



Rewiew

Asymmetric dimethylarginine (ADMA) as a target for pharmacotherapy

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Abstract:

Asymmetric dimethylarginine (ADMA) is synthesized during the methylation of protein arginine residues by protein arginine methyltransferases (PRMT) and is released during proteolysis. ADMA is a competitive inhibitor of nitric oxide synthase and may decrease NO availability. ADMA is eliminated by renal excretion or is metabolized by dimethylarginine dimethylaminohydrolase (DDAH) to citrulline and dimethylamine. Two other endogenous methylarginines are also synthesized by PRMT: N-monomethyl-L-arginine (L-NMMA) and symmetric dimethylarginine (SDMA). L-NMMA inhibits NO synthase but its concentrations in circulation are much lower than ADMA whereas SDMA is inactive. Plasma concentration of ADMA is markedly increased in patients with chronic renal failure and moderately increased in patients with many other diseases including hyperlipidemia, diabetes mellitus, arterial hypertension, hyperhomocysteinemia and heart failure. The increased concentration of ADMA is positively correlated with markers of atherosclerosis, such as carotid artery intima-media thickness and has a predictive value for acute cardiovascular events in prospective studies. Angiotensin-converting enzyme inhibitors, angiotensin AT₁ receptor antagonists, vitamin E and, according to some studies, estrogens used in hormonal replacement therapy reduce plasma ADMA concentration, which may contribute to their beneficial effect on NO synthesis and endothelial function. However, in some states associated with excess of NO, such as septic shock or excitotoxic neuronal injury ADMA may be protective by limiting toxic effect of high concentrations of NO. This article reviews the effect of pharmacotherapy on ADMA metabolism and its possible clinical implications.

Key words:

nitric oxide, nitric oxide synthase, asymmetric dimethylarginine, dimethylarginine dimethylaminohydrolase

Abbreviations: ADMA – asymmetric dimethylarginine, DDAH – dimethylarginine dimethylaminohydrolase, LDL – low-density lipoproteins, L-NMMA – N-monomethyl-L-arginine, oxLDL – oxidized low-density lipoproteins, PPAR – peroxisome proliferator-activated receptors, PPRE – peroxisome proliferator-response element, PRMT – S-adenosylmethionine: protein arginine methyltransferase, RAR – retinoid A receptor, ROS – reactive oxygen species, RXR – retinoid X receptor, SAH – S-adenosylhomocysteine, SAM – S-adenosylmethionine, SDMA – symmetric dimethylarginine

L-arginine by a family of NO synthases (NOS) including type I or neuronal NOS (nNOS), type 2 or inducible NOS (iNOS) and type 3 or endothelial NOS (eNOS). NO is involved in the regulation of vascular tone, neurotransmission in the central and peripheral nervous system, killing the invading microorganisms by macrophages and regulation of mitochondrial respiration [143]. The availability of NO in a given cell depends on many factors including expression and activity of NOS, abundance of NOS substrate, L-arginine, and its cofactor, tetrahydrobiopterin, and quenching of NO by reactive oxygen species (ROS). Studies performed during the last decade indicate that NO production may also be regulated by endogenous NOS inhibitors, in particular asymmetric dimethylarginine

Introduction

Nitric oxide (NO) is one of the most important mediators and neurotransmitters. NO is synthesized from

(ADMA). The level of NOS inhibitors may change under physiological and pathological conditions leading to NO deficiency in various disease states. Although several excellent reviews about ADMA have been published [23, 51, 85, 160, 161], none of them focused specifically on its modulation by pharmacotherapy. The aim of this paper is to provide the reader with brief overview of the synthesis and metabolism of ADMA, its biological activity, and modulation of ADMA metabolism by drugs commonly used in clinical practice.

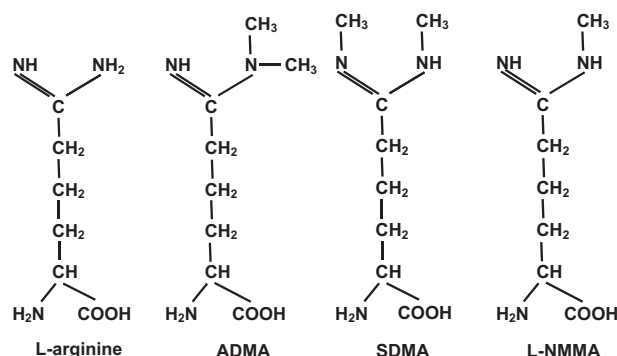


Fig. 1. Structure of endogenous methylarginines

Synthesis and metabolism of ADMA

Methylated arginine derivatives were first isolated from human urine in 1970 [77]. Two years earlier, Paik and Kim purified an enzyme capable of methylating protein arginine residues which they denoted protein methylase 1 [120]. However, the key study initiating research in this field was published in 1992 by Vallance et al. [162]. The authors identified ADMA in human plasma and urine and demonstrated that ADMA inhibited the isolated NO synthase. In addition, ADMA contracted rat aortic rings *in vitro*, inhibited endothelium-dependent relaxation in response to acetylcholine, and increased blood pressure when infused into the guinea pigs. Local infusion of ADMA into the brachial artery of human volunteers caused a dose-dependent fall in forearm blood flow. Finally, it was shown that ADMA concentration was markedly elevated in patients with chronic renal failure [162].

Apart from ADMA, two related compounds, symmetric dimethylarginine (SDMA) and N-monomethyl-L-arginine (L-NMMA) are synthesized endogenously. L-NMMA is as potent as ADMA in decreasing NOS activity but its concentration in plasma is about tenfold lower, however, intracellular concentration of L-NMMA and ADMA may be comparable at least in some tissues [28], indicating that both are important NOS regulators. SDMA at concentrations in the circulation are comparable to ADMA but it has no effect on NOS [162]. Structure of ADMA and related compounds is presented in Figure 1.

ADMA, SDMA and L-NMMA are synthesized during the methylation of protein arginine residues by S-adenosylmethionine:protein arginine methyltransferases (protein methylases, PRMT). These enzymes transfer the methyl group from S-adenosylmethionine

(SAM) to arginine thus forming methylated arginine and S-adenosylhomocysteine (SAH); the latter is subsequently hydrolyzed to homocysteine (Fig. 2). Two types of PRMT have been identified. PRMT1 methylates histones and nuclear RNA-binding proteins and yields L-NMMA and ADMA, whereas PRMT2 methylates exclusively myelin basic protein and generates L-NMMA and SDMA but not ADMA [129]. Recent studies suggest that multiple isoforms of PRMT1 and PRMT2 encoded by separate genes exist. It is estimated that about 1–4% of arginine residues in nuclear proteins are methylated and this is an irreversible reaction since protein-bound arginine residues cannot be demethylated. Free methylarginines are released

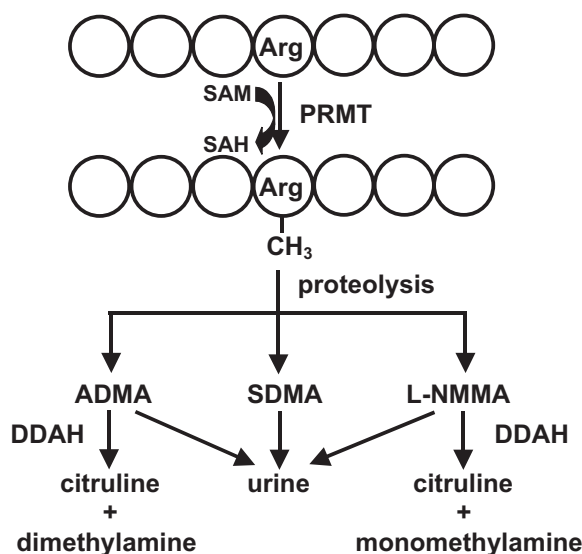


Fig. 2. Synthesis and metabolism of ADMA and related compounds. Arg – arginine

during proteolysis and are not incorporated back into proteins. Normal plasma level of ADMA is less than 1 μM ; it increases up to 10-fold in patients with end-stage renal disease and more moderately (2–3 fold) in many other pathologies (see below) [161].

All three methylarginines are eliminated by renal excretion, however, more than 90% of ADMA and L-NMMA is metabolized by dimethylarginine dimethylaminohydrolase (DDAH), which degrades them to citrulline and dimethylamine or monomethylamine, respectively (Fig. 2) [115]. DDAH exists in 2 isoforms: DDAH1 is predominantly expressed in tissues containing nNOS and DDAH2 mainly in tissues containing eNOS or iNOS [94, 155]. Pharmacological inhibition of DDAH increases ADMA concentration and reduces NO production [103], whereas transgenic DDAH overexpression has the opposite effect both *in vitro* [141] and *in vivo* [39]. DDAH is expressed in many tissues including endothelial cells, brain, pancreas, etc., however, the liver and kidney may be the principal organs responsible for ADMA metabolism. Indeed, only a small portion of ADMA extracted from the blood by the kidney is recovered in urine whereas the rest is metabolized by renal DDAH [114].

Biological activity of ADMA

ADMA inhibits NO production by isolated NOS and by intact cells with IC_{50} of 2–10 μM , which is well within (patho)physiological range [23]. ADMA is a competitive inhibitor of all three NOS isoforms and its effect is reversed by high concentration of L-arginine. ADMA may be responsible for the so-called “arginine paradox” – the observation that L-arginine improves NO generation in many experimental systems, although physiological level of this amino acid in plasma and tissues is much above K_m of NOS [35, 156].

Apart from NO, nitric oxide synthase may generate superoxide anion radical (O_2^-). This effect is called “NOS uncoupling” and is observed when the availability of NOS substrate, L-arginine, or its cofactor, tetrahydrobiopterin, is insufficient. ADMA and L-NMMA, by competing with arginine, may also induce “NOS uncoupling” leading to oxidative stress [27, 151].

Interestingly, all three methylarginines compete with cellular uptake of L-arginine by plasma membrane cationic amino acid transporter (y^+). Thus, also

SDMA may limit NO generation by reducing intracellular arginine availability [24, 25, 32]. Because ADMA is a “substrate-based” inhibitor of NOS, one cannot exclude the possibility that it alters the activity of other arginine-metabolizing enzymes such as arginine:glycine amidinotransferase (a rate-limiting enzyme in creatine synthesis), arginase (the first enzyme of the urea cycle), etc., and exerts some effects unrelated to NOS blockade. Indeed, chronic infusion of ADMA induces comparable microvascular lesions in wild-type and eNOS knockout mice [148], suggesting an NO-independent effect.

Regulation of ADMA metabolism in physiological and pathological conditions

In theory, four mechanisms may lead to accumulation of ADMA: (1) increased methylation of proteins by PRMT, (2) augmented proteolysis and release of preformed methylarginines, (3) impaired renal excretion, and (4) impaired metabolism by DDAH.

Little is known about the regulation of PRMT activity. Nevertheless, shear stress increases PRMT expression and activity and stimulates production of ADMA in cultured endothelial cells by activating a transcription factor, nuclear factor- κB (NF- κB) [118]. Interestingly, shear stress increases DDAH activity suggesting that the increase in ADMA results from the increased protein methylation and occurs despite concomitant stimulation of ADMA metabolism. Shear stress may contribute to the increase in ADMA observed in hypervolemic states such as heart failure [159], renal failure [162] or high-salt diet [53]. In addition, native low-density lipoproteins (LDL) increase PRMT activity and formation of ADMA in endothelial cells [22]. Protein methylation is augmented in proliferating cells and, indeed, ADMA concentration is higher in regenerating than in quiescent endothelial cells [7]. Finally, anti-DNA antibodies stimulate methylation of ribonucleoproteins and may underlie increased synthesis of methylarginines in patients with systemic lupus [26, 149].

The increased proteolysis may contribute to elevation of ADMA in hypercatabolic states such as endotoxemia [109], hyperthyroidism [58] and muscular dystrophy [62, 97]. Impaired urinary excretion leads

to overaccumulation of methylarginines in uremic patients [50], but may also contribute to elevation of ADMA in hemorrhagic [5] or septic shock [114].

However, there is little doubt that the most common mechanism leading to accumulation of ADMA involves impaired metabolism by DDAH. In many experimental systems accumulation of ADMA is accompanied by reduced DDAH activity despite normal DDAH protein level which suggests that specific activity of the enzyme (activity per unit of enzyme mass) decreases. Reduced sulfhydryl (-SH) group of cysteine²⁴⁹ is essential for DDAH activity and, therefore, this enzyme is extremely sensitive to oxidative stress. Indeed, a decrease in DDAH activity is often accompanied by accumulation of reactive oxygen species, and antioxidants protect the enzyme [68, 70]. Oxidative stress may also stimulate formation of ADMA as evidenced by the increased expression of PRMT1 in endothelial cells subjected to excess of ROS [68].

DDAH may also be inactivated by nitrosylation of the -SH group to the respective nitrosothiol (-S-NO) which occurs when NO is generated in excessive amounts by iNOS. This mechanism may lead to accumulation of ADMA and, consequently, may limit NO generation when iNOS is induced by proinflammatory cytokines [86, 93]. However, the effect of cytokines on DDAH is inconsistent. In cultured rat vascular smooth muscle cells, interleukin-1 β (IL-1 β) stimulates the expression of both iNOS and DDAH. The increased DDAH activity results in reduced ADMA concentration, which further augments NO production. Indeed, a specific inhibitor of DDAH, 4124W, increases the amount of ADMA and reduces NO synthesis in cells stimulated with this cytokine [158]. Thus, DDAH-ADMA pathway may either counteract [86, 93] or cooperate with iNOS [158] in regulating NO production during the inflammatory state. Impaired metabolism by DDAH may also contribute to overaccumulation of ADMA in patients with liver dysfunction [113, 157].

ADMA and related methylarginines in pathology

Accumulating evidence suggests that ADMA is involved in the pathogenesis of arterial hypertension. Infusion of ADMA increases blood pressure [2, 83].

Apart from inducing vasoconstriction, ADMA may contribute to abnormal renal sodium handling by limiting the inhibitory effect of NO on tubular Na⁺ reabsorption; the effect is observed at low doses which are hemodynamically neutral [84]. Daily urinary excretion of ADMA is increased in Dahl salt-sensitive rats if they are maintained on high-salt diet and this is accompanied by the increase in blood pressure and reduction of urinary excretion of NO metabolites [106]. In contrast, in Dahl salt-resistant rats, diet rich in NaCl has no effect on blood pressure or urinary ADMA. The increased ADMA concentration was also observed in humans with essential hypertension [54, 150]. Interestingly, in patients with salt-sensitive hypertension, high-salt diet increased plasma ADMA concentration and reduced whole-body NO production in accordance with results in Dahl salt-sensitive rats [53]. The increased ADMA has been observed in patients with white-coat hypertension [33] and in women with pregnancy-induced hypertension [125, 133]. Interestingly, urinary excretion of ADMA in spontaneously hypertensive rats (SHR) is normal [164] or lower than in control animals [106], suggesting that excess of ADMA is not secondary to the increased blood pressure but rather plays a causative role in selected forms of hypertension.

Nitric oxide, produced continuously by endothelial cells, is an essential antiatherogenic factor. NO inhibits oxidative modification of plasma lipoproteins, reduces the adhesion of monocytes to the endothelium, inhibits proliferation of vascular smooth muscle cells and suppresses platelet activity [111]. ADMA stimulates many processes involved in atherogenesis such as monocyte adhesiveness [30], expression of proinflammatory and chemotactic factors [20], accumulation of oxidatively modified LDL in macrophages [140]. Plasma concentration of ADMA is increased in patients with virtually every risk factor of atherosclerosis including hypercholesterolemia [18], hypertriglyceridemia [101], diabetes mellitus [95, 122], obesity [48] and hyperhomocysteinemia [146, 147]. Chronic infusion of ADMA induces atherosclerosis in mice [148]. In humans, plasma concentration of ADMA correlates with markers of subclinical atherosclerosis such as carotid artery intima-media thickness [174] and is a predictive factor for acute cardiovascular events in prospective studies not only in patients with renal failure [175] but also in those with intact kidney function [100, 136].

In patients with end-stage renal disease, plasma level of ADMA is much higher than in any other diseases. This is accompanied by the increase in SDMA since this isoform is also excreted in urine. In contrast, in most other diseases only ADMA but not

SDMA is elevated suggesting that impaired metabolism by DDAH plays a prominent role. In addition, due to avid binding to plasma proteins, methylarginines are poorly removed by dialysis. It is suggested that ADMA is an important uremic toxin in re-

Tab. 1. The effect of anti-RAAS treatment on plasma methylarginines and L-arginine concentrations

Animals/patients	Treatment	% change from baseline ^a				Ref.
		ADMA	SDMA	L-NMMA	L-Arginine	
Animal studies (ACEI)						
Rats with experimental hyperhomocysteinemia	Captopril 3 mg/kg/day 4 weeks	-49%	?	?	?	[152]
Rats treated with isolated human LDL	Captopril 10 mg/kg (single dose)	-15%	?	?	?	[74]
	Captopril 20 mg/kg (single dose)	-32%	?	?	?	
Rats treated with isolated human LDL	Enalapril 30–60 mg/kg	–	?	?	?	[74]
Human studies (ACEI)						
Essential hypertension n = 20	Enalapril 10 mg/day 1 week	-16%	?	?	–	[40]
Syndrome X ^b n = 10	Enalapril 10 mg/day 8 weeks	-17%	-17%	?	–	[31]
Essential hypertension n = 48	Enalapril 20 mg/day 12 weeks	-14%	?	?	?	[112]
Essential hypertension n = 7	Perindopril 4 mg/day 4 weeks	-18%	?	?	?	[65]
Essential hypertension n = 48	Zofenopril 30 mg/day 12 weeks	-24%	?	?	?	[112]
Type 2 diabetes n = 11	Perindopril 4 mg/day 4 weeks	-17%	?	?	?	[64]
Human studies (ARB)						
Essential hypertension n = 20	Eprosartan 3000 mg/day 1 week	-16%	?	?	–	[40]
Essential hypertension n = 7	Losartan 50 mg/day 4 weeks	-10%	?	?	?	[65]

^a percent change of mean or median during treatment, ^b anginal chest pain with normal coronary angiogram, “?” – not studied, “–” – no change

nal failure patients. By suppressing different NOS isoforms, it may contribute to various complications including arterial hypertension, left ventricular hypertrophy, atherosclerosis, immune deficiency and osteodystrophy [85, 98, 166].

The increased concentration of ADMA has been observed in many other diseases such as idiopathic pulmonary hypertension [82], peripheral arterial occlusive disease [19], portal hypertension [92], heart failure [159], hypopituitarism [91], erectile dysfunction [102], aging [81, 168], systemic sclerosis [44], subarachnoidal hemorrhage [76] and depression [138].

Effect of drugs on ADMA metabolism

Inhibitors of renin-angiotensin-aldosterone system (RAAS)

Angiotensin converting enzyme inhibitors (ACEI), angiotensin AT₁ receptor blockers (ARB) and aldosterone antagonists are commonly used in the treatment of arterial hypertension and heart failure. ACEI and ARB have been shown to decrease plasma ADMA in many studies (Tab. 1) however, the mechanism through which RAAS inhibitors modulate ADMA metabolism is not clear at present. Angiotensin II increases ROS formation by vascular NADPH oxidase [78]. Due to inactivation of DDAH by ROS, ACEI and ARB might improve ADMA metabolism by ameliorating oxidative stress. Indeed, in some studies serum markers of oxidative stress have been reduced by these drugs [52, 65]. Napoli et al. [112] compared the effects of two ACE inhibitors: zofenopril which contains reduced sulfhydryl groups and thus possesses direct radical-scavenging properties, and enalapril, which does not contain -SH groups and has no antioxidant activity. They demonstrated that zofenopril was much more effective in reducing ADMA concentration [112]. However, in other studies [64], no change in serum lipid peroxidation products was observed in patients treated with ACEI and, therefore, other mechanisms should be considered. Because shear stress increases ADMA and SDMA formation by endothelial cells, RAAS blockade could decrease ADMA by lowering blood pressure. Simultaneous depression of both ADMA and SDMA observed by some authors [31] would be

consistent with this possibility. However, in other studies [e.g. 64] ACEI or ARB decreased ADMA despite having no effect on blood pressure. Also, the observation that only perindopril but not bisoprolol reduces ADMA in hypertensive patients despite similar decrease in blood pressure [65] suggests that effect on blood pressure does not play a pivotal role. In most studies with ACEI or ARB no changes in renal function were observed. Thus, it is unlikely that reduction of ADMA concentration resulted from the improvement of renal excretion. Indeed, in patients with type 2 diabetes, treatment with perindopril reduced plasma ADMA but had no effect on urinary ADMA [64].

Aldosterone antagonists are increasingly recommended for the treatment of arterial hypertension and heart failure since they have been demonstrated to improve survival in several trials [89]. Unfortunately, the effect of these drugs on ADMA metabolism has not been examined so far.

Statins

Statins are competitive inhibitors of 3-hydroxy-3-methylglutarylcoenzyme A reductase, a rate-limiting enzyme in cholesterol biosynthesis. Apart from decreasing plasma cholesterol, statins increase the expression of eNOS, inhibit adherence of leukocytes and platelets to the endothelium, block proliferation of vascular smooth muscle cells and ameliorate oxidative stress.

Given the deleterious effect of hyperlipidemia and oxidative stress on DDAH activity, it should be expected that statins will normalize ADMA metabolism due to their potent antioxidant and lipid-lowering properties. Surprisingly, almost all studies performed so far have demonstrated that this is not the case. In a recent randomized study, neither simvastatin nor atorvastatin administered for 8 weeks at relatively high doses (80 and 40 mg/day, respectively) had any effect on plasma ADMA, SDMA or L-NMMA in patients with normal to moderately elevated LDL cholesterol [121]. Similarly, pravastatin (40 mg/day for 8 weeks) did not modify plasma methylarginines in 32 hypercholesterolemic men without ischemic heart disease [49]. Although ADMA level before treatment was 60% higher in hyperlipidemic than in control group and pravastatin markedly reduced LDL-cholesterol, average ADMA level decreased after treatment only by 9% which was not statistically significant. In another study, simvastatin administered

for 2 months at 20 mg/day had no effect on ADMA and SDMA in 25 asymptomatic hypercholesterolemic patients [124]. Atorvastatin (10 mg/day for 6 months) did not change ADMA or L-arginine levels in patients with type 2 diabetes [139]. Lovastatin (10 mg/day for 12 weeks) failed to reduce ADMA or SDMA in rabbits made hyperlipidemic by high-cholesterol diet [16]. A single intravenous administration of LDL increases plasma ADMA in the rat and simvastatin administered at a dose of 30–120 mg/kg/day for 3 days before LDL injection failed to modify ADMA level [72]. In addition, simvastatin had no effect on LDL- or oxLDL-stimulated ADMA formation by cultured endothelial cells *in vitro* and did not prevent LDL or oxLDL-induced decrease in DDAH activity [72].

It is unclear why, despite improving the lipid profile and oxidant-antioxidant balance, statins have no or only weak effect on ADMA concentration. It cannot be excluded that in most studies statin administration was too short to cause desirable impact on ADMA metabolism. Alternatively, the beneficial effect of statins might be offset by other unfavorable mechanisms. In particular, statins decrease paraoxonase 1 (PON1) activity [11]. PON1 degrades homocysteine thiolactone – a metabolite of homocysteine which attaches to proteins and modifies their properties [9]. Homocysteine decreases DDAH activity [147], therefore, it is possible that statins, by inhibiting PON1, unfavorably affect DDAH by homocysteine-dependent mechanism [10]. In addition, statins may impair insulin sensitivity [79] and insulin resistance is associated with the increased ADMA [145].

In contrast to these studies, a 6-week treatment with rosuvastatin (10 mg/day) decreased plasma ADMA by about 18% in 23 patients with hypercholesterolemia [99]. Yin et al. [171] have recently reported that pravastatin prevents the decrease in DDAH activity induced by glycated serum albumin in the isolated rat aortic rings, which may suggest a beneficial effect of statins on ADMA metabolism in diabetes. However, this study was performed only *in vitro*, pravastatin was used at very high concentration and aortic rings were incubated with pravastatin for only 15 min, therefore, the physiological implications of these findings are unclear.

Interestingly, baseline ADMA concentration may determine the effect of statins on endothelial function. For example, pravastatin enhanced myocardial blood flow in patients with low ADMA but not in those with elevated ADMA [67]. It may be speculated that pra-

vastatin, despite increasing eNOS expression and/or activity, cannot stimulate NO production because the enzyme is blocked by ADMA. Indeed, a combined treatment with simvastatin and L-arginine improved endothelial function in patients with high ADMA, probably because arginine competed with ADMA for binding to eNOS [15].

In summary, statins had little or no effect on ADMA in most studies, although one cannot exclude that they favorably modulate DDAH-ADMA pathway in selected groups of patients such as those subjected to extreme oxidative stress or diabetics. In addition, high ADMA concentration may offset the beneficial effect of statins on endothelial function in some patients.

Fibrates and niacin

Fibrates are the drugs of choice for the treatment of hypertriglyceridemia and/or low high-density lipoproteins (HDL)-cholesterol – two lipid abnormalities most commonly observed in patients with metabolic syndrome and type 2 diabetes. Fibrates are the synthetic agonists of peroxisome proliferator activated receptor- α (PPAR- α) – a ligand-activated transcription factor which, upon activation, heterodimerizes with the retinoid X receptor (RXR) and binds to the peroxisome proliferator response element (PPRE) in the promoter region of target genes [12].

Yang et al. [170] have demonstrated that administration of fenofibrate at doses of 30 or 100 mg/kg/day for 6 days abolished the increase in ADMA concentration induced by intravenous injection of native human LDL in the rat. The beneficial effect of fenofibrate could have resulted from its antiinflammatory and/or antioxidant activities [170]. In the subsequent study [169], the same group reported that *in vitro* fenofibrate itself had no effect on the release of ADMA by cultured endothelial cells, however, it concentration-dependently attenuated the increase in ADMA release induced by oxidized LDL. Fenofibrate significantly attenuated oxLDL-induced decrease in DDAH activity but had no effect on DDAH in cells not treated with oxLDL [169]. These results suggest that fibrates may have a beneficial effect on ADMA metabolism, especially in subjects with hyperlipidemia and/or increased oxidative stress. PPRE was identified within the promoter region of DDAH-2 gene [75], thus PPAR agonists may also directly regulate DDAH gene expression.

Until now, only one study [43] addressed the effect of fenofibrate in humans. In 25 men with hypertriglyceridemia and no symptoms of atherosclerosis, fenofibrate (200 mg/day for 6 weeks) had no effect on plasma ADMA and SDMA concentrations, however, increased plasma L-arginine by 22% which resulted in a significant increase in L-arginine/ADMA ratio. The reason for the discrepancy between these results and above-mentioned experimental studies is unclear, however, might be many-fold. First, the expression of PPAR α , especially in the liver, is much higher in rodents than in humans, which could lead to species differences in the effect of fibrates [43, 170]. Second, in experimental studies the effect of fenofibrate was examined only in animals [170] or cells [169] subjected to supranormal oxidative stress and fenofibrate was applied for a relatively short time. Most importantly, in the study of Dierkes et al. [43] direct beneficial impact of fenofibrate could be overridden by some indirect negative effects. In particular, although fenofibrate decreased plasma triglycerides, it simultaneously increased LDL-cholesterol, slightly but significantly reduced HDL-cholesterol, and markedly (by 44%) increased plasma homocysteine; all these changes might have a negative impact on ADMA. In addition, fenofibrate increased plasma creatinine suggesting reduction of creatinine clearance [43]. Although recent studies indicate that fibrates stimulate synthesis of creatine and creatinine while having no effect on glomerular filtration [61], this suggests a complex effect of these drugs on arginine metabolism since creatine is produced from arginine. In addition, fibrates stimulate protein degradation in skeletal muscles which could enhance release of preformed ADMA [119].

Niacin is well known to improve blood lipids. Recently, niacin administered at 1500–2000 mg/day for 6 weeks has been demonstrated to decrease plasma ADMA by 8–12% in 26 patients with low HDL-cholesterol [165]. The authors suggest that niacin reduces synthesis of methylarginines because the metabolism of niacin requires large amounts of methyl groups. Consequently, a methyl donor, SAM, could be depleted and become unavailable for the methylation of proteins. This hypothesis is consistent with the simultaneous decrease in SDMA observed in that study [165]. Thus, niacin might have a unique mechanism of action among other drugs in that it reduces ADMA synthesis rather than improves its metabolism.

Thiazolidinediones and other antidiabetic agents

Thiazolidinediones are synthetic agonists of peroxisome proliferator-activated receptor- γ (PPAR- γ) which mainly control adipocyte differentiation and carbohydrate metabolism. PPAR- γ agonists improve insulin sensitivity and are used as oral hypoglycemic agents in patients with type 2 diabetes [12]. *In vitro*, a PPAR- γ agonist, troglitazone, attenuated shear stress-induced up-regulation of PRMT1 and ADMA release by cultured endothelial cells [118]. This effect is brought about by preventing the shear stress-induced activation of NF- κ B. Troglitazone also prevented a shear stress-induced increase in DDAH activity but had no effect on steady-state PRMT1 expression, ADMA release and DDAH activity in cells not subjected to shear stress [118]. Recently Wakino et al. [164] have demonstrated that another PPAR- γ activator, pioglitazone (160 mg/kg/day for 4 weeks) decreased plasma ADMA concentration by about 20% in both spontaneously hypertensive rats and in control normotensive Wistar-Kyoto (WKY) rats. This effect was accompanied by the increase in renal expression of DDAH2 but not DDAH1 in both strains. Although SHR were insulin-resistant before treatment as evidenced by higher plasma insulin, triglycerides and nonesterified fatty acids and pioglitazone corrected these abnormalities, the increase in insulin sensitivity could not explain its beneficial effect on ADMA since pioglitazone did not change insulin sensitivity but decreased ADMA in WKY, and increased the expression of DDAH2 also in cultured renal tubular cell line.

To our knowledge, only one clinical study with thiazolidinediones addressed their effect on plasma ADMA. Stuhlinger et al. [145] demonstrated that rosiglitazone (4 mg/day for 4 weeks followed by 8 mg/day for additional 8 weeks) decreased plasma ADMA by 30% in 7 insulin-resistant nondiabetic hypertensive individuals. Unfortunately, the effect of PPAR- γ agonists on ADMA has not been studied either in diabetic patients or in animal models of diabetes.

Asagami et al. [6] examined the effect of metformin on plasma ADMA in patients with type 2 diabetes. Metformin was added to the treatment schedule because glycemia was inadequately controlled by diet or by diet + sulfonylurea derivative, and was administered at a dose of 1–2 g/day for 3 months. Metformin administered either alone or in combination with sulfonylurea derivatives reduced plasma ADMA by

about 30%. Metformin had no effect on either SDMA or L-arginine which resulted in a significant elevation of L-arginine/ADMA ratio. However, it is unclear if metformin reduced ADMA secondary to improving glycemic control or through the mechanism unrelated to glucose homeostasis because the effect of other hypoglycemic drugs was not evaluated in humans. The former possibility is likely since insulin supplementation prevented the elevation of ADMA in experimental streptozotocin-induced diabetes in the rat [167]. Interestingly, metformin (N,N-dimethylbiguanide) is structurally similar to ADMA and may be transported to the cells by the γ^+ system [80]. Moreover, ADMA inhibits metformin transport to the cells [42], however, the implications of this fact, if any, remain unclear at present.

Antioxidants

Oxidative stress, in particular oxidative modification of plasma lipoproteins, plays an important role in atherogenesis. However, large prospective clinical trials performed to date have demonstrated that supplementation of antioxidants such as vitamin E or vitamin C had either no effect on acute cardiovascular events or even worsened prognosis in patients with ischemic heart disease [56]. Nevertheless, several smaller studies suggest that antioxidants may improve cardiovascular prognosis in patients with enhanced oxidative stress such as those with renal failure [13].

Experimental studies indicate that various synthetic antioxidants preserve DDAH activity and reduce ADMA formation induced by different prooxidant agents such as LDL [18], hyperglycemia [95] and homocysteine [147]. Pretreatment with vitamin E (100 mg/day for 5 days) prevented the elevation of plasma ADMA induced by the injection of LDL in the rat [73]. Saran et al. [132] observed that vitamin E (800 U/day for 8 weeks) decreased plasma ADMA in 8 patients with chronic renal failure by 14%. However, ADMA concentration after treatment was still significantly higher than in the control group. In contrast to renal failure patients, vitamin E had no effect on ADMA in healthy persons. Although plasma SDMA was more than twofold higher in patients with chronic kidney disease, it was not reduced by vitamin E [132]. Morimoto et al. [110] have recently reported that hemodialysis through vitamin E-coated polysulfone membranes decreased plasma ADMA level by 27%, which was accompanied by significant reduction of oxLDL. In contrast, hemodialysis through

similar membranes not coated with vitamin E did not change ADMA or oxLDL.

A hypocholesterolemic drug, probucol, is also the most potent lipophilic antioxidant known. Jiang et al. [73] have demonstrated that probucol administered at a dose of 75 or 150 mg/kg/day for 5 days significantly inhibited the elevation of ADMA induced by LDL in the rat. *In vitro*, probucol had no effect on baseline ADMA formation by cultured endothelial cells but attenuated the increase in ADMA accumulation induced by oxLDL [126]. However, administration of probucol for 3 weeks at 100 mg/kg/day had no effect on plasma ADMA in cynomolgus monkeys [126]. In addition, although probucol (500 mg/day for 3 months) improved flow-induced (NO-dependent) relaxation of brachial artery in 9 patients with ischemic heart disease, it had no effect on plasma ADMA [153]. Thus, although some experimental studies suggest the beneficial effect of probucol on ADMA metabolism, its impact *in vivo* remains unproven at present.

Three studies addressed the effect of plant-derived antioxidants [69–71]. In cultured endothelial cells, daviditin A [70], demethylbellidifolin (DMB, 1,3,5,8-tetrahydroxanthone) [71] or 1,3,5,6-tetrahydroxanthone [69] partially prevented lysophosphatidylcholine-induced decrease in DDAH activity and increase in ADMA accumulation. *In vivo*, DMB administered at 60–120 mg/kg significantly inhibited the elevation of plasma ADMA induced by LDL in the rat [71].

Hormonal replacement therapy (HRT)

Estrogens have many beneficial effects in the cardiovascular system which results in lower incidence of ischemic heart disease in women before menopause than in men [45]. That ADMA metabolism may be regulated by estrogens is suggested by the observations that: (1) ADMA concentration is lower in females before menopause than in males, (2) in females ADMA increases after the menopause [137], (3) plasma concentration of ADMA drops markedly during normal pregnancy which is a hyperestrogenic state [125]. *In vitro*, 17β -estradiol significantly reduced ADMA concentration in the medium of cultured endothelial cells which was accompanied by the increase in DDAH activity [59]. Estradiol had no effect on DDAH1 protein expression. These data indicate that estradiol improves the specific activity of DDAH1, although its effect on the expression of DDAH2 cannot be excluded. Interestingly, in contrast

to many agents tested in other *in vitro* studies, estradiol increased baseline DDAH activity and reduced ADMA in cells not challenged with oxidized lipoproteins, inflammatory cytokines, homocysteine, etc.

The results of both animal and human studies addressing the effect of HRT on ADMA *in vivo* are inconsistent. In male rats, 17 β -estradiol administered at 0.1–0.3 mg/kg for 5 days prevented the elevation of plasma ADMA induced by subsequent injection of LDL [37]. In female rats, ovariectomy augmented the accumulation of ADMA in regenerating endothelial cells induced by balloon injury of the carotid artery, and estradiol replacement reversed this effect [63]. However, in another study neither ovariectomy nor estradiol supplementation had any effect on plasma ADMA [123]. The reasons of these discrepancies are unclear but the effect of estrogens may be tissue-specific since it has been demonstrated that estradiol decreases DDAH activity and increases ADMA as well as L-NMMA concentrations in urethra of ovariectomized rabbits [117].

The effect of HRT on endogenous methylarginines was studied in several clinical trials [59, 127, 159, 163]. Plasma ADMA concentration decreased by 19% in 15 postmenopausal women during 2 weeks after the insertion of a 100 mg ethynylestradiol implant [59]. Mean plasma SDMA concentration also decreased by almost 20% but this difference did not reach statistical significance. Teerlink et al. [154] demonstrated a 6–10% decrease in ADMA in 15 hysterectomized postmenopausal women following HRT with a conjugated equine estrogen (CEE, 0.625 mg/day for 6, 12 or 24 months). CEE had no effect on SDMA or L-arginine concentrations. In the subsequent study [127], the same group examined the effect of oral 17 β -estradiol administered either alone or in combination with progestagens in nonhysterectomized, normotensive, nonobese and otherwise healthy postmenopausal women. Estradiol administered either alone (2 mg/day) or together with a progestagen, dydrogesterone (10 mg/day for the last 14 days of each 28-day cycle) induced a significant but very modest (~5%) decrease in plasma ADMA after 4 or 12 weeks of therapy. Estradiol combined with another progestagen, trimegestone (0.5 mg/day for the last 14 days of each 28-day cycle) caused a more marked decrease in plasma ADMA (–19%). However, a concomitant decrease in plasma L-arginine was observed in all three groups. Consequently, L-arginine/ADMA ratio, which is more relevant for NO synthesis than absolute

ADMA concentration, did not change in women receiving estradiol alone or estradiol with dydrogesterone and decreased by about 20% in the group treated with estradiol and trimegestone. In a recent randomized placebo-controlled study performed on healthy postmenopausal women [163], 17 β -estradiol (1 mg/day) administered orally for 4 months or 1 year decreased plasma ADMA by 6% and 8%, respectively. The similar reduction was observed in a group receiving estradiol with progestagen, gestodene (25 μ g/day). However, arginine concentration was simultaneously decreased so that arginine/ADMA ratio did not change. Transdermally administered estradiol (50 μ g/day) caused a more modest decrease in ADMA (–4% after 1 year of therapy) but no significant change in arginine/ADMA ratio was noted also in this group. Thus, despite reducing the absolute ADMA level, the overall effect of HRT might be either neutral or even negative. In a recent study, administration of phytoestrogens (soy isoflavones 50 mg/day for 8 weeks) had no effect on plasma ADMA in 89 healthy postmenopausal women [130].

In conclusion, although four interventional studies in humans demonstrated that HRT reduced ADMA concentration, the magnitude of this reduction varied depending on specific treatment schedule (type and route of administration of estrogen, addition and type of progestagen, duration of treatment). In addition, baseline profile of risk factors (plasma lipids, homocysteine, and oxidative stress) may be important, since many of them affect ADMA and are *per se* modulated by HRT. It should be noted that large prospective trials performed so far suggest that although HRT may favorably modulate individual cardiovascular risk factors, its net effect on cardiovascular morbidity and mortality is either neutral [55] or even harmful [131].

Aspirin

Aspirin is the most widely prescribed nonsteroidal antiinflammatory drug and also possesses significant antiatherosclerotic properties due to its antioxidant and antiplatelet effects [36]. Deng et al. [41] have recently demonstrated that pretreatment with aspirin (30 mg/kg for 5 days) prevented the increase in plasma ADMA induced by intravenous administration of isolated human LDL in the rat. In addition, aspirin partially corrected the LDL-induced decrease in DDAH activity in erythrocytes [41].

ADMA accelerates senescence (irreversible growth arrest) of cultured endothelial cells [134]. The amount of ADMA increases and DDAH activity decreases progressively with aging of cultured cells whereas aspirin slows this process [14]. Interestingly, in contrast to aspirin, other nonsteroidal antiinflammatory drugs including ibuprofen and acetaminofen aggravate the deleterious effect of cell aging on ADMA metabolism. The beneficial effect of aspirin is not related to the inhibition of cyclooxygenase but rather results from its antioxidant properties [14].

Homocysteine-lowering vitamins

Homocysteine is a nonprotein amino acid, an intermediate product of methionine metabolism. The increased concentration of homocysteine is associated with several diseases including atherosclerosis, Alzheimer's disease, intrauterine growth restriction and congenital neural tube defects. Several studies have demonstrated that homocysteine stimulates ADMA formation by cultured cells [147] and that plasma ADMA level is increased in animals and humans with hyperhomocysteinemia [17, 21, 146]. Plasma concentration of homocysteine may be lowered by treatment with cofactors of homocysteine-metabolizing enzymes: folic acid, vitamin B₆ and vitamin B₁₂. However, recent randomized trials suggest that although these vitamins decrease homocysteine level, they fail to prevent acute cardiovascular events [38].

Until now, one animal [17] and four clinical studies [43, 60, 152, 173] addressed the effect of homocysteine-lowering vitamins on plasma ADMA level. In cynomolgus monkeys made hyperhomocysteinemic by high-methionine diet (2.7-fold increase in plasma homocysteine), administration of folic acid (5 mg/day), vitamin B₁₂ (4 mg/day) and vitamin B₆ (20 mg daily) for 6 months decreased plasma homocysteine to the control level but had no effect on ADMA concentration [17]. In 27 hyperhomocysteinemic patients with peripheral arterial occlusive disease, 8-week administration of folate (10 mg/day), vitamin B₁₂ (0.2 mg/day) and vitamin B₆ (20 mg/day) decreased plasma homocysteine by about 50% but had no effect on ADMA or SDMA concentrations [152]. In a double-blind randomized study performed in hyperhomocysteinemic patients [173], a mixture of vitamins B (folic acid 5 mg/day + B₆ 50 mg/day + B₁₂ 0.05 mg/day + B₁ 50 mg/day) administered for 6 weeks decreased

plasma homocysteine by almost 30% but did not alter arginine, ADMA or SDMA.

Treatment with fenofibrate usually results in a moderate elevation of plasma homocysteine and this effect may be abolished or at least reduced by concomitant administration of folic acid. Dierkes et al. [43] have demonstrated that neither fenofibrate alone nor fenofibrate plus B vitamins (0.65 mg/day of folic acid, 0.05 mg/day of vitamin B₁₂ and 5 mg/day of vitamin B₆) affected plasma ADMA or SDMA in patients with hypertriglyceridemia. However, addition of vitamins markedly reduced the fenofibrate-induced increase in homocysteine (from +44% to +13%).

Until now, only one study [60] demonstrated a successful reduction of plasma ADMA by folic acid. In that study performed on 21 subjects with hyperhomocysteinemia, folic acid was administered at 5 mg/day for 1 week followed by 1 mg/day for 37 weeks and 0.4 mg/day for the last 14 weeks (vitamins B₆ and B₁₂ were not used). Plasma homocysteine was reduced by about 50% after 6 weeks and 12 months. Median plasma ADMA at 6 weeks and 12 weeks was lower than at baseline by 70% and 85%, respectively. Although this was accompanied by a marked reduction of plasma L-arginine, NO generation was presumably improved since plasma concentration of NO metabolites significantly increased after treatment.

Omega-3 polyunsaturated fatty acids

The clinical benefit of ω -3 polyunsaturated fatty acids (n-3 PUFA) of marine origin is well recognized but the exact mechanism through which they exert cardioprotective effect is not clear [34, 57]. Raimondi et al. [128] have investigated the effect of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on plasma ADMA level in aged spontaneously hypertensive rats. It was observed that plasma ADMA was almost threefold lower in animals supplemented with EPA and DHA for 8 weeks than in the control group which received an equal amount of olive oil. In contrast, in the largest interventional study on ADMA performed so far, supplementation with n-3 PUFA at 2.4 g/day (EPA:DHA = 2:1) for 3 years did not alter plasma ADMA or SDMA in 487 hypercholesterolemic men. However, n-3 PUFA increased plasma arginine whereas a slight decrease in arginine was observed in a placebo group [47].

Retinoids

A vitamin A derivative, all-*trans*-retinoic acid (ATRA) is an agonist of retinoid A receptor (RAR) – a ligand-activated transcription factor which upon activation dimerizes with RXR and regulates the expression of target genes. In addition, ATRA may be metabolized to 9-*cis*-retinoic acid (9-*cis*RA) which is a natural ligand for RXR – a heterodimeric partner of RAR and PPAR. Recent experimental studies indicate that ATRA as well as synthetic RXR agonists exert many positive effects in the cardiovascular system, including inhibition of vascular smooth muscle cell proliferation, improvement of endothelial function, and attenuate the development of atherosclerosis in experimental models; these effects are often parallel to those of PPAR agonists [144]. Achan et al. [4] reported that ATRA and 9-*cis*RA increased DDAH-2 gene expression and enzymatic activity in cultured murine or porcine endothelial cells. In addition, ATRA reduced the accumulation of ADMA in culture medium but increased the accumulation of SDMA, probably due to enhancing protein turnover [4].

Erythropoietin

Recombinant human erythropoietin (EPO) is widely used in the treatment of anemia associated with chronic kidney disease. However, treatment with EPO may contribute to cardiovascular complications, in particular elevation of blood pressure [142]. It has been demonstrated that EPO increases the amount of ADMA and reduces DDAH activity in cultured endothelial cells [135]. The effects of EPO on ADMA and DDAH were accompanied by increased formation of reactive oxygen species and were prevented by antioxidants. In addition, a preliminary study has demonstrated that administration of EPO at a dose of 6,000 U/week increased plasma ADMA by 16% in 3 patients with renal failure who were not previously treated with this hormone. Taken together, these data suggest that erythropoietin may further impair ADMA metabolism in renal failure patients.

Is lowering of ADMA always beneficial?

Although NO deficiency is a hallmark of cardiovascular diseases, and cardiovascular researchers will pri-

marily look for the possibility to decrease ADMA level, there are some situations when NO generated in an inappropriate place or in excessive amounts is cytotoxic and ADMA might be protective by limiting NOS activity. In particular, NO generated by nNOS contributes to neuronal injury induced by stimulation of glutamate receptors during brain ischemia. nNOS-derived NO may also drive progressive neuronal death in some neurodegenerative disorders such as Alzheimer's disease [29]. It was demonstrated that preincubation with PRMT inhibitor, S-adenosylhomocysteine, aggravated injury of cerebellar neurons induced by the agonists of N-methyl-D-aspartate (NMDA) receptors [28]. SAH treatment was associated with the reduction of ADMA and L-NMMA and addition of exogenous NOS-inhibiting methylarginines abrogated NMDA-induced neuronal injury. The effect of cerebral ischemia on DDAH-ADMA pathway is unclear, however, several lines of evidence suggest that ischemia may increase ADMA concentration: (1) ischemia and/or hypoxia has been demonstrated to increase ADMA in peripheral tissues such as urethra [105], corpus cavernosum [104], lung [108] and skeletal muscle [3], (2) plasma ADMA is increased in patients with ischemic stroke [172] or acute coronary syndromes [8], (3) protein turnover is increased in injured neurons which might lead to increased release of preformed methylarginines. Thus, ADMA and/or L-NMMA might be protective by limiting NO generation in hypoperfused brain. Interestingly, Abe et al. [1] demonstrated that ADMA concentration in cerebrospinal fluid was lower in patients with Alzheimer's disease than in healthy controls. It was hypothesized that deficiency of ADMA might contribute to excessive production of NO and neuronal injury.

NO is a potent proangiogenic factor and may promote tumor vascularization [46]. ADMA inhibits angiogenesis [3], whereas overexpression of DDAH has an opposite effect [66]. In experimental studies, tumors overexpressing DDAH demonstrate increased growth rate and better vascularized phenotype [88]. High DDAH activity was also detected in some human tumors [87]. It was suggested that an increase in ADMA in patients with liver cirrhosis may be beneficial since it opposes peripheral vasodilation induced by excess of NO [96]. Finally, estrogens reduce DDAH activity in the urethra leading to accumulation of ADMA, which results in a decrease in NO-dependent urethral relaxation. The effect might be

beneficial in patients with urine incontinence often observed after the menopause [117].

Conclusions and future perspectives

After almost 15 years of research, there is little doubt that ADMA is synthesized in mammalian cells and may regulate NOS activity under physiological and pathological conditions. Although ADMA concentration is increased in many diseases in which NO deficiency has been implicated, many important questions still remain open. First, in most studies ADMA was measured only in plasma and little is known about its concentration inside the cells, which is more relevant for NOS function. Second, the increase in ADMA in most diseases (except renal failure and severe shock) is relatively small and it is unclear if this is sufficient to induce significant NOS blockade. Third, since ADMA is vigorously metabolized by the endothelium, one cannot exclude the possibility that elevation of ADMA is a consequence rather than the cause of endothelial dysfunction [107].

Keeping in mind all these uncertainties, the potential possibilities to modulate ADMA by pharmacotherapy are still of great interest. Angiotensin converting enzyme inhibitors and angiotensin AT₁ receptor antagonists are the drugs most consistently shown to reduce ADMA level in humans. In addition, reducing ADMA may contribute to the improvement of NO synthesis by some other medications including fibrates, thiazolidinediones, metformin, antioxidant vitamins, aspirin, n-3 polyunsaturated fatty acids and plant flavonoids, although the evidence of this is much weaker than for RAAS inhibitors. For some therapies like HRT, the results of various studies are inconclusive. Other drugs such as statins or homocysteine-lowering vitamins have unexpectedly no effect on ADMA in most experiments. Lack of effect or unbeneficial effect on ADMA may partially explain negative results of trials addressing the impact of HRT or homocysteine-lowering vitamins on clinical outcome. Finally, one has to remember that ADMA may also be unfavorably elevated following therapy as exemplified by erythropoietin.

Most human studies addressing the effect of pharmacotherapy on ADMA metabolism were performed on small groups of patients and were relatively short-

lasting. In addition, L-arginine was rarely measured, whereas L-arginine/ADMA ratio may be more important for NOS function than ADMA itself, and arginine concentration may be either increased [43] or decreased [60] by pharmacotherapy. In addition to classical therapies, agents affecting ADMA more specifically (e.g. PRMT inhibitors or DDAH inducers) await investigation. ADMA may also be targeted by non-pharmacological therapy such as DDAH gene transfer [39], positive airway pressure ventilation [116] or weight loss [90], however, these issues are beyond the scope of this review. Finally, the potential usefulness of agents increasing ADMA such as DDAH inhibitors should be considered in the context of unfavorable NO excess associated with septic shock, excitatory neurotoxicity or tumor angiogenesis.

Note added in proof

Recently, the second clinical study addressing the effect of fibrates on ADMA was published. In this study, Yang et al. (Eur J Clin Pharmacol, 200, 62, 179–84) have reported that fenofibrate administered at 200 mg/day for 8 weeks decreased plasma ADMA level by about 15% in patients with hypertriglyceridemia. In contrast to the study of Dierkes et al. [43], fenofibrate increased HDL-cholesterol which could account for the difference in results.

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Received:
March 13, 2006; in revised form: April 10, 2006.