Plasma Concentrations of Asymmetric-Dimethyl-Arginine in Type 2 Diabetes Associate With Glycemic Control and Glomerular Filtration Rate But Not With Risk Factors of Vasculopathy

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Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthase (NOS). Increased plasma levels of ADMA may indicate endothelial dysfunction and increased risk of angiopathy. The relation of ADMA to diabetes, glycemic control, and renal function, especially early diabetic hyperfiltration, remains unknown. We tried to evaluate whether there is an association between ADMA and glycosylated hemoglobin ($GHbA_{1c}$) on the one hand and glomerular filtration rate (GFR) on the other hand in diabetic subjects with normal or slightly increased GFR. We also studied whether plasma ADMA is associated with some risk factors of vasculopathy (hypercholesterolemia and hypertension). The study subjects consisted of 86 patients with type 2 diabetes and 65 control subjects. Plasma ADMA levels were measured by high-pressure liquid chromatography as o-pthalaldehyde (OPA) derivatives and GFR was determined by the plasma clearance of chromium 51–EDTA. The diabetic patients had lower plasma ADMA levels than the nondiabetic control subjects (0.29 ± 0.15 *v* 0.34 ± 0.16 μmol/L, $P < .03$). In the diabetic subjects, plasma ADMA concentrations were inversely correlated with $GHbA_{1c}$ ($R = -0.28$, $P = .01$). In a multivariate linear model, significant predictors of ADMA were GFR ($R = -0.32$, $P = .008$) in diabetic subjects and $GHbA_{1c}$ ($R = -0.19$, $P = .03$) and GFR ($R = -0.19$, $P = .02$) in all subjects. Plasma ADMA was not associated with risk factors of vasculopathy. We conclude that diabetic patients with a normal or slightly increased GFR have lower circulating ADMA concentrations than nondiabetic control subjects. In type 2 diabetic patients high GFR and poor glycemic control were related to low plasma ADMA concentrations.

IN THE L-arginine-nitric oxide (NO) pathway, nitric oxide synthase (NOS) converts L-arginine to NO and citrulline.[1] Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of endothelial NOS and it can modulate NO production.[2] The occurrence and clinical impacts of increased or decreased plasma ADMA levels are largely unknown. However, plasma ADMA levels seems to correlate with risk factors of atherosclerosis, such as hypercholesterolemia[3], aging,[4] and hypertension.[4-6] Elevated plasma levels of ADMA are also associated with impaired endothelium-dependent brachial artery vasodilation in young hypercholesterolemic individuals[3] and intima media thickness in healthy subjects.[4] Furthermore, we have recently shown that high ADMA is a potent predictor of acute coronary events in nonsmoking middle-aged men.[7] These findings suggest that high ADMA concentration is a potential marker for endothelial dysfunction.

Endothelial dysfunction with a reduced bioavailability of endothelium-derived NO plays an important role in the pathogenesis of diabetic vascular disease,[8,9] which is a major cause of morbidity and mortality in type 2 diabetes.[10,11] Besides being a potent vasodilatator,[12,13] NO inhibits key processes in atherogenesis, such as monocyte adhesion, platelet adhesion and aggregation, vascular smooth muscle proliferation, and interference with leukocyte–endothelial cell interaction.[2,3] In addition, NO can prevent oxidative modification of low-density lipoprotein (LDL), which is suggested to be one of the major contributors to atherosclerosis.[14] ADMA, by inhibiting NO synthase, can diminish NO bioavailability in humans. Although a significant association has been detected between glucose balance and ADMA both in animal models[15,16] and in healthy humans,[4] and a recent study of type 2 diabetic subjects found elevated plasma ADMA levels,[17] it is not fully established how ADMA relates to diabetes. Since diabetic vascular disease is related to reduced NO bioavailability, it can be hypothesized that plasma ADMA concentrations could be elevated in diabetic subjects. Although NO may be beneficial in terms of vasculopathy it may also be a harmful agent in diabetic patients by inducing renal vasodilatation and hyperfiltration, phenomena seen in early diabetic nephropathy. In fact, in early diabetic nephropathy increased filtration rates are consistently observed.[18-20] This hyperfiltration could affect ADMA concentrations due to increased renal excretion. In this study, we sought to determine how plasma ADMA relates to type 2 diabetes mellitus in patients with normal or increased filtration rates. Plasma levels of ADMA were compared between patients with type 2 diabetes and nondiabetic subjects and associations between ADMA and glycemic control, renal function, and risk factors of cardiovascular disease were evaluated.

MATERIALS AND METHODS

Subjects

Eighty-six type 2 diabetic patients and 65 controls with comparable distribution of age and gender were recruited between 1985 and 1988 from the Primary Health Care Center of the City of Tampere, Finland.

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The patients fulfilled the World Health Organization diagnostic criteria for type 2 diabetes and had a known disease duration of no more than 1 year. The study participants were evaluated after a mean period of 9.0 years (range, 7.4 to 10.7 years) follow-up. The clinical characteristics of the study participants are listed in Table 1. At the 9-year evaluation the treatment of diabetes consisted of diet and oral drug therapy in 45, insulin in 17, and combined oral drug and insulin therapy in 10 patients. The study was approved by the ethics committees of the University of Tampere and the health care center of Tampere. All subjects gave a written informed consent.

Methods

Information regarding previous and present diseases, current medication, and alcohol and tobacco consumption were obtained. Body mass index (BMI) was calculated (kg/m^2). Blood pressure (BP) was measured from both arms of the subjects using a sphygmomanometer and the mean of the 2 recordings was calculated to the nearest 2 mm Hg after 10 minutes' rest in the supine position. The mean arterial blood pressure (MAP) was calculated as twice the diastolic BP plus the systolic BP divided by 3. Hypertension was defined as systolic blood pressure greater than 160 mm Hg or diastolic blood pressure greater than 95 mm Hg. Patients on antihypertensive treatment were classified as hypertensive.

Plasma cholesterol and triglycerides were determined by the dry-slide technique (Ektachem 700 analyzer, Johnson & Johnson Clinical Diagnostics, Rochester, NY). High-density lipoprotein (HDL) cholesterol was measured with the same technique after precipitation of LDL. Blood glucose concentrations were measured with a Hitachi 717 analyzer (Japan) by the enzymatic method of Merck (USA), and glycosylated hemoglobin ($GHbA_{1c}$) measurements were made using a Mono S HR 5/5 column (Pharmacia Biotech, Uppsala, Sweden) and lithium malonate buffers, pH 5.7, according to the manufacturer's product instructions.[21] GFR was determined by the plasma clearance of chromium 51–EDTA assessed by the single-injection method.

Table 1. Patient Characteristics

Variable	Controls (n = 65)	Diabetic Patients (n = 86)
Age (yr)	64.7 ± 7.4	63.8 ± 7.1
Sex (M/F)	36/29	52/34
Present smoking (yes)	11	7*
BMI (kg/m^2)	27.8 ± 3.9	29.7 ± 4.7†
Total cholesterol (mmol/L)	5.61 ± 1.11	5.27 ± 1.00*
HDL cholesterol (mmol/L)	1.20 ± 0.46	1.09 ± 0.40
Triglycerides (mmol/L)	1.54 ± 0.80	2.06 ± 1.39†
FBGluc (mmol/L)	5.1 ± 1.2	9.4 ± 3.1
SBP (mm Hg)	152 ± 19	161 ± 21†
DBP (mm Hg)	87 ± 9	88 ± 9
MAP (mm Hg)	108 ± 12	112 ± 11*
ADMA (μmol/L)	0.34 ± 0.16	0.29 ± 0.15*
GFR (mL/min/1.73 m^2)	90 ± 19	98 ± 23*
$GHbA_{1c}$ (%)	5.7 ± 0.5	8.3 ± 1.7

NOTE. ANCOVA for continuous and χ^2-test for noncontinuos variables. Age and BMI were used as covariates for plasma lipids and ADMA: *$P \leq .05$, †$P \leq .01$ compared to controls.

Abbreviations: FBGluc, fasting blood glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; ADMA, asymmetric dimethylarginine; GFR, glomerular filtration rate; $GHbA_{1c}$, glycosylated hemoglobin.

Analysis of Asymmetric and Symmetric Dimethylarginine and Arginine

Plasma arginine and its dimethylated endogenous derivatives, ADMA and symmetric dimethylarginine (SDMA), were determined with a novel high-performance liquid chromatography (HPLC) method. Briefly, 200 μL plasma was diluted with 160 μL of distilled water and the solution was mixed with 40μL of internal standard (homo-arginine, 400 μmol/L). Arginine, ADMA, and SDMA were then absorbed on a 100-mg Bond Elut silica solid-phase extraction (SPE) column (Varian, Harbor City, CA), pretreated with 800 μL of methanol and 800 μL of distilled water. After washing, arginine, ADMA, and SDMA were eluted from the SPE column with an o-pthalaldehyde (OPA) derivatizing reagent. Derivatized arginine, ADMA, and SDMA were then separated using HPLC on a Waters Bondapak phenyl column (5 μm, 150 × 4.6 mm; Waters Oy, Helsinki, Finland) with 100 mmol/L citrate buffer, pH 6.8. Fluorescence was monitored at 328 nm (exitation) and 445 nm (emission). The HPLC equipment consisted of a LKB Pharmacia 2248 pump (Turku, Finland), a Hewlett-Packard 1050 autosampler (Espoo, Finland), and a Shimadzu RF-551 fluorescence detector (Shimadzu-Fenno Medical Oy, Vantaa, Finland). Total imprecisions for ADMA, SDMA, and arginine were no more than 12%. Samples were analyzed in duplicates. Plasma samples of participants were stored at −70°C until analyzed.

Statistical Analysis

Data are expressed as the mean ± SD unless otherwise specified. One-way analysis of covariance (ANCOVA) was used to assess the statistical differences between diabetic and control subjects in lipid and some other parameters shown in Table 1. Age and BMI were used as covariates when appropriate. Categorical variables (smoking and gender) were compared using chi-square test. Univariate correlation analysis was performed with Pearsons' correlation test for normally distributed variables and with Spearman's correlation test for non-normally distributed variables (eg, $GHbA_{1c}$). The predictors for ADMA were examined by a linear multivariate regression analysis. A probability of less than .05 was selected as the level of statistical significance. The power of the study to detect a difference in ADMA between diabetic and control subjects was 0.75 and to detect a difference in GFR was 0.86 with the present number of subjects and the probability for a type I error was $\alpha = .05$.

RESULTS

The clinical characteristics of study subjects are listed in Table 1. Forty-two control and 67 diabetic subjects had hypertension. Twenty-six of the hypertensive controls and 51 of the hypertensive diabetic subjects had received antihypertensive treatment (16 ± 8 years in controls and 15 ± 8 years in diabetic subjects). The diabetic patients had significantly higher BP values than the nondiabetic controls (Table 1). The patients had also significantly elevated GFR compared to controls (98 ± 23 and 90 ± 19 mL/min/1.73 m^2, respectively; $P = .002$). Twenty-seven diabetic patients were treated with insulin, either as a single therapy or combined with oral drug therapy. The levels of $GHbA_{1c}$ in insulin-treated patients were significantly higher than in patients treated with diet and oral drug therapy (8.9 ± 1.8 and 8.0 ± 1.5 mmol/l, respectively; $P = .02$).

The average plasma ADMA concentration was 0.31 ± 0.15 μmol/L in all subjects. The patients with type 2 diabetes had significantly lower plasma ADMA levels than nondiabetic control subjects (0.29 ± 0.15 and 0.34 ± 0.16 μmol/L, respectively; $P < .03$). Plasma SDMA concentrations tended to be

lower in diabetic patients compared to nondiabetic controls (0.79 ± 0.41 and 0.92 ± 0.51 μmol/L, respectively; $P = .13$). Plasma L-arginine concentrations in diabetic patients and control subjects were comparable (149.09 ± 30.30 and 149.96 ± 25.30 μmol/L, respectively; $P = .67$). Antihypertensive treatment had no influence on plasma ADMA levels in diabetic patients or controls.

Univariate analysis revealed a significant and inverse correlation between plasma ADMA concentrations and $GHbA_{1c}$ in the patients with type 2 diabetes ($R = -0.28$, $P = .01$; Fig 1), but not in the control subjects ($R = -0.12$, $P = .33$). Plasma ADMA concentrations were slightly lower in the insulin-treated patients compared to the diabetic patients not on insulin treatment (0.27 ± 0.11 and 0.30 ± 0.16 μmol/L, respectively; $P = .47$). There was a significant negative correlation between GFR and ADMA levels ($R = -0.29$, $P = .012$; Fig 1) in patients with diabetes mellitus, but the GFR was not associated with $GHbA_{1c}$.

In a univariate correlation analysis, plasma ADMA did not associate with plasma total cholesterol, HDL cholesterol, or triglycerides. No significant associations were found between ADMA and age, gender, or BMI nor between ADMA and smoking. Furthermore, there was no association between BP and ADMA in either the diabetic subjects or in the control subjects regardless of whether they had been on antihypertensive treatment or not.

By a multivariate linear regression analysis the significant predictors of ADMA were GFR ($R = -0.32$, $P = .01$) in diabetic patients ($R^2 = 0.13$) and $GHbA_{1c}$ ($R = -0.19$, $P = .03$) and GFR ($R = -0.19$, $P = .02$) in all subjects ($R^2 = 0.12$). The other explanatory variables in the model were age, smoking, MAP, total cholesterol, and triglycerides,

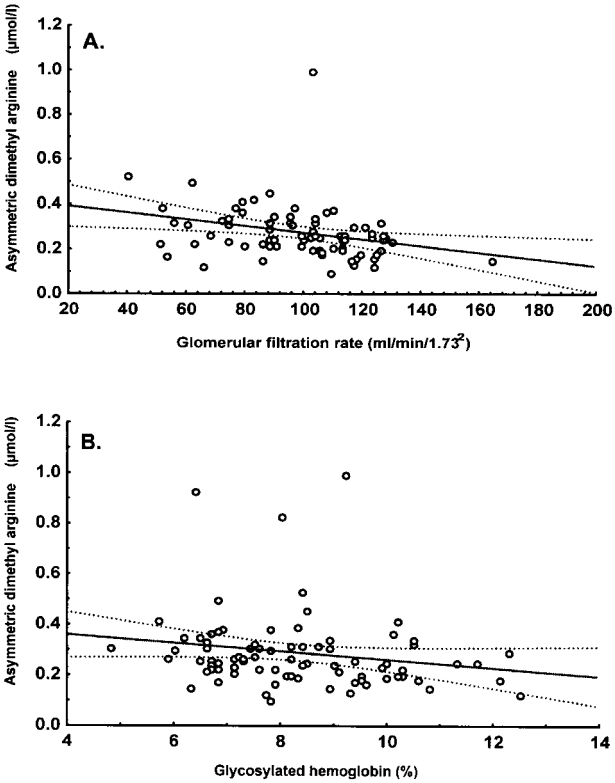

Fig 1. Correlations between ADMA levels and (A) GFR and (B) $GHbA_{1c}$ in patients with type 2 diabetes. (A) $R = -0.29$, $P = .012$; (B) $R = -0.28$, $P = .01$; dotted lines indicate 95% confidence intervals.

DISCUSSION

The main findings of this study can be summarized as follows: (1) The patients with type 2 diabetes mellitus had lower plasma ADMA levels than nondiabetic control subjects. (2) Plasma ADMA concentrations correlated inversely with $GHbA_{1c}$ in diabetic patients. (3) Plasma ADMA concentrations were inversely correlated with GFR. (4) Plasma ADMA concentrations did not correlate with BP or with the other risk factors of atherosclerosis.

The plasma levels in the control subjects in the present study were 0.34 ± 16 μmol/L. In the literature, variable ADMA concentrations have been reported. ADMA levels recorded in this study are similar to those reported in healthy controls in some previous studies,[22,23] but slightly higher than in one report[24] and lower than in some others.[4,5] These variable results are likely explained by differences in the methodology. Plasma ADMA levels have been determined by employing a variety of methods including HPLC with ultraviolet or fluorescence detection after a derivatization step, capillary electrophoresis with laser-induced fluorescence detection, and HPLC tandem mass spectrometry. This study was performed using HPLC and OPA derivatization. At present no interlaboratory comparisons have been reported. The reasons for any discrepancy in ADMA levels thus remain unexplained.

Chronic hyperglycemia may cause vascular abnormality in NO action and enhanced NO biosynthesis.[25-27] Prolonged exposure of endothelial cells to high glucose increases not only NO, but also superoxide anion production.[25,26] NO action largely depends on its relative levels and on its interaction with superoxide anions.[28] It has been speculated that NO production may overcome superoxide anion–induced NO degradation.[29] However, in experimental diabetes superoxide anion seems to curb NO modulation.[30] Whatever the reason, chronic hyperglycemia may lead to renal vasodilatation and hyperfiltration consistently seen in experimental diabetes[29] and in patients with early diabetic nephropathy.[18-20] The hyperfiltration could last for a decade or more, maybe inducing microalbuminuria and later clinical proteinuria. Concomitantly the GFR will gradually decrease, leading finally to end-stage renal disease.[31]

We measured significantly lower ADMA concentrations in diabetic patients than in control subjects. Could the increased filtration rates in our diabetic patients (compared to control subjects) have anything to do with this? Indeed, some methylarginines are in part eliminated by renal excretion[32] and ADMA is known to accumulate in patients with chronic renal failure.[32,33] Furthermore, it has recently been shown that plasma homocysteine levels are inversely related to the GFR, indicating increased clearance (of homocysteine) in patients with even slightly increased filtration rates.[34] On the basis of these considerations, we think that the low ADMA in our type 2 diabetic patients is a compensatory response to increased GFR regardless of the cause of hyperfiltration measured in these patients.

In the present study, in the diabetic patients, a low plasma ADMA value correlated on the one hand with a poor glycemic control and on the other hand with increased filtration rates. It would be tempting to speculate that these associations would be inter-related. However, we did not find any direct relation between glycemic control ($GHbA_{1c}$) and GFR in our diabetic patients. Thus other explanations must be sought. Poor glycemic control may also directly influence NO synthesis and thus be related to hyperfiltration and renal hyperperfusion as shown in early type 1 diabetes.[29,35] Another mechanism could be that long-lasting hyperglycemia may lower ADMA directly by decreasing the production of ADMA or increasing the metabolism of ADMA by as yet unknown mechanisms.

In previous studies high serum ADMA has been linked to hypertension.[5,6,36] However, in the present study no association was observed between ADMA and hypertension in diabetic patients or control subjects. Is there an explanation for these different results? The patients in this study were older than the patients in the other studies and had had hypertension for a long time; many of them also had been on long-standing antihypertensive treatment. It is possible that levels of ADMA are elevated in the early stages of hypertension in otherwise healthy young subjects. In advanced arterial hypertension and in the elderly there may be other more important mechanisms than ADMA, such as increased arterial stiffness, which influence blood pressure.

The relation between hypercholesterolemia and ADMA is not established. One clinical study has found an association,[3] while others have not.[4,37] We could also not find any association between hypercholesterolemia and ADMA in either type 2 diabetic patients or control subjects. We have previously studied the association between ADMA and hypercholesterolemia in men with mild hypercholesterolemia and in men with borderline hypertension or familial hypercholesterolemia. In these studies we did not observe elevated ADMA levels in hypercholesterolemic subjects either.[38]

Conclusion

In type 2 diabetic patients with a disease history of 9 years, circulating ADMA concentrations were lower than in nondiabetic control subjects. In type 2 diabetic patients a high GFR and poor glycemic control were related to low plasma ADMA concentrations.

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REFERENCES

1. Hecker M, Sessa WC, Harris HJ, et al: The metabolism of L-arginine and its significance for the biosynthesis of endothelium-derived relaxing factor: Cultured endothelial cells recycle L-citrulline to L-arginine. Proc Natl Acad Sci USA 87:8612-8616, 1990

2. Ito A, Tsao PS, Adimoolam S, et al: Novel mechanism for endothelial dysfunction: Dysregulation of dimethylarginine dimethylaminohydrolase. Circulation 99:3092-3095, 1999

3. Böger RH, Bode-Böger SM, Szuba A, et al: Asymmetric dimethylarginine (ADMA): A novel risk factor for endothelial dysfunction: Its role in hypercholesterolemia. Circulation 98:1842-1847,1998

4. Miyazaki H, Matsuoka H, Cooke JP, et al: Endogenous nitric oxide synthase inhibitor, a novel marker of atherosclerosis. Circulation 99:1141-1146, 1999

5. Surdacki A, Nowicki M, Sandmann J, et al: Reduced urinary excretion of nitric oxide metabolites and increased plasma levels of asymmetric dimethylarginine in men with essential hypertension. J Cardiovasc Pharmacol 33:652-658, 1999

6. Goonasekera CD, Shah V, Rees DD, et al: Vascular endothelial cell activation associated with increased plasma asymmetric dimethyl arginine in children and young adults with hypertension: A basis for atheroma? Blood Press 9:16-21, 2000

7. Valkonen VP, Päivä H, Salonen JT, et al: Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine. Lancet 358:2127-2128, 2001

8. De Vriese AS, Verbeuren TJ, Van de Voorde J, et al: Endothelial dysfunction in diabetes. Br J Pharmacol 130:963-974, 2000

9. Cosentino F, Luscher TF: Endothelial dysfunction in diabetes mellitus. J Cardiovasc Pharmacol 32:54-61, 1998 (suppl 3)

10. Pyörälä K, Laakso M, Uusitupa M: Diabetes and atherosclerosis: An epidemiologic view. Diabetes Metab Rev 3:463-524, 1987

11. Haffner SM, Lehto S, Rönnemaa T, et al: Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. N Engl J Med 339:229-234, 1998

12. Furchgott RF, Zawadzki JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288:373-376, 1980

13. Hattenbach LO, Allers A, Klais C, et al: L-arginine-nitric oxide pathway-related metabolites in the aqueous humor of diabetic patients. Invest Ophthalmol Vis Sci 41:213-217, 2000

14. Laufs U, La Fata V, Plutzky J, et al: Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. Circulation 97:1129-1135, 1998

15. Xiong Y, Lu R, Li YJ, et al: Elevation of an endogenous inhibitor of nitric oxide synthase in diabetic rat serum. Zhongguo Yao Li Xue Bao 18:511-514, 1997

16. Masuda H, Goto M, Tamaoki S, et al: Accelerated intimal hyperplasia and increased endogenous inhibitors for NO synthesis in rabbits with alloxan-induced hyperglycaemia. Br J Pharmacol 126:211-218, 1999

17. Abbasi F, Asagmi T, Cooke JP, et al: Plasma concentrations of asymmetric dimethylarginine are increased in patients with type 2 diabetes mellitus. Am J Cardiol 88:1201-1203, 2001

18. Wirta O, Pasternack A, Laippala P, et al: Glomerular filtration rate and kidney size after six years disease duration in non-insulin-dependent diabetic subjects. Clin Nephrol 45:10-17,1996

19. Chiarelli F, Cipollone F, Romano F, et al: Increased circulating nitric oxide in young patients with type 1 diabetes and persistent microalbuminuria: Relation to glomerular hyperfiltration. Diabetes 49: 1258-1263, 2000

20. Chaiken RL, Eckert-Norton M, Bard M, et al: Hyperfiltration in African-American patients with type 2 diabetes. Cross-sectional and longitudinal data. Diabetes Care 21:2129-2134, 1998

21. Jeppsson JO, Jerntorp P, Sundkvist G, et al: Measurement of hemoglobin A_{1c} by a new liquid-chromatographic assay: Methodology, clinical utility, and relation to glucose tolerance evaluated. Clin Chem 32:1867-1872, 1986

22. Causse E, Siri N, Arnal JF, et al: Determinations of asymmetrical dimethylarginine by capillary electrophoresis-laser-induced fluorescence. J Chromatogr B Biomed Sci Appl 741:77-83, 2000

23. Pi J, Kumagai Y, Sun G, et al: Improved method for simulta-

neous determination of L-arginine and its mono- and dimethylated metabolites in biological samples by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 742:199-203, 2000

24. Vishwanathan K, Tackett RL, Stewart JT, et al: Determination of arginine and methylated arginines in human plasma by liquid chromatography-tandem mass spectrometry. J Chromatogr B Biomed Sci Appl 748:157-166, 2000

25. Cosentino F, Hishikawa K, Katusic ZS, et al: High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. Circulation 96:25-28, 1997

26. Graier WF, Wascher TC, Lackner L, et al: Exposure to elevated D-glucose concentrations modulates vascular endothelial cell vasodilatory response. Diabetes 42:1497-1505, 1993

27. Cooper ME: Pathogenesis, prevention and treatment of diabetic nephropathy. Lancet 352:213-219, 1998.

28. Craven PA, DeRubertis FR, Melhem M: Nitric oxide in diabetic nephropathy. Kidney Int Suppl 60:S46-S53, 1997

29. Mattar AL, Fujihara CK, Ribeiro MO, et al: Renal effects of acute and chronic nitric oxide inhibition in experimental diabetes. Nephron 74:136-143, 1996

30. Schoonmaker GC, Fallet RW, Carmines PK: Superoxide anion curbs nitric oxide modulation of afferent arteriolar ANG II responsiveness in diabetes mellitus. Am J Physiol Renal Physiol 278:F302-309, 2000

31. Mogensen CE, Christensen CK, Vittinghus E: The stages in diabetic renal disease. With emphasis on the stage of incipient diabetic nephropathy. Diabetes 32:64-78, 1983 (suppl 2)

32. Vallance P, Leone A, Calver A, et al: Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. Lancet 339:572-575, 1992

33. Kielstein JT, Böger RH, Bode-Böger SM, et al: Marked increase of asymmetric dimethylarginine in patients with incipient primary chronic renal disease. J Am Soc Nephrol 13:170-176, 2002

34. Wirta V, Saransaari P, Wirta O, et al: Methylenetetrahydrofolate reductase gene polymorphism, hyperhomocysteinaemia and occlusive retinal vascular disease in type 2 diabetic and nondiabetic subjects. Clin Nephrol 58:171-178, 2002

35. Soper CP, Barron JL, Hyer SL: Long-term glycaemic control directly correlates with glomerular filtration rate in early type 1 diabetes mellitus before the onset of microalbuminuria. Diabet Med 15: 1010-1014, 1998

36. Holden DP, Fickling SA, Whitley GS, et al: Plasma concentrations of asymmetric dimethylarginine, a natural inhibitor of nitric oxide synthase, in normal pregnancy and preeclampsia. Am J Obstet Gynecol 178:551-556, 1998

37. Cardinale CP, Fard A, Eisenberg MS, et al: Elevated plasma level of asymmetric dimethylarginine (ADMA) is associated with atherosclerotic disease of the thoracic aorta. J Am Coll Cardiol 37: 648A, 2001 (suppl A, abstr)

38. Päivä H, Laakso J, Laine H, et al: Plasma asymmetric dimethylarginine and hyperemic myocardial blood flow in young subjects with borderline hypertension or familial hypercholesterolemia. J Am Coll Cardiol 40:1241-1247, 2002