ELSEVIER

Atherosclerosis xxx (2005) xxx–xxx

ATHEROSCLEROSIS

www.elsevier.com/locate/atherosclerosis

Asymmetric dimethyl arginine levels correlate with cardiovascular risk factors in patients with erectile dysfunction

Anthony S. Wierzbicki [a,*], Hemant Solomon [b], Peter J. Lumb [a], Kereese Lyttle [a], Michelle Lambert-Hammill [a], Graham Jackson [b]

[a] *Department of Chemical Pathology, St. Thomas' Hospital Lambeth Palace Road, London SE1 7EH, UK*
[b] *Department of Cardiology, Cardiothoracic Centre, St. Thomas' Hospital Lambeth Palace Road, London SE1 7EH, UK*

Received 11 January 2005; received in revised form 15 June 2005; accepted 21 June 2005

Abstract

Background: Erectile dysfunction is related to penile arterial endothelial nitric oxide production. Asymmetric dimethylarginine (ADMA) and E-selectin are often considered plasma markers of endothelial function.
Objective: This study investigated the relationship between these plasma markers and cardiovascular risk factors in patients with erectile dysfunction.
Methods and results: Cardiovascular risk factors, ADMA and E-selectin were assessed in 45 patients with erectile dysfunction. Plasma markers showed associations with baseline risk factors. E-selectin levels showed an inverse relationship with age ($p = 0.005$) and statin therapy ($p = 0.03$) and a weak association with concomitant β-blocker therapy ($p = 0.05$). Compared to these relatively weak associations with cardiovascular risk factors, ADMA levels showed strong associations with pulse pressure ($p < 0.001$), lack of smoking ($p = 0.002$) and lipoprotein (a) ($p = 0.004$) concentrations and weak associations with LDL-cholesterol ($p = 0.02$), and C-reactive protein levels ($p = 0.04$). ADMA levels correlated with E-selectin (partial $r = 0.76$; $p < 0.001$) after adjustment for lipoprotein (a), pulse pressure and smoking. No change in E-selectin or ADMA levels was seen after 70 days therapy with sildenafil and no relationship was found between either plasma marker and the acute pulse wave response to a single challenge dose of sildenafil.
Conclusion: ADMA levels correlate at baseline with some cardiovascular risk factors including inflammatory markers and lipoprotein (a) in patients with erectile dysfunction.

Keywords: Erectile dysfunction; Selectin; Asymmetric dimethylarginine; Cardiovascular; Risk factor; Sildenafil

1. Introduction

Erectile dysfunction is a common problem in patients with established atherosclerosis and is associated with a reduced endothelial function [1–3]. Cardiovascular risk factors including diabetes, smoking, hypertension and hypercholesterolaemia are all associated with atherosclerosis, erectile dysfunction1 and endothelial dysfunction [4]. A number of novel plasma markers have been associated with atherosclerosis and endothelial dysfunction. Erectile function is dependent on nitric oxide (NO) production by penile nerves and thus erectile dysfunction is associated with reduced plasma levels NO. The guanidino dimethyl arginine derivatives – symmetric (SDMA) and asymmetric dimethylarginine (ADMA) are derived from degradation of methylated proteins and are found in plasma [5]. ADMA, in contrast to SDMA, has been shown to inhibit NO synthase, reduce NO levels and to be associated with cardiovascular events [6]. ADMA levels are 10-fold higher inside endothelial cells. E-selectin is produced by the endothelium in response to oxidative stress and its levels are inversely associated with endothelial function [5–7]. Sildenafil as a phosphodiesterase (PDE) 5 inhibitor has acute effects on nitric oxide production

* Corresponding author. Tel.: +44 20 7188 1256; fax: +44 20 7928 4226.
E-mail address: Anthony.Wierzbicki@kcl.ac.uk (A.S. Wierzbicki).

0021-9150/$ – see front matter © 2005 Published by Elsevier Ireland Ltd.
doi:10.1016/j.atherosclerosis.2005.06.025

and hence endothelial function in the penile and general vasculatures. ADMA levels have been shown to correlate with the degree of atherosclerosis in patients with renal disease [8], insulin resistance diabetes [9,10] and coronary heart disease [11]. ADMA has been suggested to play a role in erectile dysfunction but this has never been investigated [12]. This study investigated the relationship between these markers of endothelial function, cardiovascular risk factor profiles and sildenafil-induced changes in pulse wave velocity in patients with erectile dysfunction.

2. Methods

2.1. Patient selection

Patients were recruited with ethical consent from an erectile dysfunction clinic. Erectile dysfunction was assessed by the International Index of Erectile Function (IIEF) score [13] and patients with an IIEF score <20 were recruited for the study. Baseline anthropometric data was collected and a full medication history was obtained. Initial investigations were performed after the patient had been rested and prior to phlebotomy.

2.2. Investigation protocol

Patients were studied after recruitment and again 70 days later. Patients recruited to the study were admitted to a day unit after a 12 h fast and allowed 50 min bed rest in a sitting position at constant temperature (18 °C). Baseline blood pressure and blood sampling were performed from the non-dominant arm. All blood samples were separated promptly. After a further 10 min rest photoplethysmographic measurement was conducted on the fourth finger of the hand of the other arm. The patient was given a single sildenafil 50 mg tablet and the blood pressure measurement repeated after 50 min with photo-plethysmography 10 min later. During the interim period between visits patients were randomized to 2/weekly sildenafil or daily silenafil using a card-based randomization system stratified for diabetes and coronary heart disease.

2.3. Cardiovascular risk profile

All patients had their cardiovascular risk profiles including smoking, blood pressure, fasting lipids, apolipoproteins (apo), lipoprotein (a) [Lp(a)], glucose, hemoglobin A1c (HbA_{1c}), urine albumin: creatinine ratio (ACR) assessed at baseline using standard procedures and automated laboratory techniques. All routine biochemical assays were standardised using UK national quality control schemes and measured in a single batch to reduce variation (CV < 5%). Biochemical risk factors were assessed after a 12 h fast by standard automated techniques. E-selectin (R&D Systems, Abingdon, Oxford, UK) and ADMA (DLD Diagnostika Gmbh, Hamburg, Germany) were measured in duplicate using an ELISA assays on samples stored frozen at −70 °C. Assay CVs were 6.3% for E-selectin and 7.1% for ADMA.

2.4. Pulse wave profile analysis

Blood pressure was determined in a sitting position using an Omron 705 system on each visit after a 5 min rest and prior to each determination of pulse wave profile. Pulse wave profiles were obtained using a validated, commercially available volume pulse system (Pulse Trace MP 2000; Micro Medical, Rochester, UK) which employs the principle of photoplethysmography and appropriate acquisition and analysis software for non-invasive recording and analysis of the arterial pulse [14–16]. Measurements were made on three occasions over 15 min. Data on pulse rate was recorded and the reflection and stiffness indices calculated using an in-built transfer function. Mean results were obtained for each determination and used in subsequent analyses. Pulse wave profiles from both visits were related to underlying cardiovascular risk factors.

2.5. Statistical analysis

The study design was based on the hypothesis that sildenafil administration would be associated with a 10% change in stiffness index, which has a CV of 10%, and that this would relate to a 10% difference in ADMA levels giving 90% power for a 5% significance level with 22 subjects per group. All analyses were performed using GB Stat 10.0 Dynamic Microsystems; Silver Spring, MD, USA. Variables showing non-Gaussian distributions as determined by skew and kurtosis statistics were log transformed prior to analysis. Smoking and prescribed medications were coded as dichotomous variables. Least squares multiple regression analysis was performed with IIEF score and pulse wave parameters as dependent variables and demographic characteristics, lifestyle risk factors, biochemical and physiological risk factors as dependent variables. Treatment assignment was coded as a separate dichotomous variable. All variables were included in the model and selected by forward stepwise analysis. A p-value of 0.05 was considered significant.

3. Results

3.1. Study completion

Three patients only completed one visit and asked to withdraw from the study after the initial visit. Results for the remaining patients (45) were analysed on a per protocol basis.

3.2. Baseline characteristics

Patient characteristics are shown in Table 1. Coronary heart disease was present in 24%, type 2 diabetes mellitus in 15%, 33% were current smokers and 33% were ex-smokers.

Table 1
Clinical and biochemical characteristics of patients with erectile dysfunction

Parameter	Day 1 value	Day 70 Value
Age (years)	58.5 ± 7.9	–
Body mass index (kg/m^2)	27.4 ± 3.7	27.8 ± 3.8
Cholesterol (mmol/L)	4.95 ± 0.99	4.85 ± 1.00
Triglycerides (mmol/L)	1.50(0.75–8.17)	1.55(0.50–7.15)
HDL-cholesterol (mmol/L)	1.17 ± 0.32	1.19 ± 0.30
LDL-cholesterol (mmol/L)	2.76 ± 0.84	2.68 ± 0.85
Apolipoprotein A1 (g/L)	1.46 ± 0.27	1.44 ± 0.31
Apolipoprotein B (g/L)	1.06 ± 0.25	1.03 ± 0.24
Lipoprotein (a) (g/L)	0.10(0.02–1.49)	0.08(0.02–0.92)
C-reactive protein (mg/L)	3.1(0.4–8.0)	1.9(0.4–9.7)
Fibrinogen (g/L)	3.30(1.03–3.66)	3.50(1.41–4.86)
Glucose (mmol/L)	5.4(4.1–20.1)	4.9(2.5–17.5)
HbA1c (%)	5.5(4.7–11.1)	5.5(4.6–10.6)
Albumin:creatinine ratio (mg/g creatinine)	0.80(0.2–23.1)	0.85(0.32–48.2)
IIEF score	5 (1–20)	21(1–25)***
Blood pressure (mmHg)		
Pre	133 ± 18/81 ± 12	132 ± 12/83 ± 9
Post	122 ± 12/74 ± 11***	122 ± 14/74 ± 7***
Heart rate (bpm)		
Pre	68 ± 11	65 ± 16
Post	71 ± 15	74 ± 14
Reflection index (%)		
Pre	74.0 ± 10.0	73.2 ± 10.6
Post	69.0 ± 12.4**	68.3 ± 11.4**
Stiffness index (m/s)		
Pre	11.6 ± 3.9	11.1 ± 3.7
Post	10.4 ± 4.3	9.7 ± 3.6
E-selectin (ng/mL)	521(305–813)	489(83–982)
ADMA (ng/mL)	202(110–305)	213(111–396)
ADMA (μmol/L)	1.01(0.55–1.52)	1.07(0.56–1.98)

Drug therapy on recruitment comprised anti-platelet medications in 69%, beta-blockers in 28%, angiotensin-I-converting enzyme inhibitors in 7%, statins in 51%, and fibrates in 9%.

3.3. Associations with erectile dysfunction

All patients except one described some benefit from sildenafil therapy over 70 days as IIEF scores increased (+13 points [−1 to +24] points). In multiple regression analysis change in IIEF score correlated ($r^n = 0.79$; $p < 0.001$) with initial IIEF score ($\beta = -0.65$; $p = 0.002$) and concentration of apoB ($\beta = -10.11$; $p = 0.01$). No correlation between baseline IIEF score and other cardiovascular risk factors was seen. Neither ADMA nor E-selectin levels were associated with either baseline IIEF score or change in IIEF score in response to long-term 50 mg sildenafil therapy in univariate or multivariate analysis.

3.4. Associations with baseline risk factors

Markers showed different relationships with cardiovascular risk factors and concomitant medications in patients with erectile dysfunction. These endothelium-related substances showed a degree of inter-relationship as E-selectin levels showed a strong correlation with ADMA levels (partial $r = 0.65$; $p = 0.009$) after adjustment for age, HDL-C and pulse pressure. ADMA levels correlated with E-selectin (partial $r = 0.76$; $P < 0.001$) after adjustment for Lp(a) and pulse pressure and inversely for smoking status.

Table 2
Relationship of E-selectin levels to cardiovascular risk factors in a population with erectile dysfunction as defined by forward multiple regression analysis

Parameter	Beta	S.E.	p
Beta-blocker (+)	0.29	0.14	0.05
Statin (+)	−0.28	0.13	0.03
Age (years)	−0.02	0.006	0.005
HDL-C (mmol/L)	−0.20	0.13	0.14
Ln CRP	0.12	0.08	0.15
BMI (kg/m^2)	0.03	0.02	0.08
DBP (mmHg)	−0.006	0.005	0.21

BMI = body mass index; CRP = C-reactive protein, DBP = diastolic blood pressure; Lp(a) = lipoprotein (a) $R^n = 0.70$, $p < 0.001$.

E-selectin levels (Table 2) showed a strong inverse relationship with age and with statin therapy and a weak positive correlation with concomitant β-blocker therapy. Compared to these relatively weak associations with cardiovascular risk factors, ADMA levels (Table 3) showed strong associations with pulse pressure and Lp(a) concentrations and weak associations with LDL-C, CRP levels and non-significantly with fibrate therapy. A strong negative association was seen between ADMA levels and current smoking.

3.5. Relationship with change in pulse wave velocity after sildenafil therapy

No relationship of baseline levels of E-selectin or ADMA was found with change in reflectance index, stiffness index (pulse wave velocity), pulse pressure or IIEF score in response to acute administration of 50 mg sildenafil. No difference was seen in plasma E-selectin or ADMA levels in patients receiving long-term (3 months) sildenafil therapy compared to baseline levels. No difference was seen in the acute response to a challenge dose of sildenafil at baseline or 70 days after initiation of sildenafil therapy irrespective of frequency of therapy.

Table 3
Relationship of ADMA levels to cardiovascular risk factors in a population with erectile dysfunction as determined by forward multiple regression analysis

Parameter	Beta	S.E.	p
Fibrate (+)	0.10	0.05	0.06
Smoking (+)	−0.07	0.02	0.002
DM (+)	0.07	0.03	0.07
LDL-C (mmol/L)	0.04	0.02	0.02
Ln Lp(a)	0.11	0.03	0.004
Ln CRP	0.05	0.02	0.04
PP (mmHg)	0.004	0.0009	<0.001

CRP = C-reactive protein; DM = diabetes mellitus; Lp(a) = lipoprotein (a); PP = pulse pressure; $R^n = 0.84$; $p < 0.001$.

4. Discussion

This study shows that in patients with erectile dysfunction on cardioprotective therapies ADMA concentrations correlate with E-selectin levels and show a clear relationship with pulse pressure, lack of smoking and lipoprotein (a) concentrations and weak associations with LDL-C and CRP levels. In contrast to E-selectin ADMA levels are not reduced in patients on statin therapy. No relationship of ADMA or E-selectin levels was found with baseline or post-sildenafil therapy IIEF scores. Despite acute 50 mg sildenafil therapy inducing changes in pulse pressure, reflectance and stiffness indices within 30 min the degree of systemic vascular response to sildenafil was not dependent on baseline ADMA levels. This study was designed using patients as their own controls as sildenafil therapy results in no change in IIEF score in healthy individuals and has only small effects on pulse wave parameters in this group. If a group of patients with cardiovascular risk factors but no documented erectile dysfunction had been used as controls it would be impossible to exclude the actual presence of erectile dysfunction in this group given the social stigma still associated with this condition.

No previous study has investigated ADMA levels in patients with erectile dysfunction, though a possible association has been suggested [17]. One previous study has shown that E-selectin and vascular cell adhesion molecules (CAMs) (ICAM-1 and VCAM-1) are elevated in patients with ED compared to controls [18]. There is a strong cross-correlation between selectins and CAM levels. Elevated plasma selectin and CAM levels are associated with their expression on the endothelial cell surface and cellular activation including the production of reactive oxygen species [19] This suggests that ADMA levels should also be increased in this situation and might correlate with degree of endothelial activation [20] as has been found in this study. Studies in patients with CHD suggest that in contrast to CAMs, ADMA levels are not affected by statin [21,22] or fibrate therapy [23]. However, ADMA levels are permissive for statin action on myocardial blood flow [24,25]. These findings are partially confirmed in this study as a weak but non-significant association with fibrate therapy was observed independent of blood pressure [26].

No difference was seen in E-selection or ADMA levels in relation to acute or chronic sildenafil therapy. Sildenafil, like other PDE-5 inhibitors increases NO levels by reducing catabolism but is unlikely to affect NO production from arginine and thus ADMA levels. It will however alter the plasma NO: ADMA ratio. Chronic therapy with sildenafil, in contrast to tadalafil, is unlikely to significantly influence plasma NO or ADMA levels due to its short half-life and no habituation was seen in this study. The data presented in this study suggest that IIEF and pulse wave parameters like endothelial function [27] are determined by difference and not the ratio of the arginine derivatives.

The correlation of ADMA levels with cardiovascular risk factors has been seen in patients with renal disease, diabetes with nephropathy, hypercholesterolaemia and hypertension [9,28–30] but not previously explored in patients with erectile dysfunction. The association with pulse pressure may mimic that seen in patients with left ventricular hypertrophy on dialysis [31]. Similar weak relationships have been observed between ADMA levels and LDL-C but no studies have previously examined the relationship of ADMA with Lp(a) or others plasma markers of inflammation except in renal disease [32]. The presence of an association of ADMA levels with these markers, known to show an association with endothelial function, lends further support to the hypothesis that ADMA levels can help determine endothelial function [30]. The correlation of ADMA with E-selectin levels suggest that like C-reactive protein ADMA may be related to the effects of inflammation on the endothelium [32] and indeed may regulate expression of endothelial chemokines and adhesion molecules. However, a paradoxical association was seen between ADMA levels and smoking compared to expectations based on influences on endothelial function. A similar relationship between smoking and ADMA levels was also seen in a study of 563 elderly men [33]. This may reflect statistical errors induced by multivariate or dichotonous analysis or a different mechanistic action of smoking on ADMA production.

ADMA levels correlate with carotid intima-media thickness [32] and may add additional risk information to classical risk factors [34]. Erectile dysfunction and to some extent IIEF score reflect the underlying burden of atherosclerosis [3] so a relationship with ADMA levels with risk factor burden is not unexpected. This study adds to the data suggesting that ADMA levels may be a biochemical marker capable of integrating various cardiovascular risk factors.

Acknowledgments

The authors would like to thank Pfizer for supporting this investigation.

References

[1] Solomon H, Man JW, Jackson G. Erectile dysfunction and the cardiovascular patient endothelial dysfunction is the common denominator. Heart 2003;89(3):251–3.
[2] Solomon H, Man J, Wierzbicki AS, O'Brien T, Jackson G. Erectile dysfunction: cardiovascular risk and the role of the cardiologist. Int J Clin Pract 2003;57(2):96–9.
[3] Solomon H, Man JW, Wierzbicki AS, Jackson G. Relation of erectile dysfunction to angiographic coronary artery disease. Am J Cardiol 2003;91(2):230–1.
[4] Wierzbicki AS, Chowienczyk PJ, Cockcroft JR, et al. Cardiovascular risk factors and endothelial dysfunction. Clin Sci (London) 2004;107(6):609–15.
[5] Boger RH. The emerging role of asymmetric dimethylarginine as a novel cardiovascular risk factor. Cardiovasc Res 2003;59(4):824–33.

[6] Valkonen VP, Paiva H, Salonen JT, et al. Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine. Lancet 2001;358(9299):2127–8.
[7] Vallance P, Leiper J. Cardiovascular biology of the asymmetric dimethylarginine:dimethylarginine dimethylaminohydrolase pathway. Arterioscler Thromb Vasc Biol 2004;24(6):1023–30.
[8] Kielstein JT, Frolich JC, Haller H, Fliser D. ADMA (asymmetric dimethylarginine): an atherosclerotic disease mediating agent in patients with renal disease? Nephrol Dial Transplant 2001;16(9): 1742–5.
[9] Boger RH. Asymmetrical methylarginine (ADMA) as a cardiovascular risk factor: epidemiological and prospective data. Dtsch Med Wochenschr 2004;129(15):820–4.
[10] Schiel R, Franke S, Busch M, et al. Effect of smoking on risk factors for cardiovascular disease in patients with diabetes mellitus and renal insufficiency. Eur J Med Res 2003;8(7):283–91.
[11] Lu TM, Ding YA, Lin SJ, Lee WS, Tai HC. Plasma levels of asymmetrical dimethylarginine and adverse cardiovascular events after percutaneous coronary intervention. Eur Heart J 2003;24(21): 1912–9.
[12] Maas R, Schwedhelm E, Albsmeier J, Boger RH. The pathophysiology of erectile dysfunction related to endothelial dysfunction and mediators of vascular function. Vasc Med 2002;7(3):213–25.
[13] Rosen RC, Riley A, Wagner G, et al. The international index of erectile function (IIEF): a multidimensional scale for assessment of erectile dysfunction. Urology 1997;49:822–30.
[14] Chowienczyk PJ, Kelly RP, MacCallum H, et al. Photoplethysmographic assessment of pulse wave reflection: blunted response to endothelium-dependent beta2-adrenergic vasodilation in type II diabetes mellitus. J Am Coll Cardiol 1999;34(7):2007–14.
[15] Millasseau SC, Guigui FG, Kelly RP, et al. Noninvasive assessment of the digital volume pulse Comparison with the peripheral pressure pulse. Hypertension 2000;36(6):952–6.
[16] Millasseau SC, Kelly RP, Ritter JM, Chowienczyk PJ. Determination of age-related increases in large artery stiffness by digital pulse contour analysis. Clin Sci (London) 2002;103(4):371–7.
[17] Maas R, Schwedhelm E, Albsmeier J, Boger RH. The pathophysiology of erectile dysfunction related to endothelial dysfunction and mediators of vascular function. Vasc Med 2002;7(3):213–25.
[18] Bocchio M, Desideri G, Scarpelli P, et al. Endothelial cell activation in men with erectile dysfunction without cardiovascular risk factors and overt vascular damage. J Urol 2004;171(4):1601–4.
[19] Sydow K, Munzel T. ADMA and oxidative stress. Atheroscler Suppl 2003;4(4):41–51.
[20] Smith CL, Birdsey GM, Anthony S, et al. Dimethylarginine dimethylaminohydrolase activity modulates ADMA levels, VEGF expression, and cell phenotype. Biochem Biophys Res Commun 2003;308(4):984–9.
[21] Valkonen VP, Laakso J, Paiva H, et al. Asymmetrical dimethylarginine (ADMA) and risk of acute coronary events Does statin treatment influence plasma ADMA levels? Atheroscler Suppl 2003;4(4):19–22.
[22] Paiva H, Laakso J, Lehtimaki T, et al. Effect of high-dose statin treatment on plasma concentrations of endogenous nitric oxide synthase inhibitors. J Cardiovasc Pharmacol 2003;41(2):219–22.
[23] Dierkes J, Westphal S, Martens-Lobenhoffer J, Luley C, Bode-Boger SM. Fenofibrate increases the L-arginine:ADMA ratio by increase of L-arginine concentration but has no effect on ADMA concentration. Atherosclerosis 2004;173(2):239–44.
[24] Janatuinen T, Laakso J, Laaksonen R, et al. Plasma asymmetric dimethylarginine modifies the effect of pravastatin on myocardial blood flow in young adults. Vasc Med 2003;8(3):185–9.
[25] Paiva 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 2002;40(7):1241–7.
[26] Wierzbicki AS. Lipid lowering: another method of reducing blood pressure? J Hum Hypertens 2002;16(11):753–60.
[27] Walker HA, McGing E, Fisher I, et al. Endothelium-dependent vasodilation is independent of the plasma L-arginine/ADMA ratio in men with stable angina: lack of effect of oral L-arginine on endothelial function, oxidative stress and exercise performance. J Am Coll Cardiol 2001;38(2):499–505.
[28] Chan NN, Chan JC. Asymmetric dimethylarginine (ADMA): a potential link between endothelial dysfunction and cardiovascular diseases in insulin resistance syndrome? Diabetologia 2002;45(12):1609–16.
[29] Fleck C, Schweitzer F, Karge E, Busch M, Stein G. Serum concentrations of asymmetric (ADMA) and symmetric (SDMA) dimethylarginine in patients with chronic kidney diseases. Clin Chim Acta 2003;336(1–2):1–12.
[30] Boger RH, Bode-Boger SM. Asymmetric dimethylarginine, derangements of the endothelial nitric oxide synthase pathway, and cardiovascular diseases. Semin Thromb Hemost 2000;26(5):539–45.
[31] Zoccali C, Mallamaci F, Maas R, et al. Left ventricular hypertrophy, cardiac remodeling and asymmetric dimethylarginine (ADMA) in hemodialysis patients. Kidney Int 2002;62(1):339–45.
[32] Zoccali C, Benedetto FA, Maas R, et al. Asymmetric dimethylarginine, C-reactive protein, and carotid intima-media thickness in end-stage renal disease. J Am Soc Nephrol 2002;13(2):490–6.
[33] Eid HM, Arnesen H, Hjerkinn EM, Lyberg T, Seljeflot I. Relationship between obesity, smoking, and the endogenous nitric oxide synthase inhibitor, asymmetric dimethylarginine. Metabolism 2004;53(12):1574–9.
[34] Maas R, Boger RH. Old and new cardiovascular risk factors: from unresolved issues to new opportunities. Atheroscler Suppl 2003;4(4):5–17.