Plasma Asymmetric Dimethylarginine (ADMA) Is Associated With Retinopathy in Type 2 Diabetes

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Decreased availability of nitric oxide (NO), which contributes to the development of diabetes vascular complications (1), is partially related to asymmetric dimethylarginine (ADMA). ADMA is an endogenous NO synthase inhibitor (2) and a competitive inhibitor of cellular L-arginine uptake (3). ADMA has been associated with atherosclerosis in nondiabetic populations (4) and with diabetic nephropathy in type 1 diabetes (5). In humans, the stereoisomer of ADMA, symmetric dimethylarginine (SDMA), is produced in equivalent quantities; though it does affect NO synthesis, SDMA may compete with arginine for cellular uptake (6). Our objective was to evaluate the relationship between plasma ADMA and diabetic retinopathy in type 2 diabetes.

RESEARCH DESIGN AND METHODS

— We examined 182 consecutive type 2 diabetic patients (mean age at examination 56.2 ± 6.5 years). Previously described inclusion criteria were used (7). Biochemical measurements were done using standard methods. Creatinine clearance was calculated according to the Cockroft-Gault formula. The control group consisted of 52 apparently healthy individuals matched for age and sex (mean age 54.5 ± 7.1 years). This study was approved by the local ethics committee.

All type 2 diabetic patients underwent ophthalmological evaluation. Color fundus photographs were taken as previously described (8). The final diabetic retinopathy diagnosis was based on both ophthalmoscopy and photography (Protocol of Ophthalmological Examination can be found in an online appendix (available at dx.doi.org/10.2337/dc07-1138) (9). The patients were assigned to one of three groups: *1*) no diabetic retinopathy, *2*) nonproliferative diabetic retinopathy, or *3*) proliferative diabetic retinopathy.

Plasma ADMA, SDMA, and L-arginine levels were measured by high-performance liquid chromatography (10).

We used logistic regression to study the association of ADMA, SDMA, and L-arginine with diabetic retinopathy and multivariable logistic regression to analyze whether clinical variables (sex, age of examination, duration of type 2 diabetes, A1C, arterial hypertension, lipids, BMI, and creatinine clearance) modify the odds of diabetic retinopathy occurrence. Variables independently associated with diabetic retinopathy were identified using a stepwise selection procedure. A P value <0.05 was considered statistically significant. Model fit was evaluated using the Bayesian information criterion.

RESULTS

— Nonproliferative diabetic retinopathy was diagnosed in 68 (37.3%) and proliferative diabetic retinopathy in 3 (1.6%) subjects. Both diabetic retinopathy groups were combined for further analyses. Characteristics of type 2 diabetic patients with and without diabetic retinopathy are available in Supplementary Table 1.

The ADMA level was highest in type 2 diabetic patients with diabetic retinopathy (0.60 ± 0.06 μmol/l), intermediate in those without diabetic retinopathy (0.51 ± 0.06 μmol/l), and lowest among control subjects (0.45 ± 0.05 μmol/l; $P <$ 0.001 for all comparisons). Similarly, SDMA was higher in subjects with than without diabetic retinopathy (0.45 ± 0.06 vs. 0.41 ± 0.06 μmol/l, respectively; $P < 0.001$) and lower in control subjects than in both diabetic groups (0.36 ± 0.06 μmol/l; $P < 0.001$ for both comparisons). In contrast, L-arginine levels were similar in the diabetic groups (0.80 ± 0.14 vs. 0.79 ± 0.13 μmol/l for those with and without diabetic retinopathy, respectively; $P = 0.38$); however, they were higher than in the control group (0.64 ± 0.08 μmol/l; $P < 0.0001$) (Fig. 1). Levels of ADMA, SDMA, and L-arginine were significantly correlated.

In search of a potential association among the three studied analytes and diabetic retinopathy, we checked whether their combination could form a better predictor than each of them separately. L-Arginine, both in combination and alone, was not associated with diabetic retinopathy. However, SDMA was associated with diabetic retinopathy in univariate analysis (odds ratio [OR] 1.12 [95% CI 1.06–1.18], $P < 0.0001$) but not when both ADMA and SDMA were incorporated into the model ($P = 0.35$). ADMA was the only variable consistently associated with diabetic retinopathy, both in univariate analysis (1.39 [1.26–1.53], $P < 0.0001$) and in combination with the other two analytes (1.44 [1.28–1.61], $P < 0.0001$). Thus, we used ADMA in the modeling in further analyses.

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Abbreviations: ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

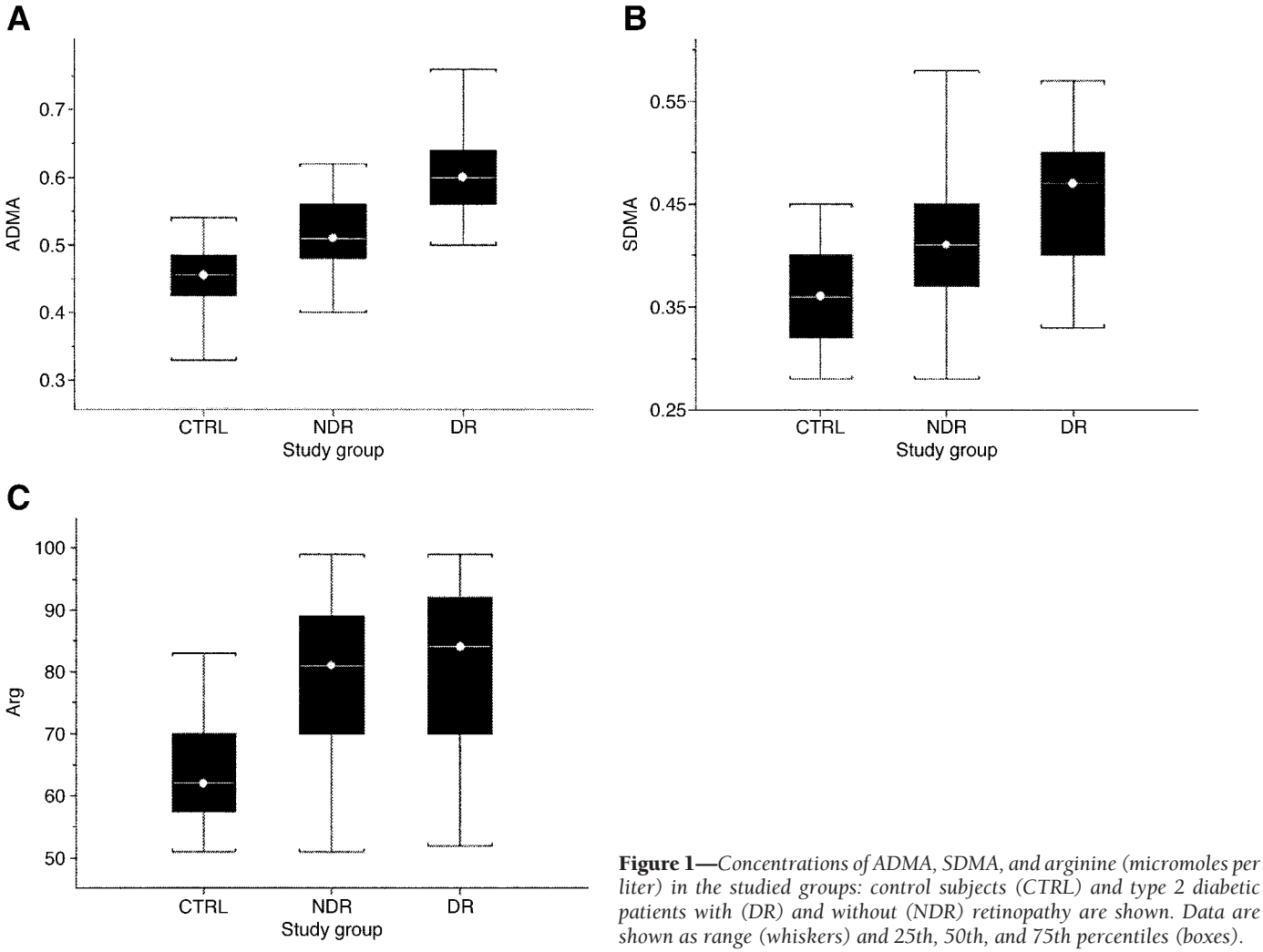

Figure 1—*Concentrations of ADMA, SDMA, and arginine (micromoles per liter) in the studied groups: control subjects (CTRL) and type 2 diabetic patients with (DR) and without (NDR) retinopathy are shown. Data are shown as range (whiskers) and 25th, 50th, and 75th percentiles (boxes).*

In a multivariable logistic regression with stepwise variable selection, plasma ADMA level was selected as a predictor of the presence of diabetic retinopathy (OR 1.80 [95% CI 1.47–2.19], $P < 0.0001$) in type 2 diabetic patients, in addition to age at examination (0.85 [0.75–0.95], $P = 0.0048$), creatinine clearance (1.24 [1.05–1.46], $P = 0.0116$), and insulin therapy (7.0 [2.08–23.61], $P = 0.0017$). Studied variables were not independent. Not unexpectedly, ADMA concentration, creatinine clearance, and age at examination were all correlated to each other, with ADMA positively correlated with age at examination ($r = 0.5$, $P < 0.0001$) and negatively correlated with creatinine clearance ($r = -0.4$, $P < 0.0001$).

ADMA also remained (OR 1.77 [95% CI 1.44–2.17], $P < 0.0001$) a significant independent predictor of the presence of diabetic retinopathy in a broader multivariable model with forced inclusion of well-proven risk factors of diabetic retinopathy into a regression equation (Supplementary Table 2).

CONCLUSIONS — This is the first report suggesting an association between elevated circulating ADMA levels and diabetic retinopathy in type 2 diabetes. Elevated ADMA levels in aqueous humor were recently described in patients with severe diabetic retinopathy (11). In type 1 diabetic subjects, there was no difference in plasma ADMA levels between patients with and without diabetic retinopathy (5). The reasons for this discrepancy might be, at least in part, associated with resistance to insulin action present in type 2 diabetes. There is evidence that ADMA may be both a result and a cause of insulin resistance (6). Interestingly, several reports indicate that resistance to insulin plays a role in the pathogenesis of diabetic retinopathy (12). Thus, one might speculate that the putative ADMA role in the pathogenesis of diabetic retinopathy is more prominent in type 2 than type 1 diabetes.

There is evidence that increased ADMA is typical of chronic renal failure. Thus, one might expect that high ADMA levels in diabetic retinopathy subjects reflect a phenomenon of the coexistence of diabetic retinopathy and diabetic nephropathy (13). The mean creatinine levels were, however, within the normal range in both diabetic groups. The unexpected apparently protective effects of age at examination and decreased creatinine clearance require comment. In the presence of colinear covariates, modeling is often a trade-off between a biased estimate with relatively high precision (when colinear covariates are removed from the model) and a less biased estimate with lower precision (with colinear covariates included) (14). It is, however, important to note that in a stepwise selection or in forced inclusion models that contained creatinine clearance and/or age at exami-

nation, association of ADMA with retinopathy became even stronger.

The potential limitation of our study is that ADMA was measured at a single time point. Thus, it did not answer the question whether elevated ADMA levels stimulate the development of diabetic retinopathy or merely constitute its marker. Prospective studies are necessary to clarify this.

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