

Diabetic Retinopathy Is Associated With Elevated Serum Asymmetric and Symmetric Dimethylarginines

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OBJECTIVE — Asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), and L-arginine directly influence nitric oxide production. Our objective was to test whether serum ADMA, SDMA, or L-arginine levels correlate with diabetic retinopathy subtype or severity.

RESEARCH DESIGN AND METHODS — A total of 162 subjects with type 1 diabetes and 343 with type 2 diabetes, of whom 329 subjects had no diabetic retinopathy, 27 had nonproliferative diabetic retinopathy (NPDR), 101 had proliferative diabetic retinopathy (PDR), and 107 had clinically significant macular edema (CSME), were recruited. Blinding diabetic retinopathy was defined as severe NPDR, PDR, or CSME. Serum ADMA, SDMA, and L-arginine concentrations were determined by mass spectroscopy.

RESULTS — In multivariate analysis, blinding diabetic retinopathy, PDR, and nephropathy were associated with significantly increased serum levels of ADMA ($P < 0.001$), SDMA ($P < 0.001$), and L-arginine ($P = 0.001$). Elevated ADMA ($P < 0.001$) and SDMA ($P < 0.001$) were also significantly associated with CSME.

CONCLUSIONS — Severe forms of diabetic retinopathy are associated with elevated serum ADMA, SDMA, and L-arginine. Further investigation is required to determine whether these findings are of clinical relevance.

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Endothelial dysfunction and impaired ocular hemodynamics underlying diabetic retinopathy development are associated with decreased nitric oxide (NO) synthase activity and NO bioavailability, resulting in vasoconstriction and increased reactive oxygen species (1). Serum asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), and L-arginine are involved in the NO pathway, directly influencing NO production. This study investigated the association between diabetic retinopathy subtypes and serum levels of ADMA, SDMA, and L-arginine in an Australian co-

hort of 505 subjects with type 1 or type 2 diabetes.

RESEARCH DESIGN AND METHODS

Subjects were recruited from ophthalmology and endocrinology outpatient clinics of three tertiary hospitals in Adelaide, South Australia. Ethics approval was obtained from the relevant Human Research Ethics Committees. The cohort consisted of 162 subjects with type 1 and 343 with type 2 diabetes. Retinopathy status for the worst eye was graded according to the Early Treatment and Diabetic Retinopathy Study criteria (2). If

either eye had clinically significant macular edema (CSME), irrespective of other diabetic retinopathy gradings, the patient was also classified as having CSME. Blinding retinopathy was defined as severe nonproliferative diabetic retinopathy (NPDR), proliferative diabetic retinopathy (PDR), or CSME.

Blood pressure and BMI were measured. Renal function tests (serum creatinine, urine albumin, and albumin-to-creatinine ratio), serum cholesterol, and A1C levels (mean of three recent levels) were obtained. Patients were classified as hypertensive if they were on antihypertension medication or had a blood pressure $\geq 140/90$ mmHg at recruitment. Hypercholesterolemia was defined as total cholesterol >5.5 mmol/l or current use of lipid-lowering medication. Nephropathy was defined as urine albumin ≥ 30 mg/day.

Serum concentrations of arginine and its dimethylated metabolites ADMA and SDMA were determined by liquid chromatography–tandem mass spectrometry of the butyl esters (3) on an Applied Biosystems 3200 Q-Trap instrument (Applied Biosystems, Scoresby, Victoria).

Statistical analyses were undertaken in SPSS (version 15.0; SPSS, Chicago, IL). A P value <0.05 was considered significant. Baseline clinical characteristics of case and control subjects were compared using the t test or χ^2 tests as appropriate. Serum ADMA, SDMA, and L-arginine concentrations were log transformed, and association with diabetic retinopathy was assessed by a hierarchical multiple regression procedure for multivariate analysis.

RESULTS — Of 505 participants, 330 had no diabetic retinopathy (105 of whom were type 1 and 225 type 2 diabetic) and 175 were classified as having blinding diabetic retinopathy (57 type 1 and 118 type 2 diabetic). In the blinding diabetic retinopathy group, 27 had severe NPDR (4 type 1 and 23 type 2 diabetic), 101 PDR (42 type 1 and 59 type 2 diabetic), and 108 CSME (26 type 1 and 82 type 2 diabetic).

Disease duration, sex, age, hypertension, hypercholesterolemia, nephropathy

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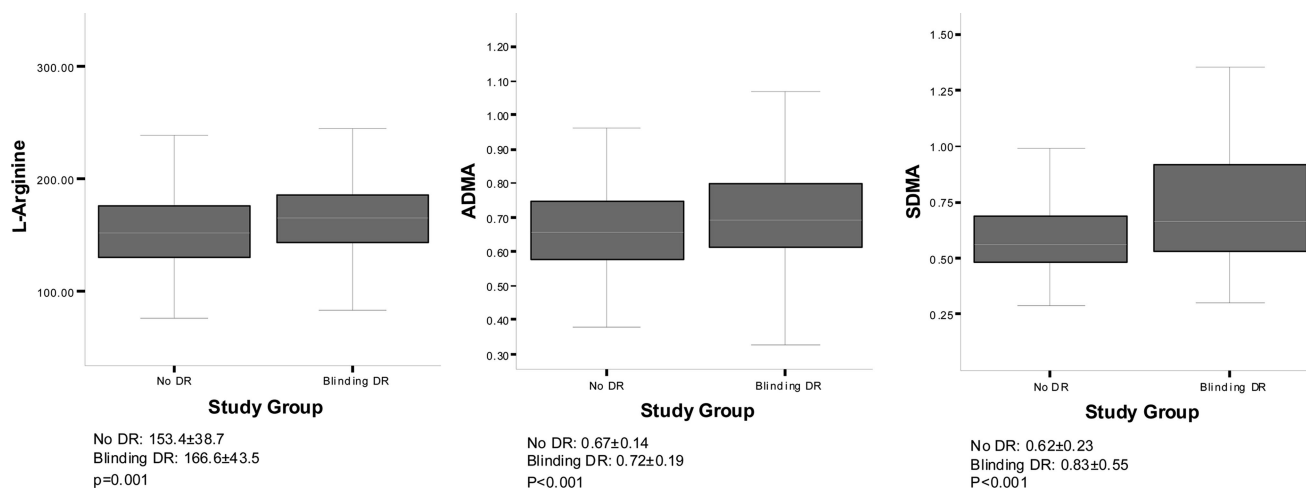


Figure 1—Box plots of untransformed concentrations of L-arginine, ADMA, and SDMA ($\mu\text{mol/l}$) in all subjects without diabetic retinopathy (DR) ($n = 330$) and blinding diabetic retinopathy ($n = 175$) are shown, regardless of type of diabetes. Data are shown as the 25th, 50th, and 75th percentiles (represented by gray boxes), range (shown as whiskers; outliers have been removed), and the median (white horizontal line). Means \pm SD and adjusted P values for each analyte are provided under the corresponding box plot. P values have been adjusted for type of diabetes, diabetes duration, age, hypertension, hypercholesterolemia, and nephropathy.

thy, and BMI were significantly correlated with diabetic retinopathy ($P < 0.05$). Blinding diabetic retinopathy (Fig. 1) and PDR were strongly associated with elevated serum ADMA ($P < 0.001$), SDMA ($P < 0.001$), and L-arginine ($P = 0.001$) after adjustment for associated covariates. In type 1 diabetic subjects, blinding diabetic retinopathy was associated with significantly increased ADMA ($P < 0.001$) and SDMA ($P < 0.001$). In patients with type 1 diabetes and PDR, there was a strong association with ADMA ($P < 0.001$) and SDMA ($P < 0.001$) and a borderline association with L-arginine ($P = 0.04$). In type 2 diabetic subjects, both blinding diabetic retinopathy and PDR were significantly associated with elevated ADMA and SDMA ($P = 0.013$ and $P < 0.001$, respectively, for blinding diabetic retinopathy and $P = 0.014$ and $P < 0.001$, respectively, for PDR). CSME was significantly associated with elevated ADMA ($P < 0.001$) and SDMA ($P < 0.001$) when both types of diabetes were combined. However, in subjects with type 1 diabetes alone, only SDMA showed a significant elevation ($P < 0.001$), and no significant association of the analytes with CSME in type 2 diabetic subjects was found.

Age, disease duration, hypertension, BMI, hypercholesterolemia, smoking, and diabetic retinopathy were found to be significantly correlated with nephropathy ($P < 0.05$). Nephropathy was associated with ADMA ($P < 0.001$), SDMA ($P < 0.001$), and L-arginine ($P = 0.001$) after

adjustment for associated covariates. All three analytes were associated with nephropathy in type 1 diabetes (ADMA, $P < 0.001$; SDMA, $P < 0.001$; L-arginine, $P = 0.034$). However, only ADMA ($P = 0.03$) and SDMA ($P < 0.001$) were associated with nephropathy in type 2 diabetes.

The mean levels of all three analytes in participants with blinding diabetic retinopathy (but with nephropathy subjects excluded [$n = 110$]) were compared with the mean levels in those with nephropathy (but with blinding diabetic retinopathy subjects excluded [$n = 68$]), and no significant differences were found ($P > 0.5$).

CONCLUSIONS—ADMA, SDMA, and L-arginine are involved in the production of NO, a key player in both microvascular damage pathogenesis and diabetic retinopathy (1). We found that all three are significantly elevated in patients with blinding diabetic retinopathy and PDR, irrespective of diabetes type. This study is the first to report an association between elevated levels of ADMA and SDMA with CSME.

Four previous studies investigated serum ADMA levels in diabetic retinopathy (4–7). Three reported elevation of ADMA in diabetic retinopathy participants (4–6). Only Malecki et al. (5) assessed the association of both SDMA and L-arginine with diabetic retinopathy in type 2 diabetes, finding an association of SDMA with diabetic retinopathy. Tarnow et al. (7) found that ADMA levels were not signifi-

cantly increased in any form of diabetic retinopathy in 600 subjects with type 1 diabetes. Our study was deliberately enriched with subjects with blinding diabetic retinopathy so differences in diabetic retinopathy phenotype affecting study power may be factors in the comparison.

The effect of nephropathy on diabetic retinopathy (8,9) could potentially be mediated by elevated dimethylarginines because all three analytes are renally cleared and ADMA and SDMA are elevated by reduced renal clearance (7,10). We observed a significant association of all three analytes with nephropathy. Serum SDMAs in patients with nephropathy, especially end-stage nephropathy, are known to be markedly higher than ADMAs (10,11). Similarly, we found higher SDMA levels compared with ADMA levels in participants with nephropathy in addition to retinopathy. One possibility is that decreased renal clearance of these analytes may lead to elevated serum concentrations directly impacting diabetic retinopathy development. Other factors that could influence ADMA include hyperglycemia-induced inhibition of dimethylarginine dimethylaminohydrolase, which degrades ADMA (12); the effects of insulin resistance (13); or medications, including oral hypoglycemic agents (13,14) and ACE inhibitors (15). Further prospective and functional studies are required to investigate the clinical and pathological significance of elevated ADMA, SDMA, and L-arginine in

diabetic retinopathy development and the relationship with nephropathy.

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