ 0.010, $P = 0.79$), but when uADP was added to eGFR, there was again an added predictive benefit with respect to progression toward ESRD ($\Delta AUCs_{macro}$ 0.029, $P = 0.03$) compared with eGFR alone (Table 3 and Supplementary Fig. 2).

There was an improvement in the prediction of progression to ESRD by adding uADP to AER (NRI 0.794, $P = 0.03$; IDI 0.115, $P < 0.0001$) but not by

Table 2—Prediction of progression based on Cox proportional hazards models using the baseline data for all variables

Variable and adjustment	Unadjusted or adjusted for sex	Adjusted for							Adjusted for the model and				
		Total cholesterol	Smoking history	HbA_{1c}	WHR	TGs	eGFR	Adjusted for PM	AER	KIM-1	L-FABP	Serum ADP
Normal AER												
uADP												
No	1.32	1.29	1.31	1.17	1.40	—	—	1.22	0.98	0.96	0.98	1.22
	(<0.001)	(0.002)	(0.001)	(0.07)	(<0.001)			(0.02)	(0.80)	(0.76)	(0.90)	(0.07)
Sex	1.39	1.36	1.39	1.24	1.42	—	—	1.25	0.99	0.97	1.01	1.26
	(<0.001)	(<0.001)	(<0.001)	(0.01)	(<0.001)			(0.01)	(0.91)	(0.81)	(0.94)	(0.04)
Microalbuminuria												
uADP												
No	1.38	—	—	1.29	1.45	1.37	—	1.34	1.01	1.31	1.02	1.30
	(0.007)			(<0.05)	(0.002)	(0.008)		(0.03)	(0.95)	(0.07)	(0.92)	(0.14)
Sex	1.46	—	—	1.35	1.46	1.42	—	1.35	1.02	1.31	1.04	1.29
	(0.001)			(0.02)	(0.001)	(0.003)		(0.02)	(0.89)	(0.06)	(0.83)	(0.15)
Macroalbuminuria												
uADP												
No	2.03	—	—	—	—	1.98	1.50	1.51	1.30	1.47	1.36	1.39
	(<0.0001)					(<0.001)	(<0.001)	(<0.001)	(0.03)	(<0.001)	(0.002)	(0.001)
Sex	2.05	—	—	—	—	2.01	1.50	1.52	1.37	1.29	1.39	1.36
	(<0.0001)					(<0.001)	(<0.001)	(<0.001)	(0.01)	(0.001)	(0.001)	(0.001)

Data are HR (*P* value). The basic models for progression for every stage derived from Cox proportional hazards models were used. The full description of the model-building process is provided in the RESEARCH DESIGN AND METHODS. No, models without any adjustments; PM, progression model; Sex, models adjusted for sex; TG, triglyceride; WHR, waist-to-hip ratio.

Table 3—ROC curve analysis for the main comparisons between uADP and AER

				Difference between AUCs					
				ADP		AER		eGFR	
ROC	AUC	SE	95% CI	Difference	*P* value	Difference	*P* value	Difference	*P* value
Progression to microalbuminuria									
uADP	0.600	0.029	0.573–0.626	—	—	0.180	<0.0001	NT	NT
AER	0.780	0.025	0.757–0.802	0.180	<0.0001	—	—	NT	NT
uADP&AER	0.780	0.025	0.757–0.802	0.180	<0.0001	4.8×10^{-5}	0.98	NT	NT
Progression to macroalbuminuria									
uADP	0.627	0.044	0.570–0.682	—	—	0.218	<0.0001	NT	NT
AER	0.845	0.033	0.799–0.884	0.218	<0.0001	—	—	NT	NT
uADP&AER	0.845	0.033	0.799–0.884	0.218	<0.0001	9.2×10^{-5}	0.09	NT	NT
Progression to ESRD									
uADP	0.842	0.029	0.798–0.880	—	—	0.057	0.04	0.010	0.79
AER	0.786	0.033	0.736–0.829	0.057	0.04	—	—	0.067	0.14
eGFR	0.853	0.030	0.809–0.890	0.010	0.79	0.067	0.14	—	—
uADP&AER	0.842	0.029	0.797–0.880	0.001	0.90	0.057	0.04	0.011	0.78
uADP&eGFR	0.882	0.023	0.841–0.915	0.040	0.14	0.096	0.01	0.029	0.03

NT, not tested; uADP&AER, Cox model formed by uADP and AER used together; uADP&eGFR, Cox model formed by uADP and eGFR used together.

adding uADP on top of the basic progression models plus AER (NRI 0.042, $P > 0.05$; IDI 0.015, $P > 0.05$). For all the other stages of DN, there was no improvement. Furthermore, in patients with baseline macroalbuminuria, addition of uADP improved the prediction of progression to ESRD compared with eGFR alone (NRI 0.637, $P < 0.001$; IDI 0.087, $P < 0.0001$), as did the addition of uADP to the basic progression models (NRI 0.674, $P < 0.001$; IDI 0.084, $P < 0.0001$) (Table 4).

Explained variability (R^2) of the Cox models for progression to ESRD was better when uADP was added either to AER alone (0.651 vs. 0.472, $P < 0.0001$) or to the basic progression model plus AER (0.818 vs. 0.772, $P = 0.007$). In addition, the mean risk increment was 0.088 (95% CI 0.0291–0.172) when uADP was added to eGFR or 0.117 (95% CI 0.056–0.200) when uADP was added to AER (Supplementary Table 3).

uADP Predicts DN Progression Independently of Serum ADP, Urinary KIM-1, or Urinary L-FABP in Patients With Macroalbuminuria

The uADP predicted progression independently of serum ADP in patients with normal AER (HR 1.26 [95% CI 1.01–1.56], $P = 0.04$) but not in patients with microalbuminuria (HR 1.29 [95% CI 0.91–1.83], $P = 0.15$). However, for patients with baseline macroalbuminuria, both variables were independent predictors of progression to ESRD (HR 1.36 [95% CI 1.13–1.64], $P = 0.001$). uADP predicted progression to ESRD independently of urinary L-FABP (HR 1.28 [95% CI 1.14–1.70], $P = 0.001$) or urinary KIM-1 (HR 1.29 [95% CI 1.18–1.80], $P = 0.02$) (Table 2).

Determinants of uADP

The common determinants of uADP levels at all baseline stages of DN were HbA_{1c}, AER, urinary L-FABP, and urinary KIM-1, whereas for patients with macroalbuminuria, age ($\beta = -0.019$, $P = 0.02$), BMI ($\beta = -0.048$, $P = 0.02$), serum ADP ($\beta = 0.015$, $P = 0.03$), eGFR ($\beta = -0.018$, $P < 0.0001$), and LDL cholesterol ($\beta = -0.196$, $P = 0.03$) also played a role (Supplementary Table 4).

CONCLUSIONS

We show that uADP is a strong and independent predictor of DN progression to ESRD in patients with type 1 diabetes. In addition, when uADP was used together with AER or eGFR, it improves the risk prediction of DN progression to ESRD. Finally, the main determinants of uADP levels were the markers of glomerular damage, tubular dysfunction, glycemic control, and serum ADP levels, but other factors may also be involved.

The main finding of this study was that uADP predicts progression to ESRD, both in Cox regression models and in the Fine and Gray competing risk analysis, independently of the progression model (with eGFR included), AER, and the tubular markers L-FABP and KIM-1, although all these variables are also associated with uADP levels at this stage. An increase in uADP in parallel with AER in patients with type 1 diabetes was previously observed only in a small study that could not explore the relationship further due to insufficient power (24). We show that uADP not only strongly predicted progression to ESRD but also is a better predictor than AER and adds a significant predictive benefit when used together with eGFR or AER. The important added value of uADP on top of either AER or eGFR is demonstrated for the calculated metrics (increment of AUC, continuous NRI, IDI, R^2 increase, and mean risk difference between models). To our knowledge, this study is the first to evaluate using a wide range of robust statistical methods the added value of uADP as a biomarker for the progression of DN in patients with type 1 diabetes. From a clinical point of view, these results are important because the risk of progression to ESRD in patients with type 1 diabetes is not easy to assess based on either AER or eGFR given each measure's limitations (25–27). Assessment of uADP on top of AER or eGFR may therefore add a predictive benefit. A comparison with other biomarkers with respect to these new metrics is difficult because of the lack of comparable data. However, the added predictive benefit of uADP was comparable to another promising biomarker, the soluble tumor necrosis factor-α receptor 1 (28).

Another finding of the present study was that in patients with type 1 diabetes and normal AER or microalbuminuria, uADP predicted progression

Table 4—Urinary ADP added predictive benefit and model performance

	Continuous NRI (95% CI)	*P* value	IDI (95% CI)	*P* value	R^2	*P* value	Mean risk difference (95% CI)	ΔTG (95% CI)
Progression to microalbuminuria								
uADP&AER vs. AER	0.062 (−0.272 to 0.235)	NS	−0.001 (−0.002 to 0.008)	NS	0.559 vs. 0.558	NS	0.000 (−0.002 to 0.008)	−0.010 (−0.050 to 0.035)
uADP&AER&PM vs. AER&PM	−0.094 (−0.286 to 0.275)	NS	−0.001 (−0.003 to 0.006)	NS	0.682 vs. 0.682	NS	−0.002 (−0.003 to 0.006)	0.006 (−0.039 to 0.035)
Progression to macroalbuminuria								
uADP&AER vs. AER	0.001 (−0.409 to 0.539)	NS	−0.003 (−0.009 to 0.022)	NS	0.680 vs. 0.680	NS	−0.003 (−0.009 to 0.022)	0.004 (−0.079 to 0.068)
uADP&AER&PM vs. AER&PM	−0.070 (−0.423 to 0.646)	NS	−0.004 (−0.011 to 0.022)	NS	0.782 vs. 0.782	NS	−0.003 (−0.010 to 0.021)	0.012 (−0.059 to 0.063)
Progression to ESRD								
uADP&AER vs. AER	0.794 (0.451 to 1.097)	0.03	0.115 (0.053 to 0.194)	<0.0001	0.652 vs. 0.472	<0.0001	0.117 (0.056 to 0.200)	0.092 (0.024 to 0.235)
uADP&eGFR vs. eGFR	0.637 (0.311 to 0.994)	<0.001	0.087 (0.030 to 0.186)	<0.0001	0.746 vs. 0.663	<0.0001	0.088 (0.029 to 0.172)	0.070 (0.042 to 0.191)
uADP&PM vs. PM	0.674 (0.261 to 0.978)	<0.001	0.084 (0.027 to 0.171)	<0.0001	0.753 vs. 0.683	<0.0001	0.083 (0.021 to 0.166)	0.034 (0.004 to 0.197)
uADP&AER&PM vs. AER&PM	0.420 (−0.032 to 0.727)	0.061	0.015 (−0.003 to 0.065)	0.052	0.818 vs. 0.805	0.008	0.020 (−0.003 to 0.064)	0.006 (−0.053 to 0.121)

Added predictive benefit was analyzed using continuous NRI, IDI, mean risk difference, and ΔTG, whereas the predictive performance of the Cox proportional hazards models was evaluated by explained variability (R^2). ΔTG, delta standardized total gain; PM, progression model; uADP&AER, Cox model formed by uADP and AER used together; uADP&eGFR, Cox model formed by uADP and eGFR used together; uADP&PM, Cox model formed by uADP and progression model used together; uADP&AER&PM, Cox model formed by uADP, AER, and progression model used together.

independently of the other clinical risk factors but not independently of AER. One possible interpretation of these results might be that uADP and AER share common pathophysiological mechanisms. Similarly to albumin, uADP is a circulating plasma protein filtered at the glomerular level (2,29). Moreover, uADP has been suggested to be a marker of vascular damage in type 2 diabetes (6,30,31)and has been previously related to the preservation of podocyte permeability and maintenance of glomerular barrier integrity (4). The current findings support a potential relationship between AER and uADP because in the present cohort, AER was a constant determinant of uADP levels across all stages of DN. This connection may be one explanation for the lack of independent predictive ability for uADP regarding AER because albuminuria is also an important predictor of progression at the earlier DN stages (32).

In addition to AER, the common determinants of uADP across all stages were HbA_{1c}, L-FABP, and KIM-1. In this regard, uADP could capture another important pathophysiological mechanism of DN on top of glomerular damage: tubular injury due to poor glycemic control. One possible explanation is that hyperglycemia may trigger not only glomerular damage but also tubular dysfunction because ADP may be lost first at the glomerular level, then later in intrarenal arteries/arterioles, and finally at all kidney levels (6). In addition, ADP has been linked to tubular damage and suggested to play a protective role in renal fibrosis (9,33,34). From these findings, we can assume that the initial ADP loss in urine possibly due to hyperglycemia is connected to glomerular dysfunction, whereas the later increase in uADP could be related to tubular dysfunction.

Finally, the study shows low BMI as a significant determinant of uADP levels only in patients with macroalbuminuria, which suggests that uADP also captures renal cachexia. This notion is supported by human studies showing that well-nourished patients undergoing hemodialysis had significantly lower serum ADP levels compared with malnourished patients, whereas in animal studies, high serum ADP levels were associated with increased energy expenditure and weight loss (35–37). Furthermore, ADP measured in the urine was mainly the

LMW isomer and had an unexpected homology with tumor necrosis factor (or cachectin), whereas in type 1 diabetes, the serum ADP increase was based on HMW ADP—the main active protective molecule (6,38,39). These data suggest that the biggest increase in ADP in serum and urine observed at the macroalbuminuric stage could be a protective mechanism against cachexia (6).

This study has some limitations, which in our view do not critically influence the results or their interpretation. One limitation is that we could not provide an analysis by sex, although there were some differences in the uADP concentrations within each sex. However, to diminish the influence of sex on the Cox regression models, all final analyses were adjusted for sex. Another limitation may be that we had performed only a single measurement of ADP concentration in the serum and urine, but it has been suggested previously that plasma ADP levels show only minimal short- and long-term variability and are not markedly influenced by normal daily activity (27). To our knowledge, any major variability of the uADP has not been shown in previously published studies. Unfortunately, we were not able to measure the ADP isoforms, but previous studies have shown that the HMW ADP is the main uADP isoform, whereas the LMW isoform is also present in the urine of patients with diabetes (6,40).

In patients with type 1 diabetes and macroalbuminuria, uADP not only is a strong independent predictor for DN progression to ESRD but also adds significant predictive benefit when used together with either AER or eGFR. This may be due to uADP capturing recognized risk factors for DN progression such as glomerular damage, tubular dysfunction, and glycemic control as well as other factors important for DN progression like cachexia.

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