

Synergistic Association of Metabolic Syndrome and Overt Nephropathy With Elevated Asymmetric Dimethylarginine in Serum and Impaired Cutaneous Microvasodilation in Patients With Type 2 Diabetes

RURIKO YAMAMOTO, MD
YOSHIMASA ASO, MD

Chronic kidney disease (CKD) is a major risk factor for cardiovascular disease as well as end-stage renal disease (1). Diabetic nephropathy is an important cause and exacerbating factor in CKD. Recent studies have demonstrated a close relationship between prevalence of the metabolic syndrome and CKD (2,3). Microvascular endothelial dysfunction resulting from impaired nitric oxide biosynthesis and bioavailability is associated with occurrence of metabolic syndrome and type 2 diabetes (4,5). de Jongh et al. (6) found cutaneous microvascular function to be related to degree of insulin resistance in obese women, suggesting that microvascular endothelial dysfunction in the skin can predict metabolic syndrome-related microangiopathy and insulin resistance. Thus, microvascular endothelial dysfunction may be a causal factor linking metabolic syndrome and CKD.

Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, causes vasoconstriction and limits blood flow, resulting in elevated blood pressure in humans (7,8). Elevated plasma ADMA concentrations are found in patients with renal insufficiency (9), diabetes (10), and metabolic syndrome (11). Accordingly, increased plasma ADMA may contribute to micro-

vascular endothelial dysfunction observed in individuals with metabolic syndrome or with CKD.

We examined cutaneous microvascular responses to heat applied locally to the skin of the dorsum of the foot in type 2 diabetic patients with or without metabolic syndrome or overt nephropathy, measuring cutaneous blood flow by laser Doppler flowmetry as well as serum ADMA concentration.

RESEARCH DESIGN AND METHODS

— We studied 105 type 2 diabetic patients (41 female and 64 male). We excluded patients who had any known CKD before diagnosis with diabetes.

Diagnosis of metabolic syndrome was based on National Cholesterol Education Panel-Adult Treatment Panel III criteria (12). We used a BMI cutoff value >25.0 kg/m² to define obesity (13), since waist circumference is unsuitable for detection of obesity in a Japanese population.

Overt nephropathy was defined as clinical proteinuria and/or a creatinine clearance <60 ml/min. We defined clinical proteinuria as urinary albumin excretion >300 mg/24 h. As an index of glomerular filtration rate, creatinine clearance was calculated using the same 24-h urine collection.

On the basis of the two definitions above, we divided the diabetic patients into four groups: group A, those with no metabolic syndrome and no overt nephropathy ($n = 38$); group B, those with metabolic syndrome but no overt nephropathy ($n = 32$); group C, those with no metabolic syndrome but with overt nephropathy ($n = 16$); and group D, those with both metabolic syndrome and overt nephropathy ($n = 19$). Cardiovascular disease was defined as coronary artery disease, stroke, and/or peripheral vascular disease.

Skin blood flow measurements

Cutaneous blood flow (flux) was recorded on the dorsum of the right foot using laser Doppler flowmetry (Perimed PF4; Perimed, Stockholm, Sweden). A thermostatic laser Doppler probe, which heats the skin locally, was placed on the dorsum of the right foot. Local temperature was increased gradually from 32°C to 44°C (14). Change in skin blood flow (Δ flux) was defined as: peak flux at 44°C (in perfusion units [PU]) – basal flux (in PU).

The ankle-brachial index was determined as the ratio of ankle systolic blood pressure to brachial systolic blood pressure (Colin Medical Technology, Komaki, Japan).

Serum concentrations of ADMA were measured by high-performance liquid chromatography. Plasma thrombomodulin, a marker of endothelial injury (15), was measured by an enzyme immunoassay (Fujirebio, Tokyo, Japan).

Data are presented as the mean \pm SD or the median (interquartile range), unless otherwise indicated. Differences were assessed by ANOVA or the Kruskal-Wallis test. Significance of differences in prevalence between groups was analyzed by a χ^2 test. A P value <0.05 was accepted as indicating statistical significance.

From the Department of Internal Medicine, Koshigaya Hospital, Dokkyo Medical University, Saitama, Japan.

Address correspondence and reprint requests to Yoshimasa Aso, MD, Department of Internal Medicine, Koshigaya Hospital, Dokkyo Medical University, 2-1-50 Minami-Koshigaya, Koshigaya, Saitama 343-8555, Japan. E-mail: yaso@dokkyomed.ac.jp.

Received for publication 26 December 2005 and accepted in revised form 14 January 2006.

Abbreviations: ADMA, asymmetric dimethylarginine; CKD, chronic kidney disease.

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

© 2006 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Table 1—Patient characteristics and laboratory data in diabetic groups categorized according to the presence of metabolic syndrome and overt nephropathy

Variable	Group A: no metabolic syndrome/ no overt nephropathy	Group B: metabolic syndrome/ no overt nephropathy	Group C: no metabolic syndrome/ overt nephropathy	Group D: metabolic syndrome/ overt nephropathy
n (female/male)	38 (14/24)	32 (18/14)	16 (6/10)	19 (4/15)
Age (years)	62.6 ± 9.7	56.8 ± 13.5	62.6 ± 10.8	60.5 ± 10.8
BMI (kg/m ²)	21.6 ± 2.6	27.6 ± 5.6†	22.9 ± 3.5§	26.2 ± 2.7†¶
Diabetes duration (years)	12.6 ± 9.0	9.9 ± 7.1	11.9 ± 7.2	12.3 ± 7.9
Fasting plasma glucose (mmol/l)	9.8 ± 2.8	9.6 ± 3.3	9.8 ± 3.5	9.8 ± 3.2
HbA _{1c} (%)	10.2 ± 2.0	9.5 ± 1.8	9.3 ± 1.7	9.6 ± 1.6
Total cholesterol (mmol/l)	4.87 ± 0.83	5.22 ± 0.73	5.39 ± 1.36	5.29 ± 0.80
Triglyceride (mmol/l)	1.54 ± 0.51	2.91 ± 1.40†	1.57 ± 0.51§	2.94 ± 0.96†#
HDL cholesterol (mmol/l)	1.40 ± 0.37	1.03 ± 0.24†	1.27 ± 0.32†	1.10 ± 0.26†
Creatinine clearance (ml/min)	82.1 ± 24.8	96.0 ± 47.4	68.7 ± 30.1†§	58.8 ± 31.4†¶
Urinary albumin excretion (mg/24h)	15.0 (11.0–29.5)	46.5 (13.5–84.0)	338 (79.5–664)†§	386 (331–766)†#
Thrombomodulin (FU/ml)	2.55 (2.20–3.00)	2.70 (2.20–3.00)	3.40 (2.75–4.00)*	3.70 (3.05–4.70)†¶
Serum ADMA (μmol/l)	0.47 ± 0.08	0.51 ± 0.08*	0.53 ± 0.09*	0.55 ± 0.10†
Ankle-brachial index	1.14 ± 0.06	1.11 ± 0.16	1.12 ± 0.15	1.16 ± 0.17
Basal flux (PU)	11.8 (6.6–16.4)	10.8 (7.8–21.5)	11.4 (8.4–16.5)	10.6 (7.4–14.6)
Peak flux (PU)	43.1 (32.0–59.6)	34.9 (25.3–43.8)	56.3 (33.4–79.2)	28.4 (20.1–31.5)*¶
Δ flux (PU)	29.9 (22.6–47.5)	17.7 (13.3–36.4)	41.9 (16.9–68.7)	15.7 (9.3–18.9)*¶
Hypertension (n [%])	6 (15.8)	22 (68.8)†	4 (25.0)#	13 (68.4)†#
Cardiovascular disease (n [%])	5 (13.2)	8 (25.0)	3 (18.8)	7 (36.8)*
Retinopathy (none /background retinopath/proliferative)	22/9/7	19/2/11	5/5/6	5/4/10
Treatment (diet/oral hypoglycemic agents/insulin)	2/26/10	4/25/3	1/12/3	0/15/4

Data are means ± SD or median (interquartile range), unless otherwise indicated. * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$ vs. group A. § $P < 0.05$, ¶ $P < 0.001$ vs. group B. ¶ $P < 0.05$, # $P < 0.001$ vs. group C.

RESULTS — Patient characteristics and laboratory data in these subgroups are summarized in Table 1. Plasma thrombomodulin was significantly higher in diabetic patients in group D than in groups A or B ($P < 0.001$, respectively). Serum ADMA was significantly higher in group D ($P < 0.001$), group B ($P < 0.05$), and group C ($P < 0.05$) than in group A. Thus, serum ADMA was highest in diabetic patients who had both metabolic syndrome and overt nephropathy. Cardiovascular disease was more prevalent in group D than in group A ($P < 0.05$).

We evaluated changes in cutaneous blood flow on the dorsum of the right foot in response to local heat in the four subgroups. Basal flux was similar between these groups. Both peak and Δ flux were significantly smaller in group D than in groups A ($P < 0.05$) or C ($P < 0.05$) (Table 1).

Serum ADMA correlated positively with urinary albumin excretion ($r = 0.22$, $P < 0.05$) and thrombomodulin ($r = 0.28$, $P < 0.01$) and negatively with HbA_{1c} ($r = -0.20$, $P < 0.05$), HDL cholesterol ($r = -0.20$, $P < 0.05$), and cre-

atinine clearance ($r = -0.28$, $P < 0.01$). By multivariate analysis, in a model that explained 45.3% of variation of serum ADMA, only HDL cholesterol ($\beta = -0.197$, $P = 0.048$) and creatinine clearance ($\beta = -0.202$, $P = 0.044$) were independent determinants of serum ADMA in patients with type 2 diabetes.

CONCLUSIONS — We found that serum concentrations of ADMA were significantly higher in diabetic patients with metabolic syndrome and overt nephropathy, metabolic syndrome but no overt nephropathy, or overt nephropathy but no metabolic syndrome than in those with no metabolic syndrome and no overt nephropathy. Plasma thrombomodulin also was significantly higher in diabetic patients with metabolic syndrome and overt nephropathy than in those with no metabolic syndrome and no overt nephropathy or overt nephropathy but no metabolic syndrome. Since ADMA is considered a novel contributor to endothelial dysfunction, these results indicate profoundly impaired endothelial dysfunction

in diabetic patients with both metabolic syndrome and overt nephropathy.

The present study showed that serum HDL cholesterol and renal function were independent determinants of serum ADMA in type 2 diabetic patients. ADMA is disposed of via two routes: renal clearance and degradation by the enzyme dimethylarginine dimethylammonihydrolase (7,8). Circulating ADMA accumulates in patients with CKD such as diabetic nephropathy. Low HDL cholesterol, a component of metabolic syndrome, was associated with high serum ADMA. Thus, serum ADMA was highest in diabetic patients with both metabolic syndrome and overt nephropathy.

The present study demonstrated for the first time that cutaneous microvasodilation measured in the foot in response to local heat was significantly lower in diabetic patients with both metabolic syndrome and overt nephropathy than in those with no metabolic syndrome and no overt nephropathy or in those with overt nephropathy but no metabolic syndrome. Although cutaneous microvasodilation in response to local heat is a complex re-

sponse involving several factors including the axon reflex and endothelial nitric oxide (16,17), such impaired vasodilation may reflect microvascular endothelial dysfunction. Thus, coexistence of metabolic syndrome and overt nephropathy is synergistically related to profound impairment of cutaneous microvascular function via elevated serum ADMA, increasing cardiovascular risk. However, a major limitation of this study is that we could not definitely exclude all patients with nondiabetic kidney disease completely, since we did not obtain renal biopsy specimens histologic diagnosis. Therefore, we referred to overt nephropathy instead of overt diabetic nephropathy.

Acknowledgments—We thank Professor T. Inukai of our department for reviewing this manuscript, Dr. K. Takebayashi for critical suggestions, and Mr. Y. Yamanouchi (SRL) for kind assistance.

References

- Muntner P, He J, Hamm L, Loria C, Whelton PK: Renal insufficiency and subsequent death resulting from cardiovascular disease in the United States. *J Am Soc Nephrol* 13:745–753, 2002
- Chen J, Muntner P, Hamm LL, Jones DW, Batuman V, Fonseca V, Whelton PK, He J: The metabolic syndrome and chronic kidney disease in U.S. adults. *Ann Intern Med* 140:167–174, 2004
- Kurella M, Lo JC, Chertow GM: Metabolic syndrome and the risk for chronic kidney disease among nondiabetic adults. *J Am Soc Nephrol* 16:2134–2140, 2005
- Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD: Obesity/insulin resistance is associated with endothelial dysfunction: implications for the syndrome of insulin resistance. *J Clin Invest* 97:2601–2610, 1996
- Calles-Escandon J, Cipolla M: Diabetes and endothelial dysfunction: a clinical perspective. *Endocr Rev* 22:36–52, 2001
- de Jongh RT, Serne EH, IJzerman RG, de Vries G, Stehouwer CD: Impaired microvascular function in obesity: implications for obesity-associated microangiopathy, hypertension, and insulin resistance. *Circulation* 109:2529–2535, 2004
- Cooke JP: Asymmetrical dimethylarginine: the Uber marker? *Circulation* 109:1813–1818, 2004
- Vallance P, Leiper J: Cardiovascular biology of the asymmetric dimethylarginine:dimethylarginine dimethylaminohydrolase pathway. *Arterioscler Thromb Vasc Biol* 24:1023–1030, 2004
- Vallance P, Leone A, Calver A, Collier J, Moncada A: Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 339:572–575, 1992
- Lin KY, Ito A, Asagami T, Tsao PS, Adimoolam S, Kimoto M, Tsuji H, Reaven GM, Cooke JP: Impaired nitric oxide synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethylarginine dimethylaminohydrolase. *Circulation* 106:987–992, 2002
- Stuhlinger MC, Abbasi F, Chu JW, Lamendola C, McLaughlin TL, Cooke JP, Reaven GM, Tsao PS: Relationship between insulin resistance and an endogenous nitric oxide synthase inhibitor. *JAMA* 287:1420–1426, 2002
- Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults: Executive summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) *JAMA* 285:2486–2497, 2001
- Aso Y, Matsumoto S, Fujiwara Y, Tayama K, Inukai T, Takemura Y: Impaired fibrinolytic compensation for hypercoagulability in obese patients with type 2 diabetes: association with increased plasminogen activator inhibitor-1. *Metabolism* 51:471–476, 2002
- Vinik AI, Erbas T, Park TS, Stansberry KB, Scanelli JA, Pittenger GL: Dermal neurovascular dysfunction in type 2 diabetes. *Diabetes Care* 24:1468–1475, 2001
- Aso Y, Inukai T, Takemura Y: Mechanisms of elevation of serum and urinary concentrations of soluble thrombomodulin in diabetic patients: possible application as a marker for vascular endothelial injury. *Metabolism* 47:362–365, 1998
- Kellogg DL Jr, Liu Y, Kosiba IF, O'Donnell D: Role of nitric oxide in the vascular effects of local warming of the skin in humans. *J Appl Physiol* 86:1185–1190, 1999
- Minson CT, Berry LT, Joyner MJ: Nitric oxide and neurally mediated regulation of skin blood flow during local heating. *J Appl Physiol* 91:1619–1626, 2001