

## Clinical Study

# Renal Function but Not Asymmetric Dimethylarginine Is Independently Associated with Retinopathy in Type 2 Diabetes

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**Background.** Asymmetric dimethylarginine (ADMA) is associated with macrovascular disease and possibly with microangiopathy in type 2 diabetes (T2DM). We tested the hypothesis that ADMA is related to diabetic retinopathy (DR) independently of macrovascular disease. **Methods.** This cross-sectional study included 127 T2DM patients selected to achieve equal distributions of patients with and without macrovascular disease in the groups with and without DR. **Results.** Patients with DR had increased ADMA, longer diabetes duration, and reduced glomerular filtration rate (GFR). ADMA correlated with GFR ( $\rho = -0.35$ ;  $P < .001$ ), diabetes duration ( $\rho = 0.19$ ;  $P = .048$ ), and age ( $\rho = 0.19$ ;  $P = .033$ ). Logistic regression analysis revealed an association of ADMA with DR. After adjustment for macrovascular disease, this association remained significant (OR 1.48; 95% CI: 1.02–2.15;  $P = .039$ ). Inclusion of GFR and T2DM duration into the model abolished this significant relationship. GFR remained the only independent predictor for DR. A 10 mL/min/1.73 m<sup>2</sup> GFR decrease was associated with DR in a multivariate model (OR 1.30; 95% CI: 1.08–1.56;  $P = .006$ ). **Conclusions.** These findings indicate an association between ADMA and DR in T2DM independent of macrovascular disease. This relationship is modified by GFR, the only parameter significantly related to DR in multivariate analysis.

## 1. Introduction

Hyperglycemia is closely linked to the risk of microvascular complications in diabetes [1]. Patients suffering from type 2 diabetes (T2DM) and diabetic retinopathy are at increased risk for developing cardiovascular disease [1–3]. Asymmetric dimethylarginine (ADMA), an endogenous competitive nitric oxide synthase inhibitor, is an emerging risk marker for future cardiovascular events in different patient groups including patients with T2DM [4]. In addition to its

relation to macrovascular complications, ADMA has also been linked to the development of diabetic microvascular complications. ADMA is elevated in patients with type 1 and type 2 diabetes with diabetic nephropathy and predicts renal impairment in these patients [5–7]. Malecki et al. found increased ADMA concentrations in subjects with T2DM and diabetic retinopathy [8]. However, it remained unclear if macrovascular disease which was associated with significantly elevated circulating ADMA in previous reports influenced this relationship. The current study was planned

to test the hypothesis that ADMA is associated with diabetic retinopathy independently of macrovascular disease and other possible confounders.

## 2. Materials and Methods

This study was conducted in accordance with the guidelines of the Declaration of Helsinki and the institutional ethics committee. It was designed as a cross-sectional study.

One hundred twenty seven patients with T2DM who referred to a specialty outpatient diabetic clinic between September 2003 and March 2004 were included. All patients were suffering from T2DM according to the American Diabetes Association criteria and were on stable antidiabetic treatment for at least 6 months. All patients were treated according to the Guidelines of the American Diabetes Association. At the time of inclusion, demographic and anthropometric data, clinical characteristics, and current medication of all patients were recorded with special attention to present cardiovascular risk factors and comorbidities. Patients were selected with regard to prevalent retinopathy and macrovascular disease to reach a proportion of around 50% patients with retinopathy and 50% patients with macrovascular disease equally distributed between the retinopathy and the no retinopathy group.

Demographic data and medical history including age, sex, smoking habits, hyperlipidemia, arterial hypertension, coronary artery disease, history of myocardial infarction, and stroke were assessed in all patients. The presence of macrovascular disease was confirmed by hospital records. The study included 60 patients with and 67 patients without diabetic retinopathy. Macrovascular disease was present in 38 patients with and in 30 patients without diabetic retinopathy. Body mass index (BMI) was calculated as weight divided by squared height. Blood pressure was determined in all subjects at rest. Arterial hypertension was diagnosed if resting blood pressure values were  $>140/90$  mmHg or patients were taking antihypertensive drugs. All patients were staged for the presence of diabetic retinopathy according to the Early Treatment of Diabetic Retinopathy Study (ETDRS) classification [9–11]. Patients who had retinal abnormalities other than diabetic retinopathy or who underwent panretinal laser treatment, vitrectomy, or anti-VEGF therapy in the past 12 months were excluded. Before retinal photography, the patient's pupils were dilated with tropicamide 1%; this procedure was repeated if the pupils did not reach at least 5 mm in diameter. Color retinal slides with a suitable 40° retinal camera were taken of two fields in both eyes. For the central field, the center of the optic disc was positioned at the nasal end of the horizontal meridian of the field of view; for the nasal field, the optic disc was positioned 1 disc diameter from the temporal edge of the field on the horizontal meridian. Two pseudostereoscopic images with a horizontal shift of 3° to 5° were then taken of each field in both eyes, resulting in eight images of each patient. The overlap of the fields recorded a retinal view of approximately 75° horizontally by 40° vertically; therefore, clinically significant lesions of diabetic retinopathy were easily detectable. Slides

were separately screened by two experienced graders in the same center, according to a validated protocol [12].

**2.1. Laboratory Investigations.** Venous blood was drawn after an overnight fast for the determination of ADMA, SDMA, L-arginine, and routine parameters. Blood levels of creatinine, HbA1c, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, and high-sensitivity C-reactive protein (CRP) were measured by standard laboratory methods in a certified laboratory. Glomerular filtration rate was calculated using the Modification of Diet in Renal Disease Study Group formula [13]. For measurement of ADMA, SDMA, and L-arginine, plasma was subjected to cation exchange solid-phase extraction and analyzed by high-performance liquid chromatography [14, 15]. The coefficients of variation for inter- and intra-assay variations tested with a pooled plasma sample were  $<3\%$  for all analytes. The detection limit for dimethylarginines was  $0.04 \mu\text{mol/L}$ .

**2.2. Statistics.** Continuous data are presented as medians (interquartile range). Categorical data are given as counts (%). Mann-Whitney  $U$  test was applied for univariate comparison of continuous data and Spearman rank correlation for assessment of associations between continuous variables. Skewed variables were log-transformed for regression analyses. Univariate and multivariate logistic regression analysis including diabetic retinopathy as dependent variable and log-transformed ADMA as well as possible confounders as independent variables was used. To assess the effect of ADMA on the prevalence of diabetic retinopathy, odds ratios (OR) and 95% confidence intervals (95% CI) for a 1 standard deviation (SD) increment in log-transformed ADMA were calculated. Variables associated with ADMA and diabetic retinopathy including the presence of macrovascular disease were treated as possible confounders in the regression analysis. Age and sex were excluded from the logistic regression analysis that included GFR, because these variables are already used in the MDRD formula. A two-sided  $P < .05$  was considered as statistically significant. Calculations were performed with SPSS for Windows (Version 16.0; SPSS, Chicago, Ill).

## 3. Results

The characteristics of the study population are presented in Table 1. Importantly, the proportion of patients with and without macrovascular disease was comparable in patients with and without diabetic retinopathy. Patients with diabetic retinopathy had significantly higher levels of ADMA and SDMA compared to those without. L-arginine was comparable between the groups. In addition, these patients had longer diabetes duration, lower GFR, and higher HDL-cholesterol. They were less likely to be smokers and tended to be older than patients without retinopathy (Table 1).

ADMA did not differ significantly between women and men ( $0.61$  ( $0.52$ – $0.93$ )  $\mu\text{mol/L}$  versus  $0.59$  ( $0.50$ – $0.68$ )  $\mu\text{mol/L}$ ;  $P = .207$ ) as well as smokers and non-smokers ( $0.58$  (IQR:  $0.50$ – $0.76$ )  $\mu\text{mol/L}$  versus  $0.58$  ( $0.49$ – $0.69$ )  $\mu\text{mol/L}$ ;  $P = .568$ ). ADMA was significantly related to

TABLE 1: Characteristics, L-Arginine, and asymmetric and symmetric dimethylarginine according to the prevalence of diabetic retinopathy.

	No retinopathy	Retinopathy	P value
N	60	67	
Female	19 (31.7%)	30 (44.8%)	.130
Proliferative retinopathy		32 (47.8%)	
Macrovascular Disease	30 (50.0%)	38 (56.7%)	.449
Smokers	25 (42.4%)	10 (19.2%)	.009
Age (years)	62 (54–69)	64 (56–73)	.051
Diabetes duration (years)	9 (4–16)	14 (10–17)	.027
HbA1c (%)	8.0 (7.0–9.0)	7.4 (7.0–8.1)	.127
Body mass index (kg/m <sup>2</sup> )	29.2 (26.4–32.3)	29.7 (25.8–34.2)	.529
Systolic blood pressure (mmHg)	141 (134–157)	144 (129–163)	.891
Diastolic blood pressure (mmHg)	83 (77–90)	80 (73–91)	.164
Total cholesterol (mg/dL)	188 (157–213)	193 (164–218)	.686
LDL cholesterol (mg/dL)	94 (74–129)	90 (69–116)	.300
HDL cholesterol (mg/dL)	44 (36–50)	50 (43–56)	.020
Triglycerides (mg/dL)	175 (115–265)	194 (147–284)	.276
Glomerular filtration rate (mL/min/1.73 m <sup>2</sup> )	79 (59–100)	59 (43–78)	<.001
L-Arginine (μmol/L)	74 (62–94)	82 (63–119)	.359
Asymmetric dimethylarginine (μmol/L)	0.55 (0.48–0.67)	0.62 (0.53–0.96)	.011
Symmetric dimethylarginine (μmol/L)	0.49 (0.43–0.63)	0.61 (0.48–0.92)	.005

Categorical variables are presented as counts (%) and continuous variables are presented as medians (interquartile range); the  $\chi^2$ -test and Mann-Whitney *U* test have been applied for comparisons between the groups.

SDMA, L-arginine, age, diabetes duration, and glomerular filtration rate in the whole study cohort. No association between ADMA and other clinical parameters could be observed (Table 2).

The univariate logistic regression analysis showed that ADMA was significantly associated with the prevalence of diabetic retinopathy before and after adjusting for macrovascular disease. However, including age and sex into the multivariate model raised the *P*-level of this association above the level of significance. Multivariate adjustment for prevalence of macrovascular disease, diabetes duration, and GFR completely abolished the association between ADMA and diabetic retinopathy (Table 3).

Because of the significant independent association between GFR and diabetic retinopathy, a secondary analysis including GFR as dependent variable was performed. GFR was significantly related to SDMA, L-arginine, diabetes duration, and HbA1c (Table 4). GFR was comparable between women and men (60 (52–86) mL/min/1.73 m<sup>2</sup> versus 72 (52–93) mL/min/1.73 m<sup>2</sup>; *P* = .255) as well as smokers and nonsmokers (72 (43–72) mL/min/1.73 m<sup>2</sup> versus 67 (53–90) mL/min/1.73 m<sup>2</sup>; *P* = .697). Both the univariate and multivariate logistic regression models showed that GFR was the only parameter independently related to the prevalence of diabetic retinopathy (Table 5).

#### 4. Discussion

The results of the present study show that circulating ADMA is related to diabetic retinopathy independently of macrovascular disease. These findings do not confirm the

TABLE 2: Spearman correlations of ADMA with other variables.

	$\rho$	P value
Age	0.189	.033
Diabetes duration	0.190	.048
HbA1c	−0.079	.412
Body mass index	0.016	.870
Systolic blood pressure	−0.133	.172
Diastolic blood pressure	−0.183	.060
Total cholesterol	−0.068	.539
LDL cholesterol	−0.040	.727
HDL cholesterol	0.024	.830
Triglycerides	−0.141	.203
Glomerular filtration rate	−0.347	<.001
L-Arginine	0.548	<.001
Symmetric dimethylarginine	0.633	<.001

hypothesis that this association is independent of other possible confounders. Interestingly, GFR was shown to be the only independent predictor for the prevalence of diabetic retinopathy in this cross-sectional analysis.

The finding that ADMA is not independently related to diabetic retinopathy in patients with T2DM was surprising regarding the recently published data by Malecki et al. [8]. Nevertheless this study missed to evaluate the prevalence of macrovascular disease which would be important. This shortfall could introduce bias, as ADMA is known to be related to macrovascular disease [4]. Macrovascular disease itself has been shown to be related to diabetic retinopathy

TABLE 3: Univariate and multivariate logistic regression analysis including diabetic retinopathy as dependent variable and asymmetric dimethylarginine (ADMA) and possible confounders as independent variables.

		Odds ratio (95% CI)	P value
Univariate model	ADMA (per 1 SD log increase)	1.50 (1.03–2.17)	.034
Multivariate model 1	ADMA (per 1 SD log increase)	1.48 (1.02–2.15)	.039
	MVD versus no MVD	1.22 (0.60–2.50)	.581
Multivariate model 2	ADMA (per 1 SD log increase)	1.36 (0.93–2.00)	.113
	Age	1.04 (1.00–1.07)	.078
	Sex (male versus female)	0.70 (0.33–1.52)	.370
	MVD versus no MVD	1.27 (0.61–2.64)	.522
Multivariate model 3	ADMA (per 1 SD log increase)	1.17 (0.73–1.90)	.518
	DM2 duration (per 1 SD log increase)	1.27 (0.80–2.01)	.306
	GFR (per 10 mL/min/1.73 m <sup>2</sup> decrease)	1.30 (1.08–1.56)	.006
	MVD versus no MVD	0.88 (0.37–2.10)	.774

GFR: glomerular filtration rate calculated with the MDRD formula; 95% CI: 95% confidence interval; DM2: diabetes mellitus type 2; MVD: macrovascular disease.

TABLE 4: Spearman correlations of GFR with other variables.

	$\rho$	P value
Diabetes duration	−0.266	.006
HbA1c	0.307	.001
Body mass index	0.085	.402
Systolic blood pressure	−0.017	.862
Diastolic blood pressure	0.247	.011
Total cholesterol	0.077	.492
LDL cholesterol	0.128	.268
HDL cholesterol	−0.136	.233
Triglycerides	0.060	.590
L-Arginine	−0.252	.005
Symmetric dimethylarginine	−0.684	<.001

as shown by Reaven et al. [16]. For this reason, the present investigation included patients with and without retinopathy who were carefully selected with regard to the prevalence of macrovascular disease. Nearly equal proportions of patients had macrovascular disease in the groups with and without retinopathy. Macrovascular disease was not a major confounder and did not abolish the significant association between ADMA and retinopathy in the multivariate regression analysis. These results differ from those reported by Malecki et al. due to the effects of sex, age, and GFR that eliminated the significant relationship between retinopathy and ADMA. GFR was the best independent predictor for the prevalence of retinopathy in our study. Interestingly, calculated creatinine clearance did not differ between patients with and without retinopathy in the study by Malecki et al. which included diabetic subjects with better renal function [8]. Only 22 patients had GFR > 90 mL/min/1.73 m<sup>2</sup> in the present study. It is possible that ADMA is independently related to retinopathy only in patients with normal renal function. The notion that moderate renal failure leads to a significant ADMA increase [17] could at least partly

explain the discrepancies between the reported findings. In addition, our study included 32 patients with T2DM and proliferative retinopathy, which reflects more prevalence of serious microvascular disease compared with the study population investigated by Malecki et al., which included only 3 patients with proliferative retinopathy [8]. Proliferative diabetic retinopathy and impaired renal function are often interrelated in patients with T2DM [18, 19]. These findings and the fact that both study cohorts differed substantially regarding the included number of patients with proliferative retinopathy provide another potential explanation for the discrepant results between the studies. In a large number of patients with type 1 diabetes, Tarnow et al. [5] did not observe a relationship between ADMA and simplex or proliferative diabetic retinopathy, findings which are in accordance with our results obtained in patients with T2DM.

Nevertheless, the divergent findings on circulating ADMA and diabetic retinopathy do not exclude a pathophysiologic role of ADMA for the development of diabetic retinopathy. As published by Sugai et al., evidence exists that ADMA concentrations in aqueous humor are associated with severe diabetic retinopathy. Nevertheless, it was also mentioned in this report that ADMA concentrations found in aqueous humor are not related to circulating ADMA [20]. This could be another explanation why circulating ADMA was not associated with diabetic retinopathy. Intraocular but not circulating ADMA might be of pathophysiologic relevance for diabetic retinopathy.

From the present data, it could be assumed that renal dysfunction, which is often found in patients with T2DM, is an important factor associated with diabetic retinopathy. This is in line with findings from previous studies, which showed a significant and independent association between retinopathy and declining renal function in T2DM. Moreover, it was shown that diabetic retinopathy can predict the deterioration of renal function [21, 22]. This suggests that reduced GFR rather appears after than before the development of retinopathy. As GFR significantly influences



TABLE 5: Univariate and multivariate logistic regression analysis including diabetic retinopathy as dependent variable and glomerular filtration rate (GFR) and possible confounders as independent variables.

		Odds ratio (95% CI)	P value
Univariate model	GFR (10 mL/min/1.73 m <sup>2</sup> decrease)	1.32 (1.13–1.54)	.001
	GFR (10 mL/min/1.73 m <sup>2</sup> decrease)	1.30 (1.08–1.56)	.006
Multivariate model	ADMA (per 1 SD log increase)	1.17 (0.73–1.90)	.518
	DM2 duration (per 1 SD log increase)	1.27 (0.80–2.01)	.306
	MVD versus no MVD	0.88 (0.37–2.10)	.881

GFR: glomerular filtration rate calculated with the MDRD formula; 95% CI: 95% confidence interval; DM2, diabetes mellitus type 2.

circulating ADMA, it is likely that elevated ADMA in patients with diabetic retinopathy appeared after the emergence of diabetic retinopathy in our study cohort.

The present investigation is limited by the cross-sectional study design which does not allow statements on causality. The strength of the present study design is the high prevalence of macrovascular disease in patients with and without retinopathy. This allowed the evaluation of the effect of macrovascular disease on the relationship between ADMA and diabetic retinopathy. Although no independent relationship between ADMA and the prevalence of diabetic retinopathy could be detected in this study, a pathophysiological link between ADMA, impaired renal function, and diabetic retinopathy cannot be excluded. Further studies are needed to evaluate the influence of ADMA on microvascular complication.

## 5. Conclusion

In conclusion, this study demonstrated that ADMA is associated with retinopathy in patients with T2DM independent of macrovascular disease. This effect is substantially modified by adjusting for GFR which remained the single independent parameter related to diabetic retinopathy in a logistic regression analysis. Our findings cannot support the hypothesis that circulating ADMA is independently associated with diabetic retinopathy but do not exclude that ADMA lies in the causative path between renal dysfunction and diabetic retinal disease.

## Conflict of Interests

The authors declared that there is no conflict of interests.

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