Elevated Plasma Asymmetric Dimethylarginine as a Marker of Cardiovascular Morbidity in Early Diabetic Nephropathy in Type 1 Diabetes

LISE TARNOW, MD, DMSC[1]
PETER HOVIND, MD[1]
TOM TEERLINK, PHD[2]

COEN D.A. STEHOUWER, MD, PHD[2]
HANS-HENRIK PARVING, MD, DMSC[1,3]

OBJECTIVE — Increased plasma concentration of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, has been associated with endothelial dysfunction, insulin resistance, and atherosclerosis in nondiabetic populations. In end-stage renal failure, circulating ADMA is elevated and a strong predictor of cardiovascular outcome. This study investigated the relation between ADMA and diabetic micro- and macrovascular complications in a large cohort of type 1 diabetic patients with and without early diabetic nephropathy.

RESEARCH DESIGN AND METHODS — ADMA concentrations in plasma were determined by a high-performance liquid chromatography method in 408 type 1 diabetic patients with overt diabetic nephropathy (252 men; mean age 42.7 years [SD 11.0], mean duration of diabetes 28 years [SD 9], median serum creatinine level 102 μmol/l [range 52–684]). A group of 192 patients with longstanding type 1 diabetes and persistent normoalbuminuria served as control subjects (118 men; mean age 42.6 years [SD 10.2], mean duration of diabetes 27 years [SD 9]).

RESULTS — In patients with diabetic nephropathy, mean ± SD plasma ADMA concentration was elevated 0.46 ± 0.08 vs. 0.40 ± 0.08 μmol/l in normoalbuminuric patients ($P < 0.001$). An increase in plasma ADMA of 0.1 μmol/l increased the odds ratio of nephropathy to 2.77 (95% CI 1.89–4.05) ($P < 0.001$). Circulating ADMA increased in nephropathy patients with declining kidney function, as indicated by elevated values in the lower quartiles of glomerular filtration rate (<76 ml $\cdot$ min^{-1} $\cdot$ 1.73 m^{-2}) ($P < 0.001$ ANOVA). Mean ADMA levels were similar in patients with or without diabetic retinopathy ($P > 0.2$). However, in 44 patients with nephropathy and history of myocardial infarction and/or stroke, ADMA was significantly elevated at 0.48 ± 0.08 μmol/l compared with 0.46 ± 0.08 μmol/l in patients without major cardiovascular events ($P = 0.05$).

CONCLUSIONS — Elevated circulating ADMA may contribute to the excess cardiovascular morbidity and mortality in early diabetic nephropathy.

Nitric oxide is synthesized by the vascular endothelium from the amino acid L-arginine by constitutive and inducible nitric oxide synthases and plays an important role as vasodilator and in the maintenance of vascular homeostasis (1). The endogenous L-arginine metabolite, asymmetric dimethylarginine (ADMA), inhibits cellular L-arginine uptake and nitric oxide synthase activity competitively. In contrast, its stereoisomer symmetrical dimethylarginine (SDMA) is produced in equivalent amounts but has no inhibitory effect on nitric oxide synthase (2).

Accumulation of ADMA has first been shown in chronic renal failure (3) and has subsequently been confirmed by other studies of advanced nondiabetic kidney disease (4,5). The idea was simple: as the kidneys fail, ADMA excretion diminishes and its concentration in plasma increases to levels sufficient to block nitric oxide generation and thereby cause cardiovascular, neurological, and other unwanted effects (3). Later reports of ADMA as a potential mediator of cardiovascular morbidity and mortality in patients with chronic renal impairment have supported this concept (6,7). However, the recent finding of a marked increase in ADMA in nondiabetic kidney disease, when glomerular filtration rate is still within the normal range (8), suggests that other mechanisms may be involved.

Although a proportion of ADMA is excreted in the urine, its major catabolism is via the enzyme dimethylarginine dimethylaminohydrolase (4), shown to be colocalized with nitric oxide synthases in various renal cell types (9). In vitro, hyperglycemia impairs dimethylarginine dimethylaminohydrolase activity in vascular smooth muscle cells and the endothelium, leading to elevated ADMA levels (10). In addition, a significant relationship has been reported between increased plasma concentrations of ADMA, type 2 diabetes, and insulin resistance, a condition closely associated with a constellation of risk factors for cardiovascular disease (11,12).

Because cardiovascular disease clusters in type 1 diabetic patients with diabetic nephropathy and, furthermore, because insulin resistance has been suggested to play a role in the pathogenesis of this microvascular complication, we aimed to study the role of ADMA in type 1 diabetes. Therefore, the objective of the present study was to investigate the relation between ADMA and diabetic micro- and macrovascular complications in a large cohort of type 1 diabetic patients

• •

Table 1—Baseline characteristics of 600 patients with type 1 diabetes with or without diabetic nephropathy

	Nephropathy	Normoalbuminuria
n	408	192
Sex (men/women)	252/156	118/74
Age (years)	42.7 ± 11.0	42.6 ± 10.2
Duration of diabetes (years)	28 ± 9	27 ± 9
BMI (kg/m^2)	24.3 ± 3.4	23.6 ± 2.5
HbA$_{1c}$ (%)*	9.4 ± 1.5	8.5 ± 1.1
Urinary albumin excretion rate (mg/24 h)†	606 (10–14,565)	8 (1–30)
Systolic blood pressure (mmHg)*	145 ± 22	132 ± 18
Diastolic blood pressure (mmHg)*	82 ± 12	76 ± 10
Serum creatinine (μmol/l)*	102 (52–684)	76 (40–116)
Serum cholesterol (mmol/l)*	5.6 ± 1.2	4.8 ± 1.0
Retinopathy (nil/simplex/proliferative)*	6/136/266	68/105/19
Insulin (IU/day)*	44 ± 14	41 ± 13
Antihypertensive therapy*	81%	13%
Plasma L-arginine concentration (μmol/l)*	72.76 ± 20.25	62.35 ± 17.43
ADMA (μmol/l)*	0.46 ± 0.08	0.40 ± 0.06
SDMA (μmol/l)*	0.59 (0.28–4.04)	0.41 (0.28–0.84)
Arg/ADMA	160 ± 47	156 ± 40

Data are *n*, means ± SD, or median (range). *$P < 0.001$; †some patients with previously persistent albuminuria at the time of investigation had a urinary albumin excretion rate below 300 mg/24 h due to ongoing antihypertensive therapy.

with or without early diabetic nephropathy.

RESEARCH DESIGN AND METHODS

— During 1993, all adult type 1 diabetic patients with diabetic nephropathy (*n* = 242) attending the outpatient clinic at the Steno Diabetes Center, who underwent measurement of glomerular filtration rate during the same year, were invited to participate in a case-control study (13,14). In 1993, blood samples were collected in 198 cases and in a group of 192 type 1 diabetic patients with long-lasting normoalbuminuria (<30 mg/24h) matched to cases with respect to sex, age, and duration of diabetes.

Since 1993, all albuminuric type 1 diabetic patients newly referred for measurement of kidney function have been invited to participate in an ongoing study of the genetics of diabetic nephropathy, and thus, the original cohort of type 1 diabetic patients with diabetic nephropathy has been expanded from 198 to 408 patients.

The diagnosis of type 1 diabetes was based on World Health Organization criteria, and diabetic nephropathy was diagnosed clinically as persistent macroalbuminuria (>300 mg/24h) in at least two of three consecutive 24-h urine collections, in the presence of diabetic reti-nopathy and the absence of other kidney or urinary tract disease (15).

The study was approved by the local ethics committee, and all patients gave their informed consent.

Clinical and laboratory investigations

Arterial blood pressure was measured twice after at least 10 min rest using a cuff of appropriate size with the patient in the supine position. Urinary albumin concentration was measured by enzyme immunoassay (16) from 24-h urine samples. Serum creatinine concentration was assessed by a kinetic Jaffé method. Glomerular filtration rate was measured in patients with diabetic nephropathy (*n* = 394) after a single injection of 3.7 MBq ^{51}Cr-EDTA by determination of radioactivity in venous blood samples taken 180, 200, 220, and 240 min after the injection (17). In all patients with at least 3 years of follow-up with yearly measurements (*n* = 293), a linear regression analysis was performed and the slope used to determine the rate of decline in glomerular filtration rate.

Diabetic retinopathy was assessed in all patients by fundus photography after pupillary dilatation and graded as nil, simplex, or proliferative retinopathy. Patients were interviewed using the World Health Organization cardiovascular questionnaire. Major cardiovascular events were diagnosed as a history of stroke and/or myocardial infarction. Smoking was defined as persons smoking one or more cigarettes/cigars/pipes per day; all others were classified as nonsmokers.

Measurements of ADMA

Plasma concentrations of arginine, ADMA, and SDMA were determined simultaneously by high-performance liquid chromatography as described previously (18). In brief, sample cleanup was performed by solid-phase extraction on polymeric cation-exchange extraction columns using monomethylarginine as internal standard. After derivatization with *ortho*-phthaldialdehyde reagent containing 3-mercaptoproprionic acid, analytes were separated by isocratic reversed-phase high-performance liquid chromatography with fluorescence detection. Analytical recovery was 98–102% and the interassay coefficient of variation was >3%. Plasma from 53 healthy volunteers contained 0.42 ± 0.06 μmol/l of ADMA on average.

Statistical analyses

Normally distributed variables are given as means ± SD. Urinary albumin excretion rate and SDMA concentrations are log transformed before analysis due to their skewed distribution and are given as medians (range). Comparisons between groups were performed using unpaired Student's *t* test or ANOVA; χ^2 test was used to compare noncontinuous variables. The relations between ADMA and diabetic nephropathy were investigated in multiple linear or logistic regression analyses. Variables included were serum creatinine and traditional risk factors (sex, duration of diabetes, BMI, HbA$_{1c}$, and blood pressure). Two-tailed *P* value ≤0.05 was considered statistically significant. No correction for multiple comparisons was performed. All calculations were performed with the commercially available software SPSS for Windows (version 10.0; SPSS, Chicago, IL).

RESULTS

— The clinical characteristics of the study groups are shown in Table 1. The two groups did not differ with regard to sex, age, or duration of diabetes. As compared with patients with normoalbuminuria, patients with diabetic nephropathy had elevated blood pres-
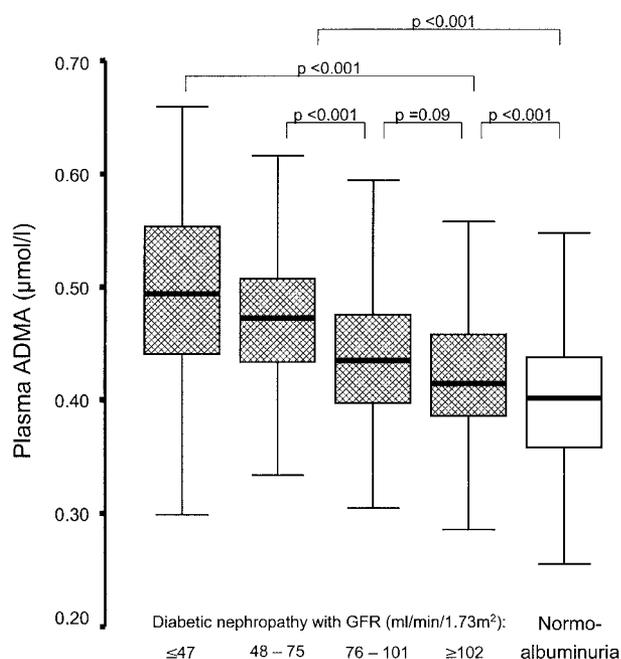
Figure 1—*Plasma ADMA concentrations in 192 type 1 diabetic patients with persistent normoalbuminuria and in 394 type 1 diabetic patients with nephropathy according to quartiles of glomerular filtration rate (GFR). Data are shown as 2.5, 25, 50, 75, and 97.5% centiles.*

sure, increased serum creatinine and HbA_{1c}, as well as increased serum cholesterol ($P < 0.001$).

Mean plasma ADMA concentration was higher in patients with overt diabetic nephropathy than in diabetic control subjects with persistent normoalbuminuria ($P < 0.001$) (Table 1, Fig. 1). Furthermore, ADMA concentrations were closely correlated to glomerular filtration rate ($r = -0.44$, $P < 0.001$) and increased in nephropathy patients with declining kidney function, as indicated by elevated values in the lower quartiles of glomerular filtration rate (Table 2, Fig. 1). Plasma concentration of ADMA did not differ between type 1 diabetic men and women (NS) but increased with age, systolic blood pressure, and serum creatinine

level (data not shown). No correlations between ADMA and either blood glucose or HbA_{1c} were observed. After adjustment for sex, duration of diabetes, BMI, HbA_{1c}, and serum creatinine level in a multiple logistic regression analyses, an increase in plasma ADMA of 0.1 μmol/l resulted in an increase in odds ratio of diabetic nephropathy of 2.77 (95% CI 1.89–4.05; $P < 0.001$). Among patients with diabetic nephropathy, circulating ADMA concentrations were higher in patients treated with antihypertensive medications: 0.47 ± 0.08 vs. 0.44 ± 0.07 μmol/l for patients taking antihypertensives and patients not treated for hypertension, respectively ($P = 0.005$). No differences were detected between patients taking ($n = 233$) or not taking ACE

inhibitors ($n = 175$; NS). Finally, smokers and nonsmokers had similar levels of ADMA (data not shown; NS).

The average decrease in glomerular filtration rate was 3.7 ml/min per year (3.6) during 6.1 years (3.0–9.6) of follow-up. There was, however, no relation between plasma ADMA at entry to the study and the subsequent rate of decline in kidney function (NS) (Fig. 2).

With respect to another microvascular complication of diabetes, ADMA levels were not found to be increased in patients with simplex or proliferative diabetic retinopathy, neither in patients with normoalbuminuria nor in nephropathic patients ($P > 0.2$; data not shown).

The prevalence of major cardiovascular events differed substantially between patients with and without diabetic nephropathy: 11% (95% CI 8–14) and 2% (0–4), respectively ($P < 0.001$). In patients with nephropathy, plasma ADMA was significantly elevated in patients with cardiovascular disease as compared with those patients without a history of nonfatal stroke and/or myocardial infarction: 0.48 ± 0.08 vs. 0.46 ± 0.08 μmol/l, respectively ($P = 0.05$). After adjusting for possible confounders (sex, blood pressure, BMI, HbA_{1c}, serum cholesterol level, smoking, and serum creatinine level), an increase in ADMA of 0.1 μmol/l conferred an increase in odds ratio of a major cardiovascular event of 1.47 (95% CI 1.02 – 2.13; $P = 0.04$).

In parallel to ADMA, plasma L-arginine and SDMA concentrations were elevated in diabetic patients with nephropathy as compared with normoalbuminuric patients (Table 1) ($P < 0.001$) and increased with declining kidney function (Table 2). As a consequence, the L-arginine/ADMA ratio did not differ significantly between patients with and

Table 2—*Arginine and its dimethylderivatives in 394 type 1 diabetic patients with diabetic nephropathy according to quartiles of glomerular filtration rate*

	Quartiles of glomerular filtration rate (<76 ml · min^{-1} · 1.73 m^{-2})			
	≤47	48–75	76–101	≥102
Serum creatinine (μmol/l)	177 (86–684)	114 (67–187)	90 (52–137)	76 (54–112)
n	98	98	98	100
Plasma L-arginine concentration (μmol/l)*	77.49 ± 21.69	75.02 ± 17.12	70.97 ± 19.73	67.32 ± 20.85
ADMA (μmol/l)*	0.50 ± 0.09	0.48 ± 0.08‡	0.44 ± 0.06†‡	0.42 ± 0.06†
SDMA (μmol/l)*	1.08 (0.54–4.04)	0.67 (0.39–1.34)	0.53 (0.33–0.80)	0.43 (0.28–0.64)

Data are *n*, means ± SD, or median (range). ANOVA: *$P < 0.001$; †$P = 0.09$, ‡$P < 0.001$.
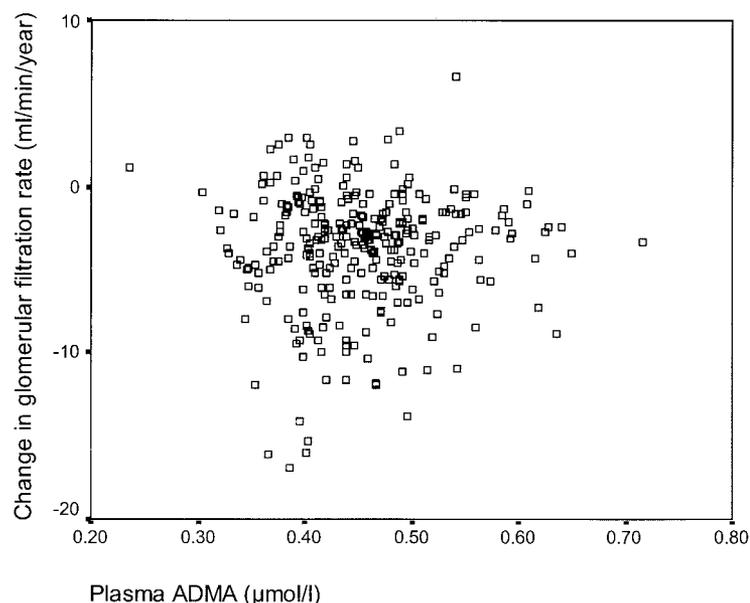
Figure 2—*Plasma ADMA and decrease in glomerular filtration rate (^{51}Cr-EDTA clearance) during a median follow-up of 6.1 years (range 3–9.6) in 293 type 1 diabetic patients with diabetic nephropathy.*

without nephropathy: 160 ± 47 vs. 156 ± 40, respectively.

CONCLUSIONS — This case-control study of large groups of well-characterized type 1 diabetic patients demonstrates that circulating ADMA is elevated in patients with diabetic nephropathy. This increase in ADMA is detectable early in the course of the diabetic kidney disease, when glomerular filtration rate is still within the normal range. Whether circulating ADMA level plays a part in the pathogenesis of diabetic nephropathy or is merely an indicator of early renal disease remains to be investigated in prospective designs. However, increased ADMA concentration does not predict subsequent decrease in renal function in patients who have already developed overt diabetic kidney disease.

No relationship was detected between ADMA and another diabetic microvascular complication: diabetic retinopathy. On the contrary, elevated ADMA levels conferred an increased risk of nonfatal stroke and myocardial infarction in patients with diabetic nephropathy, who are known to be at high risk for macrovascular morbidity and mortality.

Endothelial dysfunction has been demonstrated to occur early in the course of diabetic vascular complications (19). Endothelium-derived nitric oxide is a powerful endogenous vasodilator and plays an important role in maintenance of vascular homeostasis by inhibition of platelet aggregation, leukocyte migration, cellular adhesion, and vascular smooth muscle cell proliferation (1). In uncomplicated type 1 diabetes (20), as in type 2 diabetes (1), the release and/or bioavailibility of nitric oxide are diminished. This could result from increased oxidative stress or via hyperglycemia induced activation of protein kinase C, the polyol pathway, or accumulation of advanced glycemic endproducts. In addition to these mechanisms, the endogenous nitric oxide synthase inhibitor ADMA has recently emerged as a key factor in nitric oxide biosynthesis.

The closely related stereoisomer SDMA does not inhibit nitric oxide synthase (2). However, as arginine, ADMA, and SDMA share a common pathway for entry into the cell, high plasma concentrations of SDMA may indirectly reduce nitric oxide production by competing with arginine for cellular uptake (9). The biological and clinical relevance of this hypothesis warrants further investigation. Hitherto, in prospective follow-up studies of patients with similar levels of renal function, ADMA but not SDMA was associated with all-cause mortality and fatal/nonfatal cardiovascular events (7,21).

Increased ADMA levels in blood have been documented in several studies of patients with nondiabetic kidney diseases in advanced or terminal stages (3,4,6). Urinary excretion of ADMA decreases with increasing renal insufficiency (5). Recently, Kielstein et al. (8) reported markedly elevated ADMA concentrations in 44 nonsmoking patients with either IgA glomerulopathy or adult polycystic kidney disease with normal renal function as compared with normal subjects. In accordance with these findings, our data in 408 type 1 diabetic patients confirm and extend the observation that circulating ADMA increases early (glomerular filtration rate <76 ml $\cdot$ min^{-1} $\cdot$ 1.73m^{-2}) in the course of diabetic kidney disease. Smoking status did not affect this finding.

The contribution of defective nitric oxide bioavailability or vascular smooth muscle responsiveness to increased coronary risk in diabetes was recently investigated by forearm blood flow response in 88 (84%) predominantly normoalbuminuric type 1 diabetic patients (20). Impairment in nitric oxide release and responsiveness that could not be explained by conventional cardiovascular risk factors was reported.

In hemodialysis patients, a population known to be at extremely high absolute cardiovascular risk, plasma ADMA has been demonstrated to be higher in patients with clinically manifest atherosclerosis (6,22) and to be an independent predictor of both overall mortality and cardiovascular outcome (7). In the latter study (7) of 225 patients with end-stage renal disease surviving the first 6 months of dialysis, 120 cardiovascular events occurred during a mean follow-up of 33.4 months, including 43 nonfatal strokes and/or myocardial infarctions. In the present study of 408 type 1 diabetic patients with early diabetic nephropathy, ADMA levels were significantly higher in the group of 44 patients who had a history of stroke and/or myocardial infarction. In accordance with previous studies (23), higher ADMA levels were seen in elderly individuals and in patients treated for hypertension. The association with cardiovascular disease, however, persisted after adjustment for differences in kidney function and conventional cardiovascular risk factors.

Recently, a nested case-control study in middle-aged, predominantly nondiabetic, nonsmoking Finnish men with a previous history of coronary heart disease

found that serum ADMA concentrations in the upper quartile are associated with an increased risk of acute coronary events (21).

In conclusion, elevated circulating ADMA concentration may contribute to excess cardiovascular morbidity and mortality, even early in the course of diabetic nephropathy. The magnitude and potential reversibility of this contribution must be confirmed in prospective observational and intervention studies.

References

1. Chan NN, Vallance P, Colhoun HM: Nitric oxide and vascular responses in type 1 diabetes. *Diabetologia* 43:137–147, 2000
2. Chan NN, Chan JCN: Asymmetric dimethylarginine (ADMA): a potential link between endothelial dysfunction and cardiovascular diseases in insulin resistance syndrome? *Diabetologia* 45:1609–1616, 2002
3. Vallance P, Leone A, Calver A, Collier J, Moncada S: Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 339:572–575, 1992
4. MacAllister RJ, Rambausek MH, Vallance P, Williams D, Hoffmann KH, Ritz E: Concentration of dimethyl-L-arginine in the plasma of patients with end-stage renal failure. *Nephrol Dial Transplant* 11:2449–2452, 1996
5. Marescau B, Nagels G, Possimiers I, De Broe ME, Becaus I, Billiouw J-M, Lornoy W, De Deyn PP: Guanidino compounds in serum and urine if nondialyzed patients with chronic renal insufficiency. *Metabolism* 46:1024–1031, 1997
6. Kielstein JT, Böger RH, Bode-Böger SM, Schäffer J, Barbey M, Koch KM, Frölich JC: Asymmetric dimethylarginine plasma concentration differ in patients with end-stage renal disease: relationship to treatment method and atherosclerotic disease. *J Am Soc Nephrol* 10:594–600, 1999
7. Zoccali C, Bode-Böger SM, Mallamaci F, Benedetto FA, Tripepi G, Malatino LS, Cataliotti A, Bellanuova I, Fermo I, Frölich JC, Böger RH: Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. *Lancet* 358:2113–2117, 2001
8. Kielstein JT, Böger RH, Bode-Böger SM, Frölich JC, Haller H, Ritz E: Marked increase of asymmetrical dimethylarginine in patients with incipient primary chronic renal disease. *J Am Soc Nephrol* 13:170–176, 2002
9. Tojo A, Welch WJ, Bremer V, Kimoto M, Kimura K, Omata M, Ogawa T, Vallance P, Wilcox CS: Colocalization of dimethylating enzymes and NOS and functional effects of methylarginines in rat kidney. *Kidney Int* 52:1593–1601, 1997
10. Lin KY, 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
11. Abbassi F, Asagami T, Cooke JP, Lamendola C, McLaughlin T, Reaven GM, Stühlinger MC, Tsao PS: Plasma Concentrations of asymmetric dimethylarginine are increased in patients with type 2 diabetes mellitus. *Am J Cardiol* 88:1201–1203, 2001
12. Stühlinger MC, Abbassi 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
13. Tarnow L, Cambien F, Rossing P, Nielsen FS, Hansen BV, Lecerf L, Poirier O, Danilov S, Boelskifte S, Borch-Johnsen K, Parving H-H: Insertion/deletion polymorphism in the angiotensin-I-converting enzyme gene is associated with coronary heart disease in IDDM patients with diabetic nephropathy. *Diabetologia* 38:798–803, 1995
14. Tarnow L, Cambien F, Rossing P, Nielsen FS, Hansen BV, Lecerf L, Poirier O, Danilov S, Parving H-H: Lack of relationship between an insertion/deletion polymorphism in the angiotensin-I-converting enzyme gene and diabetic nephropathy and proliferative retinopathy in IDDM patients. *Diabetes* 44:489–494, 1995
15. Parving H-H, Andersen AR, Smidt UM, Svendsen PA: Early aggressive antihypertensive treatment reduces rate of decline in kidney function in diabetic nephropathy. *Lancet* i:1175–1179, 1983
16. Feldt-Rasmussen B, Dinesen B, Deckert M: Enzyme immunoassay: an improved determination of urinary albumin in diabetics with incipient nephropathy. *Scand J Clin Lab Invest* 45:539–544, 1985
17. Bröchner-Mortensen J, Rödbro P: Selection of routine method for determination of glomerular filtration rate in adult patients. *Scand J Clin Lab Invest* 36:35–45, 1976
18. Teerlink T, Nijveldt RJ, de Jong S, van Leeuwen PAM: Determination of arginine, asymmetric dimethylarginine, and symmetric dimethylarginine in human plasma and other biological samples by high-performance liquid chromatography. *Anal Biochem* 303:131–137, 2002
19. Jensen T, Feldt-Rasmussen B, Bjerre-Knudsen J, Deckert T: Features of endothelial dysfunction in early diabetic nephropathy. *Lancet* i:461–463, 1989
20. Chan NN, Vallance P, Colhoun HM: Endothelium-dependent and -independent vascular dysfunction in type 1 diabetes: role of conventional risk factors, sex, and glycemic control. *Arterioscler Thromb Vasc Biol* 23:1048–1054, 2003
21. Valkonen V-P, Pälvä H, Salonen JT, Lakka TA, Lehtimäki TJ, Laakso J, Laaksonen R: Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine. *Lancet* 358:2127–2128, 2001
22. Zoccali C, Benedetto FA, Maas R, Mallamaci F, Tripepi G, Malatino LS, Böger RH: Asymmetric dimethylarginine, C-reactive protein, and carotid intima-media thickness in end-stage renal disease. *J Am Soc Nephrol* 13:490–496, 2002
23. Kielstein JT, Bode-Böger SM, Frölich JC, Ritz E, Haller H, Fliser D: Asymmetric dimethylarginine, blood pressure, and renal perfusion in elderly subjects. *Circulation* 107:1891–1895, 2003