International Journal of Andrology
international journal of andrology ISSN 0105-6263

ORIGINAL ARTICLE

Asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA) and L-arginine in patients with arteriogenic and non-arteriogenic erectile dysfunction

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Keywords:
arteriogenic erectile dysfunction, asymmetric dimethylarginin, non-arteriogenic erectile dysfunction, symmetric dimethylarginine

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Received 31 August 2011; revised 10 February 2012; accepted 29 February 2012

doi:10.1111/j.1365-2605.2012.01272.x

Summary

The plasma concentration of asymmetrical dimethylarginine (ADMA), an inhibitor of nitric oxide synthase, has been linked to endothelial dysfunction. We investigated the relation between ADMA, symmetric dimethylarginine (SDMA) and L-arginine concentrations and erectile dysfunction. We compared plasma levels of ADMA, SDMA and L-arginine in 61 men in good health with erectile dysfunction of arteriogenic and non-arteriogenic origin. Diagnosis of erectile dysfunction was based on the International Index of Erectile Function Score and its aetiology was classified with penile echo-colour-Doppler in basal condition and after intracavernous injection of prostaglandin E1. The ADMA and SDMA concentrations were significantly higher in men with arteriogenic erectile dysfunction compared with those with erectile dysfunction of non-arteriogenic origin ($p < 0.05$) and the concentrations in both subgroups were significantly higher than in controls ($p < 0.001$). There was a negative correlation between ADMA and International Index of Erectile Function Score only in arteriogenic erectile dysfunction subgroup. L-arginine did not differ significantly neither between the two erectile dysfunction subgroups ($p > 0.05$) nor between each of the two erectile dysfunction subgroups and controls ($p > 0.05$). The L-arginine/ADMA and the L-arginine/SDMA ratios in arteriogenic erectile dysfunction subgroups were significantly lower than both in controls ($p < 0.05$) and in non-arteriogenic erectile dysfunction patients ($p < 0.05$); the two ratios in non-arteriogenic erectile dysfunction patients did not differ from those in the controls ($p > 0.05$). We conclude that ADMA and SDMA concentrations are significantly higher and L-arginine/ADMA ratio lower in patients who have arteriogenic erectile dysfunction compared with both patients with non-arteriogenic erectile dysfunction and controls. The negative correlation between ADMA and severity of erectile dysfunction is present only in patients with arteriogenic erectile dysfunction. This study supports the importance to always distinguish arteriogenic from non-arteriogenic erectile dysfunction patients to study the complicate erectogenic mechanisms that lead to erectile dysfunction and also to provide potential therapeutic agents for patients with arteriogenic erectile dysfunction.

Introduction

Erectile dysfunction (ED) is highly prevalent and by current estimated, 30 million men in US and 150 million men worldwide are affected and occurs in 19–64% of men aged 40–80 years, both developing and industrialized countries (Benet *et al.*, 1995). Although ED is a multifactorial process, vascular disease of the penile arteries is an

important cause of ED accounting for up to 80% of cases (Kendirci *et al.*, 2007). It has been suggested that endothelial dysfunction is linked to ED as both share a dependence on a common pathway through the nitric oxide (NO) release (Burchardt *et al.*, 2001; Hashimoto *et al.*, 2000; Widlansky *et al.*, 2003; De Angelis *et al.*, 2001). NO is the major endothelium-derived relaxing factor, playing a critical role in the maintenance of vascular tone (Palmer *et al.*,1988). Penile tumescence and erection is critically reliant on the NO release by both cavernosal nerve terminals and endothelial cells (Sàenz de Tajada *et al.*, 2004). NO is formed from the precursor amino acid, L-arginine, by enzymatic action of nitric oxide synthase (NOS). NO production may be regulated by endogenous NOS competitive inhibitor asymmetric dimethylarginine (ADMA), a L-arginine analogous. The ADMA levels in plasma are a balance between breakdown of proteins containing methylated arginine, renal excretion and the activity of hepatic dimethylarginine dimethylaminohydrolase (DDAH). The L-arginine analogous identified to day, include monomethylarginine (NMMA), symmetric dimethylarginine (SDMA) and ADMA. It was postulated that ADMA, which was identified as circulating in human plasma at a concentration 10 times greater than that of naturally occurring NMMA, might act as an important endogenous regulator of the NO pathway (Vallance *et al.*, 1992).

In view of the apparent role of NO as the key chemical element necessary for penile erection, investigators have begun to explore the incidence of ED in conditions associated to a deregulated ADMA and/or SDMA metabolism as diabetes mellitus, hypercholesterolaemia, renal failure, hypogonadism, ageing and irradiation (Schiel *et al.*, 2003; Maas *et al.*, 2005; Elesberg *et al.*, 2006; Wierzbicki *et al.*, 2006; Kielstein *et al.*, 2005; Lugg *et al.*, 1995; Garban *et al.*, 1995; Carrier *et al.*, 1995). Moreover, over the past decade the interference of these substrates with cardiovascular function has received increasing interest and several lines of evidence suggest that ADMA is involved in the pathogenesis of cardiovascular diseases (Valkonen *et al.*, 2001; Cooke *et al.*, 2004).

As ED may be an early marker for the presence of subclinical atherosclerosis (Solomon *et al.*, 2003) we studied the plasma concentrations of L-arginine, ADMA and SDMA in men with arteriogenic and non-arteriogenic ED, to assess the differences between the two ED subgroups and the healthy subjects, independently by the presence of clinical or subclinical atherosclerosis.

Materials and methods

Investigation protocol

In our centre, patients complaining of ED are currently investigated by careful history-taking and clinical andrological examination; then, a few days later, by a panel of examinations, including blood tests, complete blood picture, haemoglobin, glycated haemoglobin, glycaemia, urea, creatinine, PCR, total and HDL cholesterol, triglycerides, transaminases, testosterone, prolactin, 17-β-estradiol, urine analysis and 24-h urinary albumin excretion (microalbuminuria), the International Index of Erectile Function questionnaire (IIEF) and echo-colour-Doppler of both cavernous arteries.

The IIEF questionnaire (Rosen *et al.*, 1999) is a validated, self-administered tool (Rosen *et al.*, 1999; Rosen *et al.*, 1997), but we only evaluated the answers to the first five (erectile response dominium) of the 15 questions (IIEF–15, 1–5). Possible scores for the IIEF-5 range from 5 to 25, scores of 22–25 indicate normal erectile function whereas scores of 21 or below indicate ED (Rosen *et al.*, 1999 Rosen *et al.*, 1999).

Penile echo-colour-Doppler was performed in basal conditions and after intracavernous injection of 10 μg prostaglandin E1 (PgE1) (Aversa *et al.* 2005), and the peak systolic velocity (PSV) and end-diastolic velocity (EDV) were recorded at 5, 10, 15, 20 and 25 min after the injection in the proximal portion of the penis. Patients were classified as 'non-arteriogenic' when their PSV was ≥35 cm/sec, or ≤35 cm/sec, but >25 cm/sec with concomitant EDV ≤ 0 cm/sec, and 'arteriogenic' when their PSV was ≤20 cm/sec (Barassi A *et al.*, 2009; Barassi *et al.*, 2010). The erection quality was estimated 20 min after each injection. If a patient appeared stressed, he was given a second injection of the same dose of PgE1 and all measurements were repeated. From 30 to 60 min after the penile echo-colour-Doppler, participants were placed in a supine position and blood samples were drawn from a cubital vein into EDTA tubes. Samples were centrifuged at 4000 ***g*** for 10 min. The serum was separated and stored at −80 °C until analysis.

Patients and control group

Of a series of 590 filed ED cases studied in the period October 2008–August 2010, we randomly selected 29 arteriogenic ED patients (ED AR) and 32 non-arteriogenic ED patients (ED NON-AR), according to our exclusion criteria for this study, for a total of 61 cases (median age 48.3 years, range 36–53) with a history of at least 3 months, but not more than 1 year of ED. General exclusion criteria were: age below 30 years, congestive heart failure, diabetes, renal failure, anaemia, acute infection or rheumatic disease, evidence from the patient's clinical history of coronary artery disease, hypertension (>140/90 mm Hg in three consecutive recordings at rest), malignancy, systemic inflammatory disease, hepatopathies or arrhythmias, current smoking (Tostes *et al.*,

2008) and vitamins supplementation. Our patients did not take any therapy.

The 30 healthy controls were all blood donors (median age 45.1 years, range 34–49), and, according to their answers to the first five IIEF-15 questions (erectile response domain), did not suffer from ED. Both healthy controls and the patients of the two ED subgroups declared to develop a normal physical activity.

In accordance with the Declaration of Helsinki II, the design of the study was explained thoroughly to all the participants, and informed consent was obtained for all the test of the study from all ED patients and controls, and for administering the IIEF-15 test to controls. In particular, it was explained to the participants that no additional blood needed for this study.

Assays

From 60 to 90 min before the penile echo-color-Doppler, participants to the study were placed in a supine position and blood samples were drawn from a cubital vein into EDTA tubes. HbA1c was measured using a high performance liquid chromatography (HPLC) method (Bio-Rad). Plasma glucose, creatinine, urea, total cholesterol, HDL-cholesterol, triglycerides, hsCRP, ALT, AST, prolactin, testosterone and 17-β-estradiol were analysed with commercially available kits using Modular EVO (Roche). Plasma LDL-cholesterol was calculated with the use of Friedewald's formula. Urinary microalbumin concentration was measured in duplicate in the morning samples on a Behring Nephelometer II analyser (Siemens).

Plasma concentrations of ADMA, SDMA and L-arginine were determined by the method described by Paroni *et al.* (Paroni *et al.*, 2005). Briefly, after adding L-homoarginine as internal standard, 100 μL of plasma samples were submitted to solid phase extraction on SCX cartridges (Phenomenex STRATA SCX, 55 μm, 70 A, 100 mg/1 mL). Following three washing steps with TCA 2%, phosphate buffer pH 8.0 and methanol, respectively, cationic amino acids (ADMA, SDMA, homoarginine and L-arginine) were eluted with 1 mL of a methanol : water solution (70 : 30, v : v) containing 2% triethylamine. The eluate was evaporated to dryness under a gentle nitrogen stream and the residue was resuspendend in water. This was followed by online ortho-phthaldialdehyde derivatisation, HPLC separation (Ultrasphere Beckman ODS, 250 × 4.6 mm, 5 μm) and fluorimetric detection (λ_{ecc} 340 nm, λ_{em} 455 nm). The liquid chromatographic system included an Agilent 1200 Series Rapid Resolution chromatograph equipped with binary pump SL, thermostatted column compartment SL, high performance autosampler SL plus and a 1260 Infinity Fluorescence Detector. Within-assay and between-assay variations for all the analytes were <3.0% and <6.0%, respectively; the detection limit of the assay was 1.5 pmol injected at a signal-to-noise ratio of 3 : 1. The total run time was 32 min.

Statistical analysis

All the variables were expressed as median and range (or 25% and 75% percentiles). Normal distribution of the data was tested by the Kolmogorov–Smirnov test. The significance of differences between controls, ED AR and ED non-AR was assessed by the non-parametric test Mann–Whitney Rank sum test. Spearman correlation was used to assess the strength of association between variables. Probabilities ≤0.05 were considered to be statistically significant. All analyses were performed using the statistical software package Sigma Stat (Statistical Analysis System, version 3.0) (Jandel Scientific GmbH, Erkrath, Germany).

Results

Of the 61 men included in the study, 29 (47.5%) were classified as having ED AR and 32 (52.5%) as having ED NON-AR. Clinical and laboratory features of 30 controls and 61 patients with ED are presented in Table 1. In ED AR (median age 49.2 years, range 42–52) and ED NON-AR (median age 47.5 years, range 36–53) the median of IIEF values were 10.5 (range 5–19) and 12.8 (range 7–20) respectively. There were no significant differences between the two ED subgroups ($p > 0.05$). There were no differences neither between ED patients and controls nor between the two ED subgroups regarding all the laboratory tests ($p > 0.05$). Hormonal status of all patients was in the normal range (Table 1).

The ADMA concentration (median and range in μmol/L) in ED AR [0.682 (0.536–0.811)] and in ED NON-AR [0.554 (0.492–0.722)] was significantly higher ($p = 0.003$) compared with controls [0.445 (0.380 – 0.570)]; in ED AR it was also significantly higher ($p = 0.038$) than in ED NON-AR.

The SDMA concentration in ED AR [0.746 (0.613–0.808)] and in ED NON-AR [0.592 (0.528–0.751)] was significantly higher ($p = 0.003$ and $p \leq 0.001$ respectively) compared with controls [0.465 (0.425–0.529)]; in ED AR it was also significantly higher ($p = 0.030$) than in ED NON-AR. The results are reported in Fig. 1.

Spearman correlation analysis demonstrated a negative correlation between serum ADMA levels and IIEF score that reached the significance in ED AR ($r = -0.396$; $p < 0.01$) but without reaching the significance in ED NON-AR ($r = -0.03$; $p < 0.20$).

The L-arginine plasma concentration (median and range in μmol/L) did not differ significantly ($p > 0.05$)

Table 1 Clinical and laboratory features of controls and patients with erectile dysfunction

	Controls (n = 30)	ED (n = 61)	ED-AR (n = 29)	ED-NON AR (n = 32)
Age (years)	45.1 (34–49)	48.3 (36–53)	49.2 (42–52)	47.5 (36–53)
Systolic pressure (mmHg)	125 (118–137)	128 (116–138)	130 (118–138)	127 (116–135)
Diastolic pressure (mmHg)	82 (75–86)	85 (72–88)	87 (80–88)	83 (72–86)
Heart rate (bpm)	72 (65–81)	75 (63–88)	77 (65–88)	73 (63–85)
Body mass index (kg/m^2)	26.2 (22.3–27.4)	27.3 (21.8–28.2)	27.5 (22.5–28.2)	26.8 (21.8–27.7)
Diuresis (L)	1.6 (1.3–2.0)	1.7 (1.2–2.1)	1.8 (1.4–2.3)	1.5 (1.2–1.99)
Microalbuminuria (mg/L)	11 (11–14)	11 (11–13)	11 (11–13)	11 (11–12)
HbA_{1c} (%)	4.9 (4.7–5.2)	5.2 (5.0–5.4)	5.2 (4.9–5.3)	5.2 (5.0–5.4)
Glucose (mg/dL)	90.2 (80–96)	94.1 (85–99)	95.9 (89–102)	93.3 (84–95)
hs-PCR (mg/L)	0.9 (0.5–1.9)	1.1 (0.6–2.1)	1.3 (0.7–2.5)	0.8 (0.5–1.5)
17-β-estradiol (pg/mL)	26 (22.5–27.8)	25.5 (22.5–31.4)	25.8 (20.0–33.5)	25.5 (23.8–30.5)
Prolactin (ng/mL)	6.9 (5.8–8.5)	7.5 (6.1–9.1)	7.4 (6.6–8.4)	7.9 (6.0–9.9)
Testosterone (ng/mL)	4.9 (3.9–5.3)	4.6 (3.8–5.1)	4.5 (3.8–4.8)	4.7 (3.8–5.5)
Creatinine (mg/dL)	0.81 (0.75–0.88)	0.85 (0.79–1.00)	0.82 (0.73–0.89)	0.85 (0.78–0.92)
Urea (mg/dL)	37 (32–45)	38 (33–45)	37 (32–46)	39 (34–45)
ALT (U/L)	25 (20–32)	26 (19–39)	29 (22–43)	26 (19–34)
AST (U/L)	28 (20–34)	27 (22–31)	29 (23–32)	26 (22–31)
Total cholesterol (mg/dL)	196 (159–220)	208 (177–226)	198 (175–213)	211 (186–227)
Triglycerides (mg/dL)	118 (92–140)	112 (89–144)	116 (94–161)	112 (84–131)
HDL cholesterol (mg/dL)	53 (45–63)	55 (46–64)	52 (46–61)	57 (50–64)
IIEF-5 score	23 (22–25)	11.6 (5–20)[a]	10.5 (5–19)[a]	12.8 (7–20)[a]

Values are reported as median and range.
[a]$p < 0.001$ vs. Controls.

neither between ED AR [54.10 (46.67–65.57)] or between ED NON-AR [53.80 (40.23–62.30)] and controls [47.15 (36.20–60.50)] nor between the two ED subgroups.

The L-arginine/ADMA ratio [66.50 (52.17–90.95)] and L-arginine/SDMA ratio [72.10 (59.37–82.7)] in ED AR were significantly lower ($p = 0.002$) than in controls [100 (85.5–136.5) and 105.5 (78–144)] and than in ED NON-AR [87.70 (82.40–116.70) $p = 0.004$ and 82.90 (78–114) $p = 0.029$]; the two rations in ED NON-AR did not differ ($p > 0.05$) from the controls (Fig. 2).

Discussion and conclusions

Cells regulate NO homeostasis by different mechanisms. eNOS (type 3) and nNOS (type 1) are constitutively expressed low output enzymes whose ezymatic activity depends on Ca^{2+} levels, protein myristilation, phosphorylation and protein/protein interactions. By contrast, iNOS (type 2) is an inducible NO synthase not normally expressed in resting cells, and upon stimulation by various cytokines or bacterial lipopolysaccharide its transcription can be induced within several hours. The discovery of ADMA and demonstration of in vivo and in vitro effects on inhibiting NO synthesis has led to a large body of work attempting to discover its role in a wide range of different clinical conditions which share endothelial dysfunction as a common feature. After proteolysis of methylated proteins, ADMA is released within cells where can concur to regulate excessive iNOS activity, as well as the other NOS isoforms by competing with the NOS natural substrate arginine (Vallance *et al.*, 1992; Ueda *et al.*, 2003). The local concentrations of substrate and inhibitors, i.e. the L-arginine/ADMA concentration ratio, may be therefore an important factor, although not the only, in the pathogenesis of ED in various disease states. Presumably, the state of activation or inhibition of NOS will depend on ADMA exported from the site of origin by cationic amino acid transporter, the same used by L-arginine (CAT) (Baylis 2006; Welch *et al.*, 1997). The majority of ADMA is metabolized by the enzyme DDAH to yield citrulline and dimethyl-amine (Achan *et al.*, 2003; Tsuji *et al.*, 1986; Palm *et al.*, 2007). Intracellular ADMA that escapes metabolism by DDAH, besides to inhibit NOS, may also reduce NO generation by competing with L-arginine for cellular uptake by CAT transporter. Thus, ADMA not only blocks NOS activity but also limits the cellular uptake of L-arginine, thereby contributing to oxidative stress that further inhibits NO biogenesis (Bögerk *et al.*, 2009). SDMA, in contrast, has no direct effect on NOS. However, it may still be important by competing with L-arginine for transport across the CAT (Closs *et al.*, 1997; Bode- Böger *et al.*, 2006). Thus, also SDMA may indirectly limit NO generation.

The populations studied, ED and controls, were homogeneous with similar patterns of metabolic and inflammatory variables. It is generally accepted that ED has been

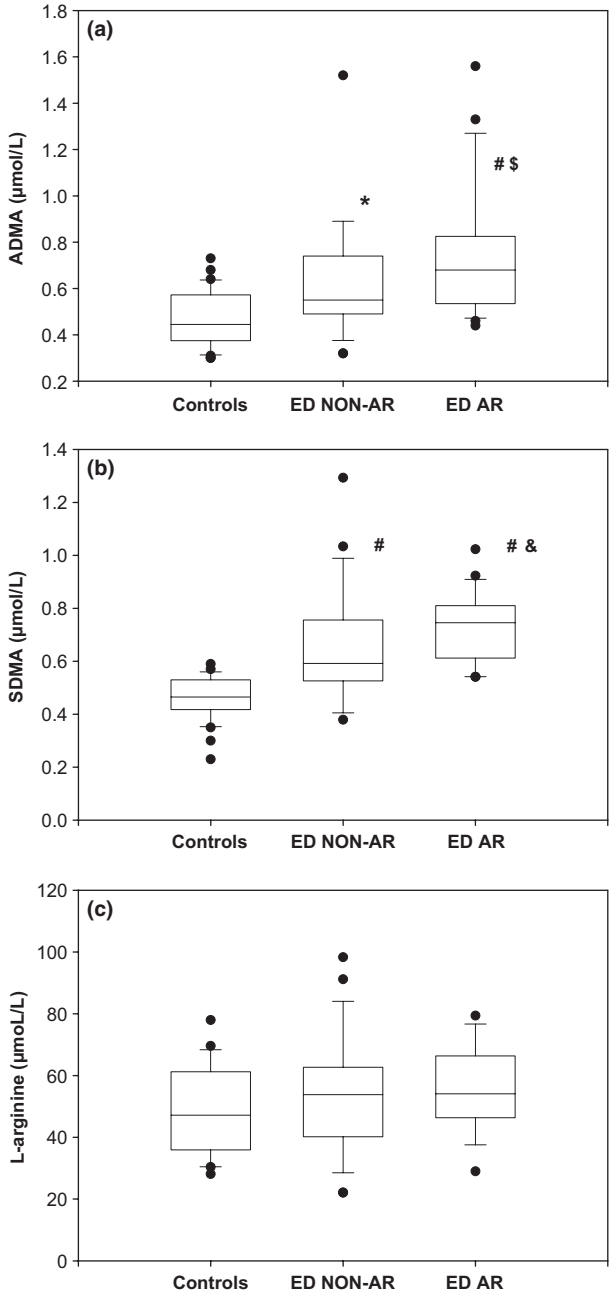

Figure 1 Plasma ADMA (a), SDMA (b) and L-arginine (c) concentrations in men with arterogenic (ED AR), non-arterogenic (ED NON-AR) erectile dysfunction and in controls. $^{*}p = 0.003$ vs. controls; $^{\#}p < 0.001$ vs. controls; $^{\$}p = 0.038$ vs. ED NON-AR; $^{\&}p = 0.030$ vs. ED NON-AR. Controls did not received intracavernous injection of PgE1. The boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. Black circles are the outlying points.

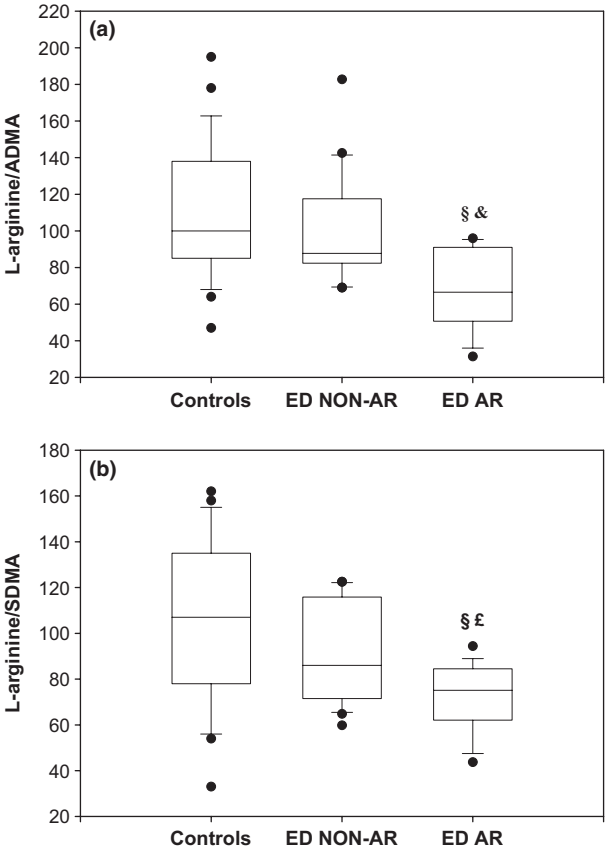

Figure 2 L-arginine/ADMA ratio (a) and L-arginine/SDMA ratio (b) in men with arterogenic (ED AR), non-arterogenic (ED NON-AR) erectile dysfunction and in controls. $^{\S}p = 0.002$ vs. controls; $^{\&}p = 0.040$ vs. ED NON-AR; $^{£}p = 0.029$ vs. ED NON-AR. Controls did not receive intracavernous injection of PgE1.

associated with advanced artheriosclerotic CAD (Solomon *et al.*, 2003; Feldman *et al.*, 2000; Takiuchi *et al.*, 2004): then, the subjects with clinical history of CAD and/or with atherosclerotic risk factors were excluded from the study. As ADMA has been associated with characteristics of the metabolic syndrome only subjects without those characteristics were included in our study.

In this study of well-characterized patients, in particular as regards the penile arteriogenic and non-arteriogenic aetiology of ED, we found a significant increase of ADMA and SDMA concentrations in both the ED subgroups compared with healthy controls and in ED AR compared with ED NON-AR. Our findings confirm recent data of Ioakeimidis *et al.* that found ADMA levels significantly higher in ED AR than in ED NON-AR patients with an independent inverse association between ADMA and PSV (Ioakeimidis *et al.* 2011).

Although elevated ADMA levels in patients with renal failure could be attributed, at least in part, to reduced

renal excretion, the precise cause of elevated ADMA levels in ED has not been identified yet. One cause could be a reduced activity of DDHA, highly expressed in the liver (Nijveldt *et al.*, 2003), whose activity may be inhibited by a large series of traditional (hypercholesterolaemia, diabetes, smoking), non-traditional (hyperhomocysteinaemia, inflammation, oestrogen deficiency, insulin resistence, hypothyroidism) risk factors and finally by oxidative stress. A fairly recent study showed that plasma reactive oxygen metabolite (ROS) concentrations were higher and plasma total antioxidant status (TAS) was lower in ED AR patients in comparison with ED NON-AR patients and in controls (Barassi *et al.*, 2009). Furthermore, through uncoupling of NOS, increased production of superoxide could further impair DDAH, leading to a perpetuating increase in ADMA concentration (Sydow *et al.*, 2003).

There were no significant differences neither between ED patients and controls nor between the two ED subgroups regarding L-arginine concentration. L-arginine/ADMA ratio and L-arginine/SDMA ratio were significantly lower in ED AR in comparison with those in the controls and in ED NON-AR. In ED NON-AR, the two ratios were not different from those in the controls. A low L-arginine/ADMA ratio (i.e. the ratio of NOS substrate and its endogenous inhibitor concentrations) is compatible with ADMA acting as a competitive inhibitor of NOS, resulting in a reduced NO synthesis. It is more difficult to explain the increased level of SDMA and the decreased L-arginine/SDMA ratio in ED patients. In fact, the role of SDMA in these pathologies is not yet completely clear. Nevertheless, the decrease of the ratio only in ED AR can contribute to limit NO generation by reducing the availability of L-arginine, the NOS substrate.

The HPLC method used for quantification is characterized by a very low imprecision, allowing reliable determination also in the presence of small concentration differences between groups. Nevertheless, we found near borderline significant differences in ADMA and L-arginine/ADMA levels between AR and NON-AR patients; then, from a diagnostic standpoint it needs caution in discerning between AR and NON-AR patients from levels near the limit of reference ranges. The concentrations of both ADMA and SDMA are tightly controlled in health, with intraindividual variations (CV_i) of 7.4% and 5.8% respectively (Blackwell *et al.*, 2007). Based on a desirable imprecision of no more than 0.5 times CV_i (Fraser *et al.*, 1999), our method meet this goal. In addition, both the generation of NO and the formation of the L-arginine analogous, by the sequence of protein methylation and proteolysis, are intracellular processes. ADMA and SDMA in plasma probably originate from cellular spillover. A limited increase in the plasma concentration may thus reflect a much larger increase of their intracellular concentration, which may be sufficient to substantially inhibit NO production. In fact, cells can concentrate L-arginine analogous up to 5–10 times than that in plasma.

This study supports the hypothesis that elevated ADMA and normal-low or low L-arginine levels may contribute, either directly or through NOS uncoupling, to the inhibition of penile NO synthesis (Maas *et al.*, 2002). In fact, also L-arginine/ADMA ratio in ED AR is significantly lower than in ED NON-AR and controls. Furthermore, the increased SDMA competes with the L-arginine entrance in the cells reducing the availability for NO synthesis. ED, but only ED AR, may be the earliest manifestation of a generalized vascular disease and only these patients may be at risk of later developing CAD.

Recently, Elesber (Elesber *et al.*, 2006) and Aktoz (Aktoz *et al.*, 2010), comparing plasma ADMA concentration and severity of ED and coronary artery disease, demonstrated a significantly negative correlation between IIEF score and ADMA levels, but none of the two studies split the ED patients in relation to the different aetiology (AR and NON-AR) according to the penile echo-colour-Doppler after intracavernous injection of prostaglandin E1. Our results showed a significantly negative correlation between serum ADMA levels and IIEF score but only in ED AR subgroup confirming the role of ADMA in ED but only in those patients with ED of arteriogenic origin.

Chen *et al.* (Chen *et al.*, 1999) reported significant improvement in sexual function when ED patients characterized by relative L-arginine deficiency (L-arginine/ADMA elevated), were treated with L-arginine supplementation in combination with phosphodiesterase type s (PDE-5) inhibitors. Quite recently, the oral supplementation with arginine or citrulline (which escapes intestinal or liver metabolism, enters the kidneys and is rapidly converted into L-arginine) has been also proposed to patients with mild ED and in heart failure (Cormio *et al.*, 2011; Orozco-Gutierrez *et al.*, 2010), thus providing the rationale for oral L-citrulline supplementation as a donor for the L-arginine/NO pathway in patients with endothelial dysfunction. From the results of our study, we can speculate that only a subgroup of ED patients, ED AR with elevated ADMA, might benefit from L-arginine or L-citrulline supplementation. For this reason, we think advisable to recognize between the ED patients those with arteriogenic aetiology.

In conclusion, NO pathway is of critical importance in the physiological regulation of erection. Elevated endogenous NOS inhibitor, like ADMA, not balanced by and increased L-arginine concentration, contributes to NO deficiency, leading impaired NO-mediated relaxation in the lower urinary tracts and corpus cavernosum. Moreover, our observations could be also interpreted as

evidence for a reduced systemic availability of L-arginine in men with ED; but only in men with ED AR, ADMA appears to be preferentially elevated and both L-arginine/ADMA ratio and L-arginine/SDMA ratio decreased; thus, confirming in this subgroup the diagnosis of vascular origin of ED obtained with the penile echo-colour-Doppler procedure. Nevertheless, the levels of ADMA and of L-arginine/ADMA are not sufficiently different to accurately discern between AR and NON-AR patients. Probably because the precise aetiology of NON-AR ED can be difficult to elucidate and may be because of a combination of organic as well as psychological factors. Taken together with the relatively small number of samples in this study, this may account for the fact that levels of ADMA and L-arginine/ADMA, even though a statistically significant difference was shown between AR and NON-AR patients, can not accurately differentiate a single patient presenting with ED in clinical setting.

Additional studies are required to define the importance of ADMA in ED, but the results demonstrate that future research should investigate separately AR from ED NON-AR patients, because they belongs to different population and accordinges to severity of arteriogenic status by using PSV values (Ioakeimidis *et al.*, 2011). This will increase understanding of the role of ADMA in the mechanisms that lead to ED and potentially provide therapeutic targets.

Finally, our results point out the importance of the determination of ADMA, SDMA and L-arginine concentration that we suggest to insert in the list of the tests for an early screening of ED patients.

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